Helsinki, 05 January 2023

Addressees
Registrant(s) of JS_Multi_910 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
27/01/2021

Registered substance subject to this decision ("the Substance")
Substance name: reaction mass of bis(2,3-epoxypropyl) terephthalate and tris(oxiranylmethyl) benzene-1,2,4-tricarboxylate
EC number: 940-592-6

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 ***10 October 2025***.

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 vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test also requested below (triggered by Annex VII, Section 8.4., column 2);

2. Short-term toxicity testing on aquatic invertebrates (Annex VII, Section 9.1.1.; test method: EU C.2./OECD TG 202).

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

3. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test also requested below (triggered by Annex VIII, Section 8.4., column 2);

4. Short-term toxicity testing on fish (Annex VIII, Section 9.1.3.; test method: EU C.1./OECD TG 203).

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

5. 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 (triggered by Annex IX, Section 8.4., column 2). For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum;

6. Long-term toxicity testing on aquatic invertebrates (Annex IX, Section 9.1.5.; test method: EU C.20./OECD TG 211);

7. Long-term toxicity testing on fish (Annex IX, Section 9.1.6.; test method: EU C.47./OECD TG 210).

The reasons for the decision(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 addressees 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. 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.

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

Reasons related to the information under Annex VII of REACH

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

1 Further mutagenicity studies must be considered under Annex VII, Section 8.4., column 2, in case of a positive result.

1.1. Triggering of the information requirement

2 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2004) and in vitro cytogenicity test (2014) and in vitro gene mutation study in mammalian cells (2014) which raise the concerns for gene mutation and chromosomal aberration.

3 Therefore, the information requirement is triggered.

4 In the comments to the draft decision you agree that the presence of positive findings in the *in vitro* gene mutation study in bacteria (2004), the *in vitro* cytogenicity study (2014) and the *in vitro* gene mutation study in mammalian cells (2014) warrant the conduct of *in vivo* follow up studies to clarify the potential for clastogenicity and mutagenicity *in vivo*.

1.2. Information requirement not fulfilled

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

2. Short-term toxicity testing on aquatic invertebrates

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

2.1. Information provided

7 You have provided a study on short-term toxicity to aquatic invertebrates (1996) with the Substance (study i).

2.2. Assessment of the information provided

8 To fulfil the information requirement, a study must comply with OECD TG 202 (Article 13(3) of REACH). Therefore, the following specifications must be met:

9 Technical specifications impacting the sensitivity/reliability of the test

a) young daphnids, aged less than 24 hours at the start of the test, are used;
b) at least five concentrations are tested. If less than five concentrations are included in the test design a justification must be provided.

10 Characterisation of exposure

c) 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 provided.

11 Reporting of the methodology and results

d) the number of immobilised daphnids is determined at 24 and 48 hours. Data are summarised in tabular form, showing for each treatment group and control, the number of daphnids used, and immobilisation at each observation;
e) the dissolved oxygen and pH measured at least at the beginning and end of the test is reported.

12 In study (i) described as short-term toxicity study on daphnids:

13 Technical specifications impacting the sensitivity/reliability of the test

a) the age of the daphnids was not specified;
b) the number of the tested concentrations was not specified.

14 Characterisation of exposure

c) no analytical monitoring of exposure was conducted.

15 Reporting of the methodology and results

d) tabulated data on the number of immobilised daphnids after 24 and 48 hours for each treatment group and control are not reported;
e) the dissolved oxygen and pH measured at least at the beginning and end of the test is not reported.

16 Based on the above,

- there are critical methodological deficiencies resulting in the rejection of the study results. Firstly, you did not specify the age of daphnids, due to which ECHA cannot assess if the sensitivity of the test was affected by the age of the applied daphnids. Secondly, you did not provide information on the tested concentrations or tabulated data. Therefore, you have not demonstrated that the provided effect values are reliable. Thirdly, you did not have analytical monitoring confirming the exposure concentrations.
- the reporting of the study is not sufficient to conduct an independent assessment of its reliability, because you did not provide relevant information on the test conditions (dissolved oxygen, pH) or tabulated data.

