EUROPEAN CHEMICALS AGENCY

~~Confidential~~

Helsinki, 19 May 2021

Addressees
Registrants of JS_D-limonene listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
13/03/2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: (R)-p-mentha-1,8-diene
EC number: 227-813-5
CAS number: 5989-27-5

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 ***27 November 2023***.

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

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

1. Extended one-generation reproductive toxicity study (Annex X, Section 8.7.3.; test method: EU B.56./OECD TG 443) by oral route, in rats, specified as follows:

- Ten weeks premating exposure duration for the parental (P0) generation;
- Dose level setting shall aim to induce systemic toxicity at the highest dose level;
- Cohort 1A (Reproductive toxicity);
- Cohort 1B (Reproductive toxicity) without extension to mate the Cohort 1B animals to produce the F2 generation

You must report the study performed according to the above specifications. Any expansion of the study must be scientifically justified.

Reasons for the request(s) are explained in the following appendix:

- Appendix entitled "Reasons to request information required under Annex X 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 to X to REACH, for registration at more than 1000 tpa.

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 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 X of REACH

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

1. Extended one-generation reproductive toxicity study

The basic test design of an extended one-generation reproductive toxicity study (EOGRTS) is a standard information requirement under Annex X to the REACH Regulation. Furthermore, column 2 of Section 8.7.3. defines when the study design needs to be expanded.

You have submitted a testing proposal for an EOGRTS according to OECD TG 443 by the oral route in rats with 2-week premating exposure duration. You have provided the following justification and specification of the study design according to the criteria described in Column 2 of Section 8.7.3, Annex X, and detailed in ECHA Guidance R.7a:

"EOGRTS without extension of Cohort 1B, without Cohorts 2A and 2B, and without Cohort 3. [...] Duration of the study: Premating period: 2 weeks Mating period: 2 weeks Postmating period 6 weeks. Route: diet as the most relevant human route of exposure. Species: Rats/Sprague Dawley".

You provided a separate document presenting your justifications for the study design and premating exposure duration.

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 considers that the proposed study design requires modification to fulfil the information requirement.

The following refers to the specifications of this required study.

Premating exposure duration and dose-level setting

The length of premating exposure period must be ten weeks to cover the full spermatogenesis and folliculogenesis before the mating, allowing meaningful assessment of the effects on fertility, unless there is substance specific information in the dossier supporting a shorter premating exposure duration as advised in the ECHA Guidance[2].

You proposed "*Premating period: 2 weeks*". As a justification for your proposal you referred to ECHA guidance Chapter 7a, which states that: "*the registrant may have additional information that may provide elements which together may support the justification, such as very low general toxicity (no effects up to the limit dose of 1000 mg/kg bw/day in any of the existing studies), fast elimination, no distribution to sex organs, accessory sex organs and brain, and no concern on germ cell toxicity/mutagenicity (no effect in germ cell mutagenicity test)*". You argued that:

i. The Substance has been demonstrated to have no effects on prostate/testes or ovaries/uterus in sub-chronic toxicity studies in rats or mice or in a 6-month study performed in dogs.
ii. If effects observed in kidneys of male rats ("*which are not relevant towards humans*") are not considered, the Substance has shown low general systemic toxicity in sub-chronic oral studies in mice and rats. The only effects observed were "*non-adverse decreased body weight gain and clinical signs at 1000 mg/kg bw/day and 1200 mg/kg*

[2] ECHA Guidance R.7a, Section R.7.6.

bw/day".

iii. The Substance is "*extensively metabolised and excreted in urine within 48 h, and mostly during the first 24 h, in the rat*". As the Substance is metabolised to more polar molecules, those are excreted more easily than the Substance.
In human volunteers "*the whole process of uptake and elimination was almost finished within 10 h after exposure*".

iv. In relation to distribution, "*the maximum radioactivity was found in liver, kidneys and blood, 1-2 h after a single oral administration of 800 mg/kg bw of [^{14}C]d-limonene in the rat, and it was negligible at 48 h in all tissues*".

v. The results of mutagenicity tests were negative.

