EUROPEAN CHEMICALS AGENCY

Helsinki, 4 November 2020

Addressees
Registrant listed in the last Appendix of this decision

Date of submission for the submitted dossier subject of a decision
20/08/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 5-hydroxy-4-propylfuran-2(5H)-one
EC number: 821-451-7
CAS number: 78920-10-2

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

DECISION ON A TESTING PROPOSAL

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), ECHA requests that you submit the information listed below by the deadline of ***11 March 2022***.

A. Requirements applicable to all the Registrants subject to Annex VII of REACH

1. In vivo mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD TG 489) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum with the Substance;

Conditions to comply with the requests

You are bound by the requests for information corresponding to the REACH Annexes applicable to your own registered tonnage of the Substance at the time of evaluation. Therefore, you have to comply with the requirements of Annex VII of REACH, if you have registered a substance at 1-10 tonnes per annum (tpa), or as a transported isolated intermediate in quantity above 1000 tpa;

Appendix A state the reasons for the request for information to fulfil the requirement set out in the respective Annex of REACH.

The Appendix entitled Observations and technical guidance addresses the generic approach for the selection and reporting of the test material used to perform the required studies and provides generic recommendations and references to ECHA guidance and other reference documents.

You must submit the information requested in this decision by the deadline indicated above in an updated registration dossier and also update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

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 for the requirements applicable to all the Registrants subject to Annex VII of REACH

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

1. In vivo mammalian alkaline comet assay (Annex VII, Section 8.4., column 2)

Examination of the testing proposal

"Mutagenicity" is an information requirement as laid down in Annex VII, Section 8.4. of the REACH Regulation. Column 2 of Annex VII, Section 8.4. provides that "Further mutagenicity studies shall be considered in case of a positive result".

The ECHA guidance R.7a states that following a positive result in an *in vitro* test, "adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed *in vivo*.

Your dossier contains a positive result for the *in vitro* gene mutation study in bacteria which raises the concern for gene mutation.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the Substance. You considered it necessary to generate information for this endpoint.

Hence, you have submitted a testing proposal for an *in vivo* mammalian alkaline comet assay.

ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. ECHA notes that 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 mutagenicity study is necessary to address the concern identified *in vitro*.

ECHA considers that the proposed test is appropriate to investigate effects on gene mutation *in vivo* as described in the ECHA Guidance[2].

According to the ECHA Guidance Chapter R.7a[3], the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a *positive in vitro* result on gene mutation.

Therefore, the proposed comet assay is a suitable test to follow up the concern on gene mutation for the Substance.

You propose to test in rats by the oral route. According to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure

[2] ECHA Guidance Chapter R.7a, Section R.7.7.6.3
[3] ECHA Guidance Chapter R.7a, Section R.7.7.6.3

and adequate exposure of the target tissues, performance of the test by the oral route is appropriate.

You propose to test in one sex (male) only, as no sex-specific toxicity is expected.

You propose to perform the test in liver and blood. 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.

You also propose to analyse blood samples in the same study. It is your discretion to perform this intended additional examination. According to OECD TG 489, it may be useful to examine multiple tissues in the same animals provided that tissue selection is justified and the laboratory has demonstrated proficiency with those tissues and competency in handling multiple tissues at the same time.

In a proposal for amendment (PfA) submitted by one of the Member State Competent Authorities (MSCAs) it was proposed to include a recommendation to perform additional *in vitro* tests according to the data requirements of REACH Annex VIII, 8.4.2. and to follow up with a combined comet assay and Mammalian Erythrocyte Micronucleus Test ("MN test", OECD TG 474) if the test according to REACH Annex VIII, 8.4.2. is positive.

In your comments to the PfA you agreed to "*first perform an in vitro chromosome aberration test to address potential aneugenicity of the Substance before an in vivo comet assay is initiated*". You further indicated that 1) "*in case the in vitro chromosome aberration test is positive for aneugenicity, [you] agree to proceed with a combined comet assay/Micronucleus test*"; and 2) "*if the in vitro chromosome aberration test is negative for aneugenicity, the in vivo comet assay is proposed to be performed*". Finally, you "*ask for permission to postpone the performance of the in vivo test until the outcome of the chromosome aberration study is available*".

