

Helsinki, 04 June 2024

**Addressee(s)**

Registrant of JS\_13052-09-0 as listed in Appendix 3 of this decision

**Date of submission of the dossier subject to this decision**

02 March 2019

**Registered substance subject to this decision ("the Substance")**Substance name: 1,1,4,4-tetramethylbutane-1,4-diyl bis(2-ethylperoxyhexanoate)  
EC/List number: 235-935-5**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 **11 December 2026**.

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

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

1. Transgenic rodent somatic and germ cell gene mutation assays (triggered by Annex VII, Section 8.4., Column 2; test method: OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; duodenum must be harvested and stored for up to 5 years. Duodenum must be analysed if the results of the glandular stomach and of the liver are negative or inconclusive.

**OR**

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

The reasons for the request(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 addressee of the decision and its corresponding information requirements based on registered tonnage band are listed in Appendix 3.

**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.

In addition, the studies relating to biodegradation and bioaccumulation are necessary for the PBT assessment. However, to determine the testing needed to reach the conclusion on the persistency and bioaccumulation of the Substance you should consider the sequence in which these tests are performed and other conditions described in this Appendix.

### **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.

Authorised<sup>1</sup> under the authority of Mike Rasenberg, Director of Hazard Assessment

Appendix 1: Reasons for the request(s)

Appendix 2: Procedure

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

Appendix 4: Conducting and reporting new tests under REACH

---

<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 1: Reasons for the request(s)**

**Reasons related to the information under Annex VII of REACH..... 4**

1. Transgenic rodent somatic and germ cell gene mutation assays or *in vivo* mammalian alkaline comet assay ..... 4

**References ..... 8**

**Reasons related to the information under Annex VII of REACH****1. Transgenic rodent somatic and germ cell gene mutation assays or *in vivo* mammalian alkaline comet assay**

- 1 Under Annex VII Section 8.4., Column 2, an appropriate *in vivo* mammalian somatic cell genotoxicity study as referred to in Annex IX, point 8.4.4, must be performed in case of a positive result in any of the *in vitro* studies referred to in Annex VII, Section 8.4. The *in vivo* study must address the concern(s) raised by the *in vitro* study results, i.e. the chromosomal aberration concern or the gene mutation concern or both, as appropriate.

*1.1. Triggering of the information requirement*

- 2 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (1999) which raise a concern for gene mutation.
- 3 You have provided in your dossier the following justification for further *in vivo* studies not being triggered: *"In accordance with Endpoint Specific Guidance Chapter R.7A, Figure R.7.7-1 "Flow chart of the mutagenicity testing strategy", no further testing (ie. no in vivo testing) need be proposed in the event of a negative mouse lymphoma assay or hprt assay, regardless to whether or not the gene mutation test in bacteria is positive or negative. This therefore implies that when considering whether an in vivo gene mutation request is required for substances requiring Annex IX test proposals due to their volume bands, a negative mouse lymphoma assay or hprt assay is sufficient evidence to waive the need for an in vivo gene mutation test"*.
- 4 In your justification, you refer to Figure R.7.7-1 of the Guidance on IR & CSA - Chapter R.7a, published June 2017, and claim that the negative *in vitro* gene mutation study in mammalian cells (OECD TG 476, 2012) provided in your dossier is sufficient to address the concern raised by the positive *in vitro* gene mutation study in bacteria (OECD TG 471, 1999).
- 5 ECHA disagrees with your interpretation of Figure R.7.7-1 of the Guidance on IR&CSA - Chapter R.7a, since it indicates that in case the *in vitro* gene mutation test in bacteria is positive, Annex VII/VIII registrants shall proceed with Annex IX requirements and *in vivo* testing.
- 6 As your dossier contains positive results for the *in vitro* gene mutation study in bacteria (OECD TG 471, 1999), the information on an *in vitro* gene mutation study in mammalian cells is not required. However, when an *in vitro* gene mutation study in mammalian cells is requested or available, as the OECD TG 476 study (2012) provided in your dossier, it is considered complementary to a gene mutation study in bacteria and it is not intended to supersede it. Therefore, a reliable positive *in vitro* gene mutation study in bacteria is sufficient on its own to trigger an *in vivo* follow-up study.
- 7 Based on the above, the negative *in vitro* gene mutation study in mammalian cells (OECD TG 476, 2012) provided in your dossier does not remove the concern for gene mutation raised by the positive *in vitro* gene mutation study in bacteria (OECD TG 471, 1999).
- 8 In your comments to the draft decision you disagree with the triggering of the information requirement. You state that *"the positive findings in the Ames test in one experiment, in one strain, only with the inclusion of metabolic activation, are of no biological relevance as they were only expressed at concentrations way above solubility leading to emulsion forming (and phase separation in other in vitro assays"*. You have provided a detailed evaluation of the *in vitro* gene mutation study in bacteria.

