KEMI
Swedish Chemicals Agency

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

N,N-diethylhydroxylamine

EC No 223-055-4

CAS RN 3710-84-7

Evaluating Member State: Sweden

Dated: 10 August 2023

Evaluating Member State Competent Authority

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Year of evaluation in CoRAP: 2019

Before concluding the substance evaluation a Decision to request further information was issued on: 9 December 2020

Further information on registered substances here:

http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances

Further information on the substance evaluation process here:

https://echa.europa.eu/regulations/reach/evaluation/substance-evaluation

DISCLAIMER

This document has been prepared by the evaluating Member State as a part of the substance evaluation process under the REACH Regulation (EC) No 1907/2006. The information and views set out in this document are those of the author and do not necessarily reflect the position or opinion of the European Chemicals Agency or other Member States. The Agency does not guarantee the accuracy of the information included in the document. Neither the Agency nor the evaluating Member State nor any person acting on either of their behalves may be held liable for the use which may be made of the information contained therein. Statements made or information contained in the document are without prejudice to any further regulatory work that the Agency or Member States may initiate at a later stage.

Foreword

Substance evaluation is an evaluation process under REACH Regulation (EC) No. 1907/2006. Under this process the Member States perform the evaluation and ECHA secretariat coordinates the work. The Community rolling action plan (CoRAP) of substances subject to evaluation, is updated and published annually on the ECHA web site[1].

Substance evaluation is a concern driven process, which aims to clarify whether a substance constitutes a risk to human health or the environment. Member States evaluate assigned substances in the CoRAP with the objective to clarify the potential concern and, if necessary, to request further information from the registrant(s) concerning the substance. If the evaluating Member State concludes that no further information needs to be requested, the substance evaluation is completed. If additional information is required, this is sought by the evaluating Member State. The evaluating Member State then draws conclusions on how to use the existing and obtained information for the safe use of the substance.

This Conclusion document, as required by Article 48 of the REACH Regulation, provides the final outcome of the Substance Evaluation carried out by the evaluating Member State. The document consists of two parts i.e. A) the conclusion and B) the evaluation report. In the conclusion part A, the evaluating Member State considers how the information on the substance can be used for the purposes of regulatory risk management such as identification of substances of very high concern (SVHC), restriction and/or classification and labelling. In the evaluation report part B the document provides explanation how the evaluating Member State assessed and drew the conclusions from the information available.

With this Conclusion document the substance evaluation process is finished and the Commission, the Registrant(s) of the substance and the Competent Authorities of the other Member States are informed of the considerations of the evaluating Member State. In case the evaluating Member State proposes further regulatory risk management measures, this document shall not be considered initiating those other measures or processes. Further analyses may need to be performed which may change the proposed regulatory measures in this document. Since this document only reflects the views of the evaluating Member State, it does not preclude other Member States or the European Commission from initiating regulatory risk management measures which they deem appropriate.

[1] http://echa.europa.eu/regulations/reach/evaluation/substance-evaluation/community-rolling-action-plan

Contents

Part A. Conclusion

1. CONCERN(S) SUBJECT TO EVALUATION

N,N-diethylhydroxylamine, here referred to as "the Substance" or "DEHA", was originally selected for substance evaluation in order to clarify concerns about:

-Suspected Mutagen

-Suspected Carcinogen

During the evaluation no other concern was identified.

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

The Substance has been assessed under Dossier evaluation[2] on several occasions following its REACH registration in 2013 (Annex X). It has no harmonised classification and labelling (CLH) in the CLP Regulation (EC 1272/2008).

In 2015, a Testing Proposal Examination (TPE) was performed and a decision with the following information requests was issued: 1. Viscosity (OECD TG 114), 2. Long-term toxicity on terrestrial invertebrates (Earthworm reproduction test, OECD TG 222) and 3. Effects on soil micro-organisms (nitrogen transformation test, EU C.2L / OECD TG 216).

In 2017, a Compliance Check (CCH) decision was issued with request for a Sub-chronic toxicity study (90-day), oral route (OECD TG 408) in rats. The registration(s) were updated with this information.

In 2021, another CCH decision was issued, following the Testing Proposal Evaluation (TPE) for an Extended one-generation reproductive toxicity study (EOGRTS, OECD TG 443) by oral route, in rats, with extension of the Cohort 1B to produce F2 generation. The deadline to update the registration(s) with this information is March 2025.

3. CONCLUSION OF SUBSTANCE EVALUATION

The evaluation of the available information on the substance has led the evaluating Member State to the following conclusions, as summarised in the table below.

Table 1

CONCLUSION OF SUBSTANCE EVALUATION Conclusions	Tick box
Need for follow-up regulatory action at EU level	√
Harmonised Classification and Labelling	√
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	

[2] https://echa.europa.eu/information-on-chemicals/dossier-evaluation-status/-/dislist/substance/100.020.960

No need for regulatory follow-up action at EU level	

4. FOLLOW-UP AT EU LEVEL

4.1. Need for follow-up regulatory action at EU level

4.1.1. Harmonised Classification and Labelling

The Substance has a self-classification as Muta. Cat. 2 (H341) by 5 notifiers among the 28 aggregated self-classifications in the C&L Inventory, representing a total 596 notifiers. The registrants self-classify DEHA as Flam. Liq. 3 (H226), Acute Tox. 4 via dermal and inhalation route, STOT SE 3 (H335, respiratory irritation) and Aquatic Chronic 2 (H411).

