~~Confidential~~

Helsinki, 19 October 2022

Addressees
Registrants of JS_216-365-6 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
08/09/2017

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: [1R-(1α,2β,5α)]-1-(isopropyl)-2-methoxy-4-methylcyclohexane
EC number: 216-365-6
CAS number: 1565-76-0

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 October 2024***.

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

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

1. *In vivo* mammalian erythrocyte micronucleus test (Annex VIII, Section 8.4., column 2; test method: OECD TG 474) in mice or rats, oral route.

Reasons for the request(s) are explained in the following appendix entitled "Reasons to request information required under Annex VIII 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 and VIII to REACH, for registration at 10-100 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.

Approved[1] under the authority of Mike Rasenberg, 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 VIII of REACH

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

1. In vivo mammalian erythrocyte micronucleus test

1.1. Submitted Testing proposal

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

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.

1.2 Assessment of the information provided in the dossier

Under Annex VIII Section 8.4., column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results for the *in vitro* cytogenicity test ("CA test", OECD TG 473, 2015) which raise the concern for chromosomal aberrations.

In your comments on the draft decision you indicated that new data have been generated to address the genetic toxicity of the Substance. In a dossier update (submission no. [redacted]) from 30 November 2021, you provided the following new data: an *in vitro* micronucleus test ("MN test", OECD TG 487, 2021), which gave negative results. Moreover, you mention that the result obtained in the MN test is supported by the other modified *in vitro* MN test (2016) available in the dossier. You therefore indicate that the "*overall conclusion*" for the *in vitro* cytogenicity information requirement is therefore negative.

1.2.1 CA test (2015)

According to OECD TG 473 (para. 44), the test material is considered to be clearly positive if, in any of the experimental conditions examined, all the following criteria are met:

a) at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control;
b) the increase is dose-related when evaluated with an appropriate trend test; and
c) any of the results are outside the distribution of the historical negative control data.

In the dossier update you referred to the CA test (2015) and concluded that:

(i.) the significant positive results were noted "*only for exposure in presence of metabolic activation"*;
(ii.) since only three concentrations were tested, "*the dose-dependency of the results was not proven with certainty"*; and
(iii.) the positive results *were "only slightly exceeding the historical control values"*.

However, ECHA notes the following:

(i.) The test system with metabolic activation shows statistically significant increases; it is noted that such a result can be obtained "*in any of the experimental conditions examined*", that is with and/or without metabolic activation. Therefore, criterion a) above is met.

(ii.) The number of test substance concentrations analysed in the CA test is in line with the requirements set in OECD TG 473; since "*at least three test concentrations*" should be evaluated (OECD TG 473, para. 21). Therefore, the increase noted in the values obtained for the three increasing tested concentrations (1.7%, 5.5% and 6.7%) is dose related. Therefore, criterion b) above is met.

(iii.) The positive results in the CA test with S9 mix fall "*outside the distribution of the historical negative control data*". Additionally, the percentage of cells with aberrations of 5.5% and 6.7% cannot be considered as "*only slightly exceeding"* the range of the historical data, which is 0 to 3.5%. Additionally, your claim contradicts the information provided in the robust study summary, where it is mentioned that "*statistically significant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with 317.2 and 555.1 µg/ml (5.5 and 6.7 % aberrant cells, excluding gaps). These values exceed the laboratory historical solvent control data (0.0–3.5 % aberrant cells, excluding gaps)*". Therefore, criterion c) above is met.

Since all the three criteria, indicated in OECD TG 473 are met, the Substance is considered able to induce chromosomal aberrations in cultured mammalian cells in this test system.

1.2.2 Conclusion

We acknowledge that the results obtained in the MN test (study from 2021) are "*clearly negative*" and that you claim that this is supported by the other modified in vitro MN test (2016) available in the dossier. However, we note that the results obtained in the CA test are "*clearly positive*", as explained above, under section 1.2.1.

We observe that the concentrations tested in the CA test, that is 181, 317 and 555 µg/mL, were actually not studied in the MN test, where lower concentrations were studied (the highest concentration tested was 76 µg/mL). Therefore, the positive results of the CA test (at 317 and 555 µg/mL) and the negative results of the MN test (up to 76 µg/mL) cannot be considered as contradictory, but can rather be considered as consistent results.

Based on the above considerations, the negative results in the MN test cannot overrule the positive results in the CA test. Therefore, the concern for chromosomal aberration (*in vitro*) remains.

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

1.3 Test selection

ECHA notes that the proposed 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 (ECHA Guidance R.7a, Section R.7.7.6.3. and Figure R.7.7-1).

1.4 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.

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.

1.5 Outcome

Your testing proposal is accepted under Article 40(3)(a) 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 summaries[2].

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 dossiers[3].

[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 2 June 2020.

ECHA held a third party consultation for the testing proposal(s) from 19 October 2020 until 3 December 2020. 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 took into account your comments and did not amend the request.

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.

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.

Appendix D: List of references - ECHA Guidance[4] and other supporting documents

Evaluation 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 documents[7]

[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]

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.