

Helsinki, 01 February 2022

Addressees

Registrants of [REDACTED] listed in the last Appendix of this decision

Date of submission of the dossier subject of a decision

12/02/2021

Registered substance subject to this decision, hereafter 'the Substance'

Substance name: [REDACTED]

List number: [REDACTED]

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

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by **8 August 2023**.

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 are explained in the following appendix entitled "Reasons to request information required under Annexes 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>.

Approved¹ under the authority of Mike Rasenberg, 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 proposals you submitted.

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

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.

Your dossier contains positive results for the *in vitro* gene mutation study in mammalian cells (OECD TG 490; [REDACTED], 2020) which raise the concerns both for gene mutation and chromosomal aberration. In this study there was a statistically significant, dose-related and reproducible increase in mutant frequency, for both small and large colonies, in the presence of metabolic activation.

1.1. Information provided to fulfil the information requirement

You have submitted a testing proposal for an *In vivo* mammalian alkaline comet assay combined with *In vivo* mammalian erythrocyte micronucleus test 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 concern identified *in vitro*.

1.2. Test selection

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

The *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) and the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) can be combined in a single study (see OECD TG 474 para. 37c; OECD TG 489 para. 33; ECHA Guidance R.7a, Section R.7.7.6.3). While the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations. A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

The combined study, together with the results of the *in vitro* mutagenicity studies, can be used to make definitive conclusions about the mechanism(s) inducing *in vivo* mutagenicity and lack thereof. Furthermore, the combined study can help reduce the number of tests performed and the number of animals used while addressing (structural and numerical) chromosomal aberrations as well as gene mutations.

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

1.3. Specification of the study design

Species

You proposed testing in the rat. According to the test method OECD TG 489, rats are routinely used in this test. Therefore, the combined test (OECD TG 489 and OECD TG 474) must be performed in rats.

You propose testing in males only, assuming that no substantial differences in the maximum tolerated dose (MTD) are identified between the sexes. According to OECD TG 489 (paragraph 31), the comet assay can be performed in either sex, however if there is data demonstrating relevant differences between males and females then a study in both sexes is encouraged. Based on the available study in the dossier (OECD TG 422) no significant differences were reported between the sexes; therefore ECHA confirms that testing in males is appropriate.

Route of administration

You proposed testing by the oral route. 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.

Tissues and cells to be examined

For the comet assay you proposed testing in the liver. You indicate that there is evidence showing that the Substance "*requires metabolic activation to its genotoxic form and therefore inclusion of a site of contact tissue is unnecessary*".

ECHA acknowledges that the positive *in vitro* results, obtained in OECD TG 490, were noted in the presence of metabolic activation only. However, in line with the test method OECD TG 489, the test must by default be performed by analysing tissues from liver, as primary site of xenobiotic metabolism, and the 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 also analyse both site-of-contact tissues to ensure a sufficient evaluation of the potential for genotoxicity at the site of contact in the gastro-intestinal tract.

For the micronucleus assessment you proposed testing the bone marrow. According to OECD TG 474, the MN test can either be performed on the bone marrow or the peripheral blood cells.

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]).

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.

In the comments to the draft decision, you agree to perform the requested study.

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 summaries².

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 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.

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 dossiers³.

² <https://echa.europa.eu/practical-guides>

³ <https://echa.europa.eu/manuals>

Appendix C: Procedure

ECHA received your testing proposal(s) on 19 February 2021 and started the testing proposal evaluation in accordance with Article 40(1).

ECHA held a third party consultation for the testing proposal(s) from 20 May 2021 until 5 July 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.

In your comments you agreed to the draft decision. ECHA took your comments into account 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 Guidance⁴ and other supporting documentsEvaluation 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)⁵

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)⁶

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.

⁴ <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

⁵ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

⁶ https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

OECD Guidance documents⁷

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.

⁷ <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
██████████	████████████████████	██████████

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.