

Helsinki, 05 December 2022

Addressee

Registrant of JS_478-27-6_Deltamin_Full as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision

02/02/2021

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

Substance name: 1,8-naphthylenediamine

EC/List number: 207-529-8

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 **12 June 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 combined with in vivo mammalian erythrocyte micronucleus test, also requested below (triggered by Annex VII, Section 8.4., column 2).

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; 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 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 list of requests above, the same information requirement is mentioned under different headings. This is because under the REACH Regulation this information is required under different conditions, dependent on the tonnage of the registration. While the reasons for the information requirement may thus differ, only one study is to be conducted. All registrants concerned must make every effort to reach an agreement as to who is to carry out the study on behalf of the others under Article 53 of REACH.

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 VII of REACH

1. In vivo mammalian alkaline comet assay combined with In vivo mammalian erythrocyte micronucleus test

- 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, 1981, 1982, 1997 & 1998; QSAR, 2003) which raise the concern for gene mutations.
- 3 Moreover, based on the results of the in vitro micronucleus test (OECD TG 487, 2018) there is also a concern for chromosomal aberrations, as explained under Section 2 below.
- 4 ECHA considers that an in vivo follow-up study is necessary to address the identified concerns.
- 5 For the assessment of the testing proposal, see Section 2.

Reasons for the decision(s) related to the information under Annex VIII of REACH**2. In vivo mammalian alkaline comet assay combined with In vivo mammalian erythrocyte micronucleus test**

- 6 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.
- 7 Your dossier contains positive results for the in vitro gene mutation study in bacteria (OECD TG 471, 1981, 1982, 1997 & 1998; QSAR, 2003) which raise the concern for gene mutations.
- 8 Moreover, as raised in a proposal for amendment (PfA) submitted by one of the Member States Competent Authorities (MSCAs), there are positive results for the in vitro micronucleus test (OECD TG 487, 2018), which raise an additional concern for chromosomal aberrations.
- 9 According to OECD TG 487, a test chemical is considered to be clearly positive if, in any of the experimental conditions examined, the following criteria are met:
- i. at least one of the test concentrations exhibits a statistically significant increase compared with the concurrent negative control;
 - ii. the increase is dose-related in at least one experimental condition when evaluated with an appropriate trend test; and
 - iii. any of the results are outside the distribution of the historical negative control data (e.g. Poisson-based 95% control limits; see para. 52).
- 10 In the dossier you conclude that the OECD TG 487 study (2018) did not lead to a clear positive or negative result and that the response has therefore been assessed as equivocal under the conditions of the test.
- 11 With reference to the OECD TG 487 study (2018) and the above-mentioned criteria, ECHA notes the following:
- i. There are several statistically significant results; in particular, the results obtained in experiment I (without metabolic activation) and experiments II (with and without metabolic activation), showed a statistically significant increase in proportion of micronuclei at various concentration levels.
 - ii. There is no dose-related increase in any of the experimental conditions tested.
 - iii. The statistically significant results noted in i. were outside and above the historical control data.
- 12 Criteria i. and iii. are thus met, while criterion ii. is not met. Therefore, the response of OECD TG 487 study (2018) is not clearly positive.
- 13 According to OECD TG 487 (see para. 60), if the response is neither clearly negative nor clearly positive, the data should be evaluated by expert judgement and/or further investigations.
- 14 You have not provided further investigations. Therefore, following the re-evaluation of the data available in the dossier, based on expert judgement, we consider that, because of the several increases noted, which are statistically significant and outside the historical control

range, there is a positive response noted in OECD TG 487 study (2018), which raises a concern for chromosomal aberrations.

- 15 This is also supported by the QSAR predictions in the Danish QSAR Database², which predict a positive response (in domain) for chromosomal aberrations in the QSAR Battery combining predictions from the CASE Ultra, Leadscope and SciQSAR models.

2.1. Information provided to fulfil the information requirement

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

- 17 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.

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

2.2. Test selection

- 19 The positive in vitro results available in the dossier indicate a concern for both chromosomal aberration and gene mutation.

- 20 The in vivo mammalian alkaline comet assay ("comet assay", OECD TG 489) 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.

- 21 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.

- 22 Therefore, as also mentioned in the PfA, the comet assay combined with the MN test is the most appropriate study for the Substance.

2.3. Specification of the study design

- 23 You did not specify the species to be used for testing. 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.

- 24 You did not specify the route for testing. 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.

- 25 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

² Danish QSAR Database: <https://qsar.food.dtu.dk/>

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.

- 26 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]).

2.3.1. Germ cells

- 27 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.

2.3.2. Cross-linking properties

- 28 You are reminded that you may decide to take into account the potential cross-linking properties of the Substance in the experimental setup of the comet assay and perform a modified comet assay in order 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 [2] 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 [3-6] in the OECD TG 489 or Pant et al. 2015 [7]). In order to ensure the robustness of the test result a specific positive control group of animals would be needed.

2.3.3. References

- [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] 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.
- [3] Merk and Speit (1999) Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. *Environ Mol Mutagen*;33(2):167-72.
- [4] Pfuhler and Wolf (1996) Detection of DNA-crosslinking agents with the alkaline comet assay. *Environ Mol Mutagen*;27(3):196-201.
- [5] Wu and Jones (2012) Assessment of DNA interstrand crosslinks using the modified alkaline comet assay. *Methods Mol Biol*;817:165-81.
- [6] 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.

- [7] 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.

Outcome

- 29 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 4 March 2021.

ECHA held a third party consultation for the testing proposal(s) from 22 April 2021 until 7 June 2021. 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.

ECHA received proposal(s) for amendment and modified the draft decision.

The deadline of the decision has been extended from 12 to 30 months from the date of the adoption of the decision. Six months were added due to the modification in the study specification (see Appendix 1). Moreover, ECHA exceptionally further extended this standard time for the submission of such information by 12 months taking into account currently longer lead times in contract research organisations.

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: Addressee 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 and VIII to REACH, for registration at 10-100 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 summaries³.
- (4) 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 shall 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 dossiers⁴.

³ <https://echa.europa.eu/practical-guides>

⁴ <https://echa.europa.eu/manuals>