Bundesministerium
Klimaschutz, Umwelt,
Energie, Mobilität,
Innovation und Technologie

SUBSTANCE EVALUATION CONCLUSION

as required by REACH Article 48

and

EVALUATION REPORT

for

3-aminopropyldimethylamine

EC No. 203-680-9

CAS RN 109-55-7

Evaluating Member State: **Austria**

Dated: 24 January 2023

Evaluating Member State Competent Authority

Austrian MSCA:
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Year of evaluation in CoRAP: 2014

Further information on registered substances can be found here:

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

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. CONCERNS SUBJECT TO EVALUATION

3-aminopropyldimethylamine (DMAPA), hereafter “the Substance”, was originally selected for substance evaluation to clarify concerns about:

- Sensitisation (human health)
- Exposure of workers
- Wide dispersive use
- High aggregated tonnage

In addition, the following concerns were identified:

- Developmental toxicity (human health)
- Repeated dose toxicity (human health)
- Environmental toxicity (ecotoxicity)

2. OVERVIEW OF OTHER PROCESSES / EU LEGISLATION

Based on the classification of the Substance, relevant EU legislation exist like the Safety and Health of Workers at Work Directive.

For more information, please see:

legislation-obligation - ECHA (europa.eu)

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 Table 3-1 below.

Table 3-1: Conclusion of Substance Evaluation

Conclusions	Tick box
Need for follow-up regulatory action at EU level	X
Harmonised Classification and Labelling	X
Identification as SVHC (authorisation)	
Restrictions	
Other EU-wide measures	
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 already the following harmonised classification according to Regulation (EC) 1272/2008: Flam. Liq. 3, Acute Tox. 4* (oral), Skin Corr. 1B and Skin Sens. 1 (index number 612-061-00-6).

During SEV, additional concerns were identified or confirmed with regards to acute dermal toxicity, skin sensitisation (sub-category) and reproductive toxicity (development). Consequently, the current entry of Regulation (EC) No 1272/2008 with the index number 612-061-00-6 should be amended.

The outcome of the evaluation performed leads to the need for an update of the CLP-Annex VI entry for the Substance (index number 612-061-00-6). Therefore, a harmonisation of classification is considered necessary to ensure adaption of classification of the Substance throughout industry sectors.

The evaluating Member State Competent Authority (eMSCA) proposes the following classification and labelling (C&L) in addition:

Table 4.1.1-1: Additional C&L proposed

ADDITIONAL C&L PROPOSED (harmonised C&L process to be initiated)	
Acute toxicity: dermal	Acute Tox. 3, H311
Specific target organ toxicity (single exposure): Irritation to the respiratory tract	STOT SE 3, H335
Sensitisation	Sub-category 1A proposed. Skin Sens. 1A, H317
Reproductive toxicity	Repr. 1B, H360D

Referring to the C&L inventory, different classifications are available. Skin Sens. 1A and Repr. 1B are not indicated among them.

Reasons for the proposal

Acute toxicity

For acute dermal toxicity several studies are available in rats and rabbits. The lowest LD50 value was obtained in rabbits with an LD50 value of ~820 mg/kg bw (unpublished study report, 1964). According to the LD50 value obtained from this study being >200 mg/kg bw but <1000 mg/kg bw the Substance meets the criteria for classification as Acute Tox. 3, H311: Toxic in contact with skin.

Specific target organ toxicity - single exposure

According to the CLP guidance (ECHA, 2017a) a classification for corrosivity is in general considered to implicitly cover the potential to cause RTI (respiratory tract irritation) and so the additional classification for STOT SE category 3 is considered to be superfluous, although it can be assigned. Nevertheless, observed local irritating effects described by clinical signs in animal studies include accelerated or irregular respiration, dyspnoea or eyelid closure. Occupational investigations indicate alterations of pulmonary functions in

workers. Therefore, additional labelling with STOT SE 3 H335 (May cause respiratory irritation) according to Regulation (EC) No. 1272/2008 seems justified.

Skin sensitisation

For this endpoint a reliable and robust LLNA (Wright et al., 2001) is given the highest weight indicating strong to moderate potency depending on the vehicle. A reliable GPMT test indicates also high potency after rechallenge. The Buehler assay found moderate potency, however the 55% responding were close to the cut-off of 60% for sub-category 1A. The classification is supported by human diagnostic patch test data that showed a relatively high and substantial incidence of reactions in relation to relatively low exposure. Therefore, the Substance meets the criteria for classification in sub-category 1A, H317: May cause an allergic skin reaction.

Reproductive toxicity

Developmental toxicity has been reported in a reliable OECD TG 414 study with the source substance DEAPA (unpublished study report, 2016b). Based on the increased post-implantation losses, lower mean number of live foetuses and several malformations concerning the skeleton a $NOAEL_{development}$ of 50 mg/kg bw/d for DEAPA (or 39 mg/kg bw/d expressed as the Substance) was determined. Therefore, the Substance meets the criteria of Regulation (EC) No 1272/2008, Table 3.7.1(a) for classification as Repr. 1B, H360D: May damage the unborn child.

The eMSCA concludes that 3-aminopropyldiethylamine (DEAPA, EC 203-236-4) can be applied as a source substance for read-across for the repeated dose and developmental toxicity endpoints. For evaluation of possible effects on fertility, a second source substance, ethylenediamine (EC 203-468-6), was used in the read-across approach of the eMSCA. Further information concerning reproductive toxicity has become available recently based on an EOGRTS requested via a compliance check for the source substance DEAPA (ECHA, 2018). A detailed evaluation of this study is outstanding, based on the submission of the study after closure of the evaluation in July 2022.

At this stage, based on the available data, the eMSCA proposes: Repr. 1B, H360D: May damage the unborn child.

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

Currently not applicable.

4.1.3. Restriction

Currently not applicable.

4.1.4. Other EU-wide regulatory risk management measures

Currently not applicable.

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 (IF NECESSARY)

Indication of a tentative plan is not a formal commitment by the evaluating Member State. 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.

Table 6-1: Follow-Up Actions

Follow-up action	Date for intention	Actor
CLH dossier	Intended for 2024/2025[2]	Member State Austria

[2] To be finally decided formally in accordance with the Austrian Chemical law

Part B. Substance evaluation

7. EVALUATION REPORT

7.1. Overview of the substance evaluation performed

Justification for evaluation

The Substance was originally selected for substance evaluation to clarify concerns about:

- Sensitisation (human health)
- Exposure of workers
- Wide dispersive use
- High aggregated tonnage

In addition, the following concerns were identified:

- Developmental toxicity (human health)
- Repeated dose toxicity (human health)
- Environmental toxicity (ecotoxicity)

Table 7.1-1: Summary of hazard assessment performed

EVALUATED ENDPOINTS	
Endpoint evaluated	**Outcome / conclusion**
Acute toxicity: oral	Acute Tox. 4, H302 confirmed
Acute toxicity: dermal	Concern confirmed: acute dermal toxicity, harmonised C&L process to be initiated Acute Tox. 3, H311
Specific target organ toxicity (single exposure)	Concern confirmed: Irritation to the respiratory tract, harmonised C&L process to be initiated STOT SE 3, H335
Skin corrosion/irritation	Skin Corr. 1, H314 confirmed
Serious eye damage/irritation	Eye Dam. 1, H318 confirmed
Sensitisation	Concern confirmed: classification for sub-category 1A proposed, harmonised C&L process to be initiated. Skin Sens. 1A, H317
Repeated dose toxicity	Concern refuted: Read-across acceptable, no classification proposed.
Mutagenicity	Concern refuted: No classification proposed
Carcinogenicity	Concern refuted: No classification proposed
Reproductive toxicity	Concern confirmed: Read-across acceptable, harmonised C&L process to be initiated. Repr. 1B, H360D

Aquatic toxicity	Concern refuted: No classification proposed
PBT/vPvB	Concern refuted: No concern identified

7.2. Procedure

Evaluation of the Substance was launched in March 2014. The Registrant(s) of this substance were contacted before start of evaluation and asked to support the evaluation by providing the original studies used for the joint registration. The Registrant(s) provided the requested studies. In a first step, the performed evaluation of the Substance was not targeted and covered all sections of the chemical safety assessment. In a second step, the main focus of evaluation was on the areas of concern. Studies provided by the Registrant(s), publicly available studies/data, studies for reference substances, QSARs and exposure modelling tools were used by the eMSCA for assessment and conclusion.

The identified concerns and proposed tests for clarification by eMSCA were communicated to the Registrant(s) after the first year of evaluation. The Draft Decision was sent to the Registrant(s) in May 2015 for comments. The Registrant(s) tried to clarify some concerns via read-across to structurally similar substances. The reviewed approach was considered to be promising by the eMSCA, but considered to be not fully robust. The Registrant(s) indicated that more data on a structurally similar substance for supporting the read-across approach is generated at that time being and would be available in the near future. Based on comments received SEv was suspended to await the new data. The generated alternative data supporting the read-across were evaluated by the eMSCA. As the new read-across approach and the data are considered to be applicable and valid, the evaluating Member State concluded the evaluation without any need to ask for new information from the registrants under Article 46(1) decision. SEv decision making was terminated in July 2022.

Based on the total of available data for the Substance, the available data were considered to be sufficient for clarifying the identified concerns indicated in the previous section and concluding on them. Evaluation was closed in July 2022.

7.3. Identity of the substance

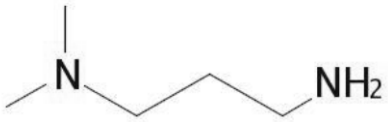
The Substance is a mono-constituent substance having the following substance identity, characteristics and structure.

Table 7.3-1: substance identity

SUBSTANCE IDENTITY	
Public name:	3-aminopropyldimethylamine
EC number:	203-680-9
CAS number:	109-55-7
CAS name:	3-dimethylaminopropylamine
IUPAC name:	N,N-dimethylpropane-1,3-diamine
Molecular formula:	C5H14N2
Molecular weight:	102.18

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

Structural formula:



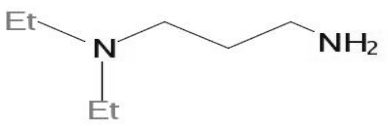
(source: European Chemicals Agency, http://echa.europa.eu/)

Main read-across substance: 3-aminopropyldiethylamine

Table 7.3-2: Substance identity of read-across substance 3-aminopropyldiethylamine

SUBSTANCE IDENTITY of read-across substance 3-aminopropyldiethylamine	
Public name:	3-aminopropyldiethylamine
IUPAC name:	N,N-diethylpropane-1,3-diamine
EC number:	203-236-4
CAS number:	104-78-9
Molecular formula:	$C_7H_{18}N_2$
Molecular weight range [g/mol]:	130.2312
Synonyms	DEAPA, 1,3-propanediamine, 3-diethylaminopropylamine

Structural formula of 3-aminopropyldiethylamine



(source: European Chemicals Agency, http://echa.europa.eu/)

Main read-across substance: ethylenediamine

Table 7.3-3: Substance identity of read-across substance ethylenediamine

SUBSTANCE IDENTITY of read-across substance ethylenediamine	
Public name:	ethylenediamine
IUPAC name:	ethane-1,1-diamine
EC number:	203-468-6
CAS number:	107-15-3
Molecular formula:	$C_2H_8N_2$
Molecular weight range [g/mol]:	60.0983
Synonyms	EDA

Structural formula of ethylenediamine

(source: European Chemicals Agency, http://echa.europa.eu/)

7.4. Physico-chemical properties

The data identified for the Substance and provided by the Registrant(s) are presented below and accessible via ECHA's dissemination site[3].

Table 7.4-1: Physico-chemical properties

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Physical state at 20°C and 101 325 Pa	liquid
Melting / freezing point at 101 325 Pa	-70°C
Boiling point at 101 325 Pa	135.1°C
Density at 25°C	0.8133 g/cm^3
Vapour pressure at 20°C	590 Pa
Water solubility at 20°C	fully miscible
Partition coefficient n-octanol/water (Log K_{ow})	-0.352
Surface tension	not surface active

[3] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14823/4/2

OVERVIEW OF PHYSICOCHEMICAL PROPERTIES	
Property	**Value**
Explosive properties	non explosive
Oxidising properties	no oxidising properties
Granulometry	not applicable
Stability in organic solvents and identity of relevant degradation products	The stability of the substance is not considered as critical.
Dissociation constant	9.33 and 5.66 at 35 °C

The studies of the physico-chemical properties were assessed by the eMSCA and are considered to be sufficiently valid and reliable for CSA purpose. Data found in literature, generated with EPISUITE, v4.1 and expert judgment predictions for these properties comply with the presented values.

7.5. Manufacture and uses

7.5.1. Quantities

Table 7.5-1: Aggregated tonnage

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

Referring to ECHA's dissemination, the following information on the registered uses are available:

This substance is registered under the REACH Regulation and is manufactured in and / or imported to the European Economic Area, at ≥ 10 000 tonnes per annum.
This substance is used by consumers, in articles, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing.

Uses at industrial sites
This substance is used in the following products: laboratory chemicals, polymers, water treatment chemicals, fuels, metal surface treatment products, pH regulators and water treatment products, lubricants and greases, pharmaceuticals and cosmetics and personal care products. This substance has an industrial use resulting in manufacture of another substance (use of intermediates).
This substance is used in the following areas: municipal supply (e.g. electricity, steam, gas, water) and sewage treatment and scientific research and development. This substance is used for the manufacture of: chemicals, textile, leather or fur, pulp, paper and paper products, rubber products.
This substance is used in the following activities or processes at workplace: industrial spraying, transfer of chemicals, closed processes with no likelihood of exposure, closed,

continuous processes with occasional controlled exposure, closed batch processing in synthesis or formulation, roller or brushing applications, transfer of substance into small containers, treatment of articles by dipping and pouring and laboratory work.

Widespread uses by professional workers
This substance is used in the following products: laboratory chemicals, fuels, pH regulators and water treatment products, lubricants and greases and polymers.
This substance is used in the following areas: building & construction work and scientific research and development. This substance is used for the manufacture of: textile, leather or fur, pulp, paper and paper products and rubber products.
This substance is used in the following activities or processes at workplace: transfer of chemicals, roller or brushing applications, non-industrial spraying, treatment of articles by dipping and pouring, closed processes with no likelihood of exposure and closed, continuous processes with occasional controlled exposure.

Article service life
ECHA has no public registered data on the use of this substance in activities or processes at the workplace.
This substance can be found in products with material based on: plastic (e.g. food packaging and storage, toys, mobile phones) and stone, plaster, cement, glass and ceramic used for large surface area articles (e.g. construction and building materials for floor coverings, isolation articles).

Consumer Uses
This substance is used in the following products: fuels and lubricants and greases.

Regarding consumer use, use of fuels, lubricants and greases are registered. Whereas human exposure to these sources might be infrequent and limited to a few number of persons, the Substance might be also present in lower concentrations or at residual and impurity levels in products (e.g. when used as intermediate). The substance is used in the preparation of some surfactants for example, such as cocamidopropyl betaine which is an ingredient in many personal care products including soaps, shampoos, and cosmetics. The presence of the Substance as an impurity in cocamidopropyl betaine is thought to be the cause of irritation experienced by some individuals like professionals and consumers handling these products. Therefore, exposure of the general public to the substance is considered to be higher than expected based on the registered uses.

For the full list of use descriptors covered by the individual registered uses, please see ECHA's dissemination site:
https://echa.europa.eu/registration-dossier/-/registered-dossier/14823

7.6. Classification and Labelling

Please find current information on classification in the C&L Inventory database at the ECHA web site. The inventory includes both harmonised classification when available and the notified self-classifications.

http://echa.europa.eu/web/guest/information-on-chemicals/cl-inventory-database.

7.6.1. Harmonised Classification (Annex VI of CLP)

The Substance has a harmonised classification according to Annex VI of CLP Regulation (Regulation (EC) 1272/2008).

