

Helsinki, 23 November 2021

Addressees

Registrants of JS_HBPA_201-244-2 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision

21/08/2020

Registered substance subject to this decision, hereafter 'the Substance'

Substance name: 4,4'-isopropylidenedicyclohexanol

EC number: 201-244-2

CAS number: 80-04-6

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)**

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by **28 February 2023**.

The requested information must be generated using the Substance unless otherwise specified.

A. Information required from the Registrants subject to Annex IX of REACH

1. *In vivo* mammalian erythrocyte micronucleus test (Annex IX, Section 8.4., column 2; test method: OECD TG 474) in mice or rats, oral route. It is at your discretion to investigate potential aneugenicity and to perform a comet assay in the liver.

Reasons for the request are explained in the following appendix entitled "Reasons to request information required under Annex IX of REACH".

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you, and in accordance with Articles 10(a) and 12(1) of REACH, the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

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 testing and reporting requirements provided under the Appendix entitled "Requirements to fulfil when conducting and reporting new tests for REACH purposes". For references used in this decision, please consult the Appendix entitled "List of references".

Appeal

This decision can be appealed to the Board of Appeal of ECHA within three months of its notification. An appeal, together with the grounds thereof, has to be submitted to ECHA in writing. An appeal has suspensive effect and is subject to a fee. Further details are described under: <http://echa.europa.eu/regulations/appeals>.

Approved¹ under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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 A: Reasons to request information required under Annex IX of REACH

This decision is based on the examination of the testing proposals you submitted.

1. In vivo mammalian erythrocyte micronucleus test

An appropriate *in vivo* somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the *in vitro* genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an *in vivo* study.

Your dossier contains positive results for the *in vitro* mammalian chromosomal aberration test (OECD TG 473) which raise the concern for chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

1.1. Information provided to fulfil the information requirement

You have submitted a testing proposal for an *In vivo* mammalian erythrocyte micronucleus test to be performed with the Substance and to also 'include staining for aneuploidy'. You also propose to incorporate 'a comet phase to investigate any potential DNA damage in the liver'.

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.

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

1.2. Test selection

According to the ECHA Guidance Chapter R.7, Section R.7.7.6.3., the *in vivo* Mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) is suitable to follow-up a positive *in vitro* result on chromosomal aberration if the Substance or its metabolite(s) will reach the target tissue.

1.3. Specification of the study design

You did not specify the species to be used for testing. According to the test method OECD TG 474, the test must be performed in mice or rats.

You did not specify the route for testing. 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.

You proposed to 'include staining for aneuploidy' as 'statistically significant increases in numerical aberrations in the form of polyploidy' were noted in the *in vitro* mammalian chromosomal aberration test (OECD TG 473). ECHA acknowledges that the MN test has the advantage of detecting both structural chromosomal aberrations (resulting from clastogenicity) and numerical chromosomal aberrations (resulting from aneuploidy).

ECHA also notes that, while aneugens can induce polyploidy, polyploidy alone does not indicate aneugenic potential and may only indicate cell cycle perturbation or cytotoxicity

(OECD TG 473). ECHA however agrees that it would be useful to include a staining to investigate potential aneugenicity, as you proposed. According to OECD TG 474, you should thus prepare two sets of slides: 1) one set of slides is stained in the standard way, and 2) another set of slides is stored until the outcome of the scoring of the first set of slides is known. In case the Substance shows an increase in the number of micronuclei (after scoring the first set of slides), the second set of slides should be stained using a centromere- or kinetochore-labelling technique (e.g. FISH or CREST). These labelling techniques can allow identifying whether the micronuclei induced by the Substance are made of whole chromosomes (indicative of aneugenicity) or fragments of chromosomes (indicative of clastogenicity).

Finally, you also proposed to combine the MN test with the comet assay to investigate any potential DNA damage in the liver. Considering that the Substance induced a positive result with metabolic activation in the OECD TG 473 study, ECHA agrees that it is relevant to include the comet assay on the liver; however this is at your discretion. In case you consider to also perform the comet assay, according to OECD TG 489, the test is usually performed in rats. Moreover, you 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).

Regarding the exposure of the target tissue, OECD TG 474 states "*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 if "*Bone marrow exposure to the test substance(s) occurred*". Accordingly, 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.

Germ cells

A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) may still 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.

In case you decide to also perform the comet assay, you may consider to collect the male gonadal cells from the seminiferous tubules in addition to the liver, 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 to Annex IX, Section 8.4., column 2, 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.

Reference

- [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.

1.4. Outcome

Under Article 40(3)(a) your testing proposal is accepted and you are requested to conduct the test with the Substance, as specified above.

Appendix B: Requirements to fulfil when conducting and reporting new tests for REACH purposes

A. 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 summaries².

B. 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 dossiers³.

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

³ <https://echa.europa.eu/manuals>

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 10 August 2020.

ECHA held a third party consultation for the testing proposal(s) from 16 December 2020 until 1 February 2021. ECHA did not receive information from third parties.

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 did not receive any comments within the commenting period.

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 D: List of references - ECHA Guidance⁴ and other supporting documentsEvaluation of available information

Guidance on information requirements and chemical safety assessment, Chapter R.4 (version 1.1., December 2011), referred to as ECHA Guidance R.4 where relevant.

QSARs, read-across and grouping

Guidance on information requirements and chemical safety assessment, Chapter R.6 (version 1.0, May 2008), referred to as ECHA Guidance R.6 where relevant.

Read-across assessment framework (RAAF, March 2017)⁵

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)⁶

Physical-chemical properties

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Toxicology

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

Environmental toxicology and fate

Guidance on information requirements and chemical safety assessment, Chapter R.7a (version 6.0, July 2017), referred to as ECHA Guidance R.7a in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7b (version 4.0, June 2017), referred to as ECHA Guidance R.7b in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.7c (version 3.0, June 2017), referred to as ECHA Guidance R.7c in this decision.

PBT assessment

Guidance on information requirements and chemical safety assessment, Chapter R.11 (version 3.0, June 2017), referred to as ECHA Guidance R.11 in this decision.

Guidance on information requirements and chemical safety assessment, Chapter R.16 (version 3.0, February 2016), referred to as ECHA Guidance R.16 in this decision.

Data sharing

Guidance on data-sharing (version 3.1, January 2017), referred to as ECHA Guidance on data sharing in this decision.

OECD Guidance documents⁷

⁴ <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

⁵ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

⁶ https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

⁷ <http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

Guidance Document on aqueous-phase aquatic toxicity testing of difficult test chemicals – No 23, referred to as OECD GD 23.

Guidance document on transformation/dissolution of metals and metal compounds in aqueous media – No 29, referred to as OECD GD 29.

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

Guidance Document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test – No 151, referred to as OECD GD 151.

Appendix E: Addressees of this decision and the corresponding information requirements applicable to them

You must provide the information requested in this decision for all REACH Annexes applicable to you.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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.