Based on the results of the Comet assay requested under this SEv and other available data, the eMSCA concludes that the Substance causes genotoxicity at the site of contact. Therefore, a harmonised classification (CLH) as Muta Cat. 2 may be warranted for the Substance. Effects observed following exposure by the oral route may also warrant a classification as STOT RE 2.

4.1.2. Identification as a substance of very high concern, SVHC (first step towards authorisation)

Not applicable.

4.1.3. Restriction

Not applicable.

4.1.4. Other EU-wide regulatory risk management measures

If the Substance would be classified as Muta. Cat. 2, it will trigger further risk management measures (RMM) under several other EU legislations, including company level RMM under the Occupational Safety and Health (OSH) legislation.

5. CURRENTLY NO FOLLOW-UP FORESEEN AT EU LEVEL

5.1. No need for regulatory follow-up at EU level

Not applicable.

5.2. Other actions

Not applicable.

6. TENTATIVE PLAN FOR FOLLOW-UP ACTIONS

Indication of a tentative plan is not a formal commitment by the evaluating MSCA. A commitment to prepare a REACH Annex XV dossier (SVHC, restrictions) and/or CLP Annex VI dossier should be made via the Registry of Intentions.

The eMSCA will not prepare a CLH proposal for the Substance at this stage.

The eMSCA notes that an EOGRTS (OECD TG 443) with the Substance is ongoing with the deadline of March 2025. The eMSCA will therefore await the outcome of this study. When the results of the OECD TG 443 are available, dependent on the outcome, the eMSCA will consider submitting a CLH proposal for the Substance.

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

N,N-diethylhydroxylamine, here also referred to as "the Substance" or "DEHA", was originally selected for substance evaluation in order to clarify concerns about:

-Suspected Mutagen

-Suspected Carcinogen

During the evaluation no other concern was identified.

Table 2

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome/conclusion**
Mutagenicity	Concern confirmed. An *in vivo* Comet assay was requested under the SEv. The study showed positive results. The eMSCA proposes harmonised C&L as Muta. Cat. 2 as the next regulatory step for the Substance. No further action under SEv.
Carcinogenicity	Concern inconclusive. No conclusive studies with the Substance are available. No further action under SEv.
Repeated dose toxicity	The Substance causes adverse effects on reproductive organs and other organs in males and female animals. These effects may warrant a classification as STOT RE 2.

7.2. Procedure

N,N-diethylhydroxylamine was included in the Community Rolling Action Plan (CoRAP) for substance evaluation (SEv) in 2019 by the competent authority of Sweden. The scope of the evaluation was human health, targeted to the concern for mutagenicity and carcinogenicity.

In 2020, a final SEv decision was sent to the registrant(s) with the request for a Mammalian *in vivo* alkaline comet assay, according to the OECD TG 489.

In 2023, the registration(s) were updated with the information requested in the SEv decision. The eMSCA evaluated the new information and concluded that no further information request under SEv was needed.

The eMSCA concludes that the data on the Substance may warrant a harmonised classification and labelling (CLH) proposal, amongst others, as Muta. Cat. 2 as the next regulatory step for the Substance. However, the eMSCA suggests awaiting the outcome of the ongoing EOGRTS with the Substance before assessing the need for submitting a CLH proposal.

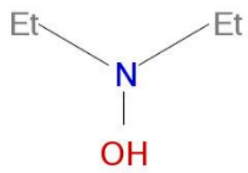
7.3. Identity of the substance

Table 3

SUBSTANCE IDENTITY	
Public name:	N,N-diethylhydroxylamine
EC number:	223-055-4
CAS number:	3710-84-7
Index number in Annex VI of the CLP Regulation:	–
Molecular formula:	C4H11NO
Molecular weight range:	89.136 g/mol
Synonyms:	N-ethyl-N-hydroxyethanamine DEHA

Type of substance X Mono-constituent ☐ Multi-constituent ☐ UVCB

Structural formula:



7.4. Physico-chemical properties

Table 4

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101.3 kPa	Liquid
Boiling point at 101.3 kPa	134°C
Vapour pressure	530 Pa at 20°C
Water solubility	450.5 g/L at 20°C
Surface tension	26.5 mN/m at 20°C
Partition coefficient n-octanol/water (Log Kow)	0.5 at 23°C and a pH 6 – 8
Dissociation constant	pKa=-0.75 at 30°C and 12.88 at 20°C
Viscosity	3.62 mPa.s (dynamic) 20°C

