~~Confidential~~

Helsinki, 23 November 2022

Addressees
Registrant(s) of JS_221-518-5 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
21/05/2018

Registered substance subject to this decision ("the Substance")
Substance name: Bis[(3,4-epoxycyclohexyl)methyl] adipate
EC/List number: 221-518-5

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 the deadline of ***2 March 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. Skin sensitisation (Annex VII, Section 8.3.)
   i. *in vitro/in chemico* skin sensitisation information on molecular interactions with skin proteins (OECD TG 442C), inflammatory response in keratinocytes (OECD TG 442D) and activation of dendritic cells (EU B.71/OECD TG 442E)(Annex VII, Section 8.3.1.); and
   ii. Only if the *in vitro/in chemico* test methods specified under point 1.(i.) are not applicable for the Substance or the results obtained are not adequate for classification and risk assessment, *in vivo* skin sensitisation (Annex VII, Section 8.3.2.; test method: EU B.42./OECD TG 429);

2. Same in vivo genetic toxicity study requested below in 6

3. Long-term toxicity testing on aquatic invertebrates (triggered by Annex VII, Section 9.1.1., column 2; test method: EU C.20./OECD TG 211)

4. Ready biodegradability (Annex VII, Section 9.2.1.1.; test method: EU C.4. A/B/C/D/E/F/OECD TG 301A/B/C/D/E/F or EU C.29./OECD TG 310)

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

5. In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.; test method: OECD TG 473) or In vitro micronucleus study (Annex VIII, Section 8.4.2.; test method: OECD TG 487)

6. In vivo genetic toxicity study (Annex VIII, Section 8.4., column 2) (triggered to be selected according to the following specifications:

i. If the results of the in vitro test requested under 5. are **negative**:

Transgenic rodent somatic and germ cell gene mutation assay (Annex VII, Section 8.4., Column 2; 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 (Annex VII, Section 8.4., Column 2; test method: EU B.62./OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum;

ii. If the results of the in vitro test 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 shall be analysed: liver, glandular stomach and duodenum.

7. Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.) to be combined with the Screening for reproductive/developmental toxicity below

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

9. Long-term toxicity testing on fish (triggered by Annex VIII, Section 9.1.3., column 2; test method: EU C.47./OECD TG 210)

10. Hydrolysis as a function of pH (Annex VIII, Section 9.2.2.1.; test method: EU C.7./OECD TG 111)

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.

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 decision
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 decision

Contents

0. Reasons common to several requests

0.1. Assessment of test material

1 To comply with this information requirement, the test material in a study must be representative for the Substance that is intended to be tested; Article 10 and Recital 19 of REACH; Guidance on IRs and CSA, Section R.4.1.

2 For the endpoints listed below, you have identified the test material as "Cycloaliphatic Epoxy Resin ERL-4221", in some cases without further information, including composition of the test materials.

- Skin sensitisation (Annex VII, Section 8.3.)
- In vitro cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2.)
- In vivo genetic toxicity study (Annex VII, VIII, Section 8.4., Column 2
- Short-term repeated dose toxicity (28 days; Annex VIII, Section 8.6.1.)
- Screening for reproductive/developmental toxicity (Annex VIII, Section 8.7.1.)

3 In the absence of composition information on the test materials, the identity of the test material and its impurities cannot be assessed and you have not demonstrated that the test materials are representative for the Substances tested.

4 Therefore, the information provided is rejected.

0.2. Assessment of the read-across approach

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

- Skin sensitisation (Annex VII, Section 8.3.)
- 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.)
- Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.)

6 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.

7 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.

8 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 toxicological properties

9 You have not provided a read-across justification document.

10 You predict the properties of the Substance from information obtained from the following source substance:

Cycloaliphatic Epoxy Resin ERL-422 1, EC No. 219-207-4

11 You provide the following reasoning for the prediction of toxicological properties: "*read-across to the source substance 7-oxabicyclo[4.1.0]hept-3-ylmethyl 7-oxabicyclo[4.1.0]heptane-3-carboxylate (CAS Number: 2386-87-0;EC Number:219-207-4) based on the presence of same functional groups and close structural similarity*".

12 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 substance.

