~~Confidential~~

Helsinki, 13 February 2024

Addressee
Registrant of JS_52434-90-9 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
21 February 2023

Registered substance subject to this decision ("the Substance")
Substance name: 1,3,5-tris(2,3-dibromopropyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione
EC/List number: 257-913-4

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 ***20 August 2027***.

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.)
   a) *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 (OECD TG 442E) (Annex VII, Section 8.3.1.); and
   b) only if the *in vitro/in chemico* test methods specified under point a) above 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. *In vitro* micronucleus study, also requested below (triggered by Annex VII, Section 8.4., Column 2).

3. Transgenic rodent somatic and germ cell gene mutation assays, **OR** *In vivo* mammalian alkaline comet assay, **OR** *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).

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

5. Growth inhibition study aquatic plants (Annex VII, Section 9.1.2.; test method: EU C.3./OECD TG 201)

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

6. *In vitro* micronucleus study (Annex VIII, Section 8.4.2., test method: OECD TG 487). The aneugenic potential of the Substance must be assessed with an additional control group for aneugenicity on top of the control group for clastogenicity, if the Substance induces an increase in the frequency of micronuclei.

7. *In vivo* genetic toxicity study (triggered by Annex VIII, Section 8.4., Column 2) to be selected according to the following specifications:

   If the results of the *in vitro* micronucleus study requested under requests 2 and 6. are **negative**:

   Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488) in transgenic mice or rats, oral route, on the following tissues: liver and glandular stomach; duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.
   OR
   In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, in other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

   If the results of the *in vitro* micronucleus study requested under requests 2 and 6. 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. For the micronucleus test:
   - centromere staining must be performed if the substance induces an increase in the frequency of micronuclei in the OECD TG 474, unless the aneugenic potential has been conclusively investigated in the *in vitro* micronucleus study requested under requests 2 and 6.;
   - target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

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

9. 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 requests 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)

Reasons common to several requests

0.1. Weight of evidence adaptation rejected

1 You have adapted the following standard information requirements by using weight of evidence in accordance with Annex XI, Section 1.2.:

- Skin sensitisation (Annex VII, Section 8.3)
- *In vitro* micronucleus study (Annex VIII, Section 8.4.2.)

2 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.

3 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.

4 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.

0.1.1. Lack of documentation justifying the weight of evidence adaptation

5 Annex XI, Section 1.2. requires that adequate and reliable documentation is provided to describe a 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.

6 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.

7 Beside this critical deficiency common to all information requirements under consideration, your weight of evidence approach has additional deficiencies.

8 Additional deficiencies that are specific for each of the information requirements individually are addressed under requests 1 and 6.

9 Additional common deficiencies are identified below.

0.1.2. Conclusion

10 Based on the above, your weight of evidence adaptation under Annex XI, Section 1.2. is rejected.

Reasons related to the information under Annex VII of REACH

1. Skin sensitisation

11 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

12 ECHA understands that you have adapted this information requirement by using Annex XI, Section 1.2. (weight of evidence) based on the following experimental data:

(i) Danish QSAR Database Results For CAS 52434-90-9 (2022) with the Substance;

(ii) OECD QSAR Toolbox (v4.5 SP1), Automated Workflow Prediction Report (2022).

13 ECHA also understands that by using the OECD QSAR Toolbox (v4.5 SP1) for source of information (ii), you intended to predict the properties of the Substance based on a read-across adaptation under Section 1.5 of Annex XI. However, your dossier does not contain an explanation why the property of the Substance may be predicted from substances that are manifestly structurally different and it does not contain any robust study summaries for the studies conducted on the source substances. These are formal conditions set out in Section 1.5 of Annex XI. Consequently, we are not able to make any evaluation of your read-across adaptation. The information provided in the OECD QSAR Toolbox (v4.5 SP1), Automated Workflow Prediction Report (2022) source of information (ii) is therefore set aside from the assessment of the information provided to fulfil this information requirement.

1.2. Assessment of the information provided

1.2.1. Assessment whether the Substance causes skin sensitisation

1.2.1.1. Weight of evidence rejected

14 As explained in Section 0.1., your documentation of the weight of evidence is not in line with the requirements of Annex XI, Section 1.2. Therefore, your adaptation is rejected. In addition, ECHA identified endpoint-specific issue(s) regarding the weight of evidence. These are addressed below.