7.5. Manufacture and uses

7.5.1. Quantities

Table 5

AGGREGATED TONNAGE (PER YEAR)				
☐ 1 – 10 t	☒ 10 – 100 t	☐ 100 – 1000 t	☒ 1000- 10,000 t	☒ 10,000-50,000 t
☒ 50,000 – 100,000 t	☒ 100,000 – 500,000 t	☐ 500,000 – 1000,000 t	☒ > 1000,000 t	☐ Confidential

7.5.2. Overview of uses

Table 6

USES	Use(s)
Uses as intermediate	–
Formulation	Formulation into mixture Mixing or blending in batch processes Transfer of substance or mixture (charging and discharging) at non-dedicated facilities
Uses at industrial sites	Polymer processing Non-reactive processing aid at industrial site Use in stripper/etchant formulation in the electronic industry Colour stabiliser for chemical products
Uses by professional workers	Colour stabiliser for film/photographic industry Coating
Consumer Uses	–
Article service life	–

7.6. Classification and Labelling

7.6.1. Harmonised Classification (Annex VI of CLP)

None.

7.6.2. Self-classification

- In the registration(s):

  Flam. Liq. 3 (H226)
  Acute Tox. 4 (H312)
  Acute Tox. 4 (H332)
  STOT SE 3 (H335)
  Aquatic Chronic 2 (H411)

- The following hazard classes are in addition notified among the aggregated self-classifications in the C&L Inventory:

  Eye Irrit. 2 (H319)
  Skin Irrit. 2 (H315)
  Acute Tox. 4 (H302)
  Acute Tox. 3 (H331)
  Skin Corr. 1C (H314)
  Muta. 2 (H341) (5 of total 596 notifiers)
  Aquatic Chronic 4 (H413)
  Not classified

7.7. Environmental fate properties

Not assessed.

7.8. Environmental hazard assessment

Not assessed.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

Not assessed.

7.9.2. Acute toxicity and Corrosion/Irritation

Not assessed.

Based on the information in the registration(s) the eMSCA notes that the Substance is irritating to the skin, eyes and respiratory tract.

7.9.3. Sensitisation

Not assessed.

7.9.4. Repeated dose toxicity

Oral route

The REACH registration dossier contains two key studies and one supporting study (a 14-day dose range-finding study to the 90-day oral toxicity study) reported under the oral repeated dose toxicity section of the registration(s).

The first key study is a 90-day oral toxicity study (2019), performed in Sprague-Dawley rats according to the OECD TG 408 as requested by the ECHA Decision (number: CCH-D-211 4366663-42-01/F), with an assigned reliability score of 1. DEHA was administered by gavage to 10 animals/sex/dose at 0, 50, 150 and 500 mg/kg bw/day. The dose-levels were selected based on the dose-range finding 14-day oral study at 100, 300 and 1000 mg/kg bw/day. Additionally, six-week recovery groups with 5 animals/sex were included in the control and high-dose groups.

There were no mortalities in the study. The only treatment-related clinical sign was ptyalism (excessive salivation) which was observed in all the animals at 500 mg/kg bw/day and occasionally in one male and one female at 150 mg/kg bw/day. This effect was not observed after cessation of treatment in the recovery group. There was a slight decrease in plasma chloride concentration and slight increase in potassium, cholesterol and triglycerides concentrations at 500 mg/kg bw/day. The mean urine volume increased

with a statistical significance in males at 150 mg/kg bw/day and in both sexes at 500 mg/kg bw/day. However, the registrant(s) reported that these effects correlated with tubular vacuolation and dilatation seen at microscopic examination but they considered these effects as non-adverse, contrary to the eMSCA.

There was a statistically significant increase in the absolute and relative weights of kidney and liver in females at 500 mg/kg bw/day. The relative liver weights were increased with statistical significance in males at 500 mg/kg bw/day. Liver hypertrophy was observed in 2 males at 500 mg/kg bw/day. Microscopic changes in the kidney (tubular vacuolation, dilation and hyaline casts) were observed in females at all doses. Changes in the liver weights, remained in females at the end of the recovery period (with statistical significance). Also, there was an increase in the absolute and relative weights of the adrenal gland in males at 500 mg/kg bw/day. Microscopic changes (cortical vacuolation) were observed in females at all doses.

The forestomach of one female had thickened mucosa and that of another female was discoloured at 500 mg/kg bw/day. Microscopic changes in the forestomach included minimal to slight, focal or multifocal vacuolar degeneration, hyperkeratosis, squamous cell hyperplasia, submucosal edema and fibroplasia (only in males) at 500 mg/kg bw/day, and to a lesser extent, hyperplasia and hyperkeratosis at 50 or 150 mg/kg bw/day. There was also a focal minimal degeneration/necrosis in the stomach of one male at 500 mg/kg bw/day.