13 We have identified the following issue(s) with the prediction(s) of toxicological properties:

0.1.1.1. Inadequate read-across hypothesis

14 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 include an explanation why the properties of the Substance may be predicted from other substances in the group, i.e. a read-across hypothesis. This hypothesis should be based on recognition of the structural similarities and differences between the substances (Guidance on IRs and CSA, Section R.6.).It should also explain why the differences in the chemical structures should not influence the toxicological properties or should do so in a regular pattern, taking into account that variations in chemical structure can affect both toxicokinetics (uptake and bioavailability) and toxicodynamics (e.g. interactions with receptors and enzymes) of substances (Guidance on IRs and CSA, Section R.6.2.1.3).

15 Your read-across hypothesis is only based on the structural similarity between the source substance(s) and the Substance, which you consider a sufficient basis for predicting the properties of the Substance. However, your hypothesis does not explain why the structural differences between the substances do not influence the toxicological properties or do so in a regular pattern.

16 While structural similarity is a prerequisite for applying the grouping and read-across approach, it does not necessarily lead to predictable or similar toxicological properties. You have not provided a well-founded hypothesis to establish a reliable prediction for a toxicological property, explaining why the structural differences do not influence toxicokinetics and toxicodynamics of the substances, and thus why the properties of the Substance may be predicted from information on the source substance(s).

0.1.1.2. Missing supporting information

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 to compare properties of the Substance and source substances.

19 As indicated above, your read-across hypothesis is based on the assumption that the structurally similar substances cause the same type of effect(s). In this context, relevant, reliable and adequate information allowing to compare the properties of the Substance and of the source substance(s) is necessary to confirm that both 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 For the source substance, you provide a repeated dose toxicity, an in vitro cytogenicity study, an in vitro gene mutation, a screening study and a prenatal developmental toxicity study used in the prediction in the registration dossier. Apart from those studies, neither your read-across justification nor the registration dossier include any robust study summaries or descriptions of data for the Substance that would confirm that both substances cause the same type of effects.

21 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

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

Reasons related to the information under Annex VII of REACH

1. Skin sensitisation

23 Skin sensitisation is an information requirement under Annex VII, Section 8.3. Under Section 8.3., Column 1, the registrants must submit information allowing (1) a conclusion whether the substance is a skin sensitiser and (2) whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

1.1. Information provided

24 You have adapted this information requirement by using a Grouping of substances and read-across approach based on experimental data from the following substances:

(i) a guinea pig maximisation test (1991) with the analogue substance Cycloaliphatic Epoxy Resin ERL-422 1, no EC number provided

1.2. Assessment of the information provided

1.2.1. Unclear test material

25 As explained in Section 0.1., the test material used for the study is not clear, and ECHA therefore cannot verify the reliability of the provided information.

1.2.2. Read-across adaptation rejected

26 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

27 As a result, the study (i) you submitted cannot be taken into account in support of the adaptation of this information requirement under Column 2 of Annex VII, Section 8.3.1.

28 On this basis, the information requirement is not fulfilled.

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

1.3. Specification of the study design

30 To fulfil the information requirement for the Substance, information on molecular interaction with skin proteins and inflammatory response in keratinocytes and activation of dendritic cells (OECD TG 442C and OECD TG 442D and EU B.71/OECD TG 442E) must be provided. Furthermore an appropriate risk assessment is required if a classification of the Substance as a skin sensitiser (Cat 1A or 1B) is warranted.

31 In case no conclusion on the skin sensitisation potency can be made for the Substance based on the existing data or newly generated in vitro data, in vivo skin sensitisation study must be performed and the murine local lymph node assay (EU Method B.42/OECD TG 429) is considered as the appropriate study for the potency estimation.

2. In vivo genetic toxicity study

32 Further mutagenicity studies must be considered under Annex VII, Section 8.4., Column 2, in case of a positive result in an in vitro gene mutation study in bacteria.

2.1. Triggering of the information requirement

33 Your dossier contains positive results for the in vitro gene mutation study in bacteria (1988) which raise the concern for gene mutations.

34 ECHA considers that an appropriate in vivo follow up genetic toxicity study is necessary to address the concern(s) identified in vitro.