1.2.1.1.1. Only one source of information (i) provided

15 Annex XI, Section 1.2. states that there may be sufficient weight of evidence from several independent sources of information based on which a conclusion on the information requirement can be drawn.

16 You have only provided one source of information.

17 Based on the above, your adaptation is rejected.

1.2.1.1.2. Inadequate documentation of the prediction (QPRF) for source of information (i)

18 Guidance on IRs and CSA R.6.1.6.3. states that the information specified in or equivalent to the (Q)SAR Prediction Reporting Format document (QPRF) must be provided to have adequate and reliable documentation of the applied method. For a QPRF this includes, among others:

- the relationship between the modelled substance and the defined applicability domain,
- the identities of close analogues, including considerations on how predicted and experimental data for analogues support the prediction.

19 You provided the following information about the prediction: "positive in domain" predictions from the Danish QSAR Database by three different models Leadscope, CASE Ultra and SciQSAR, and the consensus result from the battery mode. The information you provided about the prediction lacks the following elements: details to independently verify that the substance falls within the applicability domain as described in the documentation, and information on analogues and how their predicted and experimental data supports the prediction.

20 Therefore, it is not possible to conclude, based on any source of information alone or considered together, whether the Substance causes skin sensitisation.

1.2.1.1.3. No assessment of potency

21 To be considered compliant and enable a conclusion in cases where the substance is considered to cause skin sensitisation, the information provided must also allow a conclusion whether it can be presumed to have the potential to produce significant sensitisation in humans (Cat. 1A).

22 As the currently available data does not allow to conclude whether the Substance causes skin sensitisation (see section 1.2.1 above), this condition cannot be assessed.

1.2.1.1.4. Conclusion on your weight of evidence adaptation

23 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for skin sensitisation.

24 Based on the above, your weight of evidence adaptation under Annex XI, Section 1.2. is rejected.

25 Therefore, the information requirement is not fulfilled.

26 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

1.3. Study design

27 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 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.

28 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/in chemico 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 vitro* micronucleus study

29 Under Annex VII, Section 8.4., Column 2, further mutagenicity studies must be considered in case of a positive result.

2.1. Triggering of the information requirement

30 The Guidance on IRs & CSA, Section R.7.7.6.3., further specifies that "REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII." This is for the reason that the in vitro micronucleus study under Section 8.4.2. will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy.

31 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2008/2009, report number [redacted]). However, no adequate information from an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study, according to the requirements of Annex VIII, is available.

32 Therefore, the information requirement is triggered.

33 ECHA considers that an appropriate in vitro micronucleus study is necessary to further investigate the mutagenicity of the Substance and to help identify the most adequate follow-up in vivo study.

34 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

2.2. Information requirement not fulfilled

35 The information provided, its assessment and the specifications of the study design are addressed under request 6.

3. *In vivo* mammalian genetic toxicity study

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

3.1. Triggering of the information requirement

37 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2008/2009, report number [redacted]) which raise the concern for gene mutation.

38 Therefore, the information requirement is triggered.

39 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

3.2. Information requirement not fulfilled

40 The information provided, its assessment and the specifications of the study design are addressed under request 7.

4. Short-term toxicity testing on aquatic invertebrates

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

4.1. Information provided

42 You have adapted this information requirement by using Column 2 of Annex VII, Section 9.1.2. To support the adaptation, you have provided the following statement: "the study does not need to be conducted because a long-term aquatic toxicity study on invertebrates is proposed to be conducted".

4.2. Assessment of the information provided

4.2.1. No long-term toxicity study available in your dossier

43 Under Annex VII, Section 9.1.1., Column 2, second indent, the study may be omitted if a long-term aquatic toxicity study on invertebrates is available.

44 You have submitted a testing proposal for the information requirement on long-term toxicity on aquatic invertebrates (Annex IX, Section 9.1.5.). However, your registration dossier does not currently include a long-term aquatic toxicity study.

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

4.3. Justification for an adaptation of the short-term repeated dose toxicity study

46 In a parallel testing proposal draft decision, the registrant(s) concerned were requested to generate and submit a reliable long-term toxicity study on aquatic invertebrates (test method: OECD TG 211).