In all the groups, including controls, several animals had irregular oestrous cycles, delayed at the diestrus stage transiently or until the end of the study. This led to a high inter-individual variability in the control group and the mid-dose group. Overall, the number of cycles was slightly higher (not statistically significant) at 500 mg/kg bw/day, linked to slightly lower cycle length when compared to the controls. After the recovery period, the cycle length remained slightly higher (and number of cycles lower) at 500 mg/kg bw/day compared to controls (contrary to the observation made at the end of the treatment period). 3 of 5 females were either delayed in the diestrus or had several consecutive days in diestrus before starting a new cycle. Also, in the controls, 2 of 5 females were delayed in the diestrus stage. A treatment-related effect cannot be excluded. There was also a slight to moderate mucification of the vagina observed in two females at 500 mg/kg bw/day but not in the controls.

At 150 and 500 mg/kg bw/day, there was a statistically significant decrease in the percentage of the morphologically normal epididymal sperm (increased abnormal or no flagellum spermatozoae), mean number of testicular sperm heads and daily sperm production. This change was not statistically significant, but there was a trend (decrease) already at the lowest dose of 50 mg/kg bw/day. Motility was not affected at any dose level. The values were within the range of the historical control data, but close to the lower end values in the mid and high dose groups. They were reversible at the end of the 6-week recovery period, except for epididymal sperm count which still remained slightly lower (at the top dose) than in controls (375.9 vs. 459.8 106/g cauda: -18%). It is concluded that these effects are treatment related.

There were no test item-related changes on mean T3, T4 or TSH levels measured in control and in high dose animals at the end of the treatment period.

The registrant(s) set the highest dose (500 mg/kg bw/day) as the NOAEL in this study because according to them the effects observed described above were not adverse i.e., they were seen in the absence of degeneration/necrosis and/or of low magnitude (minimal to moderate) and generally within historical control values.

However, contrary to the Registrant(s), the eMSCA considers the observed effects at the lowest dose as adverse. In particular, the sperm parameters which were only partly reversible and observed at and above 50 mg/kg bw/day. No toxicokinetics, metabolism and distribution data are available to explain differences of toxicity between males (i.e.,

adrenals) and females (i.e., kidney and liver). In the in vivo Comet assay in rats (2022), the range-finder experiments determined the MTD of the Substance to 1750 mg/kg bw/day in males and 1000 mg/kg bw/day in females. Thus, no NOAEL can be identified based on this study. The lowest dose, 50 mg/kg bw/day, should be set as the LOAEL based on the histopathological changes observed in the kidney (females), adrenals (males) (Table 7) and sperm parameters (Table 8).

Table 7

Histopathological changes in the kidney and adrenals					
		Dose (mg/kg bw/day)			
		0	**50**	**150**	**500**
Kidney tubular dilation (females)	Grade 1	-	2	3	6
	Grade 2	-	-	-	4
Adrenal cortical vacuolation (males)	Grade 1	-	1	3	5
	Grade 2	-	-	-	2

Note: statistical significance and historical control data not available.

Table 8

Sperm parameters					
	Dose (mg/kg bw/day)				
	0 (control)	**50**	**150**	**500**	**0 (HCD)**
Morphologically normal epididymal sperm (%)	97.6	96.1 (-2%)	93.6** (-4%)	92.4** (-5%)	92.0 – 100.0
No of testicular sperm heads (10^6/g testis)	130	123 (-5%)	106* (-18%)	101** (-22%)	100.7 – 157.8
Daily sperm production rate (10^6/g testis/day)	21.3	20.2 (-5%)	17.4* (-18%)	16.6* (-22%)	16.5 – 25.9

Statistically significant from controls; *: P<0.05, **: p<0.01
Historical control data (HCD) show the individual data percentiles, 5%-95%

The other key study was a repeated dose 28-day oral toxicity study (2000) performed in Sprague-Dawley rats according to the OECD TG 407, with an assigned reliability score of 1. DEHA was administered by gavage to 5 animals/sex/dose at 0, 20, 100 and 500 mg/kg bw/day. Additional 5 animals/sex were included in the high-dose group to evaluate reversibility of effects after 2 weeks. The NOEL in this study was set at 500 mg/kg bw/day, based on clinical signs i.e. excessive salivation and reddish eye gum in males and females, reddish rhinorrhea in males and reddish urine in females, observed transiently after dosing. It should be noted that the reporting of the study is relatively poor and additional target organ changes observed at 500 mg/kg at the end of dosing or recovery period were considered incidental by the Registrant(s). They included focal myocardial degeneration in the heart, extramedullary hematopoiesis in the spleen, congestion in the thymus, liver and kidney inflammation, dilatation in the lumen of the uterus, ultimobranchial body in the thyroid.

Inhalation route

A key repeated dose 28-day inhalation toxicity study (1996) in Sprague-Dawley rats performed according to the OECD TG 412 with an assigned reliability score of 1 is reported in the registration dossier(s).

DEHA was administered via nose-only inhalation route (6 hrs/day, 5 days/week), to 15 animals/sex/dose at 0, 15, 150 and 1500 ppm (analytical concentrations of 54.6, 546.0 and 5481.8 mg/m3, respectively). Additionally, two-week recovery groups with 5 animals/sex/group were included in the study. According to the information in the registration(s) at the week 4 necropsy in the mid- and high-dose groups, reversible test-article related microscopic changes, including squamous hyperplasia in the nasal passages were observed.