17 Therefore, the requirements of OECD TG 202 are not met and the information requirement is not fulfilled.

18 In the comments to the draft decision, instead of performing a new OECD TG 202 study as requested, you propose to perform the long-term toxicity on aquatic invertebrates (OECD TG 211) requested in Section 6.

19 REACH Annex VII section 9.1.1 column 2 specifies that the short-term toxicity study does not need to be conducted if a long-term aquatic toxicity study on invertebrates is available. At present no long-term toxicity study on invertebrates is provided in the IUCLID dossier, therefore no conclusion on the compliance can currently be made. Please note that this decision does not consider updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation). You remain responsible for complying with this decision by the set deadline.

2.3. Study design and test specifications

20 Based on the information provided in the dossier, the Substance is considered as difficult to test due to its low water solubility. In particular, the Substance is a multi-constituent and

you have provided a water solubility study (OECD TG 105), where the water solubility of the Substance was determined to be 172 mg/L. However, you have also provided a growth inhibition study on aquatic plants (OECD TG 201 and OECD No 23) where you state that there was "low limit of water solubility of the test item". You provide analytical monitoring results demonstrating that the measured concentrations are notably lower than the nominal concentrations supporting your statement on low water solubility.

21 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 the Substance, it may be difficult to achieve and maintain the desired exposure concentrations. Therefore, you must monitor the test concentration(s) of the Substance throughout the exposure duration and report the results. 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. 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.

22 For multi-constituents/UVCBs, the analytical method must be adequate to monitor qualitative and quantitative changes in exposure to the dissolved fraction of the test material during the test (e.g. by comparing mass spectral full-scan GC or HPLC chromatogram peak areas or by using targeted measures of key constituents or groups of constituents).

23 If you decide to use the Water Accommodated Fraction (WAF) approach, in addition to the above, you must:

- use loading rates that are sufficiently low to be in the solubility range of most constituents (or that are consistent with the PEC value). This condition is mandatory to provide relevant information for the hazard and risk assessment (Guidance on IRs and CSA, Appendix R.7.8.1-1, Table R.7.8-3);
- provide a full description of the method used to prepare the WAF (including, among others, loading rates, details on the mixing procedure, method to separate any remaining non-dissolved test material including a justification for the separation technique);
- prepare WAFs separately for each dose level (i.e. loading rate) and in a consistent manner.

Reasons related to the information under Annex VIII of REACH

3. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

24 Appropriate in vivo mutagenicity studies must be considered under Annex VIII, Section 8.4., column 2 in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII.

3.1. Triggering of the information requirement

25 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2004) and in vitro cytogenicity test (2014) and in vitro gene mutation study in mammalian cells (2014) which raise the concerns for gene mutation and chromosomal aberration.

26 Therefore, the information requirement is triggered.

27 In the comments to the draft decision you agree that the presence of positive findings in the *in vitro* gene mutation study in bacteria (2004), the *in vitro* cytogenicity study (2014) and the *in vitro* gene mutation study in mammalian cells (2014) warrant the conduct of *in vivo* follow up studies to clarify the potential for clastogenicity and mutagenicity *in vivo*.

3.1. Information requirement not fulfilled

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

4. Short-term toxicity testing on fish

29 Short-term toxicity testing on fish is an information requirement under Annex VIII to REACH (Section 9.1.3.).

4.1. Information provided

30 You have provided a study on short-term toxicity to fish (1996) with the Substance (study i).

4.2. Assessment of the information provided

31 To fulfil the information requirement, a study must comply with OECD TG 203 (Article 13(3) of REACH). Therefore, the following specifications must be met:

32 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 provided

33 In study (i) described as a short-term toxicity study on fish:

34 Characterisation of exposure

a) no analytical monitoring of exposure was conducted.

35 Based on the above, as there was no analytical monitoring confirming the exposure concentrations, this critical methodological deficiency results in the rejection of the study results.

36 Therefore, the information requirement is not fulfilled.

37 In the comments to the draft decision, instead of performing a new OECD TG 203 study as requested, you propose to perform the long-term toxicity on fish (OECD TG 210) requested in Section 7.