35 Therefore, the information requirement is triggered.

36 This study has also been requested at higher tonnages, for assessment of the information provided (see request 6).

37 For the assessment and the specifications of the study to be performed, see request 6.

3. Long-term toxicity testing on aquatic invertebrates

38 Short-term toxicity testing on aquatic invertebrates is an information requirement under Column 1 of Annex VII to REACH (Section 9.1.1.). However, long-term toxicity testing on aquatic invertebrates must be considered (Section 9.1.1., Column 2) if the substance is poorly water soluble.

3.1. Triggering of the information requirement

39 Poorly water soluble substances require longer time to reach steady-state conditions. As a result, the short-term tests does not give a true measure of toxicity for this type of substances and the long-term test is required. A substance is regarded as poorly water soluble if, for instance, it has a water solubility below 1 mg/L or below the detection limit of the analytical method of the test material (Guidance on IRs and CSA, Section R.7.8.5).

40 In the provided OECD TG 111 (2018), the saturation concentration of the Substance in water was determined to be below the limit of detection of the analytical method (i.e., <2 mg/L).

41 Therefore, the Substance is poorly water soluble and information on long-term toxicity on aquatic invertebrates must be provided.

42 In your comments to the draft decision, you "*agree*[] *that the currently available water solubility study (EC A.6 and OECD 105), the water solubility of the test material was < 2.0 mg/L at 20°C and that indicates that the substance is poorly water soluble*". However, you consider that "*the analytical measurements in available ecotoxicology studies seem to indicate that the actual solubility of the test item in test media is clearly higher than that value*". Therefore you "*propose to conduct a new water solubility study in accordance with the OECD 105 test guideline and with OECD 123 methodological adaptations (slow-stirring) to obtain a more accurate water solubility value*". You conclude that "[i]*f the water solubility of the test item is confirmed to be higher than the previous value (< 2 mg/L), you propose not to conduct long-term aquatic toxicity studies*".

43 ECHA acknowledges your intentions to improve the physico-chemical profile of the Substance by generating further information on water solubility. For that information to be relevant to conclude on the solubility of the Substance for the purpose of aquatic toxicity testing, this information will need to be generated under conditions that are consistent with the specification set-out in the relevant test guideline (e.g., test medium composition, test

solution preparation, test conditions) as specified in the OECD GD 23, Section 7.1.1. 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.

3.2. Information provided

44 You have provided an OECD TG 202 study but no information on long-term toxicity on aquatic invertebrates for the Substance.

3.3. Assessment of the information provided

45 In the absence of information on long-term toxicity on aquatic invertebrates, this information requirement is not fulfilled.

3.4. Study design and test specifications

46 The Substance is difficult to test due to the low water solubility (<2 mg/L) and surface activity (surface tension is 52.7 mN/m). OECD TG 211 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. 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 211. 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 solutions.

4. Ready biodegradability

47 Ready biodegradability is an information requirement in Annex VII to REACH (Section 9.2.1.1.).

4.1. Information provided

48 You have provided a ready biodegradability screening study (2018) with the Substance.

4.2. Assessment of information provided

4.2.1. The provided study does not meet the information requirement

49 To fulfil the information requirement, a study must comply with the OECD TG 301 or 310 (Article 13(3) of REACH). Therefore, for a study according to OECD TG 301, the following requirements must be met:

50 Reporting of the methodology and results

a) the concentration of the inoculum, i.e. the suspended solid concentration and bacterial cell density /cells/mL) in the test vessels are reported;
b) the difference of extremes of replicate values of the removal of the test material at the plateau, at the end of the test or, if appropriate, at the end of the 10-d

window is reported;
c) the results of measurements at each sampling point in each replicate is reported in a tabular form.

51 Your registration dossier provides an OECD TG 301B study showing the following:

52 Reporting of the methodology and results

a) the concentration of the incoulum as suspended solids is reported as 30 mg/L, however, the bacterial cell density in the test is not reported;
b) the difference of extremes of replicate values of the removal of the test material at the plateau/at the end of the test/at the end of the 10-d window is not reported;
c) the results of measurements at each sampling point in each replicate is not reported in a tabular form.