The NOAEC for systemic effects was set at 150 ppm, based on slight reversible decrease in albumin and albumin/globulin (A/G) ratio in females. The NOAEC for local effects was set at 15 ppm, based on minimal to moderate nonsuppurative inflammation, minimal to mild squamous hyperplasia and mild necrosis of the nasal passages in males and females at higher doses. The eMSCA agrees with this NOAEC. On this basis and in accordance with CLP, DEHA is self-classified as Specific Target Organ Toxicity after Single Exposure (STOT-SE) Category 3 for respiratory tract irritation (H335) by the Registrants.

A range-finding study to the 28-day repeated dose inhalation toxicity study is reported as a supporting study in the registration(s).

Conclusion on repeated dose toxicity

Taken together, the eMSCA considers that the Substance causes specific target organ toxicity after repeated exposure. Effects observed following exposure by the oral route may warrant a classification as STOT RE 2, according to the CLP criteria (guidance values for STOT RE 2 are between 10 and 100 mg/kg bw/day for a 90-day study) but this option has not been considered by the Registrants.

The eMSCA also notes that the Substance administered via the oral and inhalation route causes effects such as degeneration/necrosis, hyperplasia and hyperkeratosis at the site of exposure.

In the *in vivo* rat Comet assay (2022), histopathology showed vacuolation in the testes of animals administered DEHA twice at 1750 mg/kg bw/day over 21 hours apart. The treatment-related effect of DEHA on sperm parameters will be further assessed in an EOGRTS requested under CCH.

7.9.5. Mutagenicity

An *in vivo* rat Alkaline Comet Assay with DEHA was requested under SEv in 2020 (SEV-D-2114534345-52-01/F) to address the *in vivo* genotoxicity, including the site-of-contact genotoxicity concern for the Substance. Other available *in vitro* and *in vivo* studies are summarized below.

***In vitro* genotoxicity**

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 Ames test (2001) was performed according to the OECD TG 471, 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 (2002) was performed according to the OECD TG 473, with an assigned reliability score of 1. Human lymphocytes were treated with and without S9-mix up to 5000 µg/ml DEHA. Positive and negative (only vehicle) controls were included. The results were reported as positive without metabolic activation and negative with metabolic activation. This study indicates that the Substance is an *in vitro* clastogen.

The key gene mutation study in mammalian cells (2001) was performed according to the OECD TG 476, 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 DEHA. Positive and negative (only vehicle) controls were included. The results were reported as positive without metabolic activation and negative with metabolic activation. 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 indicates that the Substance is an *in vitro* mutagen, indicating both clastogenicity and gene mutagenicity.

Another gene mutation study in mammalian cells (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 eMSCA 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 DEHA 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 DEHA or (co-)exposed to (impure) DEHA are also reported in the registration dossier(s).

In vivo **genotoxicity**

At the start of this SEv two key studies were reported under the *in vivo* genotoxicity section of the registration dossier(s), a Mammalian erythrocyte micronucleus test and an Unscheduled DNA Synthesis test. The eMSCA notes that these *in vivo* studies are not disseminated as they are not in the LR registration dossier although they are summarised in the Chemical Safety Report (CSR).

The key Unscheduled DNA Synthesis test (Unpublished study report, 2003) was performed according to the OECD TG 486, with an assigned reliability score of 1. Male Wistar rats (4/dose) were given DEHA by a single gavage administration of 800 or 2000 mg/kg bw. Positive and negative (vehicle – purified water) controls were included. The results were reported as negative.

The eMSCA notes that according to the ECHA Guidance (ECHA 2014), 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 could be reached on the indications for gene mutagenicity observed in the *in vitro* study (OECD 476, 2001) based on this study.

The key mammalian erythrocyte micronucleus test (Unpublished study report, 1995) was performed according to the OECD TG 474 with an assigned reliability score of 1. Male and female ICR mice (5/sex/dose) were given a single oral gavage dose of 375, 750 or 1500 mg/kg bw DEHA. 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 in the high dose group. There was no significant increase in micronucleated cells. Therefore, the results were reported as negative.

The eMSCA considers that the study has limited reliability. No conclusion on clastogenicity could be made based on these negative results because:

- Even though mortalities in the high dose group confirming toxicity, there were no greater than 21% reductions (in some of the groups and with no dose relationship) in the proportion of immature to total erythrocytes in the bone marrow. This implies a limited bone marrow toxicity caused by the Substance.

- The power of the study is low since only 1000 immature erythrocytes per animal were scored for the incidence of micronucleated cells. The study was indeed performed according to the then available version (from 1983) of the OECD TG 474. However, according to the current version (from 2016) of that test guideline, at least 4000 immature erythrocytes per animal needs to be scored.

- The potential for clastogenicity at the site-of-contact tissues cannot be addressed.

Two dominant lethal tests (one ambiguous and the other one 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 alkylation test, which was given a reliability score of 4, because DEHA was impure or co-administrated with other substances, namely, nitroethane and diethylamine hydrogen sulphite.