- 9 First, ECHA reiterates that under REACH a positive result in an *in vitro* gene mutation in bacteria triggers an *in vivo* genotoxicity test, even in case an *in vitro* micronucleus test and a gene mutation assay on mammalian cells are negative for the same substance.
- 10 Second, we have analysed the results of the *in vitro* gene mutation in bacteria test Ames test, provided in your comments, and we have the following observations:
- 11 For the results, observed in TA100, you state "[...] *an increased number of revertants above 2-fold of the control. The number of revertants is well above the range of HCD, seems to show a dose-response, and as such clearly represents a positive result. However, the positive results were seen at concentrations that resulted in the formation of emulsions. [...] care should be taken in interpreting a positive result that is only seen at the precipitating concentration*". Further you claim that positive results are only observed using the preincubation method with S9-mix, explaining that it "*involves concentration levels that are almost 4-times higher than during the subsequent plating phase and applied in the plate incorporation method*".
- 12 We consider that the positive result in TA100 is clearly biologically significant. In particular, we note that the first dose (625 µg/plate) inducing more than a 2-fold increase in the number of colonies (with 2.06) does not induce emulsion. Therefore, your claim that positive results are seen only at precipitating doses is not supported by the provided results.
- 13 Further, it is correct that higher concentration levels are obtained in the preincubation method compared to the standard plate incorporation method. However, the OECD TG 471 recommends the preincubation method as a different (possibly more sensitive) condition to be studied in the *in vitro* gene mutation in bacteria test.
- 14 Based on the above, we consider that the *in vitro* gene mutation in bacteria study is valid.
- 15 Therefore, the information requirement is triggered.

#### 1.2. 1.2. Information provided

- 16 You have provided an *in vivo* micronucleus study (OECD TG 474, 1999) with the Substance, giving negative results (study i).

#### 1.3. Assessment of the information provided

##### 1.3.1. Study not adequate for the information requirement

- 17 Toxicological studies must comply with a recognised test method (Article 13(3) of REACH). To address the specific concern raised by the *in vitro* positive result, an *in vivo* somatic cell genotoxicity study must be conducted according to the OECD TG 488 or 489, as indicated in the Guidance on IRs and CSA, Section R.7.7.6.3. Such study must cover the key parameters of the corresponding OECD test guideline (Article 13(3) of REACH).
- 18 Study (i) is described as an *in vivo* micronucleus test. This study is not an *in vivo* gene mutation study addressing concerns for gene mutations.
- 19 The information provided does not cover the specification(s) required by the OECD TG 488 or 489.
- 20 Based on the above, study (i) is not adequate for the information requirement.
- 21 In your comments to the draft decision you state that the Substance has "*only industrial use under well-controlled conditions without any possible consumer exposure*" and that peroxides "*upon their intermediate use in the production of plastics the peroxide is completely consumed and via covalent binding integrated in the polymer structure. As a consequence, consumers and the general public cannot be exposed to the substance*". You concluded that "*even then, the level of concerns is limited based on limited exposure, and*

*overall weight of evidence indicating lack of concern following the negative outcome of the in vitro mutation tests in mammalian cells, in alignment with the conclusions by the ECVAM experts”.*

22 In your comments to the draft decision you refer to the “*WoE approach on the whole database*”, stating, among others that “*Lack of concerns for genotoxicity is also the general picture that emerges when examining all data available within the group of the peroxyesters*”. However, you have not provided any new scientific information, in the form of robust study summaries to justify your claims. Therefore, the information in your comments is not sufficient for ECHA to make an assessment and no conclusion on the compliance of the proposed adaptation can be made.

23 Based on the above the information requirement is not fulfilled.

#### 1.4. Test selection

24 According to the Guidance on IRs & CSA, Section R.7.7.6.3., either the *in vivo* mammalian alkaline comet assay (“comet assay”, OECD TG 489) or the transgenic rodent somatic and germ cell gene mutation assay (“TGR assay”, OECD TG 488) are suitable to follow up a positive *in vitro* result on gene mutation.

#### 1.5. Study design

##### 1.5.1. Comet assay

25 In case you decide to perform the comet assay, 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, paragraph 23).

26 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.

27 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.

##### 1.5.1.1. Germ cells

28 You may consider collecting the male gonadal cells 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.

##### 1.5.2. TGR assay

29 In case you decide to perform the TGR assay, according to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

- 30 Also, according to the test method OECD TG 488, the test substance is usually administered orally.
- 31 Based on the OECD TG 488, you are requested to follow the 28+28d regimen, as it permits the testing of mutations in somatic tissues and as well as in tubule germ cells from the same animals.
- 32 According to the test method OECD TG 488, the test must be performed by analysing tissues from liver, as slowly proliferating tissue and primary site of xenobiotic metabolism, and from glandular stomach and duodenum, as rapidly proliferating tissue and site of direct 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 mutagenicity at the site of contact in the gastro-intestinal tract. However, duodenum must be stored (at or below  $-70^{\circ}\text{C}$ ) until the analysis of liver and glandular stomach is completed; the duodenum must then be analysed only if the results obtained for the glandular stomach and for the liver are negative or inconclusive.

#### *1.5.2.1. Germ cells*

- 33 You may consider collecting the male germ cells (from the seminiferous tubules) at the same time as the other tissues, to limit additional animal testing. According to the OECD 488, the tissues (or tissue homogenates) can be stored under specific conditions and used for DNA isolation for up to 5 years (at or below  $-70^{\circ}\text{C}$ ). This duration is sufficient to allow you or ECHA to decide on the need for assessment of mutation frequency in the collected germ 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.

## 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).

***Guidance for monomers and polymers***; ECHA (2023).

***Guidance on intermediates***; ECHA (2010).

All guidance documents are 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

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 23 August 2022.

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 took into account your comments and amended the request(s).

As a result of one or more changes of registration tonnage band or registration type, the request for Long-term toxicity testing on fish (triggered by Annex VIII, Section 9.1.3., Column 2; test method: EU C.47./OECD TG 210) was removed from the decision. The deadline was not changed.

ECHA also updated the reference to Annex VII, Section 8.4., Column 2 (request 1), because of the revision of the REACH Annexes (Regulation (EU) No 2022/477).

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: Addressee(s) 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 summaries (<https://echa.europa.eu/practical-guides>).

(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.

With that detailed information, ECHA can confirm 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 dossiers (<https://echa.europa.eu/manuals>).