

Helsinki, 24 June 2021

Addressees

Registrant(s) of JS_214-022-5 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision

20/09/2019

Registered substance subject to this decision ("the Substance")

Substance name: 1,3,2-dioxathiane 2,2-dioxide

EC number: 214-022-5

CAS number: 1073-05-8

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

DECISION ON A COMPLIANCE CHECK

Under Article 41 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below, by the deadline of **3 July 2023** from the date of the decision.

Requested information must be generated using the Substance unless otherwise specified.

A. Information required from all the Registrants subject to Annex VII of REACH

1. In vitro cytogenicity study in mammalian cells (test method: OECD TG 473) or In vitro micronucleus study (test method: OECD TG 487)
2. In vivo genetic toxicity study

In vivo genotoxicity study to be selected according to the following specifications:

- a. If the test results of request A.1 are negative:

Transgenic rodent somatic and germ cell gene mutation assays (OECD TG 488 from 2020) in transgenic mice or rats, oral route on the following tissues: liver, glandular stomach; duodenum must be harvested and stored for up to 5 years. The duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

OR

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

- b. If the test results of request A.1 are positive:

In vivo mammalian alkaline comet assay (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.

The reasons for the requests are provided in the following appendix entitled "Reasons to request information required under Annexes VII 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 Annex VII to REACH, for registration at 1-10 tonnes per year (tpa), or as a transported isolated intermediate in quantity above 1000 tpa.

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, when adopted under Article 51 of REACH, may be appealed to the Board of Appeal of ECHA within three months of its notification to you. Please refer to <http://echa.europa.eu/regulations/appeals> for further information.

Failure to comply

If you do not comply with the information required by this decision by the deadline indicated above, ECHA will notify the enforcement authorities of your Member State.

Authorised¹ 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 vitro cytogenicity study in mammalian cells or In vitro micronucleus study**

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. The ECHA guidance R.7a² further specifies that "REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII." This is for the reason that the *in vitro* cytogenicity test under Section 8.4.2 will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy. The obtained *in vitro* data will inform on the genotoxic concern(s) associated with the substance and help identify the most adequate follow-up *in vivo* study (requested under A.2.).

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria which raise the concern for gene mutation. However, no information from an *in vitro* cytogenicity study or an *in vitro* micronucleus study on the substance in mammalian cells is available in the dossier.

ECHA therefore considers that an appropriate *in vitro* cytogenicity or micronucleus study is necessary to further investigate the mutagenicity of the substance.

Test selection

Either the *in vitro* cytogenicity study in mammalian cells (test method OECD TG 473) or the *in vitro* micronucleus study (test method OECD TG 487) are considered suitable.

2. In vivo genotoxicity study to be selected according to the following scenarios:**a. If the test results of request A.1 are negative:**

Transgenic rodent somatic and germ cell gene mutation assay OR In vivo mammalian alkaline comet assay

b. If the test results of request A.1 are positive:

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

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.

According to the REACH integrated testing strategy, a positive result result in an *in vitro* test must be followed-up by an appropriate *in vivo* test. The ECHA guidance R.7a³ states "*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*

² ECHA Guidance R.7a, section R.7.7.6.3, p.570.

³ ECHA Guidance R.7a, section R.7.7.6.3, p.570.

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 raise the concern for gene mutation. Moreover, you did not provide any consideration explaining that the genotoxic potential of the substance cannot be expressed *in vivo*, based e.g. on lack of relevance for *in vivo* situations or the existence of threshold mechanism. ECHA therefore considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concern identified *in vitro*.

Test selection

According to the ECHA Guidance Chapter R.7a⁴, the transgenic rodent somatic and germ cell gene mutation assay (“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.

As explained above, under Appendix A, section 1, in the dossier there is no information from an *in vitro* cytogenicity study or an *in vitro* micronucleus study on the substance in mammalian cells. Therefore, by this decision, ECHA also requests an *in vitro* chromosomal aberration test (for the reasons see above Appendix A, section 1), which may raise a concern for chromosomal aberration in case of positive results.

We note that the TGR assay is not suitable to investigate chromosomal aberrations as the test method OECD TG 488 investigates specifically gene mutations. According to the ECHA Guidance R.7a, Section R.7.7.6.3, the comet assay is a genotoxicity indicator test that is suitable to follow up the positive *in vitro* result for both chromosomal aberration and gene mutation. Moreover, the *in vivo* mammalian erythrocyte micronucleus test (“MN test”, OECD TG 474) is a mutagenicity test that provides evidence on *in vivo* chromosomal mutagenicity, as this study detects both structural and numerical chromosomal aberrations.

Therefore, in case there is also a concern for chromosomal aberration, you must combine the comet assay and the MN test into a single study. The combined study can help reduce the number of tests performed and the number of animals used while addressing both structural and numerical chromosomal aberrations and gene mutations.

Therefore, based on the above, you must wait for the result of the *in vitro* test requested under A.1. and, depending on the result, to conduct either a) Comet assay or TGR, if the *in vitro* test results are negative; or b) Comet assay combined with MN test if the *in vitro* test results are positive.

Test design

- a) TGR or Comet assay (if *in vitro* test results of request A.1 are negative)
 - i.) TGR assay:

In case the TGR assay is appropriate and you decide to conduct the TGR assay, according to the OECD TG 488, the test must be performed in transgenic mice or rats and the test substance is usually administered orally.

According to the OECD TG 488, the test must be performed by analysing tissues from liver as slowly proliferating tissue and primary site of xenobiotic metabolism, glandular stomach and

⁴ ECHA Guidance Chapter R.7a, Section R.7.7.6.3

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)). In light of 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 $-70\text{ }^{\circ}\text{C}$) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

Germ cells:

You may consider to collect the male germ cells (from the seminiferous tubules) 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 $-70\text{ }^{\circ}\text{C}$). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ 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.

ii.) Comet assay:

In case you decide to perform the comet assay according to the test method OECD TG 489, the test must be performed in rats. Having considered the anticipated routes of human exposure and the need for 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.

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.

b) Comet assay combined with MN test (if *in vitro* test results of request A.1 are positive)

According to the test method OECD TG 489, the test must be performed in rats. Therefore, the combined test (OECD TG 489 and OECD TG 474) must be performed in rats. Having considered the anticipated routes of human exposure and the need for 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.* 2011⁵).

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.

⁵ 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

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 summaries⁶.

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:

- a) 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

- a) 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.
- b) 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 dossiers⁷.

⁶ <https://echa.europa.eu/practical-guides>

⁷ <https://echa.europa.eu/manuals>

Appendix C: Procedure

This decision does not prevent ECHA from initiating further compliance checks at a later stage on the registrations present.

ECHA followed the procedure detailed in Articles 50 and 51 of REACH.

The compliance check was initiated on 17 April 2020.

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

ECHA did not receive any comments within the commenting 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 modified the draft decision.

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

You did not provide any comments on the proposed amendment(s).

The Member State Committee reached a unanimous agreement on the draft decision during its MSC-74 meeting and ECHA took the decision according to Article 51(6) of the REACH Regulation.

Appendix D: List of references - ECHA Guidance⁸ 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)⁹

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)⁹

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.

OECD Guidance documents¹⁰

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

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>

¹⁰ <http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

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.

Appendix E: Addressees of this decision and their corresponding information requirements

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.