~~Confidential~~

Helsinki, 01 June 2023

Addressee(s)
Registrant(s) of BPA-EO joint submission as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
15/12/2021

Registered substance subject to this decision ("the Substance")
Substance name: 4,4'-Isopropylidenediphenol, ethoxylated
EC/List number: 500-082-2

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

DECISION ON TESTING PROPOSAL(S)

Under Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by ***9 March 2026***.

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

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

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

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

2. In vivo mammalian alkaline comet assay (triggered by Annex IX, Section 8.4.4., column 1; test method: OECD TG 489) combined with in vivo mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, or if justified, in mice, oral route. For the comet assay the following tissues must be analysed: liver, glandular stomach and duodenum.
   - centromere staining must be performed if the substance induces an increase in the frequency of micronuclei in the OECD TG 474;
   - target tissue exposure must be demonstrated if the result of the OECD TG 474 is negative.

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 addressee(s) of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes. 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 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

Reasons for the decision(s) related to the information under Annex VIII of REACH

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

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

2 Your dossier contains positive results for the *in vitro* micronucleus test (OECD TG 487, 2021) which raise the concern for chromosomal aberrations.

3 ECHA considers that the *in vivo* follow-up study is necessary to address the identified concern.

4 For the assessment of the testing proposal, see Section 2.

Reasons for the decision(s) related to the information under Annex IX of REACH

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

5 An appropriate *in vivo* mammalian somatic cell genotoxicity study is an information requirement under Annex IX, Section 8.4.4., if there is a positive result in any of the in vitro studies referred to in Annex VII or VIII, Section 8.4. The *in vivo* study must address the concerns raised by the in vitro study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

6 Your dossier contains positive results for the *in vitro* micronucleus test (OECD TG 487, 2021) which raise the concern for chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

2.1. Information provided to fulfil the information requirement

7 You have submitted a testing proposal for an in vivo mammalian erythrocyte micronucleus test to be performed with the Substance.

8 ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. You provided your considerations concluding that there were no alternative methods which could be used to adapt the information requirement(s) for which testing is proposed. ECHA has taken these considerations into account.

9 ECHA agrees that an appropriate *in vivo* follow up genotoxicity study is necessary to address the concerns identified *in vitro*.

2.2. Test selection

10 The positive *in vitro* results available in the dossier indicate a concern for chromosomal aberrations.

11 In the comments to the draft decision, you argue that the negative results obtained in the mouse lymphoma assay (OECD TG 490, 2012) with the Substance and the absence of induction of small colonies suggest that the Substance does not induce structural chromosomal damages and that the positive results obtained in the *in vitro* micronucleus test (OECD TG 489, 2021) are therefore more likely due to aneugenic than to clastogenic effects.

12 ECHA agrees that the mouse lymphoma assay is a gene mutation test that is also able to detect specific structural chromosome damages. According to OECD TG 490, the test can detect gross structural changes at the chromosomal level that affect putative growth regulating gene(s) near the TK locus and result in the induction of small colonies. However, structural chromosome damages that do not involve these putative growth regulating gene(s) would in principle not be detected in the mouse lymphoma assay. Therefore, the negative results obtained in the OECD TG 490 study (2012) cannot be used to exclude the possibility that the positive *in vitro* micronucleus test results (OECD TG 487, 2021) are due to clastogenic effects of the Substance.

13 The *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (OECD TG 474, paragraph 37c; OECD TG 489, paragraph 33; Guidance on IRs & CSA, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the

comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations.

14 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 detect effects in both distant organs, such as the bone marrow or the liver, and at site(s) of contact, such as the glandular stomach, the duodenum or the lung. Investigating several genotoxic endpoints and different tissues in a combined study is necessary to reduce the uncertainties associated with not testing all organs and to generate complementary information that provides a comprehensive overview of the genotoxic potential of the Substance. Moreover, the combined study can help limit the number of tests performed and the number of animals used.