38 REACH Annex VIII section 9.1.3 column 2 specifies that the short-term toxicity study does not need to be conducted if a long-term aquatic toxicity study on fish is available. At present no long-term toxicity study on fish is provided in the IUCLID dossier, therefore no conclusion on the compliance can currently be made. Please note that this decision does not consider updates of the registration dossiers after the date on which you were notified of the draft decision according to Article 50(1) of REACH (see section 5.4. of ECHA's Practical Guide "How to act in Dossier Evaluation). You remain responsible for complying with this decision by the set deadline.

4.3. Study design and test specifications

39 OECD TG 203 specifies that, for difficult to test substances, OECD GD 23 must be followed. As already explained above, the Substance is considered as difficult to test due to low water solubility observed in the growth inhibition study on aquatic plants. Therefore, you must fulfil the requirements described in 'Study design and test specifications' under Request 2.

Reasons related to the information under Annex IX of REACH

5. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

40 Under Annex IX, Section 8.4., column 2, the information requirement for an appropriate in vivo somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the in vitro genotoxicity studies in Annex VII or VIII and 2) there are no appropriate results already available from an in vivo somatic cell genotoxicity study.

5.1. Triggering of the information requirement

41 In relation to the first condition, your dossier contains positive results for the *in vitro* gene mutation study in bacteria (2004) and *in vitro* cytogenicity test (2014) and *in vitro* gene mutation study in mammalian cells (2014) which raise the concerns for gene mutation and chromosomal aberration.

42 In relation to the second condition, as explained under section 5.3, your dossier does not contain appropriate data from an *in vivo* somatic cell genotoxicity study.

43 In the comments to the draft decision you agree that the presence of positive findings in the *in vitro* gene mutation study in bacteria (2004), the *in vitro* cytogenicity study (2014) and the *in vitro* gene mutation study in mammalian cells (2014) warrant the conduct of *in vivo* follow up studies to clarify the potential for clastogenicity and mutagenicity *in vivo*.

44 Therefore, the information requirement is triggered.

5.2. Information provided

45 You have adapted this information requirement by applying weight of evidence (WoE) adaptation(s) under Annex XI, Section 1.2 based on the following experimental data:

(i) *In vivo* micronucleus test (OECD TG 474) conducted with the analogue substance EC 230-565-0 (1993);
(ii) *In vivo* micronucleus test (OECD TG 474) conducted with the analogue substance EC 230-638-7 (1993);
(iii) *In vivo* spermatogonial chromosome aberration test (OECD TG 483) conducted with the analogue substance EC 230-565-0 (1993);
(iv) *In vivo* spermatogonial chromosome aberration test (OECD TG 483) conducted with the analogue substance EC 230-638-7 (1993).

5.3. Assessment of the information provided

46 We have assessed this information and identified the following issue(s):

47 Annex XI, Section 1.2 states that there may be sufficient weight of evidence from several independent sources of information enabling, through a reasoned justification, a conclusion on the information requirement, while the information from each single source alone is insufficient to fulfil the information requirement.

48 The justification must have regard to the information that would otherwise be obtained from the study that must normally be performed for this information requirement.

49 According to ECHA Guidance R.4, a weight of evidence adaptation involves an assessment of the relative values/weights of the different sources of information submitted. The weight

given is based on the reliability of the data, consistency of results/data, nature and severity of effects, and relevance and coverage of the information for the given regulatory information requirement. Subsequently, relevance, reliability, coverage, consistency and results of these sources of information must be balanced in order to decide whether they together provide sufficient weight to conclude on the corresponding information requirement.

50 Annex XI, section 1.2 requires that adequate and reliable documentation is provided to describe your weight of evidence approach. This documentation must include robust study summaries of the studies used as sources of information and a justification explaining why the sources of information together provide a conclusion on the information requirement.

51 You have not included a justification for your weight of evidence adaptation, which would include an adequate and reliable (concise) documentation as to why the sources of information provide sufficient weight to conclude on the information requirements under consideration.