Table 7.6.1-1: Harmonised classification

HARMONISED CLASSIFICATION ACCORDING TO ANNEX VI OF CLP REGULATION (REGULATION (EC) 1272/2008)							
Index No	International Chemical Identification	EC No	CAS No	Classification		Spec. Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard statement code(s)		
612-061-00-6	3-aminopropyldimethylamine, N,N-dimethyl-1,3-diaminopropane	203-680-9	109-55-7	Flam. Liq. 3 Acute Tox. 4* Skin Corr. 1B Skin Sens. 1	H226 H302 H314 H317	--	--

7.6.2. Self-classification

- In the registration(s):

  Flam. Solid 1, H228

  Acute Tox. 4, H302

  Acute Tox. 4, H312

  Skin Corr. 1B, H314

  Eye Dam. 1, H318

  Skin Sens. 1B , H317

  STOT SE 3 (lung, inhalation), H335

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

  Resp. Sens 1, H334

  Met. Corr. 1, H290

7.7. Environmental fate properties

7.7.1. Degradation

Abiotic degradation

The substance is estimated to be hydrolytically stable due to the lack of hydrolysable functional groups.

Based on a calculation using AOPWIN v1.92 (EPISUITE v4.11), the substance is predicted to photodegrade indirectly by reaction with hydroxyl radicals in the atmosphere revealing a low half-life (t1/2) of about 3.428 hours. The calculation is taking into account a mean hydroxyl radical concentration of 500,000 radicals per cm^3.

The substance is expected to be found mainly in water due to high water solubility and high affinity for the water phase (96% according to Mackay Level I distribution model (Mackay Level 1 v2.11 (unpublished study report 2016c).

Based on these predictions, the substance is considered to be hydrolytically stable and to be found mainly in the aqueous phase.

Biodegradation

In the BUA Report 197 of the German Chemical Society (GDCh, 1996) it is stated that the substance is not readily biodegradable, since the pass level of 60% degradation was reached only after 10 days incubation, but has an indication for mineralization. In the Annex of the report the key study data are specified, which reflect two similar tests similar to OECD TG 301D: In one test with not adapted inoculum 0% degradation after 5 days, 56% degradation after 10 days and 65% degradation after 20 days are reported.

In the second test with adapted inoculum 0% degradation after 5 days, 60% degradation after 10 days and 69% degradation after 20 days are reported.

In the OECD SIDS (OECD, 2003) it is reported that the substance can be classified as readily biodegradable without fulfilling the 10-day window.

The aerobic biodegradation of 3-dimethylaminopropylamine has also been evaluated according to AFNOR T 90 -312 guideline (unpublished study report, 1988a). After 7 days 88% and after 28 days 100% of the substance was degraded (based on DOC removal, initial substance concentration: 38.7 mg/l).

A test on inherent biodegradability according to part C of directive 88/302/EWG (Zahn-Wellens test) using a mixture of activated sludge from domestic (70%) and industrial (30%) sewage works is available: After 15 days 100% degradation (based on DOC removal) was achieved (test substance concentration: 213 mg/l, (unpublished study report, 1990a).

To strengthen the claim of ready biodegradability also data from the structurally related substance 3-aminopropyldiethylamine (CAS 104-78-9) were included into the dossier (unpublished study report, 2005a). This substance is similar to 3-dimethylaminopropylamine: Instead of the methyl-groups 3-aminopropyldiethylamine has ethyl-groups. 90 – 100 % biodegradation were seen after 28 days based on DOC according to an OECD TG 301A test. A lag phase of 10-14 days was observed. The 10-day window is met. The concentration of the test substance was ca. 32 mg/l.

In a weight of evidence approach it is concluded that there is a high probability that the Substance is readily biodegradable.

Biodegradation in seawater was assessed according to OECD TG 306 (Biodegradability in seawater) using natural seawater as inoculum (Eide-Haugmo et al. 2009, 2012). 54.8% were degraded after 28 d (O_2 -consumption) under marine conditions.

7.7.2. Environmental distribution

A Mackay Level I distribution model (Mackay Level 1 v2.11) was used for estimating distribution of substance in the environment. It is predicted to be found mainly in water (96%) under equilibrium conditions (unpublished study report 2016c). It is concluded that the substance is not expected to evaporate significantly from water into air and does not bind significantly to soil. This is considered to be plausible based on the properties determining the distribution of the substance: moderate vapour pressure (590 Pa at 20 °C), low log K_{ow} (-0.325), low log K_{oc} (1.81), high water solubility (fully miscible) and low Henry's Law constant (< 1 Pa*m³/mol at pH 7 and 25°C) (ECHA's dissemination site[4]).

[4] https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14823/4/2

Regarding these data, the substance is considered to be “bound” mainly in the aqueous phase, if released to water once.

7.7.3. Bioaccumulation

With an estimated, pKa corrected log D of -3.03 using SPARC online calculator (unpublished study report, 2020) at pH 7 and -1.08 at pH 9 the bioaccumulation potential is considered to be low.

7.8. Environmental hazard assessment

7.8.1. Aquatic compartment (including sediment)

7.8.1.1. Fish

Short-term toxicity to fish

In the key study with *Leuciscus idus* according to German Industrial Standard DIN 38412 part 15 (similar to OECD TG 203) an LC_{50} of 122 mg/l after 96 hours for the nominal concentrations was observed (unpublished study report, 1980). The test concentrations were not monitored analytically.

Long-term toxicity to fish

No experimental long-term toxicity data on fish are available. In ToxCast from USEPA[5] no indications for endocrine disruption were identified. According to ECOSAR v1.11 a chronic value (ChV) of 111 mg/L for ECOSAR class “Aliphatic Amines” and a ChV of 997 mg/L for ECOSAR class “Neutral Organic SAR” is predicted.

7.8.1.2. Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

In a 48-hour static test with *Daphnia magna* according to EEC-Guideline 79/831/EEC method C.2 (unpublished study report, 1988b) a 48-hour EC_{50} of 59.5 mg/L was obtained. The test concentrations were not analytically monitored.

In a poorly reported Klimisch 4 study a 24h-EC_{50} value of 44.5 mg/l (nominal) was determined (according to ISO 6341 15 - water quality - determination of the inhibition of the mobility of *Daphnia magna Straus*; unpublished study report, 1988c)

Long-term toxicity to aquatic invertebrates

A NOEC of 3.64 mg/L (based on mortality/ reproduction per introduced parent and on time-weighted mean concentrations) was identified in a recent 22-d chronic reproduction test on *Daphnia magna*, according to OECD TG 211 (unpublished study report, 2017a).

The test was prolonged to 22 days, as some replicates had not produced a complete final brood. One dead animal was recorded at 3.64 mg/L, which was considered by the study authors to be inadvertent. The death of four animals in the higher concentration group (6.06 mg/L) was considered to be substance related leading to a NOEC of 3.64 mg/L. The reproduction per surviving parent revealed a LOEC of 6.06 mg/L (NOEC = 9.96 mg/L), while the NOEC for reproduction per introduced parent was 3.64 mg/L.

[5] https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID5025102#bioactivity, visited in march 2020.

A recalculation of the data after 21 days revealed a NOEC for reproduction per surviving parent as well as per introduced parent of 3.64 mg/L, while for mortality a NOEC of 6.06 mg/L was derived.

In a QSAR model ECOSAR v1.11 estimates a chronic value (ChV) for daphnia of 4.46 mg/L according to ECOSAR class “Aliphatic Amines” and 313 mg/L according to ECOSAR class “Neutral Organic SAR”.

7.8.1.3. Algae and aquatic plants

Data from a 72-hour static growth inhibition test with the green alga *Scenedesmus subspicatus* according to DIN 38412 -9 (unpublished study report, 1987a/2009) which were recalculated using ToxRatPro v2.09 revealed that the validity criteria of the recent guideline OECD 201 were not fully met, as the factor of cell number measured in the control was 14.3 and the coefficient of variation of sectional growth rates in control replicates from 0 to 72 hours was higher than 35 % (48%). Nevertheless, the coefficient of variation of average specific growth rates in control cultures was 2.9 after 72 hours. The test concentrations were not analytically monitored. After 72 hours an ErC_{50} of 64.3 mg/L and an ErC_{10} of 48.4 mg/L based on nominal concentrations were obtained. The 72h-E_bC_{50} was 53.5 mg/L and the 72h-E_bC_{10} 43.0 mg/L.

Based on nominal test concentrations using *Skeletonema costatum* a 72-h ErC_{50} of 55 mg/L was determined in an marine study performed according to ISO 10253 (Eide-Haugmo et al. 2009, 2012).

Three tests with the structurally similar substance 3-aminopropyldiethylamine (DEAPA, CAS 104 -78 -9) were provided to further assess toxicity to algae: A 72h test according to OECD test guideline 201 with *Pseudokirchneriella subcapitata* (unpublished study report, 2000a) revealed an 72h-ErC_{50} of 34 mg/l and a 72h- ErC_{10} of 26 mg/L. The NOEC for growth rate and biomass was 19.53 mg/l. The final concentrations of DEAPA were maintained within 80% of the initial concentrations.

In supportive studies with DEAPA 72h-ErC_{50} values between 100 and 150 mg/L (unpublished study report, 1990b; according to DIN 38 412-9, concentrations not measured) and 7120 mg/L (unpublished study report, 1990c; according to DIN 38 412-9 with neutralised test solutions). The test concentrations were not analytically monitored.

QSAR model ECOSAR v1.11 estimates an acute value (EC_{50}) of 104 mg/L and a chronic value (ChV) for algae of 28.1 mg/l for ECOSAR class “Aliphatic Amines” and an EC50 of 1763 mg/L and a ChV of 279 mg/L for ECOSAR class “Neutral Organic SAR”.

7.8.1.4. Sediment organisms

No data available.

7.8.1.5. Other aquatic organisms

No data available.

7.8.2. Terrestrial compartment

No data available.

7.8.3. Microbiological activity in sewage treatment systems

A non-GLP DIN test 38412 with *Pseudomonas putida* revealed an EC_{10} for aquatic micro-organisms of 69.5 mg/L (unpublished study report, 1987b).

In the provided supporting study on the read across substance 3-diethylaminopropylamine according to OECD TG 209 only EC values after 30 minutes were measured, which is not in line with the updated OECD test guideline (unpublished study report, 2005b). Nevertheless, no inhibition was observed in this test.

7.8.4. PNEC derivation and other hazard conclusions

Table 7.8.4-1: PNEC

PNEC DERIVATION AND OTHER HAZARD CONCLUSIONS		
Hazard assessment conclusion for the environment compartment	**Hazard conclusion**	**Remarks / Justification**
Freshwater	PNEC: 0.0728 mg/L	Assessment factor: 50 Extrapolation method using an assessment factor based on the lowest chronic value of 3.64 mg/L
Marine water	PNEC: 0.00728 mg/L	Assessment factor: 500 Extrapolation method using an assessment factor based on the lowest chronic value of 3.64 mg/L
Intermittent releases to water	PNEC: 0.34 mg/L	Assessment factor: 100 Extrapolation method using an assessment factor based on the lowest acute value of 34 mg/L (algae via read across data from DEAPA, CAS 104-78-9).

7.8.5. Conclusions for classification and labelling

No classification of the substance is considered warranted.

7.9. Human Health hazard assessment

7.9.1. Toxicokinetics

There are no data available on basic toxicokinetic parameters such as percentages of absorption by the oral, inhalation and dermal route, tissue distribution, bioavailability, AUC, C_{max}, T_{max}, clearance or half-life. According to Regulation (EC) No 1907/2006 the assessment of the toxicokinetic (TK) behaviour of a substance should be performed to the extent that can be derived from the relevant available information. Therefore, also data on physico-chemical properties, QSAR and supporting information from an analogue substance were used.

If the Substance reaches the lungs in its vapour state, absorption directly across the respiratory tract epithelium by passive diffusion is assumed due to its low log K_{ow} value of -0.352 and high water solubility. Based on the vapour pressure of 590 Pa inhalation exposure due to vapour is a relevant exposure pathway.

Based on the physico–chemical properties, the Substance may be able to penetrate the skin as the log K_{ow} value and high water solubility allow dermal penetration. Acute dermal toxicity studies performed on rats and rabbits indicate dermal uptake and systemic bioavailability; the dermal LD50 values were 2138.7 mg/kg bw in rabbits and > 400 to < 2000 mg/kg bw/d in rats (unpublished study report 1993b unpublished study report 1993c).

The pKa values of 9.33 and 5.6 indicate that the molecule is ionized upon oral, dermal or inhalation exposure and contact with biological tissues at physiological pH values (neutral form only at pH >9.33). According to the estimations based on the Danish QSAR Database[6] oral absorption is likely. The bioavailability score (Lipinski's Rule-of-five score) is zero, which indicates that the substance may be bioavailable. The QSAR estimate indicates 50% oral adsorption. For ethylenediamine (EDA), a structural similar substance, first pass metabolism is described in humans. Comparison of oral and i.v. data resulted in a bioavailability of about 34% after oral administration to male and female volunteers due to a first-pass effect according to Cotgreave and Caldwell (1983). In male Hilltop Wistar rats EDA bioavailability by the oral route was dose dependant with 60% at low dose (5 mg/kg bw) rising to 100% at 50 mg/kg (Yang and Tallant, 1982). Therefore some limitation of oral absorption is also likely for the Substance.

Based on systemic effects in acute and repeated dose studies tissue distribution is assumed for the Substance. Yang and Tallant (1982) reported high tissue concentrations of EDA in thyroid and bone marrow in addition to liver, kidneys and lower levels of distribution to other organs including brain in rats.

To elucidate the metabolic pathway of the Substance and further strengthen the read-across to the source substances EDA and 3-aminopropyldiethylamine (DEAPA) an *in vitro* metabolism assay with lung and liver S9-fraction of untreated male Han-Wistar rats was performed (unpublished study report 2017b, no GLP, Klimisch 1). While the *in vitro* liver S9 systems have several limitations as described in Gouliarmou et al. (2018) they are used for metabolic stability testing. The Substance was incubated with S9-fraction of rat liver and of rat lung at a nominal concentration of 50 µM, vehicle DMSO in duplicate. The incubation was performed at 37°C for 2 hours with continuous agitation. In addition to the active *in vitro* system, heat deactivated controls and buffer controls were included in the assay as well as the positive control testosterone. Under the study conditions no or limited metabolic turnover was observed for rat liver S9 while for rat lung S9 recoveries were too low for such calculations (unpublished study report, 2017b).

[6] https://qsar.food.dtu.dk/

The registrant proposed metabolisation by hydroxylation of the methyl-groups followed by oxidative dealkylation to form 3-aminopropylmonomethylamine and the primary amine 3-aminopropylamine (trimethylenediamine, 1,3-propanediamine, CAS N° 109-76-2). These metabolites may be further metabolized to desaminated oxidation products such as aldehyde or carboxylic acid derivatives. However, no experimental proof or evidence specifically for the Substance is available. The major routes of metabolism of C10-C13 primary amines involve various processes including oxidation, conjugation, and other enzyme-catalysed reactions leading to detoxification and excretion (OECD, 2011).

Dealkylation could be a pathway producing metabolites that are common breakdown products of the substances involved in the read-across approach.

The QSAR Toolbox (V 4.1) rat liver S9 metabolism simulation predicted several metabolites for the Substance and DEAPA (please see Figure 7.9.1-1 and Figure 7.9.1-2). Upon comparison of the structures of the metabolites it becomes apparent that both substances are metabolised in a similar manner. In both cases the alkylated amino group is oxidized to an N-oxide as seen in structures b for the Substance and c for DEAPA. Both substances are dealkylated to respective secondary amines 3-aminopropylmonomethylamine and N-ethylpropane-1,3-diamine, which are subsequently oxidized to their alkylated analogues of beta-alanine (N-methyl and N-ethyl metabolites). Thus they share phase I and phase II metabolic reactions.