53 Based on the above, the reporting of the study is not sufficient to conduct an independent assessment of its reliability. More specifically, the bacterial cell density in the test was not reported and it is not possible to assess if the applied cell density meets the requirements (i.e., 10^7-10^8 cells /mL) of the test guideline. In addition, the difference of extremes of relicate values of the removal of the test material was not reported for any of the specified time point during the test and as a result, the variability of the replicates values cannot be confirmed to be within the required 20% range. Further, the results of measurement at each sampling point in each replicate were not reported and it is not possible to assess whether the reported degradation values are based on the measurements in the test.

54 Therefore, the requirements of OECD 301 B are not met.

55 On this basis, the information requirement is not fulfilled.

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

Reasons related to the information under Annex VIII of REACH

5. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

57 An in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is an information requirement under Annex VIII, Section 8.4.2..

5.1. Information provided

58 You have provided:

(i) An in vivo micronucleus study with the analogue Substance ERL-4221, EC 219-207-4 (1991)
(ii) A Rat liver UDS assay (1999) with the analogue Substance EC 219-207-4
(iii) A MutaMouse TGR assay (2016) with the analogue Substance EC 219-207-4

59 Although you have not explicitly indicated it in your registration dossier, ECHA understands that you intend to adapt this information requirement under Column 2 of Annex VIII, Section 8.4.2..

5.2. Assessment of the information provided

5.2.1. Unclear test material

60 As explained in Section 0.1., the test material used for the study is not clear for the studies (i) and (iii), and ECHA therefore cannot verify the reliability of the provided information.

5.2.2. Read-across adaptation rejected

61 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

62 As a result, the studies (i), (ii) and (iii) you submitted cannot be taken into account in support of the adaptation of this information requirement under Column 2 of Annex VIII, Section 8.4.2

5.2.3. Studies (ii) and (iii) are not adequate in vivo cytogenicity test

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

64 The studies (ii) and (iii) are described as a Rat liver UDS assay and a MutaMouse TGR assay.

65 This studies (ii) and (iii) are neither a micronucleus test nor a chromosomal aberration test. Therefore, these studies do not meet the column 2 criteria.

66 On this basis, the information requirement is not fulfilled.

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

5.3. Specification of the study design

68 To fulfil the information requirement for the Substance, either *in vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method OECD TG 473) or *in vitro* micronucleus study (Annex VIII, Section 8.4.2., test method OECD TG 487) are considered suitable.

6. In vivo genetic toxicity study

69 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.

6.1. Triggering of the information requirement

70 Your dossier contains positive results for the in vitro gene mutation study in bacteria (1988) which raise the concern for gene mutations.

71 Therefore, ECHA considers that an appropriate in vivo follow up genetic toxicity study is necessary to address the concern identified in vitro.

6.2. Information provided

72 You have adapted this information requirement by using a Grouping of substances and read-across approach based on experimental data from the following substances:

(i) An in vivo micronucleus study with the analogue Substance ERL-4221, EC No. 219-207-4 (1991)
(ii) A Rat liver UDS assay (1999) with the analogue Substance EC 219-207-4
(iii) A MutaMouse TGR assay (2016) with the analogue Substance EC 219-207-4

6.3. Assessment of the information provided

6.3.1. Unclear test material

73 As explained in Section 0.1., the test material used for the study is not clear for the studies (i) and (ii), and ECHA therefore cannot verify the reliability of the provided information.

6.3.2. Read-across adaptation rejected

74 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected for studies (i), (ii) and (iii).

6.3.3. Studies (i) and (ii) are not adequate for the request

75 In order to be appropriate, according to the Guidance on IRs and CSA, Section R.7.7.6.3., the in vivo somatic cell genotoxicity study must address the specific concern raised by the in vitro positive result.

76 The study (i) is described as a micronucleus study. This study is not an in vivo somatic cell genotoxicity study addressing concerns for gene mutations.

77 Moreover, the study (ii) is described as a rat liver UDS test. This is an indicator test that detects some DNA repair mechanisms (measured as unscheduled DNA synthesis in liver cells). However, as reminded in the Guidance on IRs & CSA, R.7a, Section R.7.7.6.3 (page 571-572), the UDS test is sensitive to some (but not all) DNA repair mechanisms and not all gene mutagens are positive in the UDS test. The sensitivity of the UDS test has been

questioned (Kirkland and Speit, 2008 [1]) and its lower predictive value towards rodent carcinogens and/or in vivo genotoxicants has been confirmed in comparison with the TGR assay and comet assay (EFSA, 2017 [2]). Therefore, a negative result in a UDS assay alone is not a proof that a substance does not induce gene mutation. Moreover, though a positive result in the UDS assay can indicate exposure of the liver DNA and induction of DNA damage by the substance under investigation, it is not sufficient information to conclude on the induction of gene mutation by the substance.

