

Helsinki, 26 May 2023

Addressee

Registrant of JS_1187441-10-6 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision

19/06/2018

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

Substance name: 2-Propenoic acid, 2-methyl-, 2-hydroxyethyl ester, reaction products with phosphorus oxide

EC/List number: 810-703-1

Decision number: Please refer to the REACH-IT message which delivered this communication (in format TPE-D-XXXXXXXXXX-XX-XX/F)**DECISION ON TESTING PROPOSAL(S)**

Based on Article 40 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below by **1 September 2025**.

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

Information required from all the Registrants subject to Annex VIII of REACH

1. *In vitro* cytogenicity study in mammalian cells (Annex VIII, Section 8.4.2., test method: OECD TG 473) or *In vitro* micronucleus study (test method: OECD TG 487);
2. *In vivo* genetic toxicity study (Annex VIII, Section 8.4., column 2) to be selected according to the following specifications:
 - a. If the results of the *in vitro* test requested under A.1 are **negative**:

In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified, other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum;
 - b. If the results of the *in vitro* test requested under 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, or if justified, in mice, oral route. For the comet assay the following tissues shall be analysed: liver, glandular stomach and duodenum.

The reasons for the requests are explained in Appendix 1.

Information required depends on your tonnage band

You must provide the information listed above for all REACH Annexes applicable to you in accordance with Articles 10(a) and 12(1) of REACH. The addressee of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

You are only required to share the costs of information that you must submit to fulfil your information requirements.

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 requirements for testing and reporting new tests under REACH, see Appendix 4.

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 Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the decision

Appendix 2: Procedure

Appendix 3: Addressees of the decision and their individual information requirements

Appendix 4: Conducting and reporting new tests under REACH

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 1: Reasons for the decision

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Reasons for the decision(s) related to the information under Annex VIII of REACH

1. In vitro cytogenicity study in mammalian cells or In vitro micronucleus study

1 An in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is an information requirement under Annex VIII to REACH (Section 8.4.2.).

2 In addition, an appropriate in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is necessary to identify the adequate in vivo study, as it determines the genotoxic concern(s) associated with the Substance. Therefore, in order to decide on your testing proposal for an in vivo mammalian alkaline comet assay, an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is needed.

1.1. Information provided to fulfil the information requirement

3 You have submitted a testing proposal for an in vivo mammalian alkaline comet assay to be performed with the Substance to further investigate the mutagenicity of the Substance.

4 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.

5 Instead, you have adapted this information requirement by using Annex VIII, Section 8.4.2., Column 2. To support the adaptation, you have provided the following justification:

(i) You refer to the testing proposal by stating that "*As the COMET assay addresses all potential mode-of-actions (structural chromosome aberrations and/or gene mutations), the outcome of this in vivo study will overrule any in vitro result, therefore further in vitro testing is omitted*".

6 Under Annex VIII, Section 8.4.2., Column 2, the study usually does not need to be conducted "if adequate data from an in vivo cytogenicity test are available". The Guidance on IRs and CSA, Section R.7.7.6.3 and Table R.7.7-3 clarifies that the in vivo somatic cell cytogenicity test must be either a micronucleus test or a chromosomal aberration test, performed according to the OECD TG 474 or 475, respectively.

7 However, there is no adequate data from an *in vivo cytogenicity test* available in your dossier.

8 Therefore, the column 2 criteria are not met and your adaptation is rejected.

9 As a result, ECHA concludes that an appropriate in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study is necessary to further investigate the mutagenicity of the Substance and to help identify the most adequate in vivo follow-up study.

1.2. Specification of the study design

10 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.

1.3. Outcome

11 Under Article 40(3)(c) of REACH, you are requested to carry out the additional test, as indicated above.

2. In vivo genetic toxicity study

12 Appropriate in vivo mutagenicity studies must be considered under Annex VIII to REACH (Section 8.4., Column 2) in case of a positive result in any of the in vitro genotoxicity studies under Annex VII or VIII to REACH.

13 Your dossier contains positive results for the in vitro gene mutation study in mammalian cells which raise the concerns for gene mutations.

2.1. Information provided to fulfil the information requirement

14 You have submitted a testing proposal for an in vivo mammalian alkaline comet assay to be performed with the Substance.

15 ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity in vivo. 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.

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

2.2. Test selection

17 According to the Guidance on IRs & CSA, Section R.7.7.6.3, the in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) is suitable to follow up a positive in vitro result on gene mutation.

18 As explained above, under Section 1, in the dossier there is no adequate information from an in vitro cytogenicity study in mammalian cells or an in vitro micronucleus study, according to the requirements of Section 8.4.2., Annex VIII to REACH. Therefore, by this decision, ECHA also requests an in vitro cytogenicity study or an in vitro micronucleus study, which may raise a concern for chromosomal aberration in case of positive results.

19 In case there is also a concern for chromosomal aberration, the comet assay can be combined with an in vivo mammalian erythrocyte micronucleus test ("MN test", OECD TG 474) in a single study (see OECD TG 489 para. 33; OECD TG 474 para. 37c; Guidance on IRs & CSA, Section R.7.7.6.3). While the comet assay can detect primary DNA damage that may lead to gene mutations and/or structural chromosomal aberrations, the MN test can detect both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy). A combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

20 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.