Figure 7.9.1-1: QSAR Toolbox rat liver S9 simulator of DMAPA a) formaldehyde, b) 3-amino-N,N-dimethylpropan-1-amine N-oxide c) 3-(dimethylamino)propanoic acid, d) 3-(dimethylamino)propanal e) N-methyl-beta-alanine f) 3-aminopropylmonomethylamine g) 3-aminopropanal h) trimethylenediamine

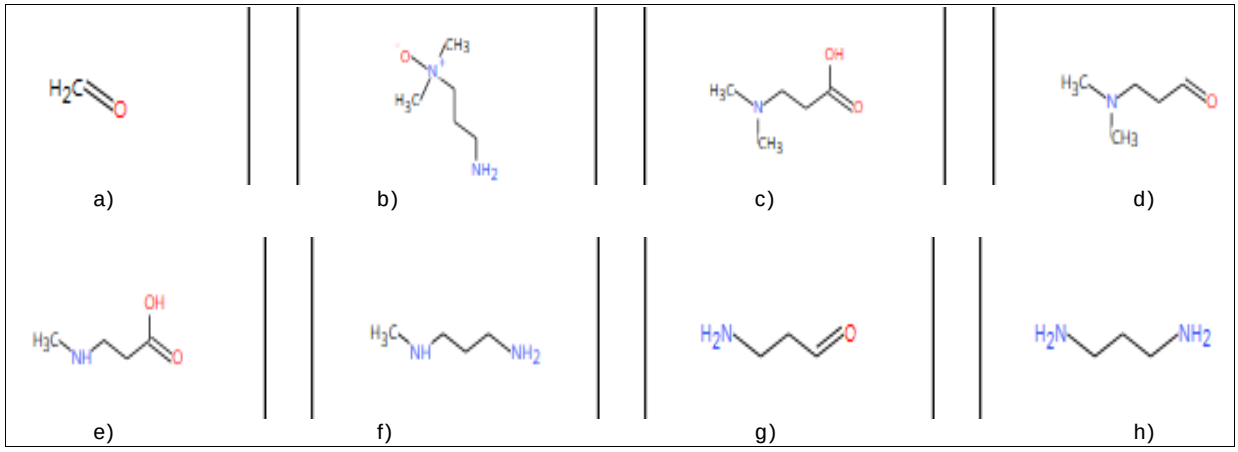
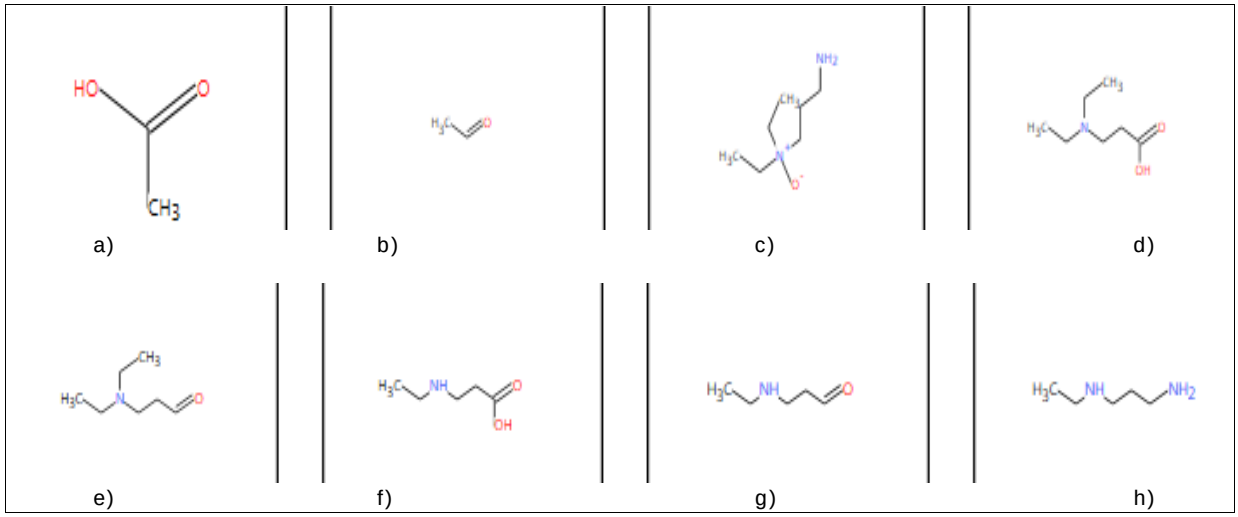


Figure 7.9.1-2: QSAR Toolbox rat liver S9 simulator of DEAPA a) acetic acid, b) acetaldehyde, c) 3-amino-N,N-diethylpropan-1-amine N-oxide, d) 3-(diethylamino)propanoic acid, e) 3-(diethylamino)propanal, f) N-ethyl-beta-alanine g) 3-(ethylamino)propanal h) N-ethylpropane-1,3-diamine



The metabolism of EDA, an analogue substance, is proposed by two main pathways (a) acetylation at one or both amino groups and (b) deamination with the intermediate aminoacetaldehyde, which is rapidly converted to glycine (NTP, 1993; Cotgreave and Caldwell, 1983). Thus mainly phase II reactions take place.

During the first 24 h after oral administration of EDA in form of aminophiline to humans urinary excretion amounted to 3% parent and 45% acetylated ethylenediamine of the applied dose. The elimination half-life was 60 min (monoexponential decrease) and plasma clearance was 589 ml/min in this study (Cotgreave and Caldwell, 1983). N-acetylethylenediamine was a major metabolite in urine.

Based on the properties, particularly water solubility and log K_{ow} and ionization of the substances at pH of urine, excretion of the Substance and/or of metabolites may occur predominantly via the urine. The Substance is not expected to accumulate in tissues.

Conclusion:

No toxicokinetic study according to OECD TG 417 was available for the Substance. Based on physico-chemical properties, QSAR estimates and information from the analogue substance EDA moderate oral absorption in animals is estimated. For EDA bioavailability in humans was only 34% after oral administration due to first pass metabolism. In addition to the oral route dermal and inhalation absorption is likely, if exposure occurs via these routes. The charged form as well as the corrosivity may modify and limit absorption processes across cell membranes. Once the substance is absorbed, it is expected to be distributed via the blood to the liver and other tissues based on the findings from toxicological studies (cf. section 7.9.2; 7.9.4).

In vitro metabolism studies with rat liver S9 showed no or limited metabolism of the Substance under the study conditions. Based on studies with primary and tertiary amines several metabolic pathways mediated by phase I and II enzymes are suggested including dealkylation, oxidation and N-acetylation leading to detoxification and excretion.

The Substance and its metabolites are expected to be excreted primarily via urine.

The Substance and the analogue substances EDA and DEAPA share common functional groups that indicate a biological similarity. No experimental proof for the formation of common metabolites is available, however QSAR data indicate similar metabolic pathways for the Substance and DEAPA with likely differences in reaction kinetics based on the methyl- and ethyl-substitution (cf. ANNEX: Read-across justification).

7.9.2. Acute toxicity and Corrosion / Irritation

Acute toxicity: oral

The Substance is already listed in Annex VI to Regulation (EC) No 1272/2008 and classified with Acute. Tox. 4 (H302, Harmful if swallowed), minimum classification. This classification is supported by the studies submitted by the registrant(s). Available animal studies are listed in Table 7.9.2-1.

Table 7.9.2-1: Acute oral toxicity studies

Study / Method	Results	Remarks / Reference
Sprague-Dawley rat m/f 5/sex/group Dose levels: 320, 630, 1000 mg/kg bw/d Test substance: DMAPA, purity not stated Oral, gavage No vehicle Observation duration: 14 d (post exposure) According to OECD 401 prior to 2002	LD50 (f) of 377.1 mg/kg bw/d (95% CL 203.3 -699.3) LD50 (m): 442.7 mg/kg bw/d (95% CL 322.8 -607.1) The LD50 was 410 mg/kg bw/d (288.1 - 584) for both sexes Mortality: 320, 630 and 1000 mg/kg bw: 3/10, 8/10 and 10/10 animals Clinical signs observed in all dose groups: decreased activity (lasting several days after dosing at all dose groups), abnormal gait, abnormal stance, flaccid body tone (immediately after dosing at day 0, all dose levels) Clinical signs not common in all dose groups: dyspnoea, chromodacryorrhea, diarrhoea, decreased body tone (at 630 mg/kg starting several days after dosing in both sexes), piloerection, prostration, tremors, salivation, discoloured urine, body drop and poor grooming were observed. Body weights increase in all dose groups, except the 320 mg/kg bw/d, where a slight body weight decrease was observed	**unpublished study report (1993a)** GLP Klimisch 2 **Key study** The observed effects are indicative for local effects. However, hypoactivity, flaccid body tone and tremors could also be a consequence of neurological dysregulation.

<table>
<tr><th>Study / Method</th><th>Results</th><th>Remarks / Reference</th></tr>
<tr><td>Hoe WIKS (SPE71) rat

10/f/group

Dose levels: 315, 500, 800, 1000, 1250 mg/kg bw

Test substance: DMAPA, purity not stated

Oral, gavage

10% aqueous solution

Observation duration: 14 d (post exposure)</td><td>LD50 (f) of 1037 mg/kg bw (CL 912 -1179)

<table><tr><td>Dose level</td><td>Mortality</td></tr><tr><td>315 mg/kg bw</td><td>0/10</td></tr><tr><td>500 mg/kg bw</td><td>1/10</td></tr><tr><td>800 mg/kg bw</td><td>0/10</td></tr><tr><td>1000 mg/kg bw</td><td>5/10</td></tr><tr><td>1250 mg/kg bw</td><td>9/10</td></tr></table>
Clinical signs (in all dose groups, severity dose dependant): abnormal gait, piloerection, dyspnoea, enlarged eyelid cleft, decreased activity. Surviving animals showed the mentioned symptoms in attenuated form and were 48 hours post-application free of clinical signs.
Macroscopic examinations of moribund animals revealed dark colouration of liver and spleen, autolysis and liquids in GI.</td><td>unpublished study report (1979)

No GLP

Klimisch 2

Supporting

The observed effects are indicative for local effects.</td></tr>
<tr><td>Mouse, rats

Test substance: DMAPA in aqua dest., purity not stated

Mouse: also administration of neutralised DMAPA

Observation duration: 24 hours, 8 days</td><td>Mouse: LD50 ~ 2 ml/kg, neutralised: LD50 ~ 7 ml/kg

Rats: LD50 ~ 2 ml/kg

(conversion into mg/kg is based on the density d= 0.8 g/cm^3 resulting in an LD50 of 1600 mg/kg bw for rats)</td><td>unpublished study report (1958)
No GLP

Mouse and rats seemed equally sensitive and the LC50 value with neutralised substance is considerable higher.

Only a summary (poor documentation, no dose levels stated) was available to MS AT, the registrant(s) indicated reliability score of 2.</td></tr>
<tr><td>rabbit: 3 animals per dose group

male/female

oral: gavage

Tested concentration: 25% aqueous neutralised solution; 6.4 ml/kg, 3.2 ml/kg, 1.6 ml/kg.

Test substance: DMAPA, purity not stated</td><td>Rabbit: LD50 between 1.6 and 3.2 ml/kg bw (1300 mg/kg and 2603 mg/kg bw).

Clinical signs only at 3.2 ml/kg: atonie, dyspnoea, tremors, tonic-clonic tremors, miosis, hypersalivation.</td><td>unpublished study report (1961a)

No GLP

Klimisch 2

Supporting

Reported documentation does not comply with current standards but individual data including histopathological examination were available.</td></tr>
</table>

In a study identified as key study (unpublished study report, 1993a; according to OECD 401 prior to 2002) the parent compound was administered by gavage to rats (m/f) at dose levels of 320 mg/kg bw, 630 mg/kg bw and 1000 mg/kg bw. The following LD50 values were determined: LD50 (f) of 377.1 mg/kg bw (95% CL 203.3 -699.3); LD50 (m) of 442.7 mg/kg bw (95% CL 322.8 -607.1). The LD50 was 410 mg/kg bw (288.1 - 584) for both sexes after acute oral administration. Clinical signs observed in all dose groups were decreased activity (lasting several days after dosing at all dose groups), abnormal gait, abnormal stance, and flaccid body tone (immediately after dosing at day 0, all dose levels). Other observations occurred primarily at higher doses with higher mortality such as dyspnoea, chromodacryorrhea, diarrhoea, piloerection, prostration, tremors, salivation, discoloured urine, body drop and poor grooming. Body weights increased in all dose groups (except the group dosed with 320 mg/kg bw, where a slight body weight decrease was observed).

In another supporting acute toxicity study the LD50 in female rats was 1037 mg/kg bw (unpublished study report, 1979). Similar an additional supporting study in rats and mice resulted in a LD50 for acute oral toxicity of approximately 1600 mg/kg bw. Main clinical signs observed were drowsiness and staggering (unpublished study report, 1958). The LC50 values reported in a study conducted with rabbits (3 animals per dose group, tested doses 6.4, 3.2 and 1.6 ml/kg) indicate an LC50 between 1.6 and 3.2 ml/kg bw (1300 mg/kg and 2603 mg/kg bw) administered as a 25% aqueous solution neutralized with H_2O (unpublished study report, 1961a).

Acute toxicity: inhalation

The Substance has a vapour pressure of 590 Pa at 20°C and registered uses for which inhalation exposure can be assumed.

Available animal studies concerning inhalation acute toxicity are summarized in Table 7.9.2-2. For acute inhalation toxicity three experimental studies were available. The most reliable study is a limit test with LC0 of >4.31 mg/L in rats that indicated local irritating effects in the respiratory tract at this concentration (unpublished study report, 1991). An older study protocol (unpublished study report, 1961b) indicated that the LC50 might be <20 mg/L for vapours, however there is uncertainty of the applied test concentrations. In the third study performed by Smith et al. (1949) no mortalities occurred after 8 hour exposure at saturated vapour pressure concentrations.

Table 7.9.2-2: Acute inhalation toxicity studies

Study/Method	Results	Remarks/Reference
Wistar rats m/f 5/sex/group Tested concentration: 4.31 mg/L Test substance: DMAPA>99.5% inhalation: vapour, nose/head only	LC50 >4.31 mg/L Clinical signs: during exposure: animals showed immediately escape attempts, eyelid closure, accelerated respiration and additionally after 15 min restlessness, after 2h irregular respiration After exposure: up to 24h accelerated respiration and ruffled fur, after 48h post	**unpublished study report (1991)** No GLP Klimisch 2 **Key study, weight of evidence** The observed effects are indicative for local effects

Study / Method	Results	Remarks / Reference
Exposure duration 4h Observation period: 14 days (post exposure) OECD 403 (1981)	exposure males showed aggressiveness (single housing necessary, reversible after 3 days). Reversible distinct eye irritation was reported (corneal stippling's, fundus not visible at the day of exposure). Body weight gain slightly retarded for female rats for day 7 – 14.	including irritation to the respiratory tract. modulated several behavioural aspects related to aggression in male rats Gross pathological examination revealed no findings. No particle sizing was performed. Limit test with 4.3 mg/L (recommended: 20 mg/L).
Rat, m/f 3 animals per dose group Test substance: DMAPA Inhalation, vapour saturated atmosphere at 20°C 30 min: 22.9 mg/L 2h: 12.0 mg/L 4h: 11.6 mg/L 8h: 13.8 mg/L Duration: 14 days Study protocol: inhalation risk test (similar to OECD 403)	At 2h exposure 1/6 animals, at 4 h 2/6 (33% mortality), at 8 h 4/6 died. No mortality at 30min LD50 value likely to be < 20 mg/L Clinical signs: 30 min and 2 h exposure: dyspnoea, eyes closed. 4 and 8 h exposure: dyspnoea, eyes closed, after 5 min brown smeared snouts, after 2h ruffled fur. 3 days post exposure: apathy, wet dirty fur, crusted eyelids, opacity; reversibility after 9 d.	unpublished study report (1961b) No GLP Nominal concentrations. No analytical verification of test atmosphere concentrations, no particle sizing. Supportive study Only a summary was available to MS AT (poor study documentation, type of inhalation exposure not specified) while registrants indicated a reliability score of 2. Study performed prior to OECD guidelines in 1958. The saturated vapour concentration is calculated with 24.8 mg/L. Local effects observed, also in the respiratory tract.
Rat, m/f, 6 animals Inhalation, vapour saturated vapour-air mixture (not stated, but assumed to be around 25 mg/l)	No mortality occurred after 8 hour inhalation of the test item	Smyth et al. (1949) Supportive study No measured concentrations were stated.

Acute toxicity: dermal

Currently, the Substance is not harmonised classified for this endpoint. The registrant(s) self-classify the Substance with Acute Tox. 4, H312 (Harmful in contact with skin). Studies for acute dermal toxicity in two species, rats and rabbits, are available and are documented in Table 7.9.2-3.