[1] Kirkland D and Speit G (2008) Evaluation of the ability of a battery of three *in vitro* genotoxicity tests to discriminate rodent carcinogens and non-carcinogens III. Appropriate follow-up testing *in vivo*. Mutat Res 654:114-32.

[2] EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger M, Knutsen HK, More S, Naegeli H, Noteborn H, Ockleford C, Ricci A, Rychen G, Silano V, Solecki R, Turck D, Younes M, Aquilina G, Crebelli R, Gurtler R, Hirsch-Ernst KI, Mosesso P, Nielsen E, van Benthem J, Carfì M, Georgiadis N, Maurici D, Parra Morte J and Schlatter J, 2017. Scientific Opinion on the clarification of some aspects related to genotoxicity assessment. EFSA Journal 2017;15(12):5113, 25 pp. https://doi.org/10.2903/j.efsa.2017.5113.

78 Therefore, the information provided does not cover the key parameter(s) required by the OECD TG 489 or by the OECD TG 488.

79 On this basis, the information requirement is not fulfilled.

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

6.4. Test selection

81 According to the Guidance on IRs & CSA, Section R.7.7.6.3, 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.

82 As explained above, under request 5, in the dossier there is no adequate information from an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study with the Substance, according to the requirements of Section 8.4.2., Annex VIII to REACH. Therefore, by this decision, ECHA also requests an in vitro cytogenicity study or an in vitro micronucleus study, which may raise a concern for chromosomal aberration in case of positive results.

83 In case 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). 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). A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

84 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.

85 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) Comet assay or TGR, if the test results of

request 5 are negative; or b) Comet assay combined with MN test if the test results of request 5 are positive. The deadline set in this decision allows for sequential testing.

6.5. Specification of the study design

*6.5.1. Comet assay or TGR assay (if the test results of request 5 is **negative**)*

6.5.2. Comet assay

86 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).

87 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.

88 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.

6.5.2.1. Germ cells

89 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.

6.5.2.2. Cross-linking properties

90 You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in the OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23 [1] in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39 [2-5] in the OECD TG 489 or Pant et al. 2015 [6]). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

[1] Nesslany *et al.* (2007) *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.

[2] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship

to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.

[3] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.

[4] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.

[5] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol Biol*;613:267-282.

[6] Pant K *et al.* (2015) Modified *in vivo* comet assay detects the genotoxic potential of 14-hydroxycodeinone, an α,β -unsaturated ketone in oxycodone. *Environ Mol Mutagen*;56(9):777-87.

6.5.3. TGR assay

91 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.

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

93 Based on the recent update of the 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.

94 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, 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. However, duodenum must be stored (at or below −70 ºC) 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.

6.5.3.1. Germ cells

95 You may consider collecting 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 ºC). 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.

*6.5.4. Comet assay combined with MN test (if the test results of request 5 are **positive**)*

96 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.

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

98 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.

99 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]).

6.5.4.1. Germ cells

100 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other 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.

6.5.4.2. Cross-linking properties

101 You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23 [2] in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39 [3-6] in the OECD TG 489 or Pant et al. 2015 [7]). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

[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.

[2] Nesslany *et al.* (2007) *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.

[3] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.

[4] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.

[5] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.

[6] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol*

Biol;613:267-282.
[7] Pant K *et al.* (2015) Modified *in vivo* comet assay detects the genotoxic potential of 14-hydroxycodeinone, an α,β -unsaturated ketone in oxycodone. *Environ Mol Mutagen*;56(9):777-87.

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

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

7.1. Information provided

103 You have provided:

(i) a sub-chronic repeated dose toxicity study (2001) with the analogue substance Cycloaliphatic Epoxide Resin ERL-4221, EC number 219-207-4.