An *in vivo* Alkaline Comet Assay with DEHA was requested under SEv in 2020 to address the genotoxicity observed in *in vitro* studies, including the site-of-contact genotoxicity concern for the Substance. The study was performed according to the OECD TG 489, using Crl:CD (SD) rats and oral gavage exposure (2022). A range-finder experiment that was performed initially showed toxicity signs including pilo-erection, elevated gait, partially closed eyelids, decreased activity, irregular breathing and a mean bodyweight loss of 3.8% (Day 1 to termination) at 1750 mg/kg bw/day in males. The maximum tolerated dose (MTD) was set to 1750 mg/kg bw/day in males and 1000 mg/kg bw/day in females. As the MTD was <2-fold between the sexes, males only were used for the study. The eMSCA notes that the RDT data demonstrated some differences between males and females (e.g., differences in target organ toxicity). The eMSCA considers that in the absence of toxicokinetics data and presence of different target organs between males and females, females could have been included in the study as recommended in the OECD TG 489 (see para. 31).

For the main Comet assay dose levels were set to 437.5, 875 and 1750 mg/kg bw/day. Six animals in each group, the control and test groups were dosed on two occasions. The second dose was administered approximately 21 hours after the first dose and 3 hours before sampling. Sections of the duodenum, glandular stomach, liver and testes from the controls and treated animals were processed for histopathological examination and assessed for necrosis and apoptosis. DNA damages were assessed by comparing the group % tail intensities (% TI) from treated animals with the concurrent control animals. The slides were also examined for any overt toxicity, e.g. an increase in background debris and/or an increase in the incidence of excessively damaged cells, i.e. Hedgehogs. These cells were excluded from the analysis, along with any cells that had unusual staining artefacts.

At 1750 mg/kg bw/day clinical signs of toxicity consisted of pilo-erection, unsteady gait, hunched posture, partially closed eyelids and decreased activity. A bodyweight loss of 1.7% was observed (Day 1 to termination). Clinical chemistry analysis showed statistically significant increases in creatinine and chloride ($p \leq 0.01$) and decreases in

potassium ($p<0.05$) and triglycerides ($p<0.01$) levels. Vacuolisation in testes was observed in all animals at the high dose.

The concurrent control mean %TI values for the duodenum, stomach, liver and testes were within the historical control range (95% confidence limits). The positive control produced a statistically significant increase in %TI in all tissues ($p\leq0.001$).

In the duodenum a statistically significant increase in the %TI was observed at 875 and 1750 mg/kg/day. An increase in the number of hedgehogs was also observed where an increase in %TI was observed. Apoptosis was reported in all animals at 875 and 1750 mg/kg bw/day. Increased mitosis was seen in two animals at 437.5 mg/kg bw/day and in one animal at 875 mg/kg bw/day. This was considered by the authors to be a reaction to the loss of cells seen in the low dose group despite apoptosis not being detectable.

In the stomach a small but statistically significant increase was observed in the %TI at 437.5 mg/kg bw/day. The individual and group %TI values were within the historical control range at this dose. A statistically significant increase was observed in the %TI at 875 and 1750 mg/kg bw/day without a dose-response relation. Increased number of hedgehogs was observed in the mid and high dose group. Apoptosis was reported at 1750 mg/kg bw/day in 3 of 6 animals.

In the liver no increase in the %TI was observed at any dose. At the high dose a small increase in the number of hedgehogs was reported. Apoptosis was detected in 5 of 6 animals. Similarly, in the testes no increase in the %TI and a small increase in the number of hedgehogs was reported at the high dose. No hedgehogs were observed in the controls. All samples were prepared using an identical method, indicating that the cause of the hedgehogs observed in duodenum and stomach was not mechanical/enzyme induced damage during sample preparation (Table 9).

Table 9

Mean Tail Intensity (%TI), percentage of Hedgehogs (%H) and number of animals with apoptosis (A) or vacuolisation (V)

	Stomach			**Duodenum**			**Liver**			**Testes**		
mg/kg/day	**%TI**	**%H**	**A**	**%TI**	**%H**	**A**	**%TI**	**%H**	**A**	**%TI**	**%H**	**V**
0	0.34	0.00	-	0.19	0.00	-	0.23	0.00	-	0.28	0.00	-
437.5	0.57*	0.00	-	0.18	0.00	-	0.08	0.00	-	0.35	0.00	-
875	12.99**	4.26	-	10.72**	4.26	6/6	0.11	0.00	-	0.23	0.00	-
1750	11.79**	9.82	3/6	16.28**	5.96	6/6	0.07	0.11	5/6	0.49	0.10	6/6
Pos. Cont.	34.00**	0.22	NE	31.48**	0.22	NE	28.33**	0.88	NE	17.98**	0.88	NE

**P≤0.001, *P≤0.05
Vehicle: water, Positive control: 200 mg/kg ethyl methanesulfonate

In the duodenum and stomach, the increases in the %TI observed at the mid and high dose were considered by the Registrant(s) to be related to the hedgehogs or cytotoxicity observed. Apoptosis was also observed in these tissues at the highest dose. It was therefore considered that the increases in %TI were cytotoxic in nature. The registrant(s) point out that according to the OECD TG 489 in the presence of hedgehogs or clear cytotoxicity, any increases in genotoxicity should be interpreted with care. They

considered increases in the %TI related to cytotoxicity and unlikely to be of genotoxic origin.