Table 7.9.2-3: Acute dermal toxicity studies

Study/Method	Results	Remarks/Reference
Sprague-Dawley rat m/f Main test: 5/sex/group, Dose level: 400 mg/kg bw, observation duration: 14 d (post exposure) Range finding test: 2/sex/group, Dose level: 1000 and 2000 mg/kg bw, observation duration: 5 d (post exposure) Test substance: DMAPA>99% Dermal, semiocclusive Vehicle: water Exposure duration 24h OECD 402 (prior to 2017 update)	Main test: LD0: 400 mg/kg bw (no clinical signs and no macroscopic lesions), no mortalities LD50 > 400 mg/kg bw and <2000 mg/kg bw Range finding test: 1000 mg/kg bw: Necrosis and/or oedema after 2 d; no deaths after 5 days, but from d 2 tremors and sedation. 2000 mg/kg bw: no clinical signs; no cutaneous reactions reported, exitus 2/2 at day 2	**unpublished study report (1993b)** GLP Klimisch 2 **Key study** One dose tested, however pre-test at 1000 and 2000 mg/kg lead to a dose selection of 400 mg/kg. The observed effects are indicative for severe local effects. No other clinical signs than tremors and sedation at 1000 mg/kg bw were reported probably related to some neurotoxic/narcotic effects.
rabbit (New Zealand White) m/f, 5 animals per dose group Test substance: DMAPA Tested concentration: 1000, 2000, 3000 mg/kg Dermal, occlusive (animals were wrapped with a rubber dam) No vehicle Exposure duration 24 h Observation period: 14 days	LD50 (m/f): 2138.7 (CI 1630-2805) mg/kg bw LD50 (m): 2396.1 (CI 1756-3269 mg/kg bw Observed clinical signs (starting at 2000 mg/kg): decreased activity, abnormal gait, abnormal stance, decreased muscle tone, dyspnoea, diarrhoea, ptosis, tremors, paralysis of hindquarters, atrophy of the hind limb, poor grooming, red urine and prostration. Macroscopic findings: observed skin necrosis (at all doses) and eschars. Animals died during study: distended and/or fluid-filled stomach and intestines,	unpublished study report (1993c) Klimisch 2 GLP No purity of the test substance reported. Test substance is only referred as a number 6398-35-1 in the report, identity in the test report not stated, bulk test article stability analysis was not performed. No LD50 for females was calculated. Number of deaths: 2000 mg/kg 2/5, 3000 mg/kg 5/5

<table>
<tr><th>Study/Method</th><th>Results</th><th>Remarks/Reference</th></tr>
<tr><td>Similar to OECD 402 (prior to 2017 update)</td><td>discoloured kidneys, lungs and liver, necrosis of the skin at application site

Terminal necroscopy: 1 male at 2000 mg/kg: discoloured kidneys</td><td></td></tr>
<tr><td>Albino rabbit

5 males per dose group

Test substance: DMAPA

No vehicle or dilution

Tested concentration:, 0.313, 0.625, 1.25, 2.5 ml/kg bw

Exposure duration: 24h

Observation period: 14 days</td><td>LD50: 1.002 ml/kg bw (Cl 0.66 – 1.58) ~ 816 mg/kg bw

<table><tr><th>Dose level</th><th>Mortality</th></tr><tr><td>2.5 ml/kg</td><td>5/5 (6h -24h post exposure)</td></tr><tr><td>1.25 ml/kg</td><td>3/5 (6h -24h post exposure)</td></tr><tr><td>0.625 ml/kg</td><td>1/5 (at 48h post exposure)</td></tr><tr><td>0.313 ml/kg</td><td>No mortalities</td></tr></table>
Survivors: occasional animal had adhesions of intestines to the abdominal wall, severe erythema and oedema of skin, eschars formation up to 14 days</td><td>unpublished study report (1964)

No GLP

No purity of the test substance reported.

Key study

Poor study documentation; level of detail similar to unpublished study report (1958) rated Klimisch 2 by the registrant(s) (cf. section oral toxicity)</td></tr>
</table>

Conclusion on acute toxicity:

Acute toxicity is likely to be a consequence of local effects due to the corrosivity of the Substance (harmonized classified as Skin Corr. 1B, H314). This is most obvious in the oral studies.

In the current Annex VI entry of Regulation (EC) No. 1272/2008 the Substance is classified as Acute Tox. 4* (H302) for acute toxicity via the oral route; the asterisk indicates that this is a minimum classification. Provided information for rats and mice resulted in a LD50 value below 2000 mg/kg bw. According to the criteria in CLP Annex I, an oral LD50 >300 but ≤ 2000 mg/kg bodyweight lead to a category 4 classification.

SEV has therefore verified, that the asterisk indicating minimum classification of the current Annex VI entry 612-061-00-6 can be removed. To facilitate consistent classification of mixtures containing the Substance, a harmonised ATE value is also needed. According to the CLP regulation (Regulation (EC) No. 1272/2008), the ATE value for a substance should be derived using the LD50, where available. The lowest LD50 value in female rats was 377 mg/kg bw and 442.7 mg/kg bw in male rats derived from the most reliable GLP study (unpublished study report, 1993a). Also higher LD50 values were reported in older studies for mice, rats and rabbits (cf. Table 7.9.2-1). Taking these data into account, and in line with table 3.1.2, Annex I of Regulation (EC) No. 1272/2008, it is proposed to assign an ATE of 380 mg/kg bw (rounded from 377 mg/kg, females shown to be more sensitive in several studies, cf. 7.9.4) for acute oral toxicity.

For acute inhalation toxicity three experimental studies were available. The most reliable study is a limit test with an LC0 of >4.31 mg/L (unpublished study report, 1991). An older study protocol indicated that the LC50 might be <20 mg/L for vapours, however there is uncertainty of the applied test concentrations. At 11.6 mg/L after 4 hour exposure 2/6 animals died (unpublished study report, 1961b). It is unclear if a concentration close to the saturated vapour concentrations calculated with 24.8 mg/L was achieved or 11.6 mg/L

were tested. Moreover 33% mortality were observed after 4 hour exposure (unpublished study report, 1961b). Therefore, in a weight of evidence it is likely that the LC50 is greater than 5 mg/l. While the LC50 might be below 20 mg/L and justify classification with Acute Tox. 4, H332 there are considerable uncertainties as outlined above. Also in the third study performed by Smith et al. (1949) no mortalities occurred after 8 hour exposure at saturated vapour pressure concentrations. Overall the presented evidence does not allow to support classification with Acute Tox. 4, H332.

In the dermal studies local effects are indicated in almost all high dose animals in all three available studies. In rats an LD50 >400 mg/kg bw was reported in a GLP compliant study similar to OECD GD 402, based on the range finding study a dose of 2000 mg/kg caused mortality in 2/2 animals tested and no death at 1000 mg/kg bw (unpublished study report, 1993b). Whereas one other GLP conform study reported an LD50 value of 2138 mg/kg bw in rabbit for combined sexes (unpublished study report 1993c), another study in male rabbits prior to GLP reported an LD50 of 816 mg/kg bw (CI 536.78 - 1285.01 mg/kg bw) (unpublished study report, 1964). The reliability cannot be fully evaluated based on limited study documentation, however, the LC50 value of this study falls within the range of table 3.1.1 for acute toxicity hazard category 3, 200 < ATE ≤ 1000. When experimental data for acute toxicity are available in several animal species, scientific judgement shall be used in selecting the most appropriate LD50 value from among valid, well-performed tests (ECHA, 2017a). While the study in rats justifies with an LD50 of >400 mg/kg bw but <2000 mg/kg bw (unpublished study report, 1993b) acute dermal toxicity hazard category 4 (300 < ATE ≤ 2000) the study in rabbits indicate with an LD50 of 2138 mg/kg bw (unpublished study report, 1993c) no classification for this endpoint. However an older study with sufficient number of animals (5 per dose group) indicate a considerable lower LD50 value of ~820 mg/kg bw.

In addition tremors and sedation as well as partial paralysis were observed in dermal acute studies in rats and rabbits in high dose animals.

According to the LD50 >200 mg/kg bw but <1000 mg/kg bw obtained from an acute toxicity study in rabbit the Substance meets the criteria for classification and labelling as Acute Tox. 3, H311: Toxic in contact with skin. An ATE of 820 mg/kg (rounded from 816 mg/kg) is proposed.

SEV has verified the concern that there is a need to harmonize the classification for dermal toxicity according to Regulation (EC) No. 1272/2008.

Corrosion / Irritation

The Substance is already listed in Annex VI to Regulation (EC) No 1272/2008 and classified with Skin Corr. 1B (H314, Causes severe skin burns and eye damage). This classification is supported by the studies listed by the registrant(s) in the Chemical Safety Report (CSR, 2017) and information compiled at the ECHA dissemination site[7] that clearly demonstrate corrosivity of the undiluted substance for standard exposure times for up to 4 hours in OECD compliant test guidelines. The available information is reliable and acceptable for the endpoint of skin and eye irritation and corrosion. The harmonised classification Skin Corr. 1B is confirmed.

As, according to the CLP Regulation, skin corrosivity shall be considered as leading to serious damage to the eyes no further testing is required according to Regulation (EC) No 1907/2006.

[7] https://echa.europa.eu/registration-dossier/-/registered-dossier/14823/7/4/2

However, an eye irritation study (unpublished study report, 1961b) is available in rabbits (2 animals) with observations after 10 minutes up to 6 days. The study shows some deviations to OECD TG 405 (old study protocol) but it confirms serious eye damage after 10 minutes. Based on these data the Substance has to be classified as Eye Dam 1, H318. However, according to ECHA (2017a) for a substance that is classified as Skin Corr. 1 also serious damage to eyes is implicit as reflected in the hazard statement for skin corrosion (H314: Causes severe skin burns and eye damage). Thus, the corresponding hazard statement (H318: Causes serious eye damage) is not indicated on the label to avoid redundancy.

Respiratory irritation/sensitisation: human data

Investigations on occupationally exposed workers are available as summarized in Brubaker et al. (1979). In the first study from 1974 (Sergant et al., 1976) workers described respiratory effects from exposure to an epoxy resin system in a ski manufacturing plant. Participants to the study were 25 mold workers and 9 controls, both subject to questionnaires and lung function tests. 10 of the exposed workers reported upper respiratory symptoms and 20 lower respiratory symptoms including cough, increased phlegm, wheezing and chest tightness via a self-reported questionnaires. Pulmonary functional tests performed before and after the work shift decreased significantly after one working day and over the week of the highly exposed group (mean exposure concentration of 0.9 ppm converted to 3.76 mg/m^3, range 0.55 -1.38 ppm) that also showed the highest prevalence of symptoms. Workers with less exposure had functional test results between control and highest exposure group (but with no changes over the week or after the first work shift on Monday).

In a follow-up investigation in 1977, substantial reductions in exposure but also in related recorded pulmonary effects were reported indicating that an average DMAPA concentration of below 0.2 ppm showed no decreased lung function changes over the work shift among the different exposure groups and controls (except pressman representing a mid exposed group). No significant group difference for lung function changes over the work shift among the worker groups was observed by an analysis of variance (Brubaker et al., 1979).

Concerning cumulative effects losses of observed FEV (forced respiratory volume, as percent of predicted by year and length of exposure), was evident in the 5 to 7 year and 2 to 4 year groups (but reductions were not significant) (Brubaker et al., 1979).

However, while the study (Brubaker et al., 1979) suits a comparison based on a baseline set in 1974 no primary data on pulmonary function testing or medical diagnoses were reported making it difficult to distinguish or diagnose the observed respiratory diseases.

Sergant et al. (1976) proposed, due to the uniform decrease in lung function of the highly exposed worker, an irritating effect rather than hypersensitivity. A study concerning aliphatic amine-induced occupational rhinitis and asthma indicated the Substance responsible for a non-irritating mechanism based on the case of a 22-year-old female with rhinitis and asthma-like symptoms exposed to this chemical at the workplace. A non-irritating concentration of the Substance was tested in a specific nasal provocation test (NPT) that is indicative of an immunoallergic or pharmacological action. However, also didecyldimethylammonium chloride gave a positive NPT making the cause of the respiratory disease less certain (Laborde-Casterot et al., 2014).

In conclusion the Substance effects pulmonary functions and can cause respiratory diseases in humans. Available information is limited by reporting, study size and co-reactivity with other chemicals. While respiratory irritation in humans is likely and supported by animal data more information would be needed to conclude on the potential for respiratory sensitization.

According to the CLP guidance (ECHA, 2017a) a classification for corrosivity is in general considered to implicitly cover the potential to cause RTI and so the additional classification for STOT SE category 3 is considered to be superfluous, although it can be assigned. Nevertheless, observed local irritating effects described by clinical signs in animal studies include accelerated or irregular respiration, dyspnoea or eyelid closure (cf. Table 7.9.2-2). Occupational investigations indicate alterations of pulmonary function. Therefore, additional labelling with STOT SE 3 H335 (May cause respiratory irritation) according to Regulation (EC) No. 1272/2008 seems justified. Information to classify for respiratory sensitisations is considered not sufficient yet.

7.9.3. Sensitisation

The Substance is listed in Annex VI to Regulation (EC) No 1272/2008 and classified as Skin Sens. 1 (H317, May cause an allergic skin reaction). A body of experimental evidence is available to characterize the skin sensitisation potential; consisting of *in vitro* tests, animal studies and human data qualifying a proposal for sub-classification.

In vitro and animal studies

An overview of the available *in vitro* and animal studies (local lymph node assay (LLNA), guinea pig maximisation tests (GMPT) and Buehler test) is compiled in Table 7.9.3-1.

Table 7.9.3-1: In vitro and animal studies on the sensitizing property of the Substance

Study / Method	Results	Reference / Remarks
In vitro test battery DPRA KeratinoSensTM h-CLAT Test material: DMAPA Results of the three methods were incorporated into the "2 out of 3" Weight of Evidence approach	Sensitizing DPRA: negative KeratinoSens: positive h-CLAT: positive The authors indicated that based on the negative protein depletion in the DPRA assay DMAPA is most likely a pro-hapten requiring metabolic activation. Mechanistic domain: pro-Schiff base former, conversion of the primary amine to an aldehyde suggested	Urbisch et al. (2016) Klimisch 2 Peer reviewed article, therefore reporting deficiency. Purity not stated Test protocols prior to OECD TG adoption.
SENS-IS Test material: DMAPA Test item (in DMSO) 50%, 10%, 15 and 0.1% Application on reconstructed human skin model in a vehicle Analysis of the expression of 17 genes (REDOX-group) and 21 genes involved in inflammation, danger signals and migration (SENS-IS group)	Results: sensitizer, SENS-IS classification: moderate Criteria for the test: If a compound induces at least 7 genes in either the REDOX or SENS-IS group of genes, it is classified as sensitizer Potency: extreme, strong, moderate or weak if found positive at 0.1%, 1%, 10% or 50% respectively. Thus DMAPA was found positive at 10%.	Cottrez et al. (2016) Klimisch 2 Peer reviewed article, therefore reporting deficiency. Purity not stated The test protocol is not peer-reviewed yet under OECD. For the status please see [8]

[8] https://tsar.jrc.ec.europa.eu/test-method/tm2011-11

Study/Method	Results	Reference /Remarks
LLNA (similar to OECD Guideline 429) Mouse (CBA/Ca) female Test material: DMAPA >99% Seven vehicles used: AOO (acetone: olive oil, 4:1), MEK (methylethylketone), DMF (dimethylformamide), PG (propylene glycol), DMSO (dimethyl sulfoxide) EtOH/w (ethanol: water, 90:10), EtOH/w (ethanol: water, 50:50)	Sensitising Negative control (AOO, MEK, DMF, PG, DMSO, EtOH/w 90:10, EtOH/w 50:50):SI 1.0 DMAPA: 0.5, 1, 2.5, 5, 10% in AOO: SI 1.3, 1.1, 3.5, 7, 13.9 0.5, 1, 2.5, 5, 10% in MEK: SI 1.6, 2.1, 3.8, 6.8, 12.6 0.5, 1, 2.5, 5, 10% in DMF: SI 1.5, 1.7, 4.4, 6.4, 15.7 0.5, 1, 2.5, 5, 10% in PG: SI 1.5, 1, 1.3, 1.4, 2.2 0.5, 1, 2.5, 5, 10% in DMSO: SI 1.4, 1.3, 2.1, 5.4, 9 0.5, 1, 2.5, 5, 10% in EtOH/w 90:10: SI 1 1.5, 1.6, 3.8, 5.9 0.5, 1, 2.5, 5, 10% in EtOH/w 50:50: SI 0.9, 1.7, 1.2, 5.5 **EC3 value** (in AOO, MEK, DMF, PG, DMSO, EthOH/w 90:10, EtOH/w 50:50): **2.2%, 1.8%, 1.7%, >10%, 3.2%, 4.1%, 7.1%**	**Wright et al. (2001)** Klimisch 2 (reliable with restriction) Key study No GLP reported Experimental result, reporting deficiency compared to full study report. Aim of the study was the determination of vehicle effects. Study prior to OECD TG adoption. According to OECD TG 429 recommended vehicles are acetone: olive oil (4:1, v/v), DMF, MEK, PG, DMSO. Depending on the vehicle EC3 values (linear interpolation) are in the range of 1.7 to >10% indicating strong to moderate skin sensitisation
Guinea pig maximisation test (EPA OPPTS 870.2600) Guinea pig, Hartley, m Test material: Batch No. G01185FNA, DMAPA >99% Induction: intradermal and epicutaneous, vehicle water Challenge: epicutaneous, occlusive, vehicle water <u>Concentration:</u>	Sensitizing Test results: 24h after challenge: 0/20 48h after challenge: 2/20 (mild dermal reactions) 24h after rechallenge: 6/20 (30%; mild dermal reactions) 48h after rechallenge: 9/20 (45%; mild to moderate dermal	ECHA dissemination website citing unpublished study report (2000b) Klimisch 1 (rated by the registrant(s)) Supporting study GLP Potency on basis of the Guinea Pig Maximisation Test after rechallenge indicate strong potency.

