

Helsinki, 17 March 2021

**Addressees**

Registrants of JS EC#264-438-6 listed in the last Appendix of this decision

**Date of submission for the jointly submitted dossier subject of a decision**

28/03/2018

**Registered substance subject to this decision, hereafter 'the Substance'**Substance name: N,N,N',N'-tetrakis(2,3-epoxypropyl)-m-xylene-*o*,*o*'-diamine

EC number: 264-438-6

CAS number: 63738-22-7

**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), you must submit the information listed below by **23 December 2022**.

The requested information must be generated using the Substance unless otherwise specified.

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

- 1. *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2; test method: OECD 489) with the option to combine it with *in vivo* mammalian erythrocyte micronucleus test (test method: OECD 474), in rats, oral route with the Substance. For the comet assay, liver, and glandular stomach and duodenum shall be analysed, for the micronucleus test, the bone marrow.**

**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 REACH, for registration at 1-10 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 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<sup>1</sup> under the authority of Christel Schilliger-Musset, Director of Hazard Assessment

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<sup>1</sup> 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**

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

**1. *In vivo* mammalian alkaline comet assay (Annex VII, Section 8.4., column 2) with the option to combine it 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 which raise the concern for gene mutations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

Therefore, you 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: "*An in vivo test in mice is proposed in which the OECD guidelines 474 and 489 are combined, in order to fully cover the information requirements on mutagenic and clastogenic properties of the substance in one study, in order to reduce the use of test animals to a minimum.*"

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.

ECHA agrees that an appropriate *in vivo* follow up genotoxicity study is necessary to address the concern identified *in vitro*.

*i. Test selection*

According to the ECHA Guidance Chapter R.7a, Section R.7.7.6.3, the comet assay (OECD TG 489) that you propose is suitable to follow up the concern for gene mutation raised by the positive *in vitro* gene mutation study in bacteria (Annex VII, Section 8.4., column 2). Therefore, the comet assay is an appropriate follow-up test for the Substance.

In your testing proposal you also indicated the intention to combine the comet assay with the *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474). ECHA considers that an *in vivo* micronucleus test is an appropriate test to investigate chromosomal aberrations (micronuclei) *in vivo* as described in the ECHA Guidance Chapter R.7a, Section R.7.7.1. and figure R.7.7-1. No *in vitro* cytogenicity study is present in the registration dossier and the additional *in vivo* micronucleus investigation you propose is not triggered under Annex VII of REACH. Therefore, it is at your discretion to combine the comet assay and the MN test into a single study. The combination of a comet assay with the MN test can help reduce the number of tests performed and the number of animals used while addressing both chromosomal aberration and gene mutation.

*ii. Test design*

You proposed testing in mice but did not provide a justification for the choice of the test species. According to the test method OECD TG 489, the test must be performed in rats.

You did not specify the route for testing. According to the test method OECD TG 489, 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 the 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.

In case you decide to perform the MN test in combination with the comet assay, you 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.* 2011<sup>2</sup>).

### *iii. Germ cells*

You may consider to collect the male gonadal cells collected from the seminiferous tubules (as described by e.g. O'Brien *et al.*<sup>3</sup>) 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.

### *iv. Outcome*

Under Article 40(3)(b) of REACH, you are requested to carry out the proposed test under modified conditions, as explained above with the Substance.

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<sup>2</sup> 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

<sup>3</sup> 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

**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<sup>4</sup>.

**B. Test material**

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.

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"<sup>5</sup>.

<sup>4</sup> <https://echa.europa.eu/practical-guides>

<sup>5</sup> <https://echa.europa.eu/manuals>

**Appendix C: Procedural history**

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 11 December 2019.

ECHA held a third party consultation for the testing proposals from 27 January 2020 until 12 March 2020. 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 REACH.

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

ECHA did not receive any comments within the notification 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 Guidance<sup>6</sup> 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)<sup>7</sup>

RAAF - considerations on multi-constituent substances and UVCBs (RAAF UVCB, March 2017)<sup>7</sup>

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 documents<sup>8</sup>

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

<sup>6</sup> <https://echa.europa.eu/guidance-documents/guidance-on-information-requirements-and-chemical-safety-assessment>

<sup>7</sup> <https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across>

<sup>8</sup> <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 the corresponding information requirements applicable to them**

You must provide the information requested in this decision for all REACH Annexes applicable to you.

<b>Registrant Name</b>	<b>Registration number</b>	<b>(Highest) Data requirements to be fulfilled</b>
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]

Note: where applicable, the name of a third party representative (TPR) may be displayed in the list of recipients whereas the decision is sent to the actual registrant.