~~Confidential~~

Helsinki, 06 June 2023

Addressees
Registrants of JS_10563-26-5_N4 as listed in Appendix 3 of this decision

Date of submission of the dossier subject to this decision
09/08/2018

Registered substance subject to this decision ("the Substance")
Substance name: N,N'-bis(3-aminopropyl)ethylenediamine
EC number/List number: 234-147-9

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 information under request 1 to 2 below by ***11 September 2025***

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

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

1. In vitro micronucleus study (Annex VIII, Section 8.4.2., test method: OECD TG 487). The aneugenic potential of the Substance must be assessed with an additional control group for aneugenicity on top of the control group for clastogenicity, if the Substance induces an increase in the frequency of micronuclei

2. Only if a negative result in Annex VII, Section 8.4.1. and Annex VIII, Section 8.4.2. is obtained, in vitro gene mutation study in mammalian cells (Annex VIII, Section 8.4.3.; test method: EU B.17./OECD TG 476 or EU B.67./OECD TG 490)

The reasons for the requests 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 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

[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 request(s)

Contents

Reasons related to the information under Annex VIII of REACH

1. In vitro micronucleus study

1 An in vitro mammalian chromosomal aberration study or an in vitro micronucleus study is an information requirement under Annex VIII, Section 8.4.2.

1.1. Information provided

2 You have adapted this information requirement by using Annex VIII, Section 8.4., Column 2. To support the adaptation, you have provided the following information:

(i) Adaptation "*In vivo micronucleus study available*";

(ii) OECD 474 study with the Substance (1992).

1.2. Assessment of the information provided

1.2.1. The provided adaptation does not meet the criteria of Annex VIII, Section 8.4., Column 2

3 Under Annex VIII, Section 8.4., Column 2, the study usually does not need to be conducted if adequate data from the corresponding in vivo study, (namely in vivo micronucleus study regarding point 8.4.2.) are available.

4 For the data from an in vivo micronucleus study to be considered adequate, the in vivo study you submitted has to meet the requirements of the OECD TG 474. Therefore, the following specifications must be met:

a) daily treatments, i.e. separated by 24h, are foreseen for repeated treatments;

b) 1 sampling time between 36 and 48h following the final treatment for peripheral blood is foreseen after 2 daily treatments;

c) the proportion of immature erythrocytes among total (immature + mature) erythrocytes and the mean number of micronucleated immature erythrocytes are reported for each group of animals.

5 In study (ii):

a) 2 treatments with a 48h interval were conducted;

b) only one post-treatment sample was done (after 48h);

c) MN induction in total blood cells were measured but mature or normochromatic erythrocytes (NCE), immature or polychromatic erythrocytes (PCE) to NCE ratio, percentage of micronucleated PCEs were not scored or reported in the dossier.

6 The information provided does not cover the specifications required by the OECD TG 474.

7 Based on the above, your adaptation is rejected, and the information requirement is not fulfilled.

8 In the comments to the draft decision, you agree to perform the requested study.

1.3. Specification of the study design

9 According to the Guidance on IR & CSA, Section R.7.7.6.3., either the in vitro mammalian chromosomal aberration ("CA") test (test method OECD TG 473) or the in vitro mammalian cell micronucleus ("MN") test (test method OECD TG 487) can be used to investigate chromosomal aberrations in vitro. However, while the MN test detects both structural chromosomal aberrations (clastogenicity) and numerical chromosomal aberrations (aneuploidy), the CA test detects only clastogenicity, as OECD TG 473 is not designed to measure aneuploidy (see OECD TG 473, paragraph 2).Therefore, you must perform the MN test (test method OECD TG 487), as it enables a more comprehensive investigation of the chromosome damaging potential in vitro. Moreover, in order to demonstrate the ability of the study to identify clastogens and aneugens, you must include two concurrent positive controls, one known clastogen and one known aneugen [1] (OECD TG 487, paragraphs 33 to 35).

1.3.1. Assessment of aneugenicity potential

10 If the result of the MN test is positive, i.e. your Substance induces an increase in the frequency of micronuclei, you must assess the aneugenic potential of the Substance.

11 In line with the OECD TG 487 (paragraph 4), you should use one of the centromere labelling or hybridisation procedures to determine whether the increase in the number of micronuclei is the result of clastogenic events (i.e. micronuclei contain chromosome fragment(s)) and/or aneugenic events (i.e. micronuclei contain whole chromosome(s)).

[1] According to the TG 487 (2016) "At the present time, no aneugens are known that require metabolic activation for their genotoxic activity" (paragraph 34).

2. *In vitro* gene mutation study in mammalian cells

12 An *in vitro* gene mutation study in mammalian cells is an information requirement under Annex VIII, Section 8.4.3., in case of a negative result in the *in vitro gene* mutation test in bacteria and the *in vitro* cytogenicity test.

2.1. Triggering of the information requirement

13 Your dossier contains (I) a negative result for *in vitro* gene mutation study in bacteria, and (II) no data or inadequate data for the other study (*in vitro* cytogenicity study in mammalian cells or in vitro micronucleus study).