15 In the comments to the draft decision, you indicate that additional groups of animals may be required for performing the combined study compared to the *in vivo* micronucleus test alone. While ECHA agrees with your comment, ECHA considers that combination with the *in vivo* comet assay is required as clastogenic effects of the Substance cannot be excluded based on the *in vitro* mutagenicity study results. As described above, this will allow investigation of both distant organs and site of contact tissues. Moreover, the combined study will still use significantly less animals than if the *in vivo* micronucleus test and *the in vivo* comet assay were performed separately.

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

2.3. Specification of the study design

17 You proposed testing in mice. 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. In the comments to the draft decision, you claim that the elimination in rats of the micronucleated cells from the circulation by the spleen can be a limiting factor to detect aneuploidy in the peripheral blood micronucleus test and that mice appear to be more sensitive than rats to certain types of aneugens because mice have a higher rate of cell division in the bone marrow than rats. However, ECHA notes that, according to OECD TG 474, splenic removal of micronucleated cells from the circulation has been clearly demonstrated not to compromise the detection of induced micronuclei in peripheral blood for both the mouse and the rat. In addition, as clastogenic effects of the Substance cannot be excluded based on the *in vitro* mutagenicity study results, your claim of a higher sensitivity of mice to some types of aneugens compared to rats should not be the only reason for deviating from the OECD TG 489 and OECD TG 474 recommendations for species selection. Therefore, the combined study must be performed in rats, or if justified, in mice.

18 You proposed testing by the oral route. 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.

19 In line with the test method OECD TG 489, the test must be performed by analysing tissues from the 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.

20 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).

21 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.

2.3.1. Assessment of aneugenicity potential

22 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. 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)).

23 In the comments to the draft decision, you agree to perform centromere staining if the Substance induces an increase in the frequency of micronuclei in the OECD TG 474 part of the study, although you note that there is no requirement to include studies for the purpose of reaching conclusions on the mechanism of action (clastogenicity and/or aneugenicity) according to REACH Annex VIII.

24 ECHA considers that the assessment of the clastogenic and aneugenic properties of the Substance is necessary to provide a comprehensive overview of the genotoxic potential of the Substance. In addition, this distinction is important for risk assessment purposes because identification of a threshold is generally considered possible for aneugenic effects and derivation of safe levels of exposure is in principle possible for them. On the other hand, clastogenic effects are considered to have no threshold.

2.3.2. Investigation of target tissue exposure

25 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".

26 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).

27 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.

2.3.3. Germ cells

28 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, and/or CA on spermatogonia/OECD TG 483, depending on the concern(s) identified) may be required

under Annex IX of REACH, in case 1) an *in vivo* genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

29 Therefore, 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, in accordance with Annex IX, Section 8.4.5, 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.

2.4. Outcome

30 Under Article 40(3)(c) you are requested to carry out the additional tests with the Substance, as specified above.

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

The Substance is listed in the Community rolling action plan (CoRAP) for the start of substance evaluation in 2025.

The information requirement for an Extended One-Generation Reproductive Toxicity Study (EOGRTS; Annexes IX or X, Section 8.7.3.) is not addressed in this decision. This may be addressed in a separate decision once the information from the sub-chronic toxicity study (90-day) requested in decision TPE-D-2114489554-35-01/F is provided, due to the fact that the results from the 90-day study are needed for the design of the EOGRTS.

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 21 April 2022.

ECHA held a third-party consultation for the testing proposals for an in vivo mammalian erythrocyte micronucleus test and an Extended One-Generation Reproductive Toxicity Study from 16 June 2022 until 1 August 2022. ECHA did not receive information from third parties on this testing proposal for an in vivo mammalian erythrocyte micronucleus test.

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

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

ECHA took into account your comments and did not amend the request(s) or the deadline. The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended 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: 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 Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

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

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

Appendix 4: Conducting and reporting new tests for REACH purposes

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

1.1. Test methods, GLP requirements and reporting

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

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

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

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

1.2. Test material

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

(1) Selection of the Test material(s)

The Test Material used to generate the new data must be selected taking into account the following:

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

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

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

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

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[3].

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