47 According to Annex VII, Section 9.1.1., Column 2, second indent and to prevent unnecessary testing, a short-term toxicity study does not need to be conducted. Therefore, to comply with the information requirement in Annex VII, Section 9.1.1., you are requested to provide a justification for adaptation, as provided in Annex VII, Section 9.1.1., Column 2, second indent.

48 In case the adopted testing proposal decision no longer contains a request for long-term toxicity study on aquatic invertebrates, you are required to provide a short-term toxicity study on aquatic invertebrates.

Therefore, you are requested to submit:

- a short-term toxicity study on aquatic invertebrates (test method: OECD TG 202) as per the study design described in section 4.4. as the long-term toxicity study on aquatic invertebrates is not requested in an adopted testing proposal decision.

4.4. Study design

49 The Substance is difficult to test due to the low water solubility (5 mg/L) and adsorptive properties (Log K_{OC} of 5.44). OECD TG 202 specifies that, for difficult to test substances, you must consider the approach described in OECD GD 23 or other approaches, if more appropriate for your substance. In all cases, the approach selected must be justified and documented. Due to the properties of Substance, it may be difficult to achieve and maintain the desired exposure concentrations. 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.

5. Growth inhibition study aquatic plants

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

5.1. Information provided

51 You have provided a Growth inhibition study on aquatic algae according to OECD TG 201 (2022) with the Substance.

5.2. Assessment of the information provided

5.2.1. The provided study does not meet the specifications of the test guideline

52 To fulfil the information requirement, a study must comply with OECD TG 201 and the specifications of OECD GD 23 if the substance is difficult to test (Article 13(3) of REACH). As explained in Section 4.4., the Substance is difficult to test. Therefore, the following specifications must be met:

Technical specifications impacting the sensitivity/reliability of the test

a) the test concentrations are below the limit of solubility of the test material in the dilution water;

Additional requirements applicable to difficult to test substances

b) if the test material is tested at the saturation concentration, evidence must be provided that all reasonable efforts have been taken to achieve a saturation concentration, which include:

1) information on the saturation concentrations of the test material in water and in the test solution, and

2) the results of a preliminary experiment demonstrating that the test solution preparation method is adequate to maximize the concentration of the test material in solution.

53 In the provided study:

Technical specifications impacting the sensitivity/reliability of the test

a) in your dossier, you report that the saturation concentration of the Substance in water was determined to be 5 mg/L based on OECD TG 105 (column elution method). In the provided study, you state that "[the Substance] *was tested for its solubility in the growth medium and formed a homogeneous white colour soluble preparation with visible particles (approximately 10%) in growth medium containing 0.01% Acetone at 100 mg/L*". You also specify that the Substance was tested at nominal concentration ranging from 50 to 327.7 mg/L.

Additional requirements applicable to difficult to test substances

b) you have not provided information on the saturation concentration of the test material in the test solution nor the results of a preliminary experiment demonstrating that the test solution preparation method is adequate to maximize the concentration of the test material in solution.

54 Based on the above, there are critical methodological deficiencies resulting in the rejection of the study results. More specifically,

- while you have not provided an estimate the saturation concentration of the test material in the test solution, the information on the water solubility of the Substance provided in your dossier as well as the result of a preliminary experiment in the test media at 100 mg/L indicate that the Substance was tested at nominal concentrations well above the limit of solubility of the test material in the dilution water.
- as the test material was likely tested above its saturation concentration in the dilution water, it is noted that the results of the analytical monitoring of exposure concentrations does not provide reliable estimates for the exposure to the test material in this study (i.e., the concentration of the test material in solution). You state that test samples were "*diluted with diluent (Acetonitrile)* [...] *to obtain the*

final concentration of approximately 5.0 µg/mL". You do not describe any procedure to remove undissolved particle before the dilution of the test sample with acetonitrile. Under these conditions, undissolved particles may have been solubilised following solvent addition and measured values are unlikely to provide a reliable estimate of dissolved concentrations under the conditions of the study.

- finally, in the absence of a reliable estimate of the saturation concentration of the test material in the test solution and of an appropriate justification that the test solution preparation method was adequate to maximize the concentration of the test material in solution, you have not justified that exposure was satisfactory in the provided study.