The eMSCA notes that DEHA, when administered by gavage, caused a clear increase in DNA damage in the duodenum and stomach, but not in the liver or testes. The increase in %TI correlated well with presence of Hedgehogs. However, there was no clear correlation between %TI and apoptosis. Apoptosis in the duodenum was reported at both the mid and the high dose. However, in the stomach apoptosis was observed only in half of the animals (3 of 6) at the high dose and not observed in the mid dose where the highest increase in %TI was detected. Apoptosis and vacuolation was also observed in the liver and testes, respectively, at the highest dose, but without any increase in the %TI or hedgehogs.

According to the OECD TG 489, to assess the biological relevance of a positive result from the Comet assay, information on cytotoxicity at the target tissue is required. Where positive or equivocal findings are observed solely in the presence of clear evidence of cytotoxicity, the study would be concluded as equivocal for genotoxicity, unless there is enough information that is supportive of a definitive conclusion. The measure of cytotoxicity in this study was apoptosis or vacuolization (testes). The eMSCA is of the view that for DEHA there is not enough information on the mechanisms of cytotoxicity and a definitive conclusion that the primary cause of increase in %TI is cytotoxicity cannot be reached.

Further, in the OECD TG 489 it is stated that no single measure of (*in vivo*) cytotoxicity can be recommended to conclude on genotoxicity. Histopathological changes such as inflammation, apoptotic or necrotic changes have been associated with increases in DNA migration. However, these changes do not always result in positive Comet findings. Consequently, no definitive list of histopathological changes that are always associated with increased DNA migration is available. Hedgehogs have previously been suggested as an indicator of cytotoxicity. However, the etiology of the hedgehogs is uncertain. Data exist which suggest that hedgehogs can be caused by cytotoxicity and/or genotoxicity.

According to the OECD TG 489, standard alkaline Comet assay is not considered appropriate to measure DNA damage in mature germ cells. Genotoxic effects as measured by the comet assay in testicular cells at different stages of differentiation have been described. However, it should be noted that gonads contain a mixture of somatic and germ cells. For this reason, positive results in whole gonad (testis) are not necessarily reflective of germ cell damage. Nevertheless, they indicate that tested chemical(s) and/or its metabolites have reached the gonad.

The eMSCA notes that data on the Substance is consistent across all the available studies, indicating site-of-contact toxicity. DEHA is an oxygen scavenger and has irritating properties. In line with these properties, cytotoxicity, characterised by histopathology, i.e., apoptosis or vacuolisation, is observed *in vivo* in the Comet assay in the examined organs. Based on the available information, it is not possible to address whether genotoxicity is a cause or a consequence of the cytotoxicity. Nonetheless, it is clear that exposure to DEHA leads to DNA damage at the site-of-contact, *in vivo*.

Regarding the potential of the Substance to induce germ-cell genotoxicity, the analysis of testes in the comet assay cannot be used to reach a conclusion. In this comet assay observed vacuolisation in the testes indicates that the gonads were exposed and confirms that the testes and consequently reproduction, may be affected. However, the lack of increase in %TI suggests that germ-cell genotoxicity potential i.e., that may warrant Muta. 1B under CLP, is unlikely.

Taking into consideration the positive results from *in vitro* mutagenicity studies, together with the results from the Comet assay, the eMSCA concludes that the potential of DEHA to induce mutagenicity at the site-of-contact cannot be disregarded. The eMSCA is of the

view that genotoxicity effects may warrant a classification as Muta. 2 (H341) according to the CLP criteria.

7.9.6. Carcinogenicity

Two old 2-year carcinogenicity studies via whole-body inhalation of a mixture of DEHA and diethylamine hydrogen sulfite, with or without nitroethane, one in rats and one in mice are reported in the registration dossier(s). Summaries of the studies are provided for information.

In the rat study, Long-Evans rats were co-exposed via inhalation to DEHA and diethylamine hydrogen sulfite, with or without nitroethane (1979). DEHA was dosed at three test concentrations ranging from 9 to 27 ppm. Nitroethane was dosed at about 10 ppm. In the first year of the study no haematology or blood chemistry effects were observed. Some gross and microscopic pathology findings were reported. One male developed a hemangioendothelioma (skin tumor) after 3 months, but no additional ones were developed later. 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, described as necrosis and early follicular degeneration was observed after 6 months exposure, but not in animals exposed 9 months or longer.

Examination of animals exposed more than 1 year indicated no significant differences between the control and test groups, except for the interstitial cell tumors of the testes. Testes tumors were observed in 4 of 47 exposed males, compared to 0 in the 25 controls. This was considered by the authors a low incidence (8,5%) to establish any conclusion on carcinogenicity.