52 In spite of this critical deficiency, ECHA has nevertheless assessed the validity of your adaptation. Your weight of evidence approach has deficiencies that are common to all information requirements under consideration and also deficiencies that are specific for these information requirements individually.

53 Relevant information that can be used to support weight of evidence adaptation for this information requirement, in case of positive results raising the concerns for both gene mutation and chromosomal aberration, includes similar information that is produced by the combination of OECD TG 489 with OECD TG 474.

54 The OECD TG 474 investigates the following key element(s):

(1) cytogenetic damage in somatic cells of animals (usually rodents).

55 The OECD TG 489 investigates the following key element(s):

(2) primary DNA damage that could lead to gene mutations and/or cytogenetic damage in somatic cells of animals (usually rodents), and

(3) DNA damage in the tissues at first site of contact, for example stomach after oral exposure and lungs after inhalation exposure.

56 We have assessed the individual sources of information with regard to relevance and reliability and identified the following issue(s):

5.3.1. Coverage of key elements investigated in the OECD TG 474

57 Sources of information (iii) and (iv) investigate germ cells, so they do not provide information on cytogenetic damage in somatic cells (key element 1).

58 Sources of information (i) and (ii) provide relevant information on cytogenetic damage in somatic cells (key element 1) but their reliability is significantly affected by the following deficiency:

59 The test materials in studies (i-ii) are different from the Substance. Therefore, ECHA understands that you use data obtained with analogue substances in a read-across approach as part of your weight of evidence adaptation. For this information to reliably contribute to the weight of evidence approaches, it would have to meet the requirements for Grouping of substances and read-across approaches.

60 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.

61 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).

62 You predict the toxicological properties of the Substance from information obtained from the following analogue substance(s):

- bis(2,3-epoxypropyl) terephthalate; EC 230-565-0
- tris(oxiranylmethyl) benzene-1,2,4-tricarboxylate; EC 230-638-7

63 You provide the following reasoning for the prediction of this information requirement: "*The two main compounds were tested separately for mutagenicity in vivo. For each compound negative results were obtained in an in vivo micronucleus study performed with rats and in an in vivo germ-cell cytogenetic study performed with mice*."

64 On the basis of this information ECHA understands that you have applied a constituent-based approach whereby you conclude on the properties of the Substance using the results obtained from independent studies conducted with source substances identified as two of the main constituents of the Substance.

65 We have identified the following issue with the prediction of toxicological properties:

66 Hazard information can be obtained from tests conducted with the Substance or from the integration of information on the individual constituents of the Substance as part of a constituent-based approach (Guidance on IRs and CSA, Section R.6.2.2.1.). Whenever a constituent-based approach is applied, the assessment must cover each relevant constituent included in the composition of the Substance to ensure that a reliable prediction can be made. In case certain constituents are considered not to be relevant for the hazard assessment, a justification must be provided.

67 The Substance is a multi-constituent substance. You have indicated that the composition of the Substance contains following constituents:

68 [redacted] You further indicate that the degree of purity of the boundary composition is between [redacted] (w/w).

69 ECHA notes that the *in vitro* study showing a concern for cytogenicity of the Substance was conducted with the Substance. However, the *in vivo* somatic cell studies (studies i-ii) that you have provided are conducted with two constituents of the Substance which only cover [redacted] (w/w) of the Substance composition.

70 You have not provided hazard data or a justification that the remaining composition of the Substance not covered by the two constituents [redacted] (w/w)) are irrelevant for the purpose of hazard identification.

71 In the absence of information or justification for the remaining composition of the Substance, no reliable conclusions on the hazardous properties of the Substance as a whole can be derived.

72 ECHA understands from your comments on the draft decision that you consider that "*the Weight of Evidence supporting the use of a constituent-based read across approach could be built for the endpoint of clastogenicity based on structural and biological similarity (common mechanism)*", in order to address the deficiencies in your adaptation identified in

the draft decision. According to the information provided in your comments, such a weight of evidence could be based on the following elements::

- comparable reactivity of all constituents of the Substance via the same mechanism supported by epoxy group reactivity as a common mechanism for DNA reactivity;
- lack of other structural alerts in OECD QSAR Toolbox endpoint specific *in vivo* mutagenicity (micronucleus) alerts by ISS and the DNA alerts for AMES, CA and MNT by OASIS profilers; and
- expected differences in bioavailability of the constituents in the Substance based on their different structure and molecular weight.

