ECHA
EUROPEAN CHEMICALS AGENCY

Helsinki, 9 December 2020

Addressees
Registrant(s) of N,N-diethylhydroxylamine listed in the last Appendix of this decision

Registered substance subject to this decision (the Substance)
Substance name: N,N-diethylhydroxylamine
EC number: 223-055-4
CAS number: 3710-84-7

Decision number: Please refer to the REACH-IT message which delivered this communication (in format SEV-D-XXXXXXXXXX-XX-XX/F)

DECISION ON SUBSTANCE EVALUATION

Under Article 46 of Regulation (EC) No 1907/2006 (REACH), you must submit the information listed below:

A. Information required to clarify the potential risk related to Mutagenicity
   1. *In vivo* Mammalian Alkaline Comet Assay (test method: OECD TG 489) in Sprague-Dawley rats, by oral route (gavage) on tissues glandural stomach, duodenum and liver as specified in Appendix A (Request A.1, Section 2.1.b), on the Substance.

Deadline
The information must be submitted by 16 December 2021.

Conditions to comply with the information requested
To comply with this decision, you must submit the information in an updated registration dossier, by the deadline indicated above. The information must comply with the IUCLID robust study summary format. You must also attach the full study report for the corresponding study in the corresponding endpoint of IUCLID.

You must update the chemical safety report, where relevant, including any changes to classification and labelling, based on the newly generated information.

You will find the justifications for the requests in this decision in the Appendix A entitled ‘‘Reasons to request information to clarify the potential risk’.
You will find the procedural steps followed to reach the adopted decision and some technical guidance detailed in further Appendices.

Appeal

This decision 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] by Christel Schilliger-Musset, Director of Hazard Assessment

[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.

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Basis for substance evaluation

The objective of substance evaluation under REACH is to allow for the generation of further information on substances suspected of posing a risk to human health or the environment ('potential risk').

ECHA has concluded that further information on the Substance is necessary to enable the evaluating Member State Competent Authority (MSCA) to clarify a potential risk and whether regulatory risk management is required to ensure the safe use of the Substance.

The ECHA decision requesting further information is based on the following:

(1) There is a potential risk to human health or the environment, based on a combination of hazard and exposure information;
(2) Information is necessary to clarify the potential risk identified; and
(3) There is a realistic possibility that the information requested would allow improved risk management measures to be taken.

The Appendix entitled 'Reasons to request information' describe why the requested information is necessary and appropriate.

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Appendix A – Reasons to request information to clarify the potential risk related to Mutagenicity

1. Potential risk

1.1 Potential hazard of the Substance

Following its assessment of the available relevant information on the Substance, the evaluating MSCA and ECHA have identified the following potential hazard(s) which must be clarified.

a) Potential mutagenicity

The available information shows that the Substance may be a clastogen whilst it cannot be excluded that it may also be a gene mutagen.

In vitro genotoxicity studies:

Three key experimental studies are reported under the *in vitro* genotoxicity section of the registration dossier(s) – a bacterial reverse mutation test (Ames test), a chromosomal aberration test and a gene mutation test in mammalian cells.

The key Ames test (year: 2001) was according to the OECD TG 471 and the GLP, with an assigned reliability score of 1. Five strains were treated (TA 1535, TA 1537, TA 98, TA 100, TA 102), with and without S9-mix at 5 concentrations of the Substance up to 5000 µg/plate. Positive and negative (vehicle - distilled water) controls were included. The results were reported as negative.

The key chromosome aberration study in mammalian cells (year: 2002) was according to the OECD TG 473 and the GLP, with an assigned reliability score of 1. Human lymphocytes were treated with and without S9-mix up to 5000 µg/ml of the Substance. Positive and negative (only vehicle) controls were included. The results were reported as positive without metabolic activation and negative with metabolic activation. This study shows that the Substance is an *in vitro* clastogen.