55 On this basis, the specifications of OECD TG 201 are not met.

56 Therefore, the information requirement is not fulfilled.

57 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

5.1. Study design

58 OECD TG 201 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" under request 4.

Reasons related to the information under Annex VIII of REACH

6. *In vitro* micronucleus study

59 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.

6.1. Information provided

60 ECHA understands that you have adapted this information requirement by using Annex XI, Section 1.2. (weight of evidence) based on the following:

(i) an *in vitro* chromosome aberration study (1982) with the Substance;

(ii) an *in vitro* chromosome aberration study (1982) with the Substance;

(iii) an *in vivo* micronucleus study (1980) with the Substance.

6.2. Assessment of the information provided

6.2.1. Weight of evidence adaptation rejected

61 In addition to the deficiencies identified in Section 0.1., ECHA identified endpoint specific issue(s) addressed below.

62 Information that can be used to support weight of evidence adaptation for the information requirement of Annex VIII, Section 8.4.3. includes similar information that is produced by the OECD TG 473 and 474. OECD TG 473 and 474 requires the study to investigate the following key parameter(s):

(1) Detection and quantification of cytotoxicity and the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in cultured mammalian cells (*in vitro*) or in mammals (*in vivo*).

6.2.1.1. Assessment whether the Substance causes structural chromosomal aberrations or micronuclei

63 The sources of information (i) and (ii) provide relevant information on detection and quantification of cytotoxicity and on the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in cultured mammalian cells (in vitro). The source of information (iii) provides relevant information on detection and quantification of cytotoxicity and on the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in mammals (in vivo).

64 However, the reliability of the sources of information (i) to (iii) is affected by the following deficiencies:

6.2.1.1.1. The provided sources of information (i and ii) do not meet the specifications of the test guideline(s)

65 In principle, to fulfil the information requirement, the study has to be an *in vitro* chromosomal aberration test or an *in vitro* micronucleus test conducted in mammalian cells. The study should normally comply with the OECD TG 473 or the OECD TG 487, respectively (Article 13(3) of REACH). Therefore, sources (i) and (ii) should be conducted consistently with the following specifications:

a) the maximum concentration tested induces 55+5% of cytotoxicity compared to the negative control, or the precipitation of the tested substance. If no precipitate or limiting cytotoxicity is observed, the highest test concentration corresponds to 10

mM, 2 mg/mL or 2 µL/mL, whichever is the lowest;
b) at least 300 well-spread metaphases are scored per concentration;
c) the positive controls induce responses compatible with those generated in the historical positive control database;
d) the negative control data is ideally within the 95% control limits of the distribution of the laboratory's historical negative control database;
e) to conclude on a negative outcome, a negative response is obtained in all three experimental conditions described in paragraph 28 of OECD TG 473, using a short-term treatment with and without metabolic activation and long-term treatment without metabolic activation.

66 In studies (i) and (ii):

a) the maximum tested concentration did not induce 55+5% of cytotoxicity compared to the negative control, and it did not induce the precipitation of the tested substance, and it was less than 10 mM, 2 mg/mL or 2 µL/mL. Therefore, it is not possible to understand whether high enough doses were selected for the studies.
b) 200 metaphases (i.e., less than 300 metaphases) were scored per concentration. Therefore, the statistical power of the studies is lower.
c) you do not report whether the positive control data is compatible with those generated in the historical positive control database. Without this data it is not possible adequately assess the studies.
d) you do not provide information on whether the negative control data was within the 95% control limits of the distribution of the laboratory's historical negative control database. Without this data it is not possible to adequately assess the studies.
e) One experimental condition described in paragraph 28 of OECD TG 473 (i.e., a short-term treatment with and without metabolic activation is missing in study (i) or too short in study (ii) (i.e., only 2 hours). Therefore, it is not possible to conclude on a negative outcome for studies (i) and (ii).

67 Based on the above, sources (i) and (ii) cannot be considered as reliable sources of information that could contribute to the conclusion whether the Substance causes structural chromosomal aberrations or micronuclei in vitro in mammalian cells investigated in the required study.