73 ECHA acknowledges your considerations on potential ways to develop or consolidate a weight of evidence adaptation for the information requirement under consideration. Your comments highlight elements that you consider could form the basis for such a weight of evidence adaptation. However, you have not provided any evidence supporting your claims and establishing, when taken together, that the remaining composition of the Substance not covered by the two constituents [redacted] (w/w)) are irrelevant for the purpose of hazard identification. Therefore, the information in your comments is not sufficient for ECHA to make an assessment of your intended weight of evidence, and the deficiencies of the currently available information, as identified above, remain.

5.3.2. Coverage of key elements investigated in the OECD TG 489

74 None of the provided sources of information investigate primary DNA damage that could lead to gene mutations and/or cytogenetic damage but rather these studies evaluated cytogenetic damage only. Therefore, these studies do not provide any information on the potential of the Substance to cause gene mutations (key element 2).

75 None of the provided sources of information investigate DNA damage in first site of contact tissues (key element 3) as sources of information (i) and (ii) investigate bone marrow erythrocytes and sources of information (iii) and (iv) investigate germ cells.

5.4. Conclusion on weight of evidence approach

76 Taken together, the sources of information provide information only on key element 1 as they provide information on cytogenetic damage in somatic cells. However, any robust conclusion on this key element is hampered by the reliability of the contribution of the information on analogue substances.

77 None of the sources of information provide information on key element 2 (DNA damage that could lead to gene mutations and/or cytogenetic damage) and key element 3 (DNA damage in first site of contact tissues).

78 It is not possible to conclude, based on any source of information alone or considered together, whether your Substance has or has not the particular dangerous properties foreseen to be investigated in an in vivo mammalian alkaline comet assay (OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (OECD TG 474).

79 You have not provided any new evidence in your comments that would alleviate the deficiencies identified above, and therefore, the conclusion of the assessment has not changed.

80 Therefore, your adaptation is rejected and the information requirement is not fulfilled.

5.5. Test selection

81 The positive in vitro results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

82 The in vivo mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (see OECD TG 474 paragraph 37c; OECD TG 489 paragraph 33; Guidance on IRs & CSA, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations. A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

83 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. Furthermore, the combined study can help reduce the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

84 Therefore, the comet assay combined with the MN test is the most appropriate study for the Substance.

85 In the comments to draft decision, you acknowledge that both endpoints, *in vivo* mutagenicity and *in vivo* clastogenicity, can be addressed by the study proposed by the current ECHA decision. You also propose to include an option on Pig-a Gene Mutation assay (adopted 30th June, 2022) which could be integrated with the erythrocyte MN assay to allow for mutagenicity and clastogenicity to be evaluated in the same assay. ECHA understands that you propose in your comments to include the option to address the data gap using a Pig-A assay combined with the MN test as an alternative to the requested *in vivo* mammalian alkaline comet assay combined with *in vivo* mammalian erythrocyte micronucleus test.

86 Considering the expected high reactivity of the Substance (epoxide) and the metabolism of epoxides in the liver, the detection of any potential effects at the site of contact such as stomach or duodenum and in distant organs such as liver is critical. However, the combined Pig-a + MN test you proposed would not allow investigations at the site of contact or in the organ such as liver as it enables to investigate gene mutation and clastogenicity/ aneugenicity in the bone marrow only. On the other hand, the combined comet and MN test as currently requested allows to investigate DNA damages at two sites of contact (stomach and duodenum) and in liver, and clastogenicity/ anaugenicity in the bone marrow, and therefore. Therefore, ECHA does not agree with your proposal to expand the test selection, as the proposed combination will not provide appropriate information for the identified concerns in light of the Substance properties.

87 Based on above, ECHA has not modified the request.

5.6. Specification of the study design

88 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.

89 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.

90 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, 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.

91 The combination of the 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]).

5.6.1. Germ cells

92 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.