In the mice study, Swiss mice were co-exposed to DEHA, nitroethane and diethylamine hydrogen sulfite (1982). Animals were exposed to DEHA with a single concentration of about 10 ppm. According to the information in the registration, the incidence of tumors, including subcutaneous tumors (principally fibrosarcomas), increased in exposed males with marginal statistical significance. The incidence of tumors in females decreased with statistical significance.

Additionally, a study in mice with a duration of 16 weeks and exposure via drinking water with an assigned reliability score of 3 is available in the public domain (Heicklen et al., 1984). In this study the effect of DEHA on the incidence of tumors induced by benzo(a)pyrene was examined. According to the study authors, as there was evidence that some forms of cancer induction involve free radical reactions, it was hypothesised that in such cases antioxidants (such as DEHA), which act as free radical scavengers, could function as anti-carcinogenic agents.

In this study groups of 20 male and 20 female CD-1(ICK)Hr mice were treated with 0, 3, 70 or 300 mg/kg bw/day DEHA from 57-112 days of age. For a four-week period between 70 to 98 days of age, all animals received (via gavage) eight 1 mg doses of benzo(a)pyrene at 4-day intervals. 137 animals survived the dosing period. Of these, 132 survived to 211 days of age at which time they were necropsied and evaluated. Tumors were observed in the lungs and squamous portion of the stomach in males and females. In this study treatment with DEHA produced no significant effect on the lung tumor incidence in either sex. An increase in stomach tumors was observed in the females. The number of animals with stomach tumors/total number of animals at 0, 3, 70 or 300 mg/kg bw/day DEHA were 4/16, 10/16, 8/16 and 11/16 in females and 2/16, 4/16, 7/15 and 3/17 in males, respectively. The authors concluded that these results suggest that DEHA may act as a promoter in female mice.

The eMSCA notes that in these two available cancer studies, animals were co-exposed to DEHA together with other substance(s). This was partly due to DEHA being used as an inhibitor of the photochemical formation of smog from nitroethane and diethylamine

hydrogen sulfite. Available data indicate that DEHA may both increase or decrease tumor incidence after co-exposure with other substances. Therefore, the concern for carcinogenicity potential of DEHA is **inconclusive**.

No further carcinogenicity test request, based on the available data, appear justified under SEv. In the absence of consumer uses and proportionality considerations, a carcinogenicity study will not be requested.

7.9.7. Toxicity to reproduction (effects on fertility and developmental toxicity)

Not assessed.

The eMSCA notes that the Substance affects male sperm parameters and female oestrous cycle in repeated dose toxicity studies (see section 7.9.4). These effects appear treatment related and may lead to toxicity to fertility.

An Extended one-generation reproductive toxicity study (OECD TG 443) with the Substance was requested under CCH in 2021. The deadline to update the registration(s) with this information on reproductive toxicity is March 2025.

7.9.8. Hazard assessment of physico-chemical properties

Not assessed.

7.9.9. Selection of the critical DNEL(s)/DMEL(s) and/or qualitative/semi-quantitative descriptors for critical health effects

Not assessed.

7.9.10. Conclusions of the human health hazard assessment and related classification and labelling

The Substance is irritating and has self-classification as Acute Tox. 4 (H312, H332, H302), STOT SE 3 (H335), Eye Irrit. 2 (H319), Skin Irrit. 2 (H315), Acute Tox. 3 (H331) and Skin Corr. 1C (H314). The eMSCA has not assessed these endpoints under this SEv.

Among the aggregated self-classifications in the C&L Inventory five notifiers (of total 596) have self-classified the Substance as Muta. Cat. 2. The eMSCA concludes that classification as Muta. Cat. 2 may be warranted for the Substance. This conclusion is based on all the available data on mutagenicity for the Substance, indicating site-of-contact mutagenicity. Effects observed following exposure by the oral route may also warrant a classification as STOT RE 2.

7.10. Assessment of endocrine disrupting (ED) properties

Not assessed.

7.11. PBT and VPVB assessment

Not assessed.

7.12. Exposure assessment

Not assessed.

7.13. Risk characterisation

Not assessed.

7.14. References

Reference to the studies cited in the registration(s) can be found on the ECHA dissemination webpage: http://echa.europa.eu/web/guest/information-on-chemicals/registered-substances.

ECHA 2014. Guidance on Information Requirements and Chemical Safety Assessment. Chapter R.7a: Endpoint specific guidance

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7.15. Abbreviations

A/G	Albumin Globulin ratio
CCH	Compliance Check
CLH	Harmonised Classification and Labelling
CoRAP	Community Rolling Action Plan
DEHA	Diethylhydroxylamine
DNEL	Derived No Effect Level
ECHA	European Chemicals Agency
eMSCA	Evaluating Member State Competent Authority
EOGRTS	Extended One-Generation Reproductive Toxicity Study
GLP	Good Laboratory Practice
LR	Lead Registrant
NOAEC	No Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
SEv	Substance Evaluation
TI	Tail Intensity
TG	Test Guideline
TPE	Testing Proposal Examination