~~Confidential~~

Helsinki, 29 November 2022

Addressees
Registrant(s) of JS_217-060-0 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
29/03/2018

Registered substance subject to this decision ("the Substance")
Substance name: Dimethyl azelate
EC number: 217-060-0

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 the deadline of **8 December 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 vitro cytogenicity study in mammalian cells (triggered by Annex VII, Section 8.4., column 2; test method: OECD TG 473); OR In vitro micronucleus study (triggered by Annex VII, Section 8.4., column 2; test method: OECD TG 487)

2. In vivo genetic toxicity (triggered by Annex VII, Section 8.4., column 2) to be selected according to the following specifications:

   If the results of the *in vitro* study requested under 1. are **negative**:

   Transgenic rodent somatic and germ cell gene mutation assay (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 (test method: OECD TG 489) in rats, or if justified, in other rodent species, oral, on the following tissues: liver, glandular stomach and duodenum.

   If the results of the *in vitro* study requested under 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 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 related to the information under Annex VII of REACH

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

1 Further mutagenicity studies must be considered under Annex VII to REACH in case of a positive result (Section 8.4., column 2).

1.1. Triggering of the information requirement

2 As raised in the proposal for amendment (PfA) submitted by one of the Member States Competent Authorities, the Guidance on IRs & CSA, Section R.7.7.6.3, further specifies that "REACH Annex VII substances for which only a bacterial gene mutation test has been conducted and for which the result is positive should be studied further, according to the requirements of Annex VIII." This is for the reason that the in vitro cytogenicity test under Section 8.4.2 will allow to further investigate the mutagenicity of the substance in accordance with the REACH integrated testing strategy.

3 Your dossier contains positive results for the *in vitro* gene mutation study in bacteria (2018); however, no adequate information from an *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study, according to the requirements of Annex VIII, is available.

4 Therefore, the *in vitro* cytogenicity study in mammalian cells or an *in vitro* micronucleus study is triggered.

5 ECHA agrees with the PfA and considers 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 follow-up *in vivo* study.

1.2. Specification of the study design

6 Either the *in vitro* cytogenicity study in mammalian cells (test method OECD TG 473) or *in vitro* micronucleus study (test method OECD TG 487) are considered suitable.

2. In vivo genetic toxicity study

7 Under Annex VII Section 8.4., Column 2, further mutagenicity studies must be considered in case of a positive result in an in vitro gene mutation study in bacteria.

2.1. Triggering of the information requirement

8 Your dossier contains positive results for the in vitro gene mutation study in bacteria (2018) which raise the concern for gene mutation.

9 Therefore, the information requirement is triggered.

2.2. Assessment of the information provided

2.2.1. No in vivo study provided

10 The Guidance on IRs and CSA, section R.7.7.6.3 states that following a positive result in an in vitro test, "adequately conducted somatic cell in vivo testing is required to ascertain if this potential can be expressed in vivo. In cases where it can be sufficiently deduced that a positive in vitro finding is not relevant for in vivo situations (e.g. due to the effect of the test substances on pH or cell viability, in vitro-specific metabolism: see also Section R.7.7.4.1), or where a clear threshold mechanism coming into play only at high concentrations that will not be reached in vivo has been identified (e.g. damage to non-DNA targets at high concentrations), in vivo testing will not be necessary.".

11 However, no data from an in vivo somatic cell genotoxicity study is available in the dossier. Moreover, you did not provide any considerations explaining that the genotoxic potential of the substance cannot be expressed in vivo, based e.g. on lack of relevance for in vivo situations or the existence of threshold mechanism.

12 ECHA considers that an appropriate in vivo follow up mutagenicity study is necessary to address the concern identified in vitro.

2.3. Test selection

13 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.

14 As explained above, under request 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 or micronucleus study, which may raise a concern for chromosomal aberration in the case of positive results.

15 As mentioned in the PfA, if 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). ECHA therefore agrees with the PfA that a combined study will thus address both the identified concerns for chromosomal aberration as well as gene mutation.

16 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.

17 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) the TGR assay or 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.

2.4. Specification of the study design

*2.4.1. Comet assay (if the test results of request 1 are **negative**)*

18 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).

19 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.

20 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.

*2.4.2. TGR assay (if the test results of request 1 are **negative**)*

21 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.

22 Also, according to the test method OECD TG 488, the test substance is usually administered orally.

23 Based on 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.

24 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, 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 ºC) 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.

*2.4.3. Comet assay combined with MN test (if the test results of request 1 are **positive**)*

25 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.

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, and from 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.

28 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] 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.

2.4.4. Germ cells

2.4.4.1. Comet assay or Comet assay combined with MN test

29 In case you perform a comet assay, 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.

2.4.4.2. TGR assay

30 In case you perform a TGR assay, 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 ºC). 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).

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

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 15 November 2021.

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.

ECHA received proposals for amendment (PfA) and modified the draft decision.

ECHA agrees with the PfA that the deadline of the decision should be extended from 12 months to 24 months to allow sequential testing. Moreover, an additional extension of 12 months from the standard deadline has been exceptionally granted to take into account currently longer lead times in contract research organisations. Therefore, ECHA has modified the deadline of the decision to 36 months.

ECHA invited you to comment on the proposed amendment(s) and referred the modified draft decision to the Member State Committee.

You did not provide any comments on the proposed amendment(s).

The Member State Committee unanimously agreed on the draft decision in its MSC-79 written procedure. ECHA adopted the decision under Article 51(6) 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.

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[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

(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 dossiers[3].

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