21 Therefore, you must wait for the results of the in vitro test requested under Request 1 and, depending on these results, to conduct either a) Comet assay, if the test results of request 1 are negative; or b) Comet assay combined with MN test if the test results of Request 1 are positive. The deadline set in this decision allows for sequential testing.

2.3. Specification of the study design

2.3.1. *Comet assay (if the test result of request 1 is **negative**)*

- 22 You proposed testing in the rat. According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified.
- 23 You proposed testing by the oral route. 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.
- 24 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.

2.3.2. *Germ cells*

- 25 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other 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.

2.3.3. *Comet assay combined with MN test (if the test result of request 1 is **positive**)*

- 26 According to the test method OECD TG 489, rats are the preferred species. Other rodent species can be used if scientifically justified. According to the test method OECD TG 474, the test may be performed in mice or rats. Therefore, the combined study must be performed in rats, or if justified, in mice.
- 27 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.
- 28 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.
- 29 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. 2011 [1]).

2.3.4. *Germ cells*

- 30 You may consider collecting the male gonadal cells from the seminiferous tubules in addition to the other 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.

Reference:

- [1] Bowen DE 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. *Muta Res*;722:7–19.

2.4. *Outcome*

- 31 Under Article 40(3)(b) your testing proposal is accepted under modified conditions, and you are requested to conduct the test with the Substance, as specified above

References

The following documents may have been cited in the decision.

Guidance on information requirements and chemical safety assessment (Guidance on IRs & CSA)

- Chapter R.4 Evaluation of available information; ECHA (2011).
Chapter R.6 QSARs, read-across and grouping; ECHA (2008).
Appendix to Chapter R.6 for nanoforms; ECHA (2019).
Chapter R.7a Endpoint specific guidance, Sections R.7.1 – R.7.7; ECHA (2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Chapter R.7b Endpoint specific guidance, Sections R.7.8 – R.7.9; ECHA (2017).
Appendix to Chapter R.7b for nanomaterials; ECHA (2017).
Chapter R.7c Endpoint specific guidance, Sections R.7.10 – R.7.13; (ECHA 2017).
Appendix to Chapter R.7a for nanomaterials; ECHA (2017).
Appendix R.7.13-2 Environmental risk assessment for metals and metal compounds; ECHA (2008).
Chapter R.11 PBT/vPvB assessment; ECHA (2017).
Chapter R.16 Environmental exposure assessment; ECHA (2016).

Guidance on data-sharing; ECHA (2017).

All Guidance on REACH is available online: <https://echa.europa.eu/guidance-documents/guidance-on-reach>

Read-across assessment framework (RAAF)

- RAAF, 2017 Read-across assessment framework (RAAF), ECHA (2017)
RAAF UVCB, 2017 Read-across assessment framework (RAAF) – considerations on multi- constituent substances and UVCBs), ECHA (2017).

The RAAF and related documents are available online:

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

OECD Guidance documents (OECD GDs)

- OECD GD 23 Guidance document on aquatic toxicity testing of difficult substances and mixtures; No. 23 in the OECD series on testing and assessment, OECD (2019).
OECD GD 29 Guidance document on transformation/dissolution of metals and metal compounds in aqueous media; No. 29 in the OECD series on testing and assessment, OECD (2002).
OECD GD 150 Revised guidance document 150 on standardised test guidelines for evaluating chemicals for endocrine disruption; No. 150 in the OECD series on testing and assessment, OECD (2018).
OECD GD 151 Guidance document supporting OECD test guideline 443 on the extended one-generation reproductive toxicity test; No. 151 in the OECD series on testing and assessment, OECD (2013).

Appendix 2: Procedure

ECHA started the testing proposal evaluation in accordance with Article 40(1) on 11 February 2020.

ECHA held a third party consultation for the testing proposal(s) from 23 March 2020 until 7 May 2020. ECHA did not receive information from third parties.

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

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 3: Addressees of this decision and their corresponding information requirements

In accordance with Articles 10(a) and 12(1) of REACH, the information requirements for individual registrations are defined as follows:

- 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;
- the information specified in Annexes VII and VIII to REACH, for registration at 10-100 tpa;
- the information specified in Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa;
- the information specified in Annexes VII to X to REACH, for registration at more than 1000 tpa.

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.

Appendix 4: Conducting and reporting new tests for REACH purposes

1. Requirements when conducting and reporting new tests for REACH purposes

1.1. 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 summaries².
- (4) Under the introductory part of Annexes VII/VIII/IX/X to REACH, where a test method offers flexibility in the study design, for example in relation to the choice of dose levels or concentrations, the chosen study design must ensure that the data generated are adequate for hazard identification and risk assessment.

1.2. 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:
 - 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.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers³.

2. General recommendations for conducting and reporting new tests

References to Guidance on REACH and other supporting documents can be found in Appendix 1.

² <https://echa.europa.eu/practical-guides>

³ <https://echa.europa.eu/manuals>