6.2.1.1.2. The provided source of information (iii) does not meet the specifications of the test guideline(s)

68 In principle, to fulfil the information requirement, the study has to be an *in vivo* chromosomal aberration test or an *in vivo* micronucleus test conducted in bone marrow or peripheral blood cells of animals, usually rodents. The study should normally comply with the OECD TG 474 or the OECD TG 475, respectively (Article 13(3) of REACH). Therefore, source (iii) should be conducted consistently with the following specifications:

a) the study includes a minimum of three dose level groups of treated animals;
b) each group includes a minimum of 5 analysable animals;
c) at least 4000 immature erythrocytes per animal are scored for the incidence of micronucleated immature erythrocytes;
d) the proportion of immature erythrocytes among total (immature + mature) erythrocytes and the mean number of micronucleated immature erythrocytes are reported for each group of animals;
e) a clear negative outcome is concluded and the data available shows that bone marrow exposure to the Substance or its metabolite(s) occurred;
f) the negative control data is ideally within the 95% control limits of the distribution of the laboratory's historical negative control database;

g) the positive controls or scoring controls induce responses compatible with those generated in the historical positive control database.

69 In study (iii):

a) the study included only 2 dose level groups of treated animals (i.e. less than three groups). Without a sufficient number of animals, the variability of the study results cannot be adequately assessed.
b) only 4 animals (i.e., less than 5 animals) were included in each group. Without a sufficient number of animals, the variability of the study results and the statistical power cannot be adequately assessed.
c) 1000 immature erythrocytes per animal (i.e. less than 4000 immature erythrocytes) were scored to determine the incidence of micronucleated immature erythrocytes;
d) the proportion of immature erythrocytes among total (immature + mature) erythrocytes and the mean number of micronucleated immature erythrocytes were not reported for each group of animals. Without a sufficient number of immature erythrocytes scored and reported, the variability of the study results cannot be adequately assessed.
e) you did not demonstrate that bone marrow exposure to the Substance, or its metabolite(s), occurred or that there is evidence that the Substance, or a relevant metabolite, will not reach the target tissue. Without this information it is not possible to adequately conclude on the study result.
f) you did not report whether the negative control did show a response within the historical control range of the laboratory. Therefore, the study results cannot be adequately assessed.
g) you did not report whether the positive control (or scoring control) did produce a statistically significant increase in the induced response when compared with the concurrent negative control. Therefore, the study results cannot be adequately assessed.

70 Based on the above, the source (iii) cannot be considered as reliable source of information that could contribute to the conclusion whether the Substance causes micronuclei in vivo in mammalian cells as normally investigated by the required study.

6.2.1.2. Conclusion on your weight of evidence adaptation

71 As explained above, the provided studies cannot be considered reliable sources of information that could contribute to the conclusion on detection and quantification of cytotoxicity and the frequency of cells with structural chromosomal aberration(s) or the frequency of micronuclei in cultured mammalian cells (in vitro) or in mammals (in vivo).

72 Therefore, it is not possible to conclude, based on any source of information alone or considered together, on the information requirement for in vitro micronucleus study.

73 As a result, your weight of evidence adaptation under Annex XI, Section 1.2. is rejected and the information requirement is not fulfilled.

74 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

6.3. Study design

75 According to the Guidance on IR & CSA, Section R.7.7.6.3., either the in vitro mammalian chromosomal aberration ("CA") test (test method OECD TG 473) or the in vitro mammalian cell micronucleus ("MN") test (test method OECD TG 487) can be used to investigate chromosomal aberrations in vitro. However, while the MN test detects both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the CA test detects only clastogenicity, as OECD TG 473 is not designed to

measure aneuploidy (see OECD TG 473, paragraph 2).Therefore, you must perform the MN test (test method OECD TG 487), as it enables a more comprehensive investigation of the chromosome damaging potential in vitro. Moreover, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen [1] (OECD TG 487, paragraphs 33 to 35).

6.3.1. Assessment of aneugenicity potential

76 If the result of the MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance.

77 In line with the OECD TG 487 (paragraph 4), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

[1] According to the TG 487 (2016) "At the present time, no aneugens are known that require metabolic activation for their genotoxic activity" (paragraph 34).

7. *In vivo* mammalian genetic toxicity study

78 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.

7.1. Triggering of the information requirement

79 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2008/2009, report number [redacted]) which raise the concerns for gene mutations.

80 Therefore, the information requirement is triggered.

81 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

7.2. Information provided

82 You have provided:

(i) an *in vivo* somatic erythrocyte micronucleus study (1980) with the Substance.