The key gene mutation study in mammalian cells (year: 2001) was according to the OECD TG 476 and the GLP, with an assigned reliability score of 1. Mouse lymphoma L5178Y cells were treated in two independent experiments with and without S9-mix up to 5000 µg/ml of the Substance. Positive and negative (only vehicle) controls were included. The results

were reported as positive without metabolic activation and negative with metabolic activation. However, in the experiments with metabolic activation there was an increase in small colonies at the top concentrations. In the experiments without metabolic activation there was an increase in both large and (more) small colonies at the top concentrations. Overall, this study shows that the Substance is an *in vitro* mutagen, indicating both clastogenicity and gene mutagenicity.

Another gene mutation study in mammalian cells (year: 1986) was reported as a supporting study with an assigned reliability score of 2. The test was performed in Chinese hamster lung fibroblasts (V79) and only without S9-mix. The results were reported as negative. However, unlike for other key studies, the results tables for this study are not available in the registration dossier(s). Therefore, the evaluating MSCA cannot verify the negative result.

An unscheduled DNA synthesis test in human lymphocytes with an assigned reliability score of 3 is also reported in the registration dossier(s). In addition to the Substance being impure, the validation criteria and the number of cells examined in this study were not reported. Furthermore, five bacterial reverse mutation assays, all with an assigned reliability score of 3, performed with only one strain and much higher than 5000 µg/plate concentrations or the impure Substance or the urine of animals (co-)exposed to the (impure) Substance are also reported in the registration dossier(s).

In vivo genotoxicity studies:

Two key experimental studies are reported under the *in vivo* genotoxicity section of the registration dossier(s) – a mammalian erythrocyte micronucleus test and an Unscheduled DNA Synthesis test.

The key mammalian erythrocyte micronucleus test (year: 1995) was according to the OECD TG 474 and the GLP, with an assigned reliability score of 1. Male and female ICR mice (5/sex/dose) were given the Substance by a single gavage administration of 375, 750 or 1500 mg/kg. Positive and negative (vehicle – distilled water) controls were included. Bone marrow was sampled at 24, 48 and 72 hours. Mortalities were observed in both males and females of the high dose group. There was no significant increase in the micronucleated cells. Therefore, the results were reported as negative. However, no conclusion on the clastogenicity observed in the *in vitro* studies (OECD 473, year:

2002 and OECD 476, year: 2001) can be made based on this negative *in vivo* study because

- The potential for clastogenicity at the site-of-contact tissues cannot be addressed by this study;
- Even though there were mortalities (in the high dose group), there were only "*slight reductions (no greater than 21%) in some groups relative to controls*"[2] and no dose-related decrease in the proportion of immature to total erythrocytes in the bone marrow. This implies a limited bone marrow toxicity caused by the Substance and therefore, the negative result of the study is of limited reliability;
- The power of the study is low since only 1000 immature erythrocytes per animal were scored for the incidence of micronucleated cells. This was indeed according to the then available version (from 1983) of the OECD TG 474 when the study was performed (in 1995); However, according to the current version (from 2016) of that test guideline, at least 4000 immature erythrocytes per animal needs to be scored.

The key Unscheduled DNA Synthesis test (year: 2003) was according to the OECD TG 486 and the GLP, with an assigned reliability score of 1. Male Wistar rats (4/dose) were given the Substance by a single gavage administration of 800 or 2000 mg/kg. Positive and negative (vehicle – purified water) controls were included. The results were reported as negative. However, according to the ECHA Guidance[3], the Unscheduled DNA Synthesis test is an indicator test measuring DNA repair of primary damage in liver cells but not a surrogate test for gene mutations *per se*. Therefore, no conclusion can be made on the indications for gene mutagenicity observed in the *in vitro* study (OECD 476, year: 2001) based on this negative *in vivo* study.