104 Although you have not explicitly indicated it in your registration dossier, ECHA understands that you intend to adapt this information requirement under Column 2 of Annex VIII, Section 8.6.1., by using a sub-chronic (90 days) study on an analogue substance.

7.2. Assessment of the information provided

7.2.1. Unclear test material

105 As explained in Section 0.1., the test material used for the study is not clear, and ECHA therefore cannot verify the reliability of the provided information.

7.2.2. Read-across adaptation rejected

106 As explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

107 As a result, the study (i) you submitted cannot be taken into account in support of the adaptation of this information requirement under Column 2 of Annex VIII, Section 8.6.1.

108 Therefore, the information requirement is not fulfilled.

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

7.3. Specification of the study design

110 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. Such an approach offers the possibility to avoid carrying out a 28-day study according to OECD TG 407, because the OECD TG 422 can at the same time fulfil the information requirement of REACH Annex VIII, Section 8.6.1 and that of REACH Annex VIII, Section 8.7.1. (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

111 For information on the study design see request 8.

8. Screening for reproductive/developmental toxicity

112 A screening 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

113 You have adapted this information requirement by using Annex VIII, Section 8.7.1., Column 2. To support the adaptation, you have provided following information:

(i) A pre-natal developmental toxicity study (2007) with the analogue substance cycloaliphatic epoxy resin ERL-4221, EC number 219-207-4.

8.2. Assessment of the information provided

114 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) is already available.

8.2.1. Unclear test material

115 As explained in Section 0.1., the test material used for the study is not clear, and ECHA therefore cannot verify the reliability of the provided information.

8.2.2. Read-across adaptation rejected

116 However, as explained in Section 0.2., your adaptation based on grouping of substances and read-across approach under Annex XI, Section 1.5 is rejected.

117 As a result, the study (i) you submitted cannot be taken into account in support of the adaptation of this information requirement under Column 2 of Annex VIII, Section 8.7.1.

118 Therefore, the information requirement is not fulfilled.

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

8.3. Specification of the study design

120 A study according to the test method EU B.64/OECD TG 422 must be performed in rats.

121 The study must be conducted with oral administration of the Substance (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

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

9. Long-term toxicity testing on fish

123 Short-term toxicity testing on fish is an information requirement under Column 1 of Annex VIII to REACH (Section 9.1.3.). However, long-term toxicity testing on fish must be considered (Section 9.1.3., Column 2) if the substance is poorly water soluble.

9.1. Triggering of the information requirement

124 In the provided OECD TG 111 (2018), the saturation concentration of the Substance in water was below the limit of detection of the analytical method (i.e. <2 mg/L).

125 Therefore, the Substance is poorly water soluble and information on long-term toxicity on fish must be provided.

126 In your comments to the draft decision, you provided similar comments as those already addressed under Request 3. ECHA's reply equally applies to this information requirement.

9.2. Information provided

127 You have provided a calculated LC50 value using ECOSAR Version 1.11 but no information on long-term toxicity on fish for the Substance.

9.3. Assessment of the information provided

128 In the absence of information on long-term toxicity on fish, this information requirement is not fulfilled.

9.4. Study design and test specifications

129 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.).

130 OECD TG 210 specifies that, for difficult to test substances, 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.

10. Hydrolysis as a function of pH

131 Hydrolysis as a function of pH is a standard information requirement in Annex VIII to REACH.

10.1. Information provided

132 You have provided a study on hydrolysis as a function of pH (2018) with the Substance.

10.2. Assessment of information provided

10.2.1. The provided study does not meet the information requirement

133 To fulfil the information requirement, a study must comply with the OECD TG 111 (Article 13(3) of REACH). For a study according to OECD TG 111, the following requirements must be met:

a) identification of hydrolysis products (tier 3) using appropriate analytical method is performed for major hydrolysis products (present at least ≥ 10 % of the applied dose).

134 Your registration dossier provides an OECD TG 111 study showing the following:

a) hydrolysis products were not identified and reported.

135 Therefore, as the hydrolysis products were not identified in your registration dossier, the provided study does not fulfil the information requirement.

136 In the comments to the draft decision, you agree that the study does not meet the information requirement. You propose to conduct a new OECD TG 111 following a simplified protocol.

137 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.

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).

All Guidance on REACH is 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 16 June 2021.

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).

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 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.

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.

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