7.3. Assessment of the information provided

7.3.1. Study not adequate for the information requirement

83 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.

84 However, the in vivo study provided is not addressing the gene mutation concern raised by the in vitro data.

85 Based on the above, the provided in vivo test is not appropriate.

86 Therefore, the information requirement is not fulfilled.

*7.3.2. Comet assay (if the test results of requests 2. and 6. are **negative**)*

87 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).

88 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.

89 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

*7.3.3. TGR assay (if the test results of requests 2. and 6. are **negative**)*

90 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.

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

92 Based on 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.

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

*7.3.4. Comet assay combined with MN test (if the test results of requests 2. and 6. are **positive**)*

94 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.

95 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.

96 In line with the test method OECD TG 489, the test must be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as sites of contact. There are several expected or possible variables between the glandular stomach and the duodenum (different tissue structure and function, different pH conditions, variable physico-chemical properties and fate of the Substance, and probable

different local absorption rates of the Substance and its possible breakdown product(s)). In light of these expected or possible variables, it is necessary to analyse both tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

97 According to the test method OECD TG 474, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen (OECD TG 474, paragraph 25, Table 1).

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

[1] Bowen 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.

7.3.4.1. Assessment of aneugenicity potential

99 If the result of the in vivo MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance unless the aneugenic potential has been conclusively investigated in the in vitro micronucleus study requested under Sections 2. and 6. In line with the OECD TG 474 (paragraph 42), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

7.3.4.2. Investigation of target tissue exposure

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

101 Therefore, to ensure that the data generated are adequate for hazard identification, you must take blood samples at appropriate times and measure plasma levels of the Substance and/or its metabolites (OECD TG 474, paragraph 40), unless exposure of the bone marrow can be demonstrated through other means, e.g. by showing a depression of immature to mature erythrocyte ratio (OECD TG 474, paragraph 48).

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

7.3.5. Germ cells

7.3.5.1. Comet assay or Comet assay combined with MN test

103 In case you perform a comet assay, you may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other aforementioned tissues in the comet assay, as it would optimise the use of animals. You can prepare the slides for male gonadal cells and store them for up to 2 months, at room temperature, in dry conditions and protected from light. Following the generation and analysis of data on somatic cells in the comet assay, you should consider analysing the slides prepared with gonadal cells. This

type of evidence may be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

7.3.5.2. TGR assay

104 In case you perform a TGR assay, you may collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below −70 ºC). 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.

105 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

8. Short-term toxicity testing on fish

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

8.1. Information provided

107 You have adapted this information requirement by using Column 2 of Annex VIII, Section 9.1.2. To support the adaptation, you have provided the following statement: "the study does not need to be conducted because a long-term aquatic toxicity study on fish is proposed to be conducted".

8.2. Assessment of the information provided

8.2.1. No long-term toxicity study available in your dossier

108 Under Annex VIII, Section 9.1.3., Column 2, second indent, the study may be omitted if a long-term aquatic toxicity study on fish is available.

109 You have submitted a testing proposal for the information requirement on long-term toxicity on fish (Annex IX, Section 9.1.6.). However, your registration dossier does not currently include a long-term aquatic toxicity study.

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

8.3. Justification for an adaptation of the short-term repeated dose toxicity study

111 In a parallel testing proposal draft decision, the registrant(s) concerned were requested to generate and submit a reliable long-term toxicity study on fish (test method: OECD TG 210).

112 According to Annex VIII, Section 9.1.3., Column 2, second indent and to prevent unnecessary animal testing, a short-term toxicity study does not need to be conducted. Therefore, to comply with the information requirement in Annex VIII, Section 9.1.3., you are requested to provide a justification for adaptation, as provided in Annex VIII, Section 9.1.3., Column 2, second indent.

113 In case the adopted testing proposal decision no longer contains a request for long-term toxicity study on fish, you are required to provide a short-term toxicity study on fish.

Therefore, you are requested to submit:

- a short-term toxicity study on fish (test method: OECD TG 203) as per the study design described in section 8.4. as the long-term toxicity study on fish is not

requested in an adopted testing proposal decision.