Based on your comments to the PfA you may consider to perform either the in vitro chromosomal aberration test in mammalian cells (test method OECD TG 473) or the in *vitro* micronucleus test in mammalian cells (test method OECD TG 487) before performing the comet assay. A positive result in the *in vitro* cytogenicity test (OECD TG 473 or 487) would indicate a concern for chromosomal aberration. In such case, it is at your discretion to combine the comet assay and the MN test into a single study. The combination of a comet assay with the MN test can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation.

In case you decide to perform the MN test in combination with the comet assay, 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[4]).

[4] Bowen D.E. 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. Mutation Research 722 7–19

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

Notes for your consideration

You may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[5]) in addition to the other aforementioned tissues, 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, 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.

[5] O'Brien, J.M., Beal, M.A., Gingerich, J.D., Soper, L., Douglas, G.R., Yauk, C.L., Marchetti, F. (2014) Transgenic Rodent Assay for Quantifying Male Germ Cell Mutant Frequency. J. Vis. Exp. (90), e51576, doi:10.3791/51576

Appendix B: Procedural history

ECHA received your registration containing the testing proposal for examination on 2 September 2019.

ECHA held a third party consultation for the testing proposal from 25 November 2019 until 9 January 2020. ECHA did not receive information from third parties.

For the purpose of the decision-making, this decision does not take into account any updates of registration dossiers after the date on which you were notified the draft decision according to Article 50(1) of REACH.

The decision making followed the procedure of Articles 50 and 51 of REACH, as described below:

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

ECHA did not receive any comments within the notification period.

ECHA notified the draft decision to the competent authorities of the Member States for proposals for amendment.

ECHA received proposal(s) for amendment and did not modify the draft decision.

ECHA invited you to comment on the proposed amendment(s) and referred the draft decision to the Member State Committee.

Your comments on the proposed amendment(s) were taken into account by the Member State Committee.

In your comment on the proposed amendment, you indicated that "*the substance was updated from Annex VII to Annex VIII*"; however, the information in REACH-IT does not show that such update has been submitted with regards to your registration.

The timeline indicated in the draft decision to provide the information requested is 12 months from the date of adoption of the decision. Based on your comments to the proposal(s) for amendment, ECHA has modified and set the deadline of the decision to 16 months.

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-71 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix C: Observations and technical guidance

1. This testing proposal examination decision does not prevent ECHA from initiating compliance checks at a later stage on the registrations present.

2. Failure to comply with the requests in this decision, or to otherwise fulfil the information requirements with a valid and documented adaptation, will result in a notification to the enforcement authorities of your Member State(s).

3. Test guidelines, GLP requirements and reporting

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

   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.

   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: 'How to report robust study summaries'[6].

4. Test material

   Selection of the test material(s)

   The registrants of the Substance are responsible for agreeing on the composition of the test material to be selected for carrying out the tests required by the present decision. The test material selected must be relevant for all the registrants of the Substance, i.e. it takes into account the variation in compositions reported by all members of the joint submission. The composition of the test material(s) must fall within the boundary composition(s) of the Substance.

   While selecting the test material you must take into account the impact of each constituent/impurity is known to have or could have 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.

   Technical reporting of the test material

   The composition of the selected test material must be reported in the respective endpoint study record, under the Test material section. The composition must include all constituents of the test material and their concentration values. Without such detailed reporting, ECHA may not be able to confirm that the test material is relevant for the Substance and to all the registrants of the Substance.

   Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[7].

[6] https://echa.europa.eu/practical-guides
[7] https://echa.europa.eu/manuals

5. List of references of the ECHA Guidance and other guidance/ reference documents[8]

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 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)[9]

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.

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

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

Appendix D: List of the registrant to which the decision is addressed and the corresponding information requirement applicable to them

Registrant Name	Registration number	(Highest) Data requirements to be fulfilled
[redacted]	[redacted]	[redacted]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.