Study / Method	Results	Reference / Remarks
Induction: Intradermal: 0.1% and 0.1% in FCA Topical induction (day 8): 5% Challenge (day 22): 1% Rechallenge (day 15 after challenge): 1%	reactions, fissuring on one animal) Neg. control: 24h, 48h after challenge: 0/20 24h after rechallenge: 0/20 Pos. control: yes	No positive reaction in the rechallenge phase in control animals.
Guinea pig maximisation test guinea pig, Dunkin-Hartley, m/f OECD 406 (1981) Test material: DMAPA Induction: intradermal and epicutaneous, vehicle water Challenge: epicutaneous, occlusive, vehicle water <u>Concentration:</u> Induction: Intradermal: 0.25% and 0.25% in FCA Topical induction (day 9): 5% Challenge (day 22): 1%	Not sensitizing Test results: 6h after challenge: 0/20 (scratch marks on the application area 3/20) 24h after challenge: 0/20 (scratch marks on the application area 6/20) 48h after challenge: 0/20 (scratch marks on the application area 5/20) Neg. control: 6h, 24h and 48h after challenge: 0/10 Pos. control: yes (results not reported) 8d: application of sodium laurylsulfate (SLS)	ECHA dissemination website citing unpublished study report (1986) Klimisch 1 (rated by the registrant(s) Supporting study GLP No scores according to the M&K grading scale is reported in the summary Study not included in the CSR SLS application only for non-irritating chemicals recommended according to TG.
similar to guinea pig maximisation test guinea pig, Hartley, f Test material: DMAPA >99% Induction: intradermal and epicutaneous, vehicle water Challenge: epicutaneous, occlusive, vehicle water Concentration: Induction: Intradermal: 1-5% and 1-5% in FCA	Sensitizing Test results: 24h after challenge: 15/15 (score: 1/4, 2/10, 1/3) 48h after challenge: 14/15 (score: 1/13, 2/1, 0/1) Neg. control: 24h after challenge: 1/3 (dermal irritation, score 2: moderate and confluent erythema)	Unpublished study report (1984) Klimisch 2 Supporting study TSCA GLP Test protocol prior to OECD adoption but followed the Magnusson and Kligman test design; Intradermal induction dose not stated. Authors concluded based on the high response “extreme”

Study/Method	Results	Reference /Remarks
Epicutaneous (day 7): 1% Challenge (day 21): 5% Rechallenge (day 28): 5%	48h after challenge: 0/3 Pos. control: dinitrochlorobenzene	sensitisation potential. Pretesting indicated that 5% did not produce dermal irritation. Challenge duration 48 hours deviating from TG 24 hours.
Buehler test Guinea pig, Hartley, 10 m/ 10 f Test material: DMAPA (liquid) Induction: epicutaneous, occlusive Challenge: epicutaneous, occlusive Induction: Epicutanous: 10% in 80% ethanol, 3 times for 6 h once a week Challenge (day 29 and 37): 3% and 1% (rechallenge), vehicle acetone Evaluation after 24h and 48h	Sensitizing Test results: 24h after challenge: 11/20, score 1.7 48h after challenge: 7/20, score 1.3 24h after rechallenge: 4/20 48h after rechallenge: 8/20 Neg. control (3%/1%): yes Skin irritation was observed in the vehicle control at challenge Pos. control: yes	ECHA dissemination website citing unpublished study report (1992) Klimisch 1 (rated by the registrant(s)) Supporting study GLP 55% were responding at 10% topical induction dose. Challenge: Severity score 1.7/1.3 after 24/48 h Rechallenge: Severity score 0.6/0.8 after 24/48 h According to TG challenge exposure should be the highest non-irritant dose.
Prior to an OECD TG, but follow the principles of the later TG Guinea pig, 10 m/f Test material: DMAPA Induction: epicutaneous, open, vehicle den. ethanol Induction: Epicutaneous: 1x50% and 9x10% Challenge: Day 13: 1% epicutaneous	Test results: 1st reading: 10/10 sanguine scabs, scarring, one animal died two days after application 2nd reading (after re-exposure of 1%):0/9 1% DMAPA in ethanol provoked no skin reactions: 0/3	Unpublished study report (1962) Klimisch 3 Supporting study No GLP No positive controls reported. Severe clinical signs including one death. Sever skin reactions reported, purity not stated

Several animal studies confirm the sensitising properties of the Substance. The animal studies reported in Table 7.9.3-1 represent guideline studies as well as studies based on testing principles that are similar to current test guidelines for skin sensitisation. According to the CLP Regulation (EC) No 1272/2008 the LLNA (OECD 429), GPMT and Buehler test (OECD 406) can be used for classification as well as sub-categorisation of skin sensitisation.

In summary four *in vitro* studies, one LLNA study, three guinea pig maximisation tests (GPMT), one Buehler test and one old protocol dated 1962 with limited reliability were available and reported in the registration data.

Rubisco et al. (2016) investigated the Substance in three *in vitro* tests (prior to OECD TG adoption) comprising of DPRA, KeratinoSens™ and h-CLAT indicative of 3 important key events in an Adverse Outcome Pathway for skin sensitisation. The results of the three non-animal test methods are used in a 2 out of 3 weight of evidence (WoE) integrated testing strategy (ITS) approach. The Substance was identified as skin sensitizer as it was positive in h-CLAT and KeratinoSens™.

In another *in vitro* study by Cottrez and co-workers the SENS-IS protocol, based on overexpression of genes involved in sensibilisation reactions, was followed. The Substance tested positive in this *in vitro* assay, however the protocol is not yet peer-reviewed nor adopted under OECD (Cottrez et al. 2016).

One LLNA, currently regarded as the preferred animal test for predicting skin sensitisation, is available for the Substance. The study was conducted as being equivalent to OECD TG 429 (but no GLP, Klimisch 2) and investigated the effects of vehicles. The reported EC3 values in the LLNA range between 1.7% and >10% in seven tested vehicles (Wright et al. 2001). OECD TG 429 recommended following vehicles in order of preference: acetone/olive oil (4:1 v/v) (AOO), dimethylformamide (DMF), methyl ethyl ketone (MEK), propylene glycol (PEG) and dimethyl sulphoxide (DMSO). The lowest EC3 value obtained with the Substance was 1.7% in DMF, other EC3 values in AOO, MEK, PG, DMSO were 2.2%, 1.8%, >10% and 3.2%, respectively. The study demonstrates that the choice of vehicles influence the relative potency of the Substance. While the results for the vehicle DMF and MEK qualify the Substance for sub-category 1A based on the EC3 values 1.7% and 1.8% (≤2% according to Table 3.4.3 of the CLP Regulation (EC) No 1272/2008) the EC3 values for AOO, DMSO and PEG were >2%.

In an unpublished study report (1984; GLP) following the Magnusson & Kligman GPMT test design a high sensitization response was observed at a high topical induction dose of 5%, but due to lack of reporting of the intradermal induction dose (only a range between 1% and 5% was stated) this test cannot be used for sub-categorisation according to CLP (unpublished study report, 1984). In a second GLP compliant guinea pig maximisation test according to OECD 406, summarized at the ECHA dissemination Website (citing an unpublished study report, 1986), no sensitization with an intradermal induction dose of 0.25% was reported. However, local irritation was induced and additional sodium lauryl sulphate was applied prior to topical induction of 5% of the Substance. The OECD TG recommends this procedure only for non-skin irritating test item. 1 % of the Substance in water was applied in the challenge phase on day 22, no rechallenge was performed.

The third GPMT according to EPA OPPTS 870.2600 (skin sensitisation) and GLP showed >60% response in test animals after an intradermal induction dose of 0.1% at rechallenge at the 48-hour observation period. In this assay a high number of responses were only seen after rechallenge (unpublished study report, 2000b). The reason for an increased sensitization rate at rechallenge is not fully understood according to Franklin et al. (1996), but a booster effect caused by the challenge stimulus may enhance an already established immunological memory arising from the induction. There were no positive skin reactions in the rechallenge phase in control animals. If the results of the rechallenge are considered, assignment to sub-category 1A (≥30% responding at ≤1% intradermal induction dose

according to Table 3.4.3 of the CLP Regulation (EC) No 1272/2008) would be justified according to this study (unpublished study report, 2000b).

In a GLP conform Buehler assay (unpublished study report, 1992) sensitisation for DMAPA was observed for a 10% induction concentration in ethanol to 55% responding. According to Table 3.4.4 of the CLP Regulation (EC) No 1272/2008 this would qualify for sub-categorisation 1B (≥30% to <60% responding at 0.2% to ≤20% topical induction dose).

Conclusion in vitro and animal data:

The reported *in vitro* and animal studies are relevant in terms of classification and generally confirm the sensitising properties of the Substance except one GPMT study. Though in this GPMT study the intradermal induction concentration was higher compared to a positive GPMT (topical induction and challenge dose were the same) the difference might be explained by the absence of a rechallenge phase.

Concerning potency considerations the reliable and robust LLNA is given the highest weight indicating strong to moderate potency depending on the vehicle. The results for the guideline recommended vehicles DMF and MEK with EC3 values of 1.7% and 1.8%, respectively, qualify the Substance for sub-category 1A (cf. Table 3.4.3 of the CLP Regulation (EC) No 1272/2008).

A reliable and GLP compliant GMPT test indicates also high potency, however, the number of positive responses occurred only after rechallenge with 30% of the animals responding after 24h and 45% after 48h observations. These results would be supportive to classify the Substance in sub-category 1A (≥30% responding at ≤1% intradermal induction dose according to Table 3.4.3 of the CLP Regulation (EC) No 1272/2008).

The third test design (Buehler assay) indicates a moderate potency, however the 55% responding animals were close to the cut-off of 60% for sub-category 1A (cf. Table 3.4.4 of Regulation (EC) No 1272/2008 criteria ≥30% to <60% responding at 0.2% to ≤20% topical induction dose).

Human data

As a third line of evidence human data including patch tests with the Substance involving several thousand dermatitis patients from dermatological clinics in several countries in Asia, Europe and North America are available as summarized in Table 7.9.3-2.

Diagnostic patch test data are generally seen as the primary source of clinical information on the occurrence of skin sensitisation and are considered to represent the most robust and important human data for classification.

Table 7.9.3-2: Human data on the sensitizing property of the Substance

Type of data	Test substance	Relevant study information *(as applicable)*	Observation	Reference
Patch tests, consecutive (unselected) patients				
Patch test data, consecutive patients	DMAPA 1% aq. (supplement to the European standard series, cocamidoproylbetaine (CAPB) and oleamidoproypl dimethylamine (OPD), 0.5% aq. with 0.12% DMAPA impurity)	285 dermatitis patients, data obtained prior to 1995. In addition CAPB and OPD were tested at the Department of Dermatology, University Bari and ISPE[9], Milano, Italy.	**8 %** were tested positive for DMAPA. OPD and CAPB positive patients were also tested positive to DMAPA.	Foti et al. (1995)
Patch test data, consecutive patients	DMAPA 1% aq. (Societa Italiana Dermatologia Allergolocia, Professionale e Ambientale (SIDAPA) test series)	5140 patients, mean age 47.9 (age range 4 to 93 years), data obtained in 2018 from 11 clinics in Italy	**1.3 %** were tested positive (31 m/37 f, mean age 47.5, range 9-81); no occupational cases; clinical relevance was observed in 55 of the 68 DMAPA-positive patients and were related to repeat use of skin cleaners containing betaines.	Foti et al. (2020)
Patch test data, consecutive patients	DMAPA in 1% aq. (included in North American contact Dermatitis Group (NACDG) screening series and other surfactants: CAPB, OPD, AA)	10 877 patients with suspected allergic contact dermatitis, tested between 2009 and 2014, retrospective analysis; University of Louisville School of Medicine, KY, U.S.	**1.7 %** (189 patients) tested positive (1+reactions), 14 patients equivocal, 22 patients irritation. Significant overlap for positive responses for CAPB, (amidoamine) AA, and OPD	Fowler et al. (2015)
Patch test data, consecutive patients	DMAPA, 1% aq. (in addition CAPB purified (Tegobetaine F50) 1% aq. And European standard series)	429 patients 429 with suspected contact allergy, tested between 2005 and 2006; (age range 9 to 81 years) in the Department of Dermatology, Peking University Third Hospital, China	**2.3 %** (10/429) tested positive. Of those, 8 were relevant. 6 patients also reacted to CAPB.	Li (2007)

[9] Institute of Skin and Product Evaluation

Type of data	Test substance	Relevant study information *(as applicable)*	Observation	Reference
Patch tests, pre-selected CAPB+ consecutive patients				
Patch test, pre-selected/ un-selected patients	DMAPA 1% aq. (CAPB 1% aq., commercial grade: 0.3% active ingredient, CAPD purer grade in addition to European standard series)	Study of 30 pre-selected CAPB 1% aq. Positive patients. Study group of 1200 eczematous patients; 46 (3.8%) tested positive to CAPB; 30 patients underwent subsequent testing for DMAPA. Department of Dermatology, University Bari and ISPE, Milano, Italy	DMAPA: 100% tested positive of pre-selected CAPB+ (30/30), all relevant (or **2.5%** consecutive eczematous patients patients) CAPB 0.5% aq. Or 1% aq. Of purer grade test in the pre-selected patient group gave 10% and 53% positive responses, respectively.	Angelini et al. (1995)
Patch test, pre-selected/ unselected patients	DMAPA 0.05%, 0.1% and 1% in pet.	Study of 12 selected patients (out of 46 positive patients reacted to CAPB 1% aq. Out of 1190 consecutive eczematous patients). Department of Dermatology, University of Milan, Division of Dermatology, General Hospital, Benevento, Italy	DMAPA: 100% of CAPB positive tested (12/12). (or prevalence of **1%** consecutive patients) 9 positive tested patients had + or ++ reactions to DMAPA already at 0.05%.	Pigatto et al. (1995)
Patch tests, selected occupational patients				
Patch test data, selective patients	DMAPA in 1% pet. Two different purchases tested (and other coconut fatty acid derivatives)	1092 patients suspected of having occupational skin diseases tested at the Finnish Institute of Occupational Health between 2002 and 2009.	11/1092 tested positive (1.01 %) Table 3 in the publication indicated 0.7% and 0.5% for the different sources. (11% (121/1092) showed an irritant reaction based on the DMAPA patch test)	Suuronen et al. 2012
Patch test data, selective patients,	DMAPA 1% aq. (36 hairdresser) DMPA 1% pet.	79 hairdresser with dermatitis patch tested results from the hairdresser	0% DMAPA positive: 0/36 and 0/79	Uter (1999)