Two dominant lethal tests (one ambiguous and the other negative), a micronucleus test (negative), a drosophila sex-linked recessive lethal test (weakly positive) and a test for histidine alkylation in haemoglobin and urine (negative) are also reported in the registration dossier(s). However, all these tests were assigned a reliability score of 3 (except the histidine akylation test which was given a reliability score of 4) because the

[2] According to the paragraph 31 of the OECD TG 474 (current version, 2016), "*The highest dose may also be defined as a dose that produces toxicity in the bone marrow (e.g. a reduction in the proportion of immature erythrocytes among total erythrocytes in the bone marrow or peripheral blood of more than 50%, but to not less than 20% of the control value*" [emphasis added].
[3] ECHA Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance. Version 6.0 – July 2017.

Substance was impure or co-exposed with other substances (nitroethane and diethylamine hydrogen sulphite).

Supporting information:

Two carcinogenicity studies (duration: 2 years) via whole-body inhalation, one each in Swiss mice and Long-Evans rats, with an assigned reliability score of 4 are reported in the registration dossier(s).

In the mice study, the Substance (at only one test concentration: 10.3 ± 3.7 ppm) was co-exposed with nitroethane and diethylamine hydrogen sulfite. "*The incidence of all tumors, as well as subcutaneous tumors (principally fibrosarcomas), increased in exposed males with marginal significance. The incidence of any tumors in exposed females decreased with marked statistical significance*".

In the rats study, the Substance (at three test concentrations ranging 9 to 27 ppm) was co-exposed with nitroethane and/or diethylamine hydrogen sulfite. "*Very early one test animal developed a hemangioendothelioma, but no additional ones developed later. Also hydrometra of the uterus, a condition common in old virgin female rats, was found in four exposed and one control female. Chronic tracheitis was found in five exposed and two control animals. Thyroid lesions were seen in the exposed animals after 6 months exposure, but not in animals exposed 9 months or longer. Examinations for animals exposed more than 1 year indicated no significant differences between the control and test groups, except for interstitial cell tumors of the testes which showed up in 4 of the 47 exposed males that were examined compared to 0 in the 25 control males*".

Since there was co-exposure in the above two studies, the effects observed cannot be entirely attributed to the Substance exposure and it can neither be confirmed nor excluded that the potential mutagenicity of the Substance could be the mode of action for the effects.

A key repeated dose 90-day oral toxicity study (year: 2019) in Sprague-Dawley rats performed according to the OECD TG 408 and the GLP, with an assigned reliability score of 1 is reported in the registration dossier(s). The Substance was administered by gavage to 10 animals/sex/dose at 0, 50, 150 and 500 mg/kg bw/day. Forestomach squamous cell hyperplasia was observed in 1 female in the low-dose group, in 2 females and 1 male in

the mid-dose group, and 2 females and three males in the high-dose group. Also forestomach fibroplasia was observed in one female in the high-dose group. You suggest that "*These changes in the forestomach and in the stomach might be linked to the pH of the dose formulations (between 10 and 11)*". However, the evaluating MSCA considers that the potential mutagenicity of the Substance at the site-of-contact could be the mode of action for these effects.

Also in the 90-day oral study, in the mid- and high-dose groups there was a statistically significant decrease in morphologically normal epididymal sperm, mean number of testicular sperm heads and daily sperm production. Even though not statistically significant, there was a trend towards such effects already in the low-dose group. This information provides evidence that the Substance reaches the germ cells which is important when considering classification of substances for germ cell mutagenicity according to the criteria in the CLP Regulation.

A key repeated dose 28-day inhalation toxicity study (year: 1996) in Sprague-Dawley rats performed according to the OECD TG 412 and the GLP, with an assigned reliability score of 1 is reported in the registration dossier(s). The Substance was administered via nose-only inhalation route to 15 animals/sex/dose at 0, 15, 150 and 1500 ppm. In the mid- and high-dose groups, "*At the week 4 necropsy, reversible test-article related microscopic changes…including"* squamous hyperplasia in the nasal passages were observed. The evaluating MSCA considers that the potential mutagenicity of the Substance at the site-of-contact could be the mode of action for these effects.

