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Helsinki, 15 June 2022

Addressees
Registrants of JS_84650-59-9 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
11/11/2020

Registered substance subject to this decision ("the Substance")
Substance name: Star anise, Illicium verum, ext.
EC number: 283-518-1

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 ***21 March 2024***.

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

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

1. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays also requested below (triggered by Annex VII, Section 8.4., column 2)

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

2. *In vivo* genetic toxicity study (triggered by Annex VIII, Section 8.4., column 2):

   In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified in other rodents, oral route, on the following tissues: liver, glandular stomach and duodenum;

   or

   Transgenic rodent somatic and germ cell gene mutation assay (test method: OECD TG 488 from 2020) 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.

Your originally proposed test using the Substance is rejected, according to Article 40(3)(d):

In vivo mammalian erythrocyte micronucleus test (EU B.12./OECD TG 474)

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 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 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.

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Appendix 1: Reasons for the decision

Contents

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

1. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result in an *in vitro* gene mutation study in bacteria (Section 8.4., Column 2).

2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 2018) which raise the concern for gene mutations.

3 ECHA considers that an *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 VIII of REACH

2. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

5 Appropriate *in vivo* mutagenicity studies must be considered under Annex VIII to REACH (Section 8.4., Column 2) in case of a positive result in any of the *in vitro* genotoxicity studies under Annex VII or VIII to REACH.

6 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 2018) which raise the concern for gene mutations.

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 received third party information concerning the testing proposal during the third party consultation. The third party information indicated that in the dossier there is a positive Ames study (OECD TG 471) and a negative *in vitro* micronucleus assay (OECD TG 487). Therefore, in relation to the proposed study, they emphasized that *"any in vivo studies performed must be scientifically relevant and in line with ECHA Guidance, in order to avoid unnecessary animal testing*". ECHA addressed the selection of the study in section 2.2. below.

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

2.2. Test selection

11 You proposed to perform an *In vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474). However, the MN test investigates chromosomal aberration and is not suitable to follow up a positive *in vitro* result on gene mutation.

12 Therefore, taking also the third party comments into consideration, ECHA notes that the MN test is not an appropriate follow-up test for the Substance.

13 According to the Guidance on IRs & CSAa, Section R.7.7.6.3, either the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) or the Transgenic rodent somatic and germ cell gene mutation assay ("TGR assay", OECD TG 488) are suitable to follow up a positive *in vitro* result on gene mutation.

2.3. Specification of the study design

2.3.1. Comet assay

14 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, para. 23).

15 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.

16 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, 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.

2.3.1.1. Germ cells

17 You may consider to collect 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.

2.3.2. TGR assay

18 According to the test method OECD TG 488, the test must be performed in transgenic mice or rats; and the test substance is usually administered orally.

19 Based on the recent update of OECD TG 488 (2020), you are requested to follow the new 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.

20 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, 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.

2.3.2.1. Germ cells

21 You may consider to collect the male germ cells (from the seminiferous tubules) at the same time as the other tissues, in order 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 duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ 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

22 Your testing proposal is rejected under Article 40(3)(d) of REACH. Under Article 40(3)(c) you are requested to carry out the additional test 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).

All Guidance on REACH is 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

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 4 March 2021.

ECHA held a third party consultation for the testing proposal(s) from 22 April 2021 until 7 June 2021. ECHA received information from third parties (see Appendix 1, Section 2).

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. One of the registrants of the Substance which was originally addressed by the draft decision submitted in its comments that it disagrees with the selection of the information. It therefore separately submitted information on mutagenicity, in accordance with Article 11(3)(c) of REACH. This decision therefore no longer addresses this registrant.

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.

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Appendix 3: Addressees 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.

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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 identify all the constituents as far as possible as well as their concentration (OECD GLP (ENV/MC/CHEM(98)16) and EU Tests Methods Regulation (EU) 440/2008 (Note, Annex). 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

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].

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