ECHA has examined your arguments and has identified the following shortcomings:

i. Lack of effects on reproductive organs in repeated dose studies without mating of the animals does not exclude that effects on functional fertility could occur.

ii. In the dossier, a NOAEL of 600 mg/kg bw/day is reported for male and female rats in a 90-day study, and in a 90-day mouse study the NOAEL was 500mg/kg bw/day. This contradicts your claim of (very) low general toxicity. Furthermore, in several studies, kidney effects have been observed in male rats (e.g., 90-day study with LOAEL 150 mg/kg bw/day). Although the mechanisms related to the observed effects are considered not relevant for humans, they show that the substance can cause systemic toxicity effects.

iii. Your toxicokinetic data and systemic effects reported in the dossier and noted above (ii) indicate that the substance and its metabolites may be sufficiently present in the body to cause systemic effects.

iv. As the substance has been detected in liver, kidneys and blood, there is an additional indication of systemic distribution. Furthermore, the provided data does not demonstrate absence of distribution to sex organs, accessory sex organs or brain.

Based on the shortcomings listed above, you have not demonstrated that ten-week premating exposure is not necessary for a meaningful assessment of the fertility effects and you have not provided substance-specific justifications that would meet the prerequisites of a shorter duration of premating exposure. Therefore, ECHA does not agree with your proposal.

Therefore, ten weeks premating exposure duration is required.

In order to be compliant and not to be rejected due to too low dose levels, the highest dose level must aim to induce systemic toxicity, but not death or severe suffering of the animals, to allow comparison of reproductive toxicity and systemic toxicity. The dose level selection should be based upon the fertility effects with the other cohorts being tested at the same dose levels. A descending sequence of dose levels should be selected in order to demonstrate any dose-related effect and to establish NOAELs.

If there is no existing relevant data to be used for dose level setting, it is recommended that results from a range-finding study (or range finding studies) are reported with the main study.

You must provide a justification with your study report that demonstrate that the dose level selection meets the conditions described above.

Cohorts 1A and 1B

Cohorts 1A and 1B belong to the basic study design and shall be included.

Species and route selection

You proposed testing by oral route in rats. ECHA agrees with your proposal.

Outcome

Under Article 40(3)(b) of REACH, you are requested to carry out the proposed test under modified conditions, as explained above with the Substance.

Further expansion of the study design

The conditions to include the extension of Cohort 1B are currently not met. Furthermore, no triggers for the inclusion of Cohorts 2A and 2B (developmental neurotoxicity) and Cohort 3 (developmental immunotoxicity) were identified. However, you may expand the study by including the extension of Cohort 1B, Cohorts 2A and 2B and/or Cohort 3 if relevant information becomes available from other studies or during conduct of this study. Inclusion is justified if the available information meets the criteria and conditions which are described in Column 2, Section 8.7.3., Annex X. You may also expand the study due to other scientific reasons in order to avoid a conduct of a new study. The study design, including any added expansions, must be fully justified and documented. Further detailed guidance on study design and triggers is provided in ECHA Guidance[3].

[3] ECHA Guidance R.7a, Section R.7.6.

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[4].

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[5].

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

Appendix C: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 16 March 2020, following the necessary clarification of the identity of your substance.

ECHA held a third party consultation for the testing proposal(s) from 25 May 2020 until 9 July 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.

In your comments on the draft decision, you have requested a prolongation of the deadline, requesting a deadline of at least 30 months. You have provided a schedule obtained from your CRO, which specifies certain timelines in the overall execution of the study (22 months in total), with the finalisation of the study in April 2023.

Furthermore, you justify your request by referring to the time needed for

i) validation of "all critical steps with the laboratory and the co-registrants in order to facilitate acceptance of the new generated data and their corresponding sharing costs [...] also for the validation of the study design and the doses to be tested in those studies"
ii) the update of your dossier and CSR.

ECHA has considered your arguments and has only partially granted the request based on the indication on study duration by the CRO and set the deadline to 30 months.

ECHA took into account your comments and did not amend the request(s) but amended the deadline.

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 Guidance[6] 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)[7]

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)[7]

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.

[6] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[7] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

OECD Guidance documents[8]
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.

[8] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

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.