93 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.

94 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.

[1] Bowen DE 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. *Muta Res.*;722:7–19.

6. Long-term toxicity testing on aquatic invertebrates

95 Long-term toxicity testing on aquatic invertebrates is an information requirement under Annex IX to REACH (Section 9.1.5.).

6.1. Information provided

96 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following information: "In accordance with column 2 of REACh (Regulation (EC) No 1907/2006) Annex IX, the long-term toxicity testing on invertebrates (required in section 9.1.5) does not need to be conducted based on the findings of the Chemical Safety Assessment; the substance does not fulfill classification criteria according to the applicable regulations and does not fulfill the criteria for vPvB or PBT."

6.2. Assessment of the information provided

6.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

97 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to aquatic invertebrates under Column 1. It must be understood as a trigger for providing further information on aquatic invertebrates if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

98 Your adaptation is therefore rejected and the information requirement is not fulfilled.

99 In the comments to the draft decision, you agree to perform the requested study.

6.3. Study design and test specifications

100 OECD TG 211 specifies that, for difficult to test substances, OECD GD 23 must be followed. As already explained above, the Substance is considered as difficult to test due to low water solubility observed in the growth inhibition study on aquatic plants. Therefore, you must fulfil the requirements described in 'Study design and test specifications' under Request 2.

7. Long-term toxicity testing on fish

101 Long-term toxicity testing on fish is an information requirement under Annex IX to REACH (Section 9.1.6.).

7.1. Information provided

102 You have adapted this information requirement by using Column 2 of Annex IX, Section 9.1. To support the adaptation, you have provided following information: "In accordance with column 2 of REACh (Regulation (EC) No 1907/2006) Annex IX, the long-term toxicity testing on fish (required in section 9.1.6) does not need to be conducted based on the findings of the Chemical Safety Assessment; the substance does not fulfill classification criteria according to the applicable regulations and does not fulfill the criteria for vPvB or PBT."

7.2. Assessment of the information provided

7.2.1. Annex IX, Section 9.1., Column 2 is not a valid basis to omit the study

103 Annex IX, Section 9.1., Column 2 does not allow omitting the need to submit information on long-term toxicity to fish under Column 1. It must be understood as a trigger for providing further information on long-term toxicity to fish if the chemical safety assessment according to Annex I indicates the need (Decision of the Board of Appeal in case A-011-2018).

104 Your adaptation is therefore rejected.

105 Therefore, the information requirement is not fulfilled.

106 In the comments to the draft decision, you agree to perform the requested study.

7.1. Study design and test specifications

107 To fulfil the information requirement for the Substance, the Fish, Early-life Stage Toxicity Test (test method OECD TG 210) is the most appropriate (Guidance on IRs and CSA, Section R.7.8.2.).

108 OECD TG 211 specifies that, for difficult to test substances, OECD GD 23 must be followed. As already explained above, the Substance is considered as difficult to test due to low water solubility observed in the growth inhibition study on aquatic plants. Therefore, you must fulfil the requirements described in 'Study design and test specifications' under Request 2.

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 request(s).

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: Addressees 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]

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 all constituents of each Test Material and their concentration values and other parameters relevant for the property to be tested.

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

This information is needed to assess 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[3].

2. General recommendations for conducting and reporting new tests

2.1. Environmental testing for substances containing multiple constituents

Your Substance contains multiple constituents and, as indicated in Guidance on IRs & CSA, Section R.11.4.2.2, you are advised to consider the following approaches for persistency, bioaccumulation and aquatic toxicity testing:

- the "known constituents approach" (by assessing specific constituents), or
- the "fraction/block approach, (performed on the basis of fractions/blocks of constituents), or
- the "whole substance approach", or
- various combinations of the approaches described above

Selection of the appropriate approach must take into account the possibility to characterise the Substance (i.e. knowledge of its constituents and/or fractions and any differences in their properties) and the possibility to isolate or synthesize its relevant constituents and/or fractions.

References to Guidance on REACH and other supporting documents can be found in Appendix 1.

[3] https://echa.europa.eu/manuals