The available and current information is not sufficient to draw a conclusion on the hazard. Further information is needed on mutagenicity.

1.2 Potential exposure

According to the information you submitted in all registration dossiers, the aggregated tonnage of the Substance manufactured or imported in the EU is in the range of 1000 – 10000 tonnes per year.

Furthermore, you reported that among other uses, the Substance is used by industrial workers as:

- a processing aid

- colour stabiliser for chemical products (fuel, resins, etc.) and in film/photographic industry, and for de-colourisation of phenols
- polymer processing

The Substance is also used by professional workers as:

- Use in stripper/etchant formulation in the electronic industry
- Use in coating

Therefore exposure to industrial and professional workers cannot be excluded.

1.3 Identification of the potential risk to be clarified

Based on all information available in the registration dossier(s) and information from the published literature, the Substance may be a mutagen.

The information you provided on manufacture and uses demonstrates a potential for exposure of industrial and professional workers.

Based on this hazard and exposure information the substance poses a potential risk to human health.

As explained in Section 1.1 above, the available information is not sufficient to conclude on hazard. Consequently further data is needed to clarify the potential risk related to mutagenicity.

1.4 Further risk management measures

If the mutagenicity of the Substance is confirmed, the evaluating MSCA will analyse the options to manage the risk(s). The results of Request A.1 will, amongst other relevant and available information, be used by the evaluating MSCA to assess whether the Substance should be classified as germ cell mutagen as defined in the CLP Regulation.

The potential classification of the Substance as germ cell mutagen (Cat. 1B or 2) based on Request A.1 would have consequences for the classification of mixtures containing the Substance due to cut-off/concentration limits triggering classification.

If classified as germ cell mutagen Cat. 1B, the evaluating MSCA will also assess whether the Substance should be proposed for identification as a substance of very high concern (SVHC) under Article 57 of REACH, which would lead to stricter risk management measures than those currently in place.

2. How to clarify the potential risk

2.1 Request A.1 (*In vivo* Mammalian Alkaline Comet Assay; test method: OECD TG 489)

a) Aim of the study

The aim of the *in vivo* Mammalian Alkaline Comet Assay ("comet assay") is to follow up on the clastogenicity and potential gene mutagenicity observed in the *in vitro* studies with the Subtance as described under Section 1.1.

b) Specification of the requested study

Species and route of exposure

The comet assay must be performed in Sprague-Dawley rats via oral gavage since in the 90-day oral study (year: 2019) performed in this strain of rats the Substance caused site-of-contact effects (forestomach hyperplasia) and also reached the germ cells.

The following tissues must be investigated:

Glandular stomach and duodenum: As set out in the OECD TG 489, the glandular stomach and duodenum are recommended as tissues to examine site-of-contact effects after oral exposure. Moreover, according to the test guideline, duodenum may be considered more relevant for humans. In view of the following possible variables; different tissue structure and function of the stomach and duodenum; different pH conditions; probable different absorption rates of the substance and possible breakdown product(s) between these two tissues; the evaluating MSCA considers that it is necessary to sample both tissues to increase the reliability of the analysis of genotoxicity at the site-of-contact.

and

Liver: As set out in the OECD TG 489, the liver is recommended as the primary site of xenobiotic metabolism, and an often highly exposed tissue to both parent substance and metabolites.

You are reminded that, if positive results from an *in vivo* somatic cell study are available, “the potential for germ cell mutagenicity should be considered on the basis of all available data, including toxicokinetic evidence”.