Type of data	Test substance	Relevant study information *(as applicable)*	Observation	Reference
hairdresser	(79 hairdresser) hairdresser's series including CAPB 1% aq. With <15 ppm DMAPA	series (1996 to 1999) of the Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, Germany.	DMAPA purity >98% 7 patients showed positive patch test results to CAPB	
Patch tests, selected patients				
Patch test, selected patients with difficult-to-treat atopic dermatitis (AD)	DMAPA in 1% pet or aq. From patch test series	Retrospective analysis of 190 patients with a clinical diagnosis of AD. Population group tested with DMAPA: 42 patients (children and adults) from 2012 to 2015. Department of Dermatology, University Medical Centre of Amsterdam, The Netherlands	17% (7/42) tested positive: 18% (5/28) children and 14% (2/14) adults	Boonstra et al. (2018)
Patch test, selected patients	DMAPA (0.1% pet.) (amidoamine 0.1% aq.)	Study on 14 previously positive CAPB patch tested patients that underwent a provocation use test with CAPB containing products for 6 wk. Thereafter 9 patients were patch tested with DMAPA at the Family & Occupational Dermatology, KY, U.S.	0/9 tested positive for DMAPA 6/9 positive to amidoamine (AA)	Fowler et al. (1997)
Patch test, selected patients	DMAPA 1%, 0.1%, 0.01%, 0.001% aq. And/or co-exposure with sodium lauryl sulfate (SLS) 0.2% or CAPB 1%.	6 patients with positive patch test with CAPB (Tegobetaine L7™) were subsequently tested with DMAPA. St. John's Institute of Dermatology, St Thomas's Hospital, London, UK	1/6 patient tested positive DMAPA at 1% Both SLS and CAPB increased the number to 3 and 4 positive tested patients and onset of reactions at lower DMAPA concentrations (0.01% and 0.1% for SLS and CAPB.	McFadden et al. (2001)

Type of data	**Test substance**	**Relevant study information** *(as applicable)*	**Observation**	**Reference**
Patch testing, selected patients	DMAPA 1% aq. Purified amidoamine (AA) 0.1%, 0.25% and 0.5% aq.	10 patients with contact allergy to CAPB 1% aq. (FIRMA) were selected and patch tested with DMAPA and AA at the Unit of Dermatology, University Bari and ISPE, Milano, Italy	10/10 DMAPA positive 4/10 AA 0.1% positive 10/10 AA 0.5% and 0.25% positive AA may trigger a cross-reaction to DMAPA via enzymatic hydrolysis according to the authors	Foti et al. (2003)
Patch test, selected patients	DMAPA 1% aq. Patch tested also 5 other surfactants included in the NACDG screening series	47 previously patch tested surfactant-positive patients were selected and tested for DMAPA. Department of Dermatology, University of Minnesota Medical School; Occupational and Contact Dermatitis Clinic, MN, U.S.	DMAPA tested positive in 34% of the patients (mild to moderate reactions). Co-reactivity among certain surfactants (CAPB, AA, OPD) and DMAPA were observed	Grey et al. (2016)
Case reports				
Patch test data, single patient	DMAPA 1% pet., purity >99%	1 patient, Non-atopic woman, suspected occupational hand dermatitis Patch test with modified European standard series, series of antimicrobials and cosmetics.	Only DMAPA tested positive (except known nickel allergy)	Kanerva et al. (1996)
Patch test data, single patient	DMAPA 1% aq. And DMAPA 1% pet.	1 patient, non-atopic dermatitis, suspected caused by shampoos (but not traced and analysed for DMAPA)	Positive reaction DMAPA 1% aq. And equivocal reaction for DMAPA 1% pet.	Uter (1999)
Patch test data, single patient	DMAPA 1% aq.	1 patient (woman) with a 4-month history of severe recalcitrant eyelid	Positive	Knopp and Watsky

Type of data	Test substance	Relevant study information *(as applicable)*	Observation	Reference
		dermatitis		(2008)
Patch test data, single patient	European stand series including ethylenediamine (0.2% pet.) Subsequent testing with serval amines including DMAPA 1% pet.	1 patient (production foreman in a CAPB manufacturing plant developed itchy face and swelling of parts of torso)	DMAPA and EDA tested positive	Speight et al. (1993)
Patch test data, single patient	Chemicals used at work including ethanolamines and DMAPA 0.1% and 1% pet.	1 patient (process operator in a chemical factory including CAPB manufacturing with complains of red scaly face and right palm.	DMAPA tested positive at 1% pet.	Speight et al. (1993)

In summary 6 diagnostic patch test studies, 7 other patch test studies, and 4 case studies were identified for the Substance (cf. Table 7.9.3-2). Of the 6 diagnostic patch test studies in 2 studies with consecutive patients a pre-selection of CABP positive patients due to probably the Substance impurities took place.

In a medical setting for diagnosis of allergic contact dermatitis, also clinical relevance is important. According to a simple definition of relevance according to Lachapelle and Maibach (2009) a positive patch test reaction is “relevant” if the allergen is traced. However, while important for clinical diagnosis the CLP guidance does not relate prevalence to clinical relevance. However, according to the guidance, the evaluation of human data, should be balanced with respect of exposure against the clinical evidence regarding the frequency of skin sensitisation (ECHA, 2017a).

For diagnostic testing of contact allergy in humans, the Substance is used e.g., in the SIDAPA (Societa Italiana Dermatologia Allergolocia, Professionale e Ambientale) baseline series. SIDAPA contains the Substance 1% aqueous (aq.). The Substance is also included in other patch test series such as the American Core Series AC-1000, Epoxy Series E-1000 or Cosmetic Series C-1000 or has been included in the NACDG screening series.

Diagnostic patch testing is the gold standard to diagnose contact allergy to a substance and is performed according to international standards by dermatologists (Johansen et al. 2015 cited in ECHA, 2017a). An important factor when assessing the prevalence (number of positive tested patients in relation to the group tested) in diagnostic patch tests is how the group of patients are defined, i.e., selected patients versus consecutive (unselected) patients. According to the ECHA (2017a) data from the testing of unselected, consecutive dermatitis patients is more standardised than testing which is undertaken on a specific patient group with a distinct diagnose or worker group (selected patients). Selected patients can also have dermatitis suspected of having contact allergy to cosmetics or special occupational groups (aimed testing).

Some studies with consecutive or selected patients investigated the role of the Substance in CAPB or OPD positive patch tested patients and there has been a debate over whether

the real allergens are the impurities the Substance and/or amidoamine. The Substance at a concentration of 1% (as used in standard patch test series) confirmed the positive finding in many (e.g. Angelini et al., 1995, Suuronen et al., 2012, Grey et al., 2016, Foti et al., 1995) but not all CAPB positive patients (e.g. Li, 2007, Uter, 1999, Fowler et al. 1997) indicating that DMAPA is an important impurity responsible for contact allergies of CAPB or coconut fatty acid derivatives. However, Suuronen et al (2012) pointed out that irritancy of all of the CAPB-related substances hamper reproducibility and interpretation. Also co-reactivity for this class of surfactants were demonstrated in several studies (e.g. Grey et al., 2016, Fowler et al. 2015). Repeated and prolonged use of surfactants can cause irritant as well as allergic contact dermatitis according to Fowler et al. (2015).

For workplace studies a prevalence of <1% in patch test studies is indicative of a **low to moderate frequency** with selected workers with known exposure or dermatitis (ECHA, 2017a). However, dermatitis patients (unselected, consecutive) **≥1.0% represents a high frequency**. "The figure of 1% for consecutive (i.e. unselected) dermatitis patients is based on the generally agreed consideration that a contact allergy frequency of ≥1% in such patients is of high concern" according to the CLP guidance (ECHA, 2017a). Another qualifier for high frequency for occurrence of skin sensitisation from human patch test data is the number of published cases ≥100 (ECHA, 2017a).

A high frequency of positive patch test reactions with DMAPA were generally seen in clinical patch tests with unselected, large patient groups: recent data from Italy indicate a high frequency of 1.3% positive reactions (SIDAPA series) in 5,140 patients (Foti et al. 2020) and a retrospective analysis of 10,877 patients between 2009 and 2014 (included in North American Standard Series tested as baseline of the North American Contact Dermatitis Group) resulted in 1.7% positives (Fowler et al., 2015). Also in China a prevalence of 2.3% of 429 consecutive patients with suspected allergic contact dermatitis tested in 2005 to 2006 indicate a high frequency. An older study dated back prior to 1995 with a smaller patient group of 285 reported 8% DMAPA positive tested in Italy (Foti et al., 1995).

A "pre-selection" of CAPB positive patch tested patients indicate 2.5% positive reactions to the Substance in 1,200 consecutive patients in Italy tested prior to 1995 (Angelini et al., 1995) which is higher than the 2020 reported prevalence of 1.3% for Italy (Foti et al., 2020) probably due to lower levels of the Substance contained in cosmetic products and CAPB nowadays. However, the prevalence reported by Angelini et al. (1995) could even be higher because "CAPB negative" but the Substance positive were missed in this study design. Another similar study from Italy with CAPB positive patch tested pre-selected patients reported a prevalence of 1% in 1,190 consecutive eczematous patients studied around the same time. In the 12 positive tested patients the Substance was applied in the patch tests at 0.05%, 0.1% and 1% (in pet.). Positive reactions in 9 patients were already seen at 0.05% (Pigatto et al., 1995).

Selected patients with difficult to treat atopic dermatitis showed a high prevalence (17%) in the study group of 42 patients (including children) tested between 2012 and 2015 (Boonstra et al., 2018). This studies indicate a high frequency (≥2% according to ECHA, 2017a) for selected dermatitis patients.

Two studies were performed in the occupational sector, one in Finland, the other in Germany studying 1,092 occupational workers with skin disease and 79 hairdresser, respectively. While none of the hairdresser tested between 1996 and 1999 showed a response to the patch test with the Substance (Uter, 1999), the Finnish study performed by FIOH indicate that 11 from 1,092 patients had allergic reactions to the Substance (1% prevalence). The later study also includes hairdresser and was performed in the period 2002 to 2009. Two different sources of the Substance were tested and some patients were tested twice. In many cases results were consistent, however severity scores for the different sources differ in four cases and positive reactions in two cases. In the table of the publication the prevalence is given with 0.7% and 0.5% for the two sources (Suuronen et al., 2012). The workplace exposure were from hair care products, hair colours and perm wave solutions for hairdressers as well as liquid soaps in the other professions. Most of the

occupational patients were engaged in much hand washing or contact with other skin irritants in their work reflected in hand eczema in all of the positive tested patients including one patient with scalp dermatitis (Suuronen et al., 2012).

The Substance was patch tested at 0.1% pet. in 9 patients after they were CAPB positive patch tested and underwent a provocation use test with CAPB containing products for 6 weeks in U.S.A. None reacted to the Substance (Fowler et al., 1997). Similar in a study performed in the UK 1 out of 6 positive patch tested CAPB patients also reacted to the Substance 1% aq. (McFadden et al., 2001). Therefore, the authors speculated that the Substance is of low importance for CAPB contact allergy, if impurities are relatively low. In addition, co-administration of SLS or CAPB increased the number of positive patients and additional the onset of responses at lower concentrations (0.1 and 0.01% DMAPA aq.) (McFadden et al. 2001). On the other hand in a study with CAPB positive patch tested patients all reacted to the Substance (10/10) as well as purified amidoamine (AA). According to the authors this provides evidence that AA may trigger a cross-reaction to the Substance via enzymatic hydrolysis (Foti et al., 2003).

Another study from U.S. tested previously surfactant positive patch tested patients with DMAPA 1% aq. The Substance tested positive in 34% of the patients. Co-reactivity among certain surfactants (CAPB, AA, OPD) and the Substance were observed in this study as well (Grey et al., 2016).

Also several case studies reported non-atopic dermatitis including scalp and eyelid in workers and non-occupational exposed patients including workers from a CAPB manufacturing plant (Knopp and Watsky, 2008, Kanerva et al., 1996, Speight et al., 1993).

Conclusion: The available human data identified are all relevant in terms of classification and confirm the sensitising properties of the Substance. The available diagnostic patch test data including large patients group, different time frames of almost three decades and different global regions (Europe, Asia and North America) are seen as important information for the skin sensitisation assessment and sub-categorisation. The three recent studies in more than 15,000 patients all indicate a high frequency (>1%) of positive allergic skin reactions (Fowler et al., 2015, Foti et al., 2020, Li, 2007). In occupational investigations with around 1,200 patients the prevalence of the Substance positive reactions was low to moderate (<1%). Variations in positive patch test frequency may be related to age, gender or region according to ECHA (2017a). Also difficulties due to skin irritating effects of the Substance in patch tests were reported (Suuronen et al., 2012).

Reported case studies confirm the general picture observed in the other patch tests with dermatitis patients. Overall, the number of reported cases of tested dermatitis patients showing positive reactions to the Substance is well above 100 reported cases (cf. Table 7.9.3-2).

These findings show a **high frequency of occurrence of sensitization** for the Substance in humans. For deciding on the appropriate sub-category considerations of exposure against the clinical evidence regarding the frequency of skin sensitisation is also important.

In addition to these patch test studies Basketter et al. (2014) developed a scheme for potency assessment for skin sensitization relative to humans with 6 categories. the Substance was assigned to category 2 “a frequent cause of contact allergy, but of less significance compared with category 1”. For this category it is assumed that regular contact with moderate concentrations is likely to sensitize perhaps 1% to 10% of those so exposed. High or low exposure and doses can enhance or decrease the sensitization rates. Human repeated insult patch test NOEL values typically fall within the range 25 to 500 µg/cm^2 for chemicals in category 2 according to Basketter et al. (2014). This assignment would correspond to sub-categorisation 1A CLP Regulation (EC) No 1272/2008 Annex I, 3.4.2.2.2.1 (human repeated insult patch test positive responses at ≤500 µg/cm^2).

Human exposure

Table 3.3 of the CLP guidance (ECHA, 2017a) provides scores representing weightings whose purpose is to enable derivation of exposure indices reflecting the relative importance of dose versus frequency of exposure for classification into sub-categories. An additive exposure index of 1-4 equates to low exposure, whereas 5 to 6 reflects high exposure according to the guidance document. Low exposure corresponds to sub-categorisation 1A and high exposure to 1B.

Table 7.9.3-3: Relatively high or low exposure according to CLP guidance (ECHA, 2017a)

Exposure data	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1% < 500 µg/cm^2 (score 0)	≥ 1% ≥ 500 µg/cm2 (score 2)
Repeated exposure	< once /daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	< 100 exposures (score 0)	≥ 100 exposures (score 2)

The following considerations are taken into account for the derivation of the additive exposure index for this substance:

Referring to the current registrations, mainly industrial and professional uses are registered. These uses cover handling of the pure and higher concentrations of the substance (>> 1%). Personal protective equipment is required, if potential human contact cannot be excluded fully, as the substance is corrosive and sensitizing. No patch studies are available for these groups of users.

Exposure to the Substance as impurity in end products

Nevertheless, significantly more people (general public and professionals) are expected to be exposed to residual amounts of the Substance remaining as impurities in end products (<< 1%) considering the use as intermediate for the manufacture of other chemicals. The Substance is used for the manufacture of Cocamidopropyl betaine (CAPB) for example and several other related amidopropyl betaines. They are zwitterions and used mainly as surfactants in cosmetics like hair shampoos. Non-cosmetic uses of CAPB cover household cleaning products, including laundry detergents, hand dishwashing liquids, and hard surface cleaners. Depending on the manufacturer, residual of the Substance can range from 0.0003% to 0.02%[10] (Moreau et al., 2004).

Several studies indicated that the found contact allergy is not caused by CAPB itself, but rather by the impurities like the Substance (Foti et al, 2003; Fowler et al, 2004; McFadden, 2001; Moreau et al, 2004). A “pre-selection” of CAPB positive patch tested patients indicate 2.5% positive reactions to the Substance in 1200 consecutive patients in Italy tested prior to 1995 (Angelini et al., 1995) which is higher than the 2020 reported prevalence of 1.3%

for Italy (Foti et al., 2020) probably due to lower levels of the Substance contained in cosmetic products and CAPB nowadays.

In a study with CAPB positive patch tested patients all reacted to the Substance (10/10) as well as to purified amidoamine (AA). According to the authors this provides evidence that AA may trigger a cross-reaction to the Substance via enzymatic hydrolysis (Foti et al., 2003). The Substance was patch tested at 0.1% pet. in 9 patients after they were CAPB positive patch tested and underwent a provocation use test with CAPB containing products for 6 weeks in U.S.A. None reacted to the Substance (Fowler et al., 1997). Similar in a study performed in the UK 1 out of 6 positive patch tested CAPB patients also reacted to the Substance 1% aq. (McFadden et al., 2001). Therefore, the authors speculated that the Substance is of low importance for CAPB contact allergy, if impurities are relatively low. In addition co-administration of SLS or CAPB increased the number of positive patients and additional the onset of responses at lower concentrations (0.1 and 0.01% DMAPA aq.) (McFadden et al., 2001).

Considering the presence of residuals of the Substance in end products (<< 1%, score 0) for general public and professionals (e.g. hairdressers, cleaning persons), daily exposure several times per day (score 2) and a high total number of exposure events per person (score 2) are anticipated leading to an exposure index of 4 in total. An additive exposure index of 1-4 equates to low exposure corresponding to sub-categorisation 1A.

