~~Confidential~~

Helsinki, 01 June 2021

Addressees
Registrants of JS_116_09_6 listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision
12/06/2019

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: Hydroxyacetone
EC number: 204-124-8
CAS number: 116-09-6

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 ***8 December 2022***.

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 alkaline comet assay (Annex VIII, Section 8.4., column 2; test method: OECD TG 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD TG 474) in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

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

- 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 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 VIII of REACH

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

1. In vivo mammalian alkaline comet assay combined with in vivo mammalian erythrocyte micronucleus test

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* gene mutation study in bacteria and the *in vitro* mammalian chromosomal aberration test, which raise the concerns for gene mutations and chromosomal aberrations.

1.1. Information provided to fulfil the information requirement

You have 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*. 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 genotoxicity study is necessary to address the concerns identified *in vitro.*

1.2. Test selection

You have proposed to perform an *In vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489).

However, the positive *in vitro* results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

In your comments to the draft decision you indicate that the results obtained for the Ames studies were equivocal as the "*results were borderline and not reproducible in two different tests and at least some findings are likely to be related to the reaction of the test item with the vehicle DMSO.*"

We indeed note that the positive results for TA 100 and TA 97, with and without metabolic activation, were obtained in two different experiments. However, the positive result in TA 97 was one of the tests performed with a different vehicle that is water and not DMSO.

We also note that the overall conclusion for the *in vitro* gene mutation study in mammalian cells is negative. However, in the dossier it is reported that "*a statistically significant increase in mutant frequency was detected at the concentrations 2 mg/mL and 1 mg/mL*". Therefore it is not clear whether the result obtained is "*clearly negative*". Nonetheless, this result does not remove the remaining concern raised by the results obtained in the Ames studies.

Furthermore, we note that in the dossier you state that based on these Ames results "*there are indications for a mutagenic potential*" of the Substance. Moreover, in your testing proposal

you yourself claim that the Substance "*was positive in a bacteria gene mutation (OECD 471, GLP) and in an in vitro mammalian cytogenicity assay (OECD 473, GLP)*" and that based on these positive results you propose to perform the comet assay.

Therefore, based on the above, ECHA considers that there are both concerns for gene mutations and chromosomal aberrations due to the positive *in vitro* results (OECD TGs 471 and 473) in the dossier.

According to the ECHA Guidance R.7a, Section R.7.7.6.3, the comet assay is a suitable test to follow up the positive *in vitro* result for chromosomal aberration and gene mutation. However, only the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) detects both structural and numerical chromosomal aberrations, and therefore provides direct evidence of *in vivo* chromosomal mutagenicity. As indicated in the ECHA Guidance, it is possible to combine the comet assay and the MN test into a single study. The combination study can help reduce the number of tests performed and the number of animals used while providing useful information on the potential of the Substance to induce chromosomal aberration and gene mutation.

In your comments to the draft decision you agree that the comet assay is a suitable *in vivo* follow-up test for the Substance, in line with both REACH Article 40(3) and ECHA's guidance documents. However, you do not agree that the comet assay should be combined with the MN test, mainly because you consider the argumentation by ECHA, on requesting two tests, is neither scientifically valid nor in line with the REACH regulation or ECHA's guidance document; in addition you state that there are no substance specific findings that justify the combination with the MN test.

However, we note that at the 70th meeting of the Member States Committee (MSC-70) (June 2020)[2], it was agreed that the combined study of the comet assay and the MN test would be most suitable when both concerns for chromosomal aberration and gene mutation exist and no *in vivo* genotoxicity data are available in the dossier. During this MSC-70 meeting it was also concluded that the combination study can be considered as one single study.

Therefore, ECHA is following the approach agreed at MSC-70, that is to request a combination study, as based on the data available in the dossier and as explained above, there are both concerns for gene mutation and chromosomal aberration and there are no *in vivo* genotoxicity studies available in the dossier.

We acknowledge that the ECHA guidance still needs to be updated and aligned with this current practice agreed at MSC-70. This information concerning the need to perform a combination study (comet assay and MN test) has been communicated in the ECHA website, following the MSC-70 agreement, under the recommendations to registrants concerning the mutagenicity information requirement[3].

In your comments you also state that "*the CROs have so far limited or even no historical control data for the MN test in the rat*". However, we are aware that the main CROs have performed the comet assay in combination with the MN test on rats in a regulatory framework.

Therefore, based on the above, the comet assay combined with the MN test is the most appropriate study for the Substance.

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[2] Minutes of the of the 70th Meeting of the Member State Committee (MSC-70), 10-12 June 2020, web conference: https://echa.europa.eu/documents/10162/28685870/MinutesofMSC-70_adopted-1.pdf/2972d2e5-6a5b-67ce-efc8-1a67a8e025a9

[3] ECHA website, Support, Recommendations to registrants, Standard information requirements, Mutagenicity: https://echa.europa.eu/standard-information-requirements-recommendations

1.3. Specification of the study design

You did not specify the species to be used for testing. According to the test method OECD TG 489, the test must be performed in rats. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be conducted in the rat.

You did not specify the route for testing. 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.

The combination of OECD TGs 489 and 474 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 *[1]*).

In your comments to the draft decision you state that *"the substance has some irritation potential which will trigger the maximum dose to be administered as the forestomach is a key target organ for a substance presenting a direct mutagen".* You also note that this might result in *"negative findings"* in a MN test performed in parallel with the comet assay *"as potential higher doses could have been tested in a separate MN test".*

As explained above the comet assay has to be performed on liver, glandular stomach and duodenum. It is at your discretion if you also want to perform the comet assay on the forestomach. As for the MN test you can either perform it on the bone marrow or the peripheral blood cells, according to OECD TG 474. As regards the dosing we note that both OECD TGs 489 and 474 indicate that the study should aim to identify the maximum tolerated dose (MTD) or doses not inducing too high toxicity in the target organs of the comet assay or in the bone marrow. Therefore, this *"irritation potential"* should not have an impact on the performance of the combination study.

Germ cells

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.

Reference:

[1] Bowen DE 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. *Muta. Res.;*722:7–19.

1.4. Outcome

Under Article 40(3)(b) your testing proposal is accepted under modified conditions 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[4].

B. Test material

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 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.

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 9 June 2020.

ECHA held a third party consultation for the testing proposal(s) from 23 November 2020 until 7 January 2021. 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.

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.