For this reason, it is recommended to prepare slides from single cell/nuclei suspensions from gonadal tissues and store them under suitable conditions for an appropriate amount of time. In case a positive result is obtained from any of the somatic tissues in the comet assay, it is recommended to analyse the gonadal slides.
With respect to possible outcomes, a positive result in whole gonads is not necessarily reflective of germ cell damage since gonads contain a mixture of somatic and germ cells. However, for the Substance there is already information from the 90-day oral study that it reaches the germ cells. Nevertheless, such positive result would provide further supporting information that the Substance and/or its metabolite(s) have not only reached the gonads but also caused genotoxic effects.
A negative or inconclusive result in whole gonads cannot be used to conclude on the germ cell genotoxicity as the sensitivity of the comet assay in gonadal cells has not been validated to detect germ cell genotoxicity.

To address the missing information identified above, the OECD TG 489 will allow to identify information both on clastogenicity and gene mutagenicity, which are required to conclude on the mutagenic properties, and to confirm whether the observed genotoxic mode of action is of potential risk for the Substance.

Request for the full study report
You must submit the full study report which includes:

- a complete rationale of test design and
- interpretation of the results
- access to all information available in the full study report, such as implemented method, raw data collected, interpretations and calculations, consideration of uncertainties, argumentation, etc.

This will enable the evaluating MSCA to fully and independently assess all the information provided, including the statistical analysis, and to efficiently clarify the mutagenic hazard of the Substance.

c) Alternative approaches and how the request is appropriate to meet its objective

The request is:

- appropriate, because it will provide information which will clarify clastogenicity and potential gene mutagenicity of the Substance. This will enable the evaluating MSCA to conclude on the somatic cell mutagenicity of the Substance;
- the least onerous measure because there is no equally suitable alternative method available to obtain the information that would clarify the potential hazard. There are no possible alternatives that could generate the same information (site-of-contact clastogenicity and gene mutagenicity). Consequently that there is no experimental study available at this stage that will generate the necessary information and does not need to test on vertebrate animals.

2.2 References relevant to the requests (which are not included in the registration dossier)

None. All the studies referred to are available in the registration dossier(s).

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Appendix B: Procedure

This decision does not imply that the information you submitted in your registration dossier(s) are in compliance with the REACH requirements. ECHA may still initiate a compliance check on your dossiers.

12-month evaluation

- Due to initial grounds of concern for Carcinogenicity and for Mutagenicity, the Member State Committee agreed to include the Substance (EC No 223-055-4, CAS RN 3710-84-7) in the Community rolling action plan (CoRAP) to be evaluated in 2019. The competent authority of Sweden ('the evaluating MSCA') was appointed to carry out the evaluation.

- In accordance with Article 45(4) of REACH, the evaluating MSCA carried out its evaluation based on the information in the registration dossier(s) you submitted on the Substance and on other relevant and available information.

- The evaluating MSCA completed its evaluation considering that further information is required to clarify the following concerns: Mutagenicity. After concluding on the mutagenicity concern the evaluating MSCA will consider whether further information is required to clarify the concern for Carcinogenicity.

- Therefore, it submitted a draft decision (Article 46(1) of REACH) to ECHA on 18 March 2020.

Decision-making

ECHA notified you of the draft decision and invited you to provide comments.

The decision making followed the procedure of Articles 50 and 52 of REACH as described below. For the purpose of this decision-making, dossier updates made after the date the draft of this decision was notified to you (Article 50(1) of REACH) will not be taken into account.

(i) Registrant(s)' commenting phase

ECHA did not receive any comments from you by the end of the commenting period.

(ii) Proposals for amendment by other MSCAs and ECHA and referral to the Member State Committee

The evaluating MSCA notified the draft decision to the competent authorities of the other Member States and ECHA for proposal(s) for amendment.

Appendix C: Technical Guidance to follow when conducting new tests for REACH purposes

Test methods, GLP requirements and reporting

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.

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.

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[4].

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 toxicity, the selected Test Material must contain that constituent/ impurity.

2. *Information on the Test Material needed in the updated dossier*

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

a) 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.
b) The reported composition must include all constituents of each Test Material and their concentration values.

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 "How to prepare registration and PPORD dossiers"[5].

[5] https://echa.europa.eu/manuals