

Helsinki, 11 March 2021

Addressees

Registrants of JS_220-482-8 listed in the last Appendix of this decision

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

13/11/2018

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

Substance name: Diethyl bis(2-hydroxyethyl)aminomethylphosphonate

EC number: 220-482-8

CAS number: 2781-11-5

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), ECHA requests that you submit the information listed below by the deadline of **19 December 2022**.

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

A. 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 489) combined with *in vivo* mammalian erythrocyte micronucleus test (test method OECD 474); in rats, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

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:

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

How to comply with your information requirements

To comply with your information requirement, 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 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 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 (Annex VIII, Section 8.4., column 2)

Under Annex VIII Section 8.4., column 2 of REACH, the performance of an appropriate *in vivo* somatic cell genotoxicity study must be considered if there is a positive result in any of the *in vitro* genotoxicity studies in Annex VII or VIII.

Your dossier contains positive results for the *in vitro* cytogenicity test and *in vitro* gene mutation study in mammalian cells which raise the concerns for gene mutations and chromosomal aberrations. Moreover, no data from an *in vivo* somatic cell genotoxicity study is available in the dossier.

An appropriate *in vivo* genotoxicity study to follow up the concerns on gene mutations and/or chromosomal aberrations is not available for the Substance. 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.

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

ECHA notes that the proposed test is an appropriate test to investigate effects on gene mutations and chromosomal aberrations *in vivo*².

In case there are positive results *in vitro* studies showing concern for both chromosomal aberration and gene mutation, the ECHA Guidance Chapter R.7a³ identifies the following tests as options for a follow-up *in vivo* study. The mammalian erythrocyte micronucleus test ("MN test", OECD TG 474), the mammalian bone marrow chromosomal aberration test ("CA test", OECD TG 475) or the *in vivo* mammalian alkaline comet assay ("comet assay", OECD TG 489) are suitable to follow up a positive *in vitro* result showing chromosomal aberration. The MN test and CA test are able to detect chromosomal aberrations, whereas the comet assay is an indicator assay detecting putative DNA lesions. The transgenic rodent somatic and germ cell gene mutation assay ("TGR", OECD TG 488) and the *in vivo* comet assay are suitable to follow up a positive *in vitro* result showing gene mutation. The TGR assay is able to detect gene mutations, whereas the comet assay is an indicator assay detecting putative DNA lesions.

As also indicated in the ECHA Guidance, it is possible 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 you reduce the number of tests performed and the number of animals used while providing useful information on the potential of the Substance to induce chromosomal aberration and gene

² ECHA Guidance R.7a, Section R.7.7.6.3. and Figure R.7.7-1

³ ECHA Guidance Chapter R.7a, Section R.7.7.6.3

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

ii. Test design

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

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 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.

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.* 2011⁴).

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.*⁵) 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)(a) of REACH, you are requested to carry out the proposed test with the Substance:

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.

⁴ 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

⁵ 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: 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 proposal 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 C: 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 must 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'⁶.

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.

Technical instructions are available in the manual "How to prepare registration and PPORD dossiers"⁷.

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

⁷ <https://echa.europa.eu/manuals>

5. List of references of the ECHA Guidance and other guidance/ reference documents⁸

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 in this decision.

ECHA Read-across assessment framework (RAAF, March 2017)⁹

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.

OECD Guidance documents

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

Guidance Document on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption – No 150, referred to as OECD GD 150.

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>

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]

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.