

Helsinki, 11 November 2021

Addressees

Registrant(s) of JS_609-251-6 as listed in the last Appendix of this decision

Date of submission of the dossier subject to this decision

04/02/2021

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

Substance name: Oligomeric reaction products of 4,4'-propane-2,2-diylidiphenol and 2-methyloxirane and 2-(chloromethyl)oxirane

EC number: 460-230-6

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 **18 May 2023**.

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 vivo* mammalian alkaline comet assay (triggered by Annex VII, 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 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". 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 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.

Your dossier contains positive results for the *in vitro* gene mutation study in bacteria ([REDACTED], OECD 471, 1997), *in vitro* gene mutation study in mammalian cells ([REDACTED], OECD 476, 2012) and *in vitro* mammalian chromosomal aberration test [REDACTED] OECD 473, 2012), which raise the concerns for both gene mutation and chromosomal aberration.

The ECHA guidance R.7a² states that following a positive result in an *in vitro* test, "*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.*"

However, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier. Moreover, you did not provide any considerations 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 considers that an appropriate *in vivo* follow up mutagenicity study is necessary to address the concerns identified *in vitro*.

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.

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

Test design

According to the test method OECD TG 489, the test must be performed in rats. Therefore, ECHA considers that 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 comet analysis alongside the requirements of tissue sampling for the mammalian erythrocyte micronucleus test (see OECD TG 489, e.g. Bowen *et al.* 2011³).

Potential cross-linking properties

You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order to detect cross links. Therefore, you may consider preparing and analysing two sets of slides: one set of slides submitted to the standard experimental conditions (as described in OECD TG 489); the other set of slides submitted to modified experimental conditions that enable the detection of DNA. The modified experimental conditions may utilise one of the following options: (1) increase of electrophoresis time, e.g. as described in reference 23⁴ in the OECD TG 489; (2) treatment of isolated cells (either in suspension or embedded in the slides) with a chemical (e.g. MMS); or (3) treatment of isolated cells (either in suspension or embedded in the slides) with ionising radiation (options 2 and 3 are described e.g. in references 36-39⁵ in the OECD TG 489 or Pant⁶ *et al.* 2015). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

Germ cells

³ 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

⁴ Reference 23 of OECD TG 489 (2016): (23) Nesslany, F, Zennouche N, Simar-Meintieres S, Talahari I, NKili-Mboui E-N, Marzin D (2007), *In vivo* comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, Vol. 630/1, pp. 28-41.

⁵ References 36 to 39 of OECD TG 489 (2016): (36) Merk, O., G. Speit (1999), Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity, *Environmental and Molecular Mutagenesis*, Vol. 33/2, pp. 167-72; (37) Pfuhrer, S., H.U. Wolf (1996), Detection of DNA-crosslinking agents with the alkaline comet assay, *Environmental and Molecular Mutagenesis*, Vol. 27/3, pp. 196-201; (38) Wu, J.H., N.J. Jones (2012), Assessment of DNA interstrand crosslinks using the modified alkaline comet assay, *Methods in Molecular Biology*, Vol. 817, pp. 165-81; (39) Spanswick, V.J., J.M. Hartley, J.A. Hartley (2010), Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay, *Methods in Molecular Biology*, Vol. 613, pp. 267-282.

⁶ Pant K, Roden N, Zhang C, Bruce C, Wood C, and Pendino K (2015) Modified *In Vivo* Comet Assay Detects the Genotoxic Potential of 14-Hydroxycodone, an α,β -Unsaturated Ketone in Oxycodone. *Environmental and Molecular Mutagenesis* 56, 777-787.

You may consider to collect the male gonadal cells collected 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.

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 summaries⁷.

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 dossiers⁸.

⁷ <https://echa.europa.eu/practical-guides>

⁸ <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 25 March 2021.

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.

As no amendments were proposed, ECHA adopted the decision under Article 51(3) of REACH.

Appendix D: List of references - ECHA Guidance⁹ 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)¹⁰

RAAF - considerations on multiconstituent substances and UVCBs (RAAF UVCB, March 2017)¹¹

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 documents¹²

⁹ <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

¹⁰ <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

¹¹ https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

¹² <http://www.oecd.org/chemicalsafety/testing/series-testing-assessment-publications-number.htm>

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.

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 |
|------------------------|----------------------------|----------------------------------------------|
| ██████████ | ██████████ | ██████████ |

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.