~~Confidential~~

Helsinki, 18 January 2021

Addressees
Registrants of JS_Benzalacetone listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
26 March 2020

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 4-phenylbutenone
EC number: 204-555-1
CAS number: 122-57-6

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 ***25 April 2022***.

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

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

1. In vivo mammalian alkaline comet assay (triggered by Annex VII, Section 8.4., column 2) as requested below;

B. Requirements applicable to all the Registrants subject to Annex VIII of REACH

1. In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2) as requested below;

C. Requirements applicable to all the Registrants subject to Annex IX of REACH

1. In vivo mammalian alkaline comet assay (Annex IX, 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;

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

- Appendices entitled "Reasons to request information required under Annexes VII to IX of REACH", respectively.

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 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;
- 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.

For certain endpoints, ECHA requests the same study from registrants at different tonnages. In such cases, only the reasoning why the information is required at lower tonnages is provided in the corresponding Appendices. For the tonnage where the study is a standard information requirement, the full reasoning for the request including study design is given. Only one study is to be conducted; the registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the other registrants under Article 53 of REACH.

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". In addition, you should follow the general recommendations provided under the Appendix entitled "General recommendations 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 VII of REACH

1. In vivo mammalian alkaline comet assay

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

Under Annex VII, Section 8.4., column 2 of REACH, further mutagenicity studies must be considered in case of a positive result in an *in vitro* gene mutation study in bacteria.

ECHA guidance R.7a, Section R.7.7.6.3 (p.570) states that, following a positive result in an *in vitro* test, an "*adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary*".

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, which raises the concern for gene mutations. You also submitted a negative *in vivo* micronucleus study (OECD TG 474). However this study does not address the concern on gene mutation.

Therefore, you have submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance.

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

The assessment of the information provided, the selection of the requested test, the test design and the outcome are addressed in request C.1.

Appendix B: Reasons to request information required under Annex VIII of REACH

1. In vivo mammalian alkaline comet assay

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

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 required under Annex VII or VIII.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, *in vitro* cytogenicity tests and *in vitro* gene mutation study in mammalian cells which raise the concerns for gene mutations and chromosomal aberrations. Moreover, you also submitted a negative *in vivo* micronucleus study (OECD TG 474). This study addresses the concern on chromosomal aberrations *in vivo*. However it is not appropriate to address the concern on gene mutations.

Therefore, you have submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance.

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

The assessment of the information provided, the selection of the requested test, the test design and the outcome are addressed in request C.1.

Appendix C: Reasons to request information required under Annex IX of REACH

1. In vivo mammalian alkaline comet assay

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

Under Annex IX Section 8.4., column 2 of REACH, the information requirement for an appropriate *in vivo* somatic cell genotoxicity study is triggered if 1) there is a positive result in any of the *in vitro* genotoxicity studies required under Annex VII or VIII and 2) there are no appropriate results already available from an *in vivo* somatic cell genotoxicity study.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria, *in vitro* cytogenicity tests and *in vitro* gene mutation study in mammalian cells which raise the concerns for gene mutations and chromosome aberrations. Moreover, you also submitted a negative *in vivo* micronucleus study (OECD TG 474). This study addresses the concern on chromosomal aberrations *in vivo*. However it is not appropriate to address the concern on gene mutations.

Therefore, you submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance.

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 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.*

i. Test selection

ECHA notes that the proposed test is appropriate to investigate effects on gene mutations *in vivo* (ECHA Guidance R.7.7.6.3., Figure R.7.7-1).

According to the ECHA Guidance R.7.7.6.3., the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up a positive *in vitro* result on gene mutation.

ii. Test design

You did not specify the species to be used for testing and the route for testing.

According to the test method OECD TG 489, the test must be performed in rats. 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.

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.

iii. 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.

Therefore, you may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*[2]) 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, 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.

iv. Outcome

Under Article 40(3)(a) of REACH, you are requested to carry out the proposed test with the Substance.

In your comments to the draft decision you agree to perform the study however you do not agree with the proposal to collect the male gonadal cells and to prepare the slides for further analysis. You indicate that "*This is not appropriate as the study design of a Comet assay according to OECD Test Guideline 489 is not suitable for a robust assessment of potential genotoxicity of a substance in germ cells*."

ECHA notes that the collection of the male gonadal cells, as specified under section iii. (*Germ cells*) above, is a recommendation for you to consider. This may be useful if the assessment of the potential for germ cell mutagenicity of the Substance is needed.

[2] 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 D: 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[3].

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[4].

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

Appendix E: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 16 October 2019

ECHA held a third party consultation for the testing proposals from 23 March 2020 until 7 May 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(s).

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 F: List of references - ECHA Guidance[5] 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)[6]

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.

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[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.

[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment
[6] https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across
[7] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316
[8] http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm

Appendix G: 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) 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.