

Helsinki, 17 January 2023

Addressee

Registrant of JS_22288-43-3 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision

26/01/2022

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

Substance name: 1,1,3,3-tetramethylbutyl 2-ethylperoxyhexanoate

EC/List number: 244-894-2

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 **24 January 2025**.

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

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), same as under "3."

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

2. In vivo mammalian alkaline comet assay (triggered by Annex VIII, Section 8.4., column 2), same as under "3."

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

3. In vivo mammalian alkaline comet assay (triggered by Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, or if justified, other rodent species, oral route, on the following tissues: liver, glandular stomach and duodenum.

The reasons for the decision(s) 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 addressees of the decision and their corresponding information requirements based on registered tonnage band are listed in Appendix 3.

In the requests above, the same study has been requested under different Annexes. This is because some information requirements may be triggered at lower tonnage band(s). In

such cases, only the reasons why the information requirement is triggered are provided for the lower tonnage band(s). For the highest tonnage band, the reasons why the standard information requirement is not met and the specification of the study design are provided. Only one study is to be conducted.

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

Contents

Reasons for the decision(s) related to the information under Annex VII of REACH	4
1. In vivo mammalian alkaline comet assay	4
Reasons for the decision(s) related to the information under Annex VIII of REACH	5
2. In vivo mammalian alkaline comet assay	5
Reasons for the decision(s) related to the information under Annex IX of REACH	6
3. In vivo mammalian alkaline comet assay	6
References	9

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

1. In vivo mammalian alkaline comet assay

- 1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., Column 2).
- 2 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2004) which raise the concern for gene mutations.
- 3 ECHA considers that an in vivo follow-up study is necessary to address the identified concern.
- 4 For the assessment of the testing proposal, see Section 3.

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

2. In vivo mammalian alkaline comet assay

- 5 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.
- 6 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2004) which raise the concern for gene mutations.
- 7 ECHA considers that an in vivo follow-up study is necessary to address the identified concern.
- 8 For the assessment of the testing proposal, see Section 3.

Reasons for the decision(s) related to the information under Annex IX of REACH**3. In vivo mammalian alkaline comet assay**

9 An appropriate in vivo somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if (1) there is a positive result in any of the in vitro genotoxicity study under Annex VII or VIII to REACH and (2) there are no results available from an in vivo study.

10 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 2004) which raise the concern for gene mutations. Moreover, no data from an in vivo somatic cell genotoxicity study is available in the dossier.

11 In your dossier you mention that:

- 1) *"Although there is a marginal positive Ames result, the available data show a lack for genotoxicity in mammalian based cell assays"* and
- 2) *"although positive results are commonly obtained for peroxyesters in the Ames test, their possible genotoxic potential is in vivo not expressed"*.

12 ECHA notes the following:

13 According to OECD TG 471, one of the criteria to determine a positive result is "a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation". In the provided Ames study (2004) there is a reproducible increase (over two-fold), noted in three experiments, in at least one concentration (at 1000 µg per plate) in the presence of S9 mix, for the *E. coli* WP2 uvr A strain. Therefore, the result of the Ames study is considered to be a clear positive result.

14 We further note that at Annex IX, the in vivo somatic cell genotoxicity study is triggered if there is a positive in vitro result in any of the in vitro genotoxicity studies in Annex VII or VIII and there are no results available from an in vivo study already. As explained above, both of these criteria are met. Therefore, the negative result obtained in the OECD TG 476 study does not supersede the positive result obtained in the OECD TG 471 study.

15 You also refer to the Ames data of other peroxyesters and indicate that even though they gave positive results, genotoxicity was not expressed in vivo. However, you yourself state that the genotoxicity mechanism caused by "this chemical class is not clear".

16 You have not provided any justification nor documentation to explain how and why this information can be used to predict the outcome on mutagenicity for the Substance.

17 In the absence of such documentation, ECHA cannot assess the relevance of such claims in respect to the mutagenic properties of the Substance.

3.1. Information provided to fulfil the information requirement

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

19 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.

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

3.2. Test selection

21 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.

3.3. Specification of the study design

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 (OECD TG 489, para. 23).

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.

3.3.1. Germ cells

25 A subsequent germ cell genotoxicity study (TGR/OECD TG 488) may still be required under Annex IX of REACH, in case 1) an in vivo genotoxicity test on somatic cell is positive, and 2) no clear conclusion can be made on germ cell mutagenicity.

26 Therefore, 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, in accordance with Annex IX, Section 8.4., column 2, 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.

3.3.2. Cross-linking properties

27 You are reminded that you may decide to consider the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay 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 23 [1] 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 36-39 [2-5] in the OECD TG 489 or Pant et al. 2015 [6]). To ensure the robustness of the test result a specific positive control group of animals would be needed.

- [1] Nesslany *et al.* (2007) In vivo comet assay on isolated kidney cells to distinguish genotoxic carcinogens from epigenetic carcinogens or cytotoxic compounds *Muta Res*;630(1-2):28-41.
- [2] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.
- [3] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.
- [4] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.
- [5] Spanswick *et al.* (2010) Measurement of DNA interstrand crosslinking in individual cells using the Single Cell Gel Electrophoresis (Comet) assay. *Methods Mol Biol*;613:267-282.
- [6] Pant K *et al.* (2015) Modified *in vivo* comet assay detects the genotoxic potential of 14-hydroxycodone, an α,β -unsaturated ketone in oxycodone. *Environ Mol Mutagen*;56(9):777-87.

3.4. Outcome

- 28 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test, 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 15 February 2022.

ECHA held a third party consultation for the testing proposal(s) from 10 May 2022 until 27 June 2022. ECHA did not receive information from third parties.

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

The deadline of the decision is set based on standard practice for carrying out OECD TG tests. It has been exceptionally extended by 12 months from the standard deadline granted by ECHA to take into account currently longer lead times in contract research organisations.

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 Annexes VII, VIII and IX to REACH, for registration at 100-1000 tpa.

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.

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 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.

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>