EUROPEAN CHEMICALS AGENCY

Helsinki, 6 February 2020

Addressees
Registrants of [redacted] listed in the last Appendix of this decision

Date of submission for the jointly submitted dossier subject of a decision
25 October 2018

Registered substance subject to this decision, hereafter 'the Substance'
Substance name: 1,3,2-dioxathiolane 2,2-dioxide
EC number: 600-809-4
CAS number: 1072-53-3

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) and on the testing proposals you submitted, ECHA requests that you submit the information listed below by the deadline of ***11 November 2021***.

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

1. The *in vivo* genotoxicity study also requested at B.1. below (triggered by Annex VII, Section 8.4., column 2).

B. Requirements applicable to all 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) in rats, oral route, on the following tissues: liver, glandular stomach and duodenum using the Substance

   OR

   Transgenic rodent somatic and germ cell gene mutation assays (Annex VIII, Section 8.4., column 2; test method EU B.58./OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver, glandular stomach using the Substance; 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.

Conditions to comply with the request

Each addressee of this decision is bound by the requests for information corresponding to the REACH Annexes applicable to their own registered tonnage of the Substance at the time of evaluation of the jointly submitted dossier.
To identify your legal obligations, please refer to the following:

- 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;
- you have to comply with the requirements of Annexes VII and VIII of REACH, if you have registered a substance at 10-100 tpa.

Registrants are only required to share the costs of information that they must submit to fulfil the information requirements for their registration.

When a study is required under several Annexes of REACH, the reasons are provided in the corresponding appendices of this decision. 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 in accordance with Article 53 of REACH.

Appendix A and B state the reasons for the requests for information to fulfil the requirements set out in Annexes VII and VIII 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

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

   OR

   Transgenic rodent somatic and germ cell gene mutation assays (Annex VII, Section 8.4., column 2).

Mutagenicity is an information requirement in Annex VII to 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 technical dossier contains the following studies with the registered substance that show positive results with and without metabolic activation:

- *In vitro* gene mutation study in bacteria, performed according to OECD TG 471.
- *In vitro* gene mutation study in mammalian cells, performed according to OECD TG 490.

The positive results indicate that the Substance is inducing gene mutations under the conditions of the tests.

These positive findings regarding gene mutation cannot be addressed by other *in vitro* tests (e.g., OECD TG 487 or 473), nor by other types of testing (e.g., toxicokinetic studies), which do not provide any information related to the specific gene mutation concern raised by the positive in vitro gene mutation test in bacteria.

In addition, the existing *in vivo* studies are not addressing the gene mutation concern for the reasons described under Section B.1.

An appropriate *in vivo* genotoxicity study to follow up the concern on gene mutations is not available for the Substance and you considered it necessary to generate information for this endpoint. You have therefore submitted a testing proposal for an *in vivo* mammalian alkaline comet assay to be performed with the Substance.

The examination of your proposal for the type of test as well as of the test design are addressed in Appendix B, Section 1 below.

Appendix B: Reasons for the requirements applicable to all the Registrants subject to Annex VIII of REACH

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

   OR

   Transgenic rodent somatic and germ cell gene mutation assays (Annex VIII, Section 8.4., Column 2)

Mutagenicity is a standard information requirement in Annex VIII to the REACH Regulation. Column 2 of Annex VIII, Section 8.4. provides that "Appropriate in vivo mutagenicity studies shall be considered in case of a positive result in any of the genotoxicity studies in Annex VII or VIII."

As already explained in Appendix A, Section 1 above, the provided information on an in vitro gene mutation study in bacteria, performed according to OECD TG 471, and an in vitro gene mutation study in mammalian cells, performed according to OECD TG 490, show positive results indicating that the Substance is inducing gene mutations under the conditions of the tests.

In the absence of an appropriate in vivo genotoxicity study to follow up the concern on gene mutations you considered it necessary to generate information for this endpoint. You have therefore 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(s) for which testing is proposed. ECHA has taken these considerations into account.

The proposed test is an appropriate test to further investigate effects on gene mutations *in vivo* as described in the ECHA Guidance R.7a, Section R.7.7.6.3. and figure R.7.7-1. According to this Guidance document, the transgenic rodent somatic and germ cell gene mutation assays ("TGR assay", OECD TG 488) is also suitable to follow up a positive *in vitro* result on gene mutation. The TGR assay is able to detect permanent gene mutations, whereas the comet assay is an indicator test to detect putative DNA lesions.

Under Article 40(3)(a) of the REACH Regulation you are therefore requested to carry out one of the following studies 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.

or

Transgenic rodent somatic and germ cell gene mutation assays (test method: EU B.58./OECD TG 488) 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. The test material used should be freshly prepared.

Information about the design of the studies

The Substance should be dissolved in water in order to avoid potential interference of the vehicle with the genotoxicity of the Substance.

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

In case you decide to perform a comet assay, according to the test method OECD TG 489, the assay must be performed in rats. Having considered the anticipated routes of human exposure and adequate exposure of the target tissues, performance of the test by the oral route is appropriate.

In line with the test method OECD TG 489, the comet assay 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)). Following 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.

In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats and the Substance is usually administered orally. In line with this test method, 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)). Following 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 °C) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for glandular stomach and liver are negative or inconclusive.

Germ cells

In case you decide to perform the comet assay, 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, 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.

In case you decide to perform the TGR, you may consider to collect the male germ cells 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 °C). Following the generation and analysis of data on somatic cells, you should consider analysing the collected germ cells.

[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

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.

Appendix C: Procedural history

ECHA received your registration containing the testing proposal for examination on 7 November 2018.

ECHA held a third party consultation for the testing proposal from 24 January 2019 until 11 March 2019. 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 the REACH.

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

ECHA notified you of the draft decision and invited you to provide comments within 30 days of the notification.

In your comments you agreed to the draft decision. ECHA took your comments into account and did not amend the requests.

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: 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 shall 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'[3].

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.

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

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"[4].

5. List of references of relevant ECHA Guidance documents[5]

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.

[4] https://echa.europa.eu/manuals
[5] https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment

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

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