Table 7.9.3-4: Derivation of additive exposure index

Exposure data	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1% (score 0)	-
Repeated exposure	-	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	-	≥ 100 exposures (score 2)
Total score Additive exposure index		4

Conclusion:

There is sufficient information available for evaluation of skin sensitisation of the Substance. For sub-categorisation the following evidence is listed in Regulation (EC) No. 1272/2008, Annex I, 3.4.2.2.2.1 concerning human data:

(a) positive responses at ≤ 500 µg/cm^2 (HRIPT, HMT — induction threshold);

(b) diagnostic patch test data where there is a relatively high and substantial incidence of reactions in a defined population in relation to relatively low exposure;

(c) other epidemiological evidence where there is a relatively high and substantial incidence of allergic contact dermatitis in relation to relatively low exposure.

A classification as Skin Sens 1A is indicated by the use of animal data and supported by human diagnostic patch test data that showed a relatively high and substantial incidence of reactions in relation to relatively low exposure (score: 4 in total).

Regarding human exposure, daily exposure to residuals of the Substance in end products (several times per day, high total number of exposure events per person) are expected leading to an exposure index of 4 in total. This assignment would correspond to sub-categorisation 1A.

Therefore, SEV has verified the concern that there is a need to specify the harmonized classification for skin sensitisation according to Regulation (EC) No. 1272/2008. Based on the available evidence classification for sub-category 1A is justified resulting in Skin Sens. 1A, H317.

7.9.4. Repeated dose toxicity

For repeated dose toxicity (RDT) two studies (28 days, 2 to 10 days) with the Substance are summarised in Table 7.9.4-1. No other studies of longer duration are available for the Substance, therefore read-across to DEAPA und EDA was proposed by the registrant(s). While read-across to DEAPA is more robust based on assumed similar toxicokinetic, data for EDA are also compiled for completeness. Please see also ANNEX: Read-across justification.

Table 7.9.4-1: Studies on repeated dose toxicity

Study/Method	Results	Remarks/ Reference
OECD TG 407 (old protocol before update in 2008) rat (Wistar Hoe: f(SPF71)) male/female 5/sex/dose; except high dose f: 10 Test substance: DMAPA Dose levels 0, 10, 50, 250 mg/kg bw/d Administration route: gavage Vehicle: water Study duration: 28 days	NOAEL: 50 mg/kg bw/d 250 mg/kg bw/d: **f**: clinical signs: decreased activity, stilted gait, swollen abdomen, respiratory sounds, gasping and panting, mostly seen in females that died intercurrently (4 animals). f: clinical chemistry: ↑ Aspartate aminotransferase (AST), ↓ total protein m: clinical signs: 1 animal irregular respiration Macroscopic and microscopic findings: 4/10 f died intercurrently: discoloration of lungs with multiple red spots and foamy content. 1 f also with a small spleen. Histopathology of 4 f: lesions included congestion of organs, pulmonary haemorrhage and oedema, consistent of cardiorespiratory failure as cause of death. 1 f exhibited marked loss of lymphatic follicles of the spleen and lymphatic sheath atrophy (probably reflecting chronic stress due to treatment).	**Unpublished study report (1996)** Klimisch 2 key study GLP No functional observations conducted in the fourth exposure week. No thyroid hormone measurements (optional) Organ weights: prostate and seminal vesicles with coagulating glands as a whole, thymus, brain: not investigated Histopathology: brain, spinal cord, eye, thymus, thyroid,

Study/Method	Results	Remarks/ Reference
	1 male with clinical signs: focal balloon degeneration of the stratum corneum of the forestomach´s squamous epithelium with granulocytic infiltration of the submucosa. All dose groups: Bw and bw gain normal in all groups No compound related effect on haematology or urine analysis (pH, volume, specific weight). In all dose groups in females ↓ uric acid, (hypouricemia) but no dose dependency, urea unchanged No compound related effects on organ weights.	prostrate and seminal vesicles with coagulating glands, vagina, urinary bladder, lymph nodes peripheral nerve, skeletal muscles and bone with bone marrow: not investigated Female rats seem to be more sensitive.
No TG 4 Rabbits, 4 cats Test substance: DMAPA Dose: 1 ml/kg bw (rabbit ~800 mg/kg), 0.8 ml/kg (cat, ~640 mg/kg) (conversion into mg/kg is based on the density d= 0.8 g/cm^3) Administration route: gavage Vehicle: water, neutralised with HCL Study duration: Rabbit: 4, 5 or 10 days Cats: 2 and 4 days	Rabbits: 3/4 died after 4 doses/days at ~800 mg/kg. clinical signs and effects: atony, atypical gait, diarrhoea; reduced feed intake, haemorrhage (stomach) 2/4: kidney impairment Cats: 1/4 died after 2 doses/days at ~640 mg/kg Clinical signs and effects: emesis, diarrhoea, reduced feed intake; Intercurrent moribund animal: atony, haemorrhagic gastritis, impairment of the kidney, no effect on blood urea	Unpublished study report (1961a) Klimisch 3 No/prior to GLP Supportive information Insufficient documentation
OECD TG 408 (1998) Rats (Sprague-Dawley rat, RjHan:SD)	NOAEL: 50 mg/kg bw/d Expressed as DMAPA (corrected for molecular weight: 102.178 g/mol / 130.23 g/mol= 0.78; $NOAEL_{DEAPA}$*0.78 = **39** mg/kg bw/day)	Unpublished study report (2016a) Klimisch 1 key study GLP

Study/Method	Results	Remarks/ Reference
10/sex/dose, additionally 5/sex/dose for control and high dose Test substance: Diethylamino-propylamine (DEAPA) Purity: >99.85% Dose levels 0, 50, 250, 750 mg/kg bw/d Administration route: 5 mL/kg/d, gavage Vehicle: solution of hydrochloric acid (pH-neutralized) Study duration: 90 days followed by a 30 day recovery period	750 mg/kg: 1 f humanely killed in wk 11 based on severe clinical signs; At necropsy enlarged spleen, lesions in stomach, red discoloration of thymus. Vacuoles in white matter of brain, in pars nervosa of pituitary gland, in renal tubules, in choroid plexus, in the spleen and GALT (Gut-associated lymphoid tissue). Lesions partly associated with stress comprised of: atrophy of the ovaries, uterus and vagina, pancreas degranulation, increased adipose tissue in the bone marrow associated with decreased cellularity of the hematopoietic cells and thymus atrophy. Clinical signs: 750 mg/kg: 5/14 f; 2/15 m: thin appearance, hunched posture, piloerection, abnormal reddish colour urine (vagina), loud breathing, dyspnoea. 250 mg/kg: loud breathing (2/10 m), red discoloration of vagina (1/10 f) 50 mg/kg: hunched posture and piloerection (1/10 f) for 2 d FOB, motor activity m+f: 750 mg/kg: ↑ mean number of horizontal movements and rearing. f: ↓ mean landing foot splay 50 and 250 mg/kg: m+f: ↑ mean number of rearing movements Body weight: 750 mg/kg: m: ↓ bw gain (stat. sign.-23%) and bw -6% not stat. sign.); f: no test-item related effect. Oestrous cycle: trend in ↑ cycle length at 250 and 750 mg/kg (not stat. sign.) Hematology: 750 mg/kg: m: ↓RBC, ↓haemoglobin+↓PCV (tendency also at ≥250 mg/kg), ↑reticulocytes, ↑neutrophils, ↓eosinophils; ↑prothrombin time (also increased in f to a lesser extent). MCV, MCH no effects; all effects reversible 750 mg/kg: f: ↑ neutrophils, ↓ eosinophils indicative of stress leukogram. Biochemistry: ↓ Na (-1% f ≥250 mg/kg, m: 750 mg/kg), ↓ Cl (-2% m at 750 mg/kg), ↑ P (m+f: 8-15% ≥250 mg/kg), ↓ mean protein and albumin (-5%), creatine (-9%) (m: 750 mg/kg), ↑ triglyceride (f: +38-58%, ≥250 mg/kg),	Range finding study included Functional observations battery at wk 13, reactivity to manipulation and different stimuli and motor activity assessed; investigation of oestrous cycle, Hematology, bone marrow, blood biochemistry. T3, T4 and TSH were not determined. pituitary gland and thyroid weight was not determined (deviation to updated TG 408) While the study authors suggested a NOAEL of 750 mg/kg in males and 250 mg/kg in females based on clinical signs of pour conditions in four females, eMSCA is of the opinion that findings from motor activity, biochemistry, histopathology in brain, organ weight changes and clinical signs justify a NOAEL of 50 mg/kg bw/d.

Study/Method	Results	Remarks/ Reference
	↑AST (m: +23% 750 mg/kg), ↑ALP m+f: +88% and +62% 750 mg/kg), uric acid not measured. Urine: 750 mg/kg: hematuria (2/10 m+f), Glucosuria (3/10 m), no consistent effect on pH or volume or specific gravity No effects on seminology or ophthalmology. Organ weights: 750 mg/kg: rel. ↑ kidney (m+f, stat. sign. and not reversible in male -17%), abs.&rel.↓ thymus (m+f, not stat. sign, but correlate with histopat.) Histopathology: 750 mg/kg: vacuoles in kidneys, brain, pars nervosa, spleen, mesenteric lymph node and GALT. Diffuse vacuoles in the pars nervosa contain vasopressin accord. to IHC[11]. In f after recovery not fully reversible; hyperkeratosis in forestomach, fully reversible after recovery; thymus, lymphoid atrophy (in 1/10 m+f –could be related to stress) 250 mg/kg: 2/10 f vacuoles in brain (choroid plexus), hyperkeratosis in forestomach	
Similar to OECD TG 408 (1998) Rats (Fischer 344) 10/sex/dose, Test substance: CAS # 333-18-6 Ethylene-diamine.2HCl (EDA) Purity: little impurities Dose levels 0, 50, 260, 1040 mg/kg bw/d Correction factor: 0.4518 for EDA	$NOAEL_{EDA}$: 23 mg/kg bw/d (rational by study author: ↑ ALT in m, ↑ MCV in f at 118 mg/kg bw/d), females more susceptible to EDA toxicity Expressed as DMAPA (corrected for molecular weight: 102.178 g/mol / 60.098 g/mol= 1.7; $NOAEL_{EDA}$*1.7= **37.4** mg/kg bw/day) Diet & water: f: ↓ reduction at high dose, ↑ at low dose (all stat. signif.) Body weight: 470 mg/kg: m+f: ↓ bw gain (-28 to -38% stat. sign.) Organ weights: 470 mg/kg: m+f: ↓ in abs. and rel. (-8% m, -7% f) liver weights; ↓ abs. heart weight, spleen: ↓ abs. m, ↑ rel. in f	Yang et al. (1983) Klimisch 2 Supportive study No GLP Spacing of top dose not optimal Deviation to OECD 408 (2018): T3, T4 and TSH were not determined. Body weight ranges were given, but no mean values, therefor max. weight variation

[11] Immunohistochemistry staining

Study/Method	Results	Remarks/ Reference
base: doses 23, 118, 470 mg/kg Administration route: **diet** Vehicle: solution of hydrochloric acid (pH-neutralized to pH~8) Study duration: 90 days	m: tracheitis m+f: pH in urine decreased m+f,liver: hypertrophy with associated karyomegaly and multinucleated hepatocyte (m:2/10, f:7/10), in females more pronounced. This lesion may be indicative for hepatotoxicity as it is seen already after 3 months (grading is lacking) Hematology: 470 mg/kg, **m**: ↓ red blood cells, ↑MCV, ↓glucose, ↑AST, ↑ALT ↑ALP; **f**: ↓ red blood cells, haematocrit, ↑ MCV, MCH, ↓glucose, ↑ ALP, ↑ AST, ↑ALT 118 mg/kg: **f** ↑MCV; **m** ↑ALT Based on comparative studies HCl does not alter the toxicity profile of EDA for the oral route according to study authors.	of 20% at study begin could not be evaluated. No functional observations performed. No epididymis weights and sperm parameters investigated. Weights of uterus, ovaries, thymus, prostate, pituitary gland and thyroid were not determined. Ophthalmoscopic examination not performed. Histopathology not reported or investigated: spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, pancreas, lungs, aorta, ovaries, uterus, cervix, vagina, testes, mammary gland, urinary bladder, lymph nodes, peripheral nerve, skeletal muscle, and bone, with bone marrow, skin and eyes. No information on prothrombin time
Similar to OECD TG 408 (1998) Rats (Fischer 344) 10/sex/dose, Test substance: CAS # 333-18-6 Ethylene-diamine.2HCl (EDA) Purity: >98%	$LOAEL_{EDA}$: 100 mg/kg bw/day (based on eye lesions) LOAEL expressed as DMAPA: 170 mg/kg bw/d Mortality: 35% at 800 mg/kg Clinical signs (immediately after dosing at 600 and 800 mg/kg): burrowing, gasping, sneering and squinting of both eyes, thin appearance, hunched posture, rough hair coat, cage bedding wetter suggestive of increased urination	Unpublished study report (1982b) Klimisch 2 Supportive study no GLP 270 and 360 mg/kg judged to be above the MTD based on mortality, clinical signs and reduced bw.

Study/Method	Results	Remarks/ Reference
Dose levels 0, 100, 200, 400, 600, 800 mg/kg bw/d Administration route: gavage Vehicle: solution of hydrochloric acid (pH-neutralized) Study duration: 90 days; 5 days/wk	Terminal bw changes: ↓ from highest to lowest doses M/F: ~-45/-19% at 800 mg/kg, -28/-12% at 600 mg/kg, -12/-5% 400 mg/kg -7/-2% at 200 mg/kg, -1/+7% (M/F) at 100 mg/kg Ophthalmoscopic results at 6 and 12 weeks: cataracts, iris injection, conjunctivitis, cloudy cornea and posterior chamber haemorrhage at 800 mg/kg; 600 mg/kg: cataracts, 600 and 400 mg/kg: retinal atrophy, loss of vascular integrity in the fundus and posterior chamber haemorrhage Histopathology: 100 mg/kg: eye: 3/10 f retina atrophy (minimal to moderate) 200 mg/kg: eye: 3/10 m retina atrophy (severe to mild) 400 mg/kg: eyes: 1 m+f haemorrhage in posterior chamber; 12th wk: retinal atrophy and loss of vascular integrity 1 m 3/10 m, 7/10 f: eye retina atrophy and/or dysplasia (severe to mild), renal tubular regeneration 600 mg/kg: eyes: 6th week: 3/10 M haemorrhage; 4/10 F cataracts, 1/10 F haemorrhage; 12th wk: haemorrhage progressed to retinal atrophy and additional cataracts in animals (except 2 males with no eye lesions), cloudy appearing lens, renal tubular degeneration, necrosis, regeneration 800 mg/kg: 100% cataracts and some animals iris infection, conjunctivitis and cloudy cornea, haemorrhage, retina atrophy, synechia, proteinaceous fluid, uterus atrophy, renal tubular degeneration/necrosis/regeneration Organ weights: increase in rel. kidney weights +12-18% at 100, 200 and 400 mg/kg f, in m +14 and 16% at 200 and 400 mg/kg/bw, respectively with increases at higher doses, no changes in rel. liver weights, changes in thymus at high dose> MTD	Deviation to OECD 408 (2018): T3, T4 and TSH were not determined. No functional observations performed. No epididymis weights and sperm parameters investigated. Weights of uterus, ovaries, prostate, pituitary gland and thyroid were not determined. Not clear which tissues were investigated in histopathology. No hematology, urinalysis and clinical chemistry investigated. Statistical method lacking, unclear if performed

In a GLP compliant 28-day study according to OECD TG 407 at oral doses (gavage) of 10, 50 and 250 mg/kg bw/day (no neutralisation) the Substance caused statistically significant hypouricemia in female Wistar rats in all dose groups (unpublished study report, 1996). Body weight gain and organ weights were unaffected by treatment, however the study protocol did not follow the most recent OECD TG 407 (dated 2008) and thus the following organs were not investigated: brain, spinal cord, eye, thymus, thyroid, prostrate and seminal vesicles with coagulating glands, vagina, urinary bladder, lymph nodes peripheral nerve, skeletal muscles and bone with bone marrow. Also functional observations were lacking in the fourth week. The NOAEL was set at 50 mg/kg bw/d (based on severe general signs of toxicity and death of animals in the next dose level). The data indicate a steep

dose response with 4/10 dead female animals at 250 mg/kg bw and almost no effects at the mid dose of 50 mg/kg bw/d (please see Table 7.9.4-1). Cardiorespiratory failure were identified as cause of death (congestion of organs, pulmonary haemorrhage and edema). One male at 250 mg/kg bw/d showed clinical signs and GI-tract effects indicative of local irritation (unpublished study report, 1996). In the reproduction/developmental toxicity screening test (cf. section 7.9.7) local effects in the GI in males (Wistar) occurred in addition to lung and kidney changes that lead also to a NOAEL of 50 mg/kg bw/d. However, systemic toxicity in females were absent up to the highest tested dose of 200 mg/kg bw/d in this study (unpublished study report, 1999).

