~~Confidential~~

Helsinki, 11 August 2022

Addressees
Registrant(s) of DMT(2-EHTG) as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
08/02/2022

Registered substance subject to this decision ("the Substance")
Substance name: 2-ethylhexyl 10-ethyl-4,4-dimethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate
EC number: 260-829-0

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 ***19 May 2025***.

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

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

1. In vivo mammalian alkaline comet assay (triggered by Annex IX, Section 8.4., column 2; test method: OECD TG 489) in rats, 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.

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.

Authorised[1] 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 IX of REACH

1. In vivo mammalian alkaline comet assay

1 An appropriate *in vivo* somatic cell genotoxicity is an information requirement under Annex IX to REACH (Section 8.4., Column 2) if both of the following criteria are met: (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.

2 Regarding the first criterion, your dossier contains positive results from the *in vitro* gene mutation study in mammalian cells (OECD TG 476, 2019) which raise the concern for gene mutations.

3 In your comments to the draft decision, you indicate that your initial interpretation of positive results in the above OECD TG 476 study (2019) may not be correct and that "*The weight-of-evidence indicates DMTE [the Substance] is not mutagenic. The available in vitro data for DMTE indicate an EQUIVOCAL ability to induce gene mutations : it is clearly negative in bacteria but a very weak response has been observed in Chinese hamster ovary (CHO) cells. This response occurred only at a concentration that was at or above approached the limit of toxicity (22 to 27% relative survival [RS], compared to the maximum recommended limit of 10-20% RS) and only marginally exceeded the laboratory's HCD [historical control data]. There is no evidence that DMTE causes gross chromosome damage*."

4 However, ECHA disagrees that the ability of the Substance to induce gene mutations is equivocal. ECHA considers the positive OECD TG 476 study (2019) with the Substance as valid. In the first repeat experiment, the mutant frequency at the highest test concentration of 0.0625 µl/ml fulfilled two of the three criteria of OECD TG 476 for a clearly positive result, i.e. (i) at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control, and (ii) any of the results are outside the distribution of the historical negative control data. Although the third criterion of a positive trend test was not met, a concentration-related increase in the mutant frequency could still be observed, both in the absence and presence of metabolic activation. These positive results were also reproduced in a second repeat experiment, confirming that the Substance induces gene mutations in mammalian cells. The levels of cytotoxicity achieved in both repeat experiments were also acceptable as relative survival was above 10% at all test concentrations. ECHA further notes that concentrations higher than 0.0625 µl/ml could have been tested since the observed relative survival at this concentration was 22 and 27% in the first and second repeat experiments, respectively, while the highest test concentration should aim to achieve between 20 and 10% relative survival according to OECD TG 476. While the results already indicate that the Substance is positive in this study, testing at higher concentrations may have induced a higher mutant frequency and a clearer concentration-response relationship.

5 Furthermore, although the *in vitro* gene mutation test in bacteria and the *in vitro* gene mutation test in mammalian cells both investigate gene mutations, they are considered complementary as they cover different gene mutation mechanisms. Therefore, the negative results obtained in the *in vitro* gene mutation study in bacteria with the Substance (1996) provided in your dossier and referred to in your comments cannot be used to supersede the positive results obtained in the *in vitro* gene mutation study in mammalian cells (OECD TG 476, 2019) and do not remove the concern for gene mutation.

6 Finally, according to the Guidance on IRs & CSA, Section R.7.7.4.1, results from methods testing different genotoxic endpoints, i.e. gene mutations or chromosomal aberrations, should not be combined in an overall weight-of-evidence analysis, but should be subjected to such analysis separately for each endpoint. The negative OECD TG 473 study (2019) with the Substance provided in your dossier and referred to in your comments investigates chromosomal aberration and not gene mutation. Therefore, this study does not remove the concern for gene mutation raised by the positive OECD TG 476 study (2019).

7 Regarding the second criterion, no data from an *in vivo* somatic cell genotoxicity study with the Substance is available in the dossier.

1.1. Information provided to fulfil the information requirement

8 You have submitted a testing proposal for a combined *in vivo* mammalian erythrocyte micronucleus test and *in vivo* mammalian alkaline comet assay to be performed with the Substance.

9 ECHA requested your considerations for alternative methods to fulfil the information requirement for Genetic toxicity *in vivo*. In particular, you now disregard the *in vivo* studies with the analogue substance dimethyltin dichloride (EC no. 212-039-2) submitted in your dossier (*in vivo* micronucleus study, 1991; *in vivo* UDS study, 1998), because you consider the read-across hypothesis as not valid. This concurs with ECHA's assessment in its prior decision CCH-D-2114372123-58-01/F of 13 November 2017.

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

1.2. Test selection

11 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) that you propose is suitable to follow up the concern for gene mutation raised by the positive *in vitro* gene mutation study in mammalian cells. Therefore, the comet assay is an appropriate follow-up test for the Substance.

12 You also propose to combine the comet assay with an *in vivo* mammalian erythrocyte micronucleus test ("MN test", OECD TG 474).

13 According to the Guidance on IRs & CSA , Section 7.7.1 and figure R.7.7-1, the MN test is an appropriate test to investigate effects *in vivo*, on chromosomal aberrations (micronuclei). However, your dossier contains negative results for the *in vitro* cytogenicity test, therefore there is no concern for chromosomal aberrations (*in vitro*). It is therefore at your discretion to perform the MN test in combination to the comet assay.

1.3. Specification of the study design

14 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).

15 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.

16 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. In your testing proposal justification, you refer to the IWGT conclusions that only one site-of-contact gastro-intestinal tract tissue (stomach or duodenum/jejunum) needs to be tested and therefore propose comet analysis in the duodenum only. However, 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.

17 In case you consider to also perform the MN test, 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]).

1.3.1. Germ cells

18 A subsequent germ cell genotoxicity study (TGR/OECD TG 488, or CA on spermatogonia/OECD TG 483) 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.

19 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.

20 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.

1.4. Outcome

21 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 19 December 2019.

ECHA held a third party consultation for the testing proposals from 27 January 2020 until 12 March 2020. ECHA received third party comments, but these did not include scientifically valid information or studies addressing the hazard endpoint addressed by this decision.

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 took into account your comments and did not amend the request or the deadline.

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.

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.

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
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[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[2].

(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

Before generating new data, you must agree within the joint submission on the chemical composition of the material to be tested (Test Material) which must be relevant for all the registrants of the Substance.

(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 variation in compositions reported by all members of the joint submission,
- 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 and whether it is suitable for use by all members of the joint submission.

Technical instructions on how to report the above is available in the manual on How to prepare registration and PPORD dossiers[3].

[2] https://echa.europa.eu/practical-guides
[3] https://echa.europa.eu/manuals