14 The *in vivo* micronucleus study provided in the dossier is rejected for the reasons provided in request 1.

15 The result of the request 1 will determine whether the present requirement for an *in vitro* mammalian cell gene mutation study in accordance with Annex VIII, Section 8.4.3. is triggered.

16 Consequently, you are required to provide information for this information requirement, if the in vitro micronucleus study provides a negative result.

2.2. Information provided

17 You have provided the following information on the Substance:

(i) an *in vitro* gene mutation study in mammalian cells (1992)

(ii) an *in vitro* gene mutation study in mammalian cells (1991)

(iii) an *in Vitro* Sister Chromatid Exchange Assay in Mammalian Cells (1992)

2.3. Assessment of the information provided

2.3.1. The provided studies do not meet the specifications of the test guideline(s)

18 To fulfil the information requirement, a study must comply with the OECD TG 476 or the OECD TG 490 (Guidance on IRs and CSA, Table.7.7-2) (Article 13(3) of REACH). Therefore, the following specifications must be met:

a) at least 4 concentrations are evaluated, in absence and in presence of metabolic activation;

b) for the Mouse Lymphoma Assay (MLA), the concurrent positive control meets the acceptability criteria recommended by the MLA Expert Workgroup of the International Workshop for Genotoxicity Testing (IWGT) in terms of mutant frequency and/or small colony induction and described in paragraph 58 of OECD TG 490;

c) for the Mouse Lymphoma Assay (MLA), the concurrent negative control meets the acceptability criteria recommended by the MLA Expert Workgroup of the International Workshop for Genotoxicity Testing (IWGT) in terms of mutant frequency, cloning efficiency and suspension growth and described in paragraph 57 of OECD TG 490;

d) for the Mouse Lymphoma Assay (MLA), as described in paragraph 63 of OECD TG 490, a test chemical is considered to be clearly positive if, in any of the experimental conditions examined (see paragraph 33), the increase in mutation frequency above the concurrent background exceeds the Global Evaluation Factor (GEF) and the increase is concentration related (e.g. using a trend test).

19 In the provided studies:

a) in studies (i) and (ii), only 3 concentrations (i.e., less than 4 concentrations) were evaluated in absence and in presence of metabolic activation;

b) in study (ii), the mutant frequency and/or small colonies induced by the concurrent positive control do not meet the acceptability criteria recommended by the MLA Expert Workgroup of the International Workshop for Genotoxicity Testing (IWGT) and described in paragraph 58 of OECD TG 490 (i.e., the positive control (89 x 10^{-6} and 238 x 10^{-6} without and with S9, respectively) is lower than the recommended 300 x 10^{-6} spontaneous mutants above the negative control. No information on the number and proportion of small colonies induced by the positive control is provided so it is not possible to confirm whether the positive control the corresponding IWGT criteria are fulfilled);

c) in study (ii), the response of the negative control does not meet the acceptability criteria recommended by the MLA Expert Workgroup of the International Workshop for Genotoxicity Testing (IWGT) and described in paragraph 57 of OECD TG 490 (i.e., the negative control values (21 x 10^{-6} and 19 x 10^{-6} without and with S9, respectively) are lower than the recommended 50 -170 x 10^{-6} spontaneous mutants for the microwell version of the assay);

d) Although a statistically significant increase in mutant frequency is obtained with the test substance with S9, the responses are below the GEF according to IWGT criteria and no dose-response is observed (all test concentrations result in about the same induction level of 90-98 x 10^{-6}) in study (ii). In study (i) also no dose-

response is observed and, mutant frequency even decreases at the highest concentration with still acceptable toxicity levels.

20 The study (iii) is a study performed according to OECD TG 479 which became obsolete in 2014 and which does not address the parameters required by OECD TG 476/490.

21 The information provided does not cover the specification(s) required by the OECD TG 476/490.

22 Therefore, the information requirement is not fulfilled.

23 In the comments to the draft decision, you agree to perform the requested study.

2.4. Specification of the study design

24 To fulfil the information requirement for the Substance, either the in vitro mammalian cell gene mutation tests using the hprt and xprt genes (OECD TG 476) or the thymidine kinase gene (OECD TG 490) are considered suitable.

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 (2012).
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 14 March 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.

In you comments, you provided additional information which addresses the issues identified in the draft decisions for the following information requirements:

- growth inhibition study on aquatic plants,
- short term toxicity testing on fish, and
- long term toxicity testing on fish.

ECHA takes note that you have updated your registration dossier on 11 January 2023 (submission number: [redacted]). Therefore, ECHA has removed the requests for the above mentionned information requirements.

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;

Registrant Name	Registration number	Highest REACH Annex applicable to you
[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, in this case the purity and presence of impurities.

With that detailed information, ECHA can confirm whether the Test Material is relevant for

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

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 (https://echa.europa.eu/manuals).

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