~~Confidential~~

Helsinki, 19 January 2023

Addressees
Registrant of 101_JS_TAT as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
21/05/2021

Registered substance subject to this decision ("the Substance")
Substance name: N,N-dimethyl-N'-(2,2,6,6-tetramethylpiperidin-4-yl)propane-1,3-diamine
EC/List number: 278-817-9

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 ***26 January 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. In vivo mammalian alkaline comet assay or Transgenic rodent somatic and germ cell gene mutation assays 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 or Transgenic rodent somatic and germ cell gene mutation assays also requested below (triggered by Annex VIII, Section 8.4., column 2)

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

3. In vivo genetic toxicity study (triggered by Annex IX, Section 8.4., column 2)

   In vivo mammalian alkaline comet assay (test method: OECD TG 489) in rats, or if justified in other rodents, oral route, on the following tissues: liver, glandular stomach and duodenum.

   OR

   Transgenic rodent somatic and germ cell gene mutation assays (test method: OECD TG 488) in transgenic mice or rats, oral route on the following tissues: liver and glandular stomach; germ cells and 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.

4. Sub-chronic toxicity study (90-day) (Annex IX, Section 8.6.2.; test method: OECD TG 408) by oral route, in rats.

5. Pre-natal developmental toxicity study (Annex IX, Section 8.7.2.; test method: OECD TG 414) by oral route, in one species (rat or rabbit).

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.

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 VII of REACH

1. In vivo mammalian alkaline comet assay; or Transgenic rodent somatic and germ cell gene mutation assays

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, 2012) 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; or Transgenic rodent somatic and germ cell gene mutation assays

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, 2012) 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; or Transgenic rodent somatic and germ cell gene mutation assays

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, 2012) which raise the concern for gene mutations. Moreover, no data from an in vivo somatic cell genotoxicity study is available in the dossier.

3.1. Information provided to fulfil the information requirement

11 You have submitted a testing proposal for an In vivo Mammalian Erythrocyte *Pig-a* Gene Mutation Assay to be performed with the Substance. You also proposed to combine this study with the sub-chronic toxicity study (90 day) addressed in section 4 below.

12 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.

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

3.2. Test selection

14 The proposed Pig-a assay can be used to further investigate genotoxic potential detected by *in vitro* systems. However, based on the information provided in the dossier, the genotoxic effect observed in the *in vitro* test (positive result in the Ames test) is observed only without metabolic activation, which means that the genotoxic effect is due to the parent compound (and not to the metabolites). The Pig-a assay may not detect the effect of the parent substance as it cannot be ruled out that only the metabolite(s) would reach the bone marrow (i.e. the target organ of this assay).

15 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) is suitable to follow up a positive in vitro result on gene mutation. Moreover, the potential effect of the parent (non-metabolised) substance on target tissue(s) can be detected in the comet assay or in the TGR assay, as first site of contact tissues are analysed in these assays.

16 Therefore, either the comet assay or the TGR assay is an appropriate follow-up test for the Substance.

3.3. Specification of the study design

3.3.1. Comet assay

17 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).

18 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.

19 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.

3.3.1.1. Germ cells

20 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.

21 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. TGR assay

22 According to the test method OECD TG 488, the test must be performed in transgenic mice or rats.

23 According to the test method OECD TG 488, test substance is usually administered orally.

24 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.

25 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 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.

3.3.2.1. Germ cells

26 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.

27 Therefore, you must collect 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, in accordance with Annex IX, Section 8.4., column 2, to decide on the need for assessment of mutation frequency in the collected germ cells. This type of evidence would be relevant for the overall assessment of possible germ cell mutagenicity including classification and labelling according to the CLP Regulation.

3.3.3. Combining a comet assay or a TGR assay with a sub-chronic toxicity study (90 day)

28 You may consider to combine a comet assay or a TGR assay with a repeated dose toxicity study, as long as this will not impair the validity of and the results from each individual study.

29 If you decide to combine both assays you should consider a number of practical aspects, which may prove challenging, such as:

(i) The selection of dosing, which should use the maximum tolerated dose (as defined in OECD TG 489, para. 36 / OECD TG 488, para. 28), and which should avoid administration via feed or drinking water (see OECD TG 489, para. 12 and Annex 3(2) / OECD TG 488, para. 27 and 29).

(ii) The historical control values should take into account the different age of test animals.

(iii) Careful consideration should be given to the tissue sampling in the comet assay/ TGR assay alongside the requirements of tissue sampling for other types of toxicological assessments. There could be some minor changes in the sampling regimen of the combined assays, however they should not adversely affect the sensitivity of these assays. For e.g. harvesting 24 hours after the last dose, which is typical of a general toxicity study, is not appropriate for the comet assay, where samples are usually collected 2-6 h after the last treatment (see OECD TG 489, para. 33 / OECD TG 488, para. 27).

(iv) For a combination with a comet assay, you must ensure that for a negative study there is evidence demonstrating the exposure of, or toxicity to, the target tissues (see OECD TG 489, para. 34).

(v) For a combination with a TGR assay, you must also ensure that a) the performance of the repeated dose toxicity study is not adversely affected by using a transgenic rodent strain (see OECD TG 488, para. 7) and b) the schedule of the repeated dose toxicity study is compatible with the TGR assay.

3.4. Outcome

30 Your testing proposal is rejected under Article 40(3)(d) of REACH. Under Article 40(3)(c) you are requested to carry out the additional test with the Substance, as specified above.

4. Sub-chronic toxicity study (90-days)

31 A sub-chronic toxicity study (90 day) is an information requirement under Annex IX to REACH (Section 8.6.2.).

4.1. Information provided to fulfil the information requirement

32 You have submitted a testing proposal for a Sub-chronic toxicity study (90 day) according to OECD TG 408 with the Substance.

33 ECHA requested your considerations for alternative methods to fulfil the information requirement for Repeated dose toxicity. 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.

34 ECHA agrees that a 90-day study is necessary.

4.2. Specification of the study design

35 You proposed testing in the rat. ECHA agrees with your proposal because the rat is the preferred species according to the OECD TG 408. Therefore, the study must be conducted in the rat.

36 You proposed testing by the oral route. ECHA agrees with your proposal because this route of administration is appropriate to investigate systemic toxicity; Guidance on IRs and CSA, Section R.7.5.4.3.2.

4.3. Outcome

37 Your testing proposal is accepted under Article 40(3)(a) and you are requested to conduct the test with the Substance, as specified above.

5. Pre-natal developmental toxicity study

38 A pre-natal developmental toxicity (PNDT) study (OECD TG 414) in one species is an information requirement under Annex IX to REACH (Section 8.7.2.).

5.1. Information provided to fulfil the information requirement

39 You have submitted a testing proposal for a PNDT study according to OECD TG 414 by the oral route with the Substance.

40 ECHA requested your considerations for alternative methods to fulfil the information requirement for Developmental toxicity. 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.

41 ECHA agrees that a PNDT study in a first species is necessary.

5.2. Specification of the study design

42 You proposed testing in the rat as a first species. You may select between the rat or the rabbit because both are preferred species under the OECD TG 414 (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

43 You proposed testing by the oral route. ECHA agrees with your proposal because this route of administration is the most appropriate to investigate reproductive toxicity (Guidance on IRs and CSA, Section R.7.6.2.3.2.).

5.3. Outcome

44 Your testing proposal is accepted under Article 40(3)(a) 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 13 July 2021.

ECHA held a third party consultation for the testing proposal(s) from 26 August 2021 until 11 October 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.

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 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
[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