114 In your comments to the draft decision, you state that you agree with ECHA's evaluation.

8.4. Study design

115 OECD TG 203 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" under request 4.

9. Hydrolysis as a function of pH

116 Hydrolysis as a function of pH is an information requirement under Annex VIII to REACH (Section 9.2.2.1.).

9.1. Information provided

117 You have provided a hydrolysis study according to OECD TG 111 (2022) with the Substance.

9.2. Assessment of the information provided

9.2.1. The provided study does not meet the specifications of the test guideline

118 To fulfil the information requirement, a study must comply with OECD TG 111 (Article 13(3) of REACH). This TG is designed as a tiered approach and each tier is triggered by the results of the previous tier. Therefore, the following specifications must be met:

Technical specifications impacting the sensitivity/reliability of the test

b) a test material concentration below 0.01M or half the saturation concentration (whichever is the lower) is used;

c) the recoveries in the buffer solutions range between 90-110 %. In case, it can be demonstrated that reaching such level is technically challenging, recoveries > 70% are acceptable for non-labelled test materials but a justification needs to be provided;

Identification of hydrolysis products (Tier 3)

d) all major hydrolysis products observed in Tier 2 testing (i.e. at least those representing > 10% of the applied dose) must be identified using an appropriate analytical method (Tier 3).

119 In the provided study:

Technical specifications impacting the sensitivity/reliability of the test

a) in the Tier 1 and Tier 2 tests, you report that the test material concentration was c.a. 20.6 mg/L. In section 4.8 of IUCLID, you provide a water solubility estimate of 5 mg/L based on OECD TG 105;

b) in the Tier 2 test, you report that recoveries were < 10% at all temperature tested (20, 35 and 50°C) at the end of the test (i.e., 24h at 20 and 35°C and 20h at 50°C);

Identification of hydrolysis products (Tier 3)

c) you have not quantified the presence of degradation products in the Tier 2 test and you state that "*no hydrolysis products were detected based on HPLC*".

120 Based on the above there are critical methodological deficiencies resulting in the rejection of the study results. More specifically:

- the test was conducted at a concentration that was well above the saturation concentration of the test material. Under such conditions, it is unclear if the dissipation of the parent substance observed during the test is due to hydrolysis or to other loss processes such as precipitation of undissolved material;
- the recoveries in the Tier 2 test were well below the minimum acceptable value from the test guideline. To be considered reliable a hydrolysis study should provide information on the concentration of the parent and (if present) of hydrolysis products so that mass balance information ranges between 90-110 % (or at least > 70% if justified by technical difficulties). In the absence of such information, it is not demonstrated that dissipation of the parent is due to hydrolysis rather than to other loss processes (e.g. precipitation, adsorption);
- while you claim that fast hydrolysis occurred in this study, you indicate that no hydrolysis products could be detected (and therefore no identification of hydrolysis products could be conducted). Therefore, the requirements of the Tier 3 test are not met.

121 On this basis, the specifications of OECD TG 111 are not met.

122 Therefore, the information requirement is not fulfilled.

123 In your comments to the draft decision, you state that you agree with ECHA’s evaluation.

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 24 August 2022.

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

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

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

ECHA received proposal(s) for amendment and modified the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

The Member State Committee unanimously agreed on the draft decision during its MSC-84 meeting. ECHA adopted the decision under Article 51(6) of REACH.

As a result of one or more changes of registration tonnage band or registration type, the requests for

- In vivo mammalian genetic toxicity study in somatic cells (Annex IX, Section 8.4.4.) or Analysis of male germ cells tissues (Annex IX, Section 8.4.5.),
- Simulation testing on ultimate degradation in surface water (Annex IX, Section 9.2.1.2.), Soil simulation testing (Annex IX, Section 9.2.1.3.),
- Sediment simulation testing (Annex IX, Section 9.2.1.4.),
- Identification of degradation products (Annex IX, Section 9.2.3.),
- Bioaccumulation in aquatic species (Annex IX, Section 9.3.2),
- Long-term toxicity on terrestrial invertebrates (triggered by Annex IX, Section 9.4.1., Column 2),
- Effects on soil micro-organisms (Annex IX, Section 9.4.2.), and
- Long-term toxicity on terrestrial plants (triggered by Annex IX, Section 9.4.3., Column 2)

were removed from the decision. The deadline was not changed.

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

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

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

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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 (https://echa.europa.eu/practical-guides).

(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.

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

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