A second non-guideline study with only a very short duration in rabbit und cats with one high dose resulted in severe local effects in the stomach and kidney (assumed also to be the cause of reported deaths) (unpublished study report, 1961a).

In a reliable 90-day gavage study according to GLP and OECD TG 408 (protocol 1998) with SD rats with the analogue DEAPA (pH-neutralized) a NOAEL of 50 mg/kg bw/d (equivalent to a $NOAEL_{DMAPA}$ = 39 mg/kg bw/day) was considered by eMSCA based on findings from motor activity, biochemistry, histopathology in brain, organ weight changes and clinical signs at the next higher dose of 250 mg/kg bw/d. Mean body weight at termination was lower for males at 750 mg/kg (-6% compared to control), but not statistically significant. No treatment related effect on mean body weight was observed for females.

For motor activity at 750 mg/kg a higher mean number of horizontal movements and rearing in males (+24% and +31%, respectively) and females (+22% and +76% compared to controls, respectively) were recorded. At 50 and 250 mg/kg bw/d between +20% and +44% of rearing movements were noted in male and female rats. In view of the magnitude of observed changes at 50 mg/kg bw/d, and mainly due to the contribution of one male and one female, effects at this dose level were not considered severe enough for NOAEL setting. Lower mean landing foot splay values were noted in females at the highest dose.

While there was no statistically significant effect on mean oestrous cycle length or mean number of cycles a trend towards an increase in mean oestrous cycle length was observed in females given 250 mg/kg bw/d or 750 mg/kg bw/d.

Mean red blood cell count, mean haemoglobin and packed cell volume were statistically significant lower at 750 mg/kg bw/d in males, the latter two parameters with a decreasing tendency also at 250 mg/kg bw/d. Reticulocytes were increased. The statistical significance of these effects was mainly due to one high dose male and values were close to minimum reported historical control data. At 750 mg/kg bw/d lower mean eosinophil and higher neutrophil counts were recorded in males and females (indicative of a stress leukogram). Prolonged prothrombin time was observed in males and to a lesser extent in some females.

When compared with mean control values, statistically significant blood biochemistry changes were observed in both sexes (cf. Table above). Changes in the markers and electrolytes of the renal function (Na+, Cl-, inorganic phosphorus, proteins and albumin) are not fully consistent, but a relationship to the histopathological findings in kidneys and/or in the pituitary gland cannot be ruled out. Also haematuria in 2/10 males and females as well as glucosuria in males were detected at the highest dose (unpublished study report, 2016a).
Relative kidney weights were increased at 750 mg/kg bw/d by +14% to +17% compared to control in females and males, respectively, which was not reversible in the treatment free period despite body weight changes recovered (a relationship of these kidney weight changes to microscopic vacuolation of renal tubules could not be established according to the study authors).

Absolute and relative thymus weights were decreased at 750 mg/kg by up to -24% (statistical significance not reached) for both sexes. This effect was considered to be related

to test item administration and/or stress and correlated with increased incidence and severity of microscopic lymphoid atrophy.

Histopathological findings in several organs were reported. At the highest dose (750 mg/kg bw/d), vacuoles were observed in kidneys (tubules and, at lesser severity, in the glomeruli), brain (choroid plexus), pars nervosa (pituitary gland), spleen, mesenteric lymph node and GALT (Gut Associated Lymphoid Tissue). The brain vacuolisation (choroid plexus) occurred also at the mid-dose (250 mg/kg bw/d) in 2/10 females indicating that the brain could be more sensitive to this effect (cf. ECHA, 2018). The vacuoles in the pars nervosa suggested to contain vasopressin based on immunohistochemical staining and this may be consistent with the enlarged neuronal ends containing an increased amount of vasopressin. Vacuoles were reversible except in 1/5 and 2/5 female in kidney and brain, respectively at the highest dose (unpublished study report, 2016a).

Two further supportive 90-day studies (dated 1982 and 1983) - similar in design but many parameters missing compared to the updated OECD TG protocol - were available with EDA (administrated as ethylenediamine-dihydrochlorid (pH neutralized) via gavage (Unpublished study report, 1982b) and in the diet (Yang et al., 1983).
For the dietary study 23, 118 and 470 mg/kg bw/d EDA-dihydrochlorid (expressed as EDA) were administered to Fischer 344 rats. At the highest dose body weight gain in male and females was statistically significantly depressed by 28% and 38%, respectively. In females water consumption was decreased at all dose levels, feed intake was increased. Absolute and relative liver weights were decreased in both sexes as well as some other organ weight changes (not always consistent or related to decreased final body weights). High dose animals also showed decreased red blood cell, females also lower haematocrit and haemoglobin values.
ALP, AST and ALT activities were elevated at the mid and/or high dose. At 470 mg/kg histopathological lesion occurred in the liver (increase in the size of hepatocytes and hepatocyte nuclei, increased variation in nuclear size and shape, and an increase in the number of multinucleate hepatocytes). Males in the high does group also had tracheitis. Urine pH was decreased, but the volume or other urinary parameters were not affected, however based on the nature of the provided information (scientific article) the study documentation is deficient and not every investigated parameter was reported. According to the study authors the substance was used as urine acidifier in human and veterinary medicine (Yang et al., 1983). A NOAEL of 23 mg/kg bw/d (expressed as EDA) was set based on treatment related effects in the mid and high dose group. Females appear to be more sensitive to EDA toxicity, especially in relation to the observed liver lesions (Yang et al., 1983).

In a reliable 90-d study with EDA-dihydrochlorid by gavage, Fisher 344 rats received 0, 100, 200, 400, 600 or 800 mg/kg bw/d (expressed as EDA) for 5 days/week Unpublished study report (1982b). The study was performed for the National Toxicology Program in U.S.A. The two highest dose levels were clearly above the MTD based on mortalities, severe clinical findings and body weight reductions. However, also at 200 mg/kg bw/d and 400 mg/kg bw/d in male body weight decrease >10%. Relative thymus weight decreased from 400 mg/kg bw/d in males and 600 mg/kg bw/d in females in presence of considerable systemic toxicity. While effects on kidneys consistent of renal tubular lesions were observed in both sexes; renal tubular regeneration occurred at a lower dose of 400 mg/kg bw/d in females. Eye was the second target organ of EDA toxicity with effects at the 100 mg/kg bw/d after 90 days, at higher doses also after 6 weeks. The lesions were described as proteinaceous fluid in the anterior and subsequent posterior chamber. Histopathology of the retina ranged from atrophy to less severe retina resetting and focal cellular losses. Also mineralized debris in the lens and distorted cells near the lenticular surface as well as irregular appearance of the lens were described. In most cases the iris was adherent to the anterior surface of the lens. No other studies performed with the Substance or DEAPA reported similar eye effects, while ophthalmological changes were not investigated in the dietary study with EDA.

In another sub-acute study (12 repeated doses on 5 days per week) kidney and lymphoid depletion occurred also at 600 mg/kg bw/d (expressed as EDA; without mortality and clinical signs) as well as reported effects on the eyes (pale) at 800 mg/kg bw/d (accompanied by severe systemic toxicity) within this short exposure regime (Unpublished study report, 1982).

Inconsistency of findings of the two studies with EDA (Yang et al., 1983 and Unpublished study report, 1982b) could be due to differences in rat strains, exposure routes (bolus/gavage versus diet) and subsequent differences in toxicokinetic and that the dietary 90-day study lacks the ophthalmological investigation. Nevertheless, also a study of shorter duration with the same rat strain confirm the EDA targets kidney and eyes by gavage (Unpublished study report, 1982b). In mice no ophthalmic effects were detected after 90-day repeated exposure of EDA (OECD, 2001).

Conclusion:

The repeated dose toxicity of the Substance was investigated in a 28-day study with a NOAEL of 50 mg/kg bw/d in Wistar rats. Compared to the updated OECD TG many parameters were not investigated. Hypouricemia was evident in all dose groups in females, probably related to alterations of hepatocellular or renal functions (decreased uric acid production or increased renal clearance). Other target organ was lung in the high dose group probably related to the corrosivity of the substance. There was a clear gender difference in susceptibility of toxic effects of the Substance with females being more sensitive. This sex difference was also observed in the 90-day studies with the source substance for the read-across DEAPA as well as EDA. These two substances fill in the data gap for longer term studies i.e., 90 days and were administered in a neutralized form (in contrast to the study of the Substance).

The study complying with most of the parameters and reporting standards of the updated OECD TG 408 guideline was performed with DEAPA in SD rats. Target organ system at lower doses was the nervous system with histopathological lesions in the brain as well as increases in rearing movements in a functional observational battery. A trend for increases in oestrous cycle length could point towards EAS modulating effects in addition to effects on the pituitary (pars nervosa, vasopressin system) observed at a higher dose. Also lesions in immune related organs were detected. The NOAEL was set at 50 mg/kg bw/d. The urinary system including kidney were affected by both substances, DEAPA and EDA with increases in organ weights and/or histopathological lesions and alteration of renal functions. Also in the OECD TG 421 screening reprotoxicity study with the Substance kidney lesions occurred in males (unpublished study report, 1999).

All three substances increased serum levels of one or more blood biochemical parameters indicative of liver injury (ALP, AST and / or ALT) but only EDA showed marked liver toxicity with decreased liver weights and histopathological alterations only after dietary administration. For EDA a dietary NOAEL of 23 mg/kg bw/d was reported. Severe ophthalmic changes in the eyes occurred at a lower dose (LOAEL 100 mg/kg bw/d) after gavage in Fischer rats, ophthalmological findings were not investigated in the dietary studies with EDA and were not seen in DEAPA or the Substance RDT studies.

These studies show that the Substance and its analogue substances can cause not only local but a multitude of systemic effects when administered in a neutralized form. However, observed adverse effects levels are above the guidance value range according to Regulation (EC) No. 1272/2008 for the purpose of classification for repeated dose toxicity.

7.9.5. Mutagenicity

The provided *in vitro* mutagenicity studies by the registrant(s) with the Substance (Ames test, *in vitro* Mammalian Cell Gene Mutation Test, *in vitro* Micronucleus Test, *in vitro*

Mammalian Chromosome Aberration) are of sufficient quality, render all negative results with respect to mutagenicity and therefore do not indicate concern.

7.9.6. Carcinogenicity

The available genotoxicity/mutagenicity studies tested with the Substance are consistently negative. No specific carcinogenicity study is available that could identify non-genotoxic carcinogens. An *in vitro* cell transformation assay according to EU Method B21 (BALB/c 3T3 cell transformation assay) was performed with the Substance. No changes in the morphological and growth properties of the investigated cells were identified (unpublished study report, 1982).

The source substance EDA administrated as dihydrochlorid in F344 rats in the diet did not show evidence for a carcinogenic potential. The NOAEL was 9 mg/kg bw/d expressed as EDA for chronic toxicity (OECD, 2001, CSR, 2017, Hermansky et al., 1999) based on a LOAEL of 45 mg/bw/d (effects on bw, erythrocyte count, haemoglobin, haematocrit, serum albumin, relative organ weight increase in liver and kidney (highest dose) and hepatocellular changes). Ophthalmoscopic examinations were not performed. In a dermal carcinogenicity study in mice with EDA there was also no evidence of carcinogenicity (OECD, 2001).

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

For effects on fertility two studies, one screening test according to OECD TG 421 with the Substance and one two-generation toxicity study (Yang et al., 1984) for EDA are available. Read-across from EDA was proposed by the registrant(s). Please see ANNEX: Read-across justification. The two studies are summarized in Table 7.9.7-1.

Table 7.9.7-1: Studies on effects on fertility

Study/Method	Results	Remarks/ Reference
OECD TG 421 (1995) rat (Wistar, Chbb:THOM) male/female Test substance: DMAPA, purity >99% 10/sex/dose Test substance: DMAPA Dose levels 0, 50, 200 mg/kg bw/d	$NOAEL_{parental, female}$: >200 mg/kg bw/d for fertility and general systemic toxicity $NOAEL_{parental, male}$: 50 mg/kg bw/d for general systemic toxicity $NOAEL_{developmental, F1}$ >200 mg/kg bw/ 200 mg/kg bw: F0, male: ↓ food consumption (-6%), ↓ bw gain (-35% stat. signif. versus control), ↓ bw lower but not statistically signif., clinical signs in 2 m (piloerection, respiratory sounds). Forestomach (1/10), lungs (3/10), kidney (2/10) lesions. No effect on bw or bw gain in F0 females	Unpublished study report (1999) Klimisch 2 GLP Remark: No systemic toxicity in female rats, whereas in the 28-d RDT study 4/10 female died at a dose of 250 mg/kg bw/d Parameters of the updated (2016) TG are missing. Thus lactation period and additional endocrine disrupter relevant endpoints were not covered (e.g. oestrous cycle, AGD, thyroid), no clinical biochemistry (T4, TSH).

Study/Method	Results	Remarks/ Reference
Administration route: gavage Vehicle: water Study duration: ~54 d Pre-mating period: 14 d	No other substance related pathological findings in investigated organs (vagina, cervix uteri, ovaries, uterus, oviducts, seminal vesicles, coagulating glands, prostate gland, pituitary gland, liver, kidney). Male and female mating index: 100%, fertility index 90% to 100%. Gestation index: 78%, 100%, 100% and 90% (0, 10, 50 and 200 mg/kg). Mean number and % of liveborn pups not affected. Viability index (0-4 d), sex ratio and pups bw showed no differences amongst groups. Unilateral microphthalmia in one high dose pup found	The highest dose level should be chosen with the aim of inducing toxic effects but not death or severe suffering according to OECD TG 421, however no systemic effects were observable in females.
Similar to OECD TG 416 rat (Fischer 344 [rat]) male/female (13 M and 26 F per dose level F0 and F1, control 26 M and 52 F) two-generation reproductive toxicity oral: feed Test substance: CAS # 333-18-6 EDA.2HCl Purity >99% Dose levels 0, 50, 150 and 500 mg/kg bw/day Exposure: for two generations (daily)	First parental generation (P0) NOAEL $_{P0\ EDA}$ = 23 mg/kg bw/d (based on: ↓ bw gain in f (-6%)) NOAEL expressed as DMAPA: 39 mg/kg bw/d Second parental generation (P1) NOAEL $_{P1\ EDA}$: 118 mg/kg bw/d (based on: ↓ bw gain (m+f), ↓ diet consumption, kidney organ weights in f, histopathology in liver) NOAEL $_{fertility}$: >226 mg/kg bw/d Effect on bw gain: 226 mg/kg: ↓ bw gain F0, F1; ↓ food consumption; F1 male only, changes stat. signif. 118 mg/kg ↓ bw gain F0 female (-5%) stat. signif. Reproductive indices comparable in all groups (fertility index, gestation index, pub survival index 4, 14, 21, live pups per litter, gestation length)	Yang et al. (1984) Klimisch 2 No information on GLP, peer review literature paper with poor reporting. Many parameters and endpoints were not assessed and/or reported as listed below. Information lacking on time-to-mating, number of implantations and corpora lutea, post-implantation loss, litter size, sex ratio, oestrus cyclicity, sexual maturation (age at vaginal opening and preputial separation, anogenital distance, pup development litter weight data, litter size. Weights of: uterus, ovaries, epididymides, prostate, seminal vesicles (+ coagulating glands) thyroid, adrenals, thymus were not reported. Not reported which tissues were investigated for histology

Study/Method	Results	Remarks/ Reference
Complete necropsies on 5 weanlings F1/F2 per dose group 10 adults/sex/dose group of F1 and on 20 rats/sex of control group; Standardization of litter size to 10 pups Pre-mating dosing duration: 100 d Correction factor: 0.4518 for EDA based dosing: 23, 118, 226 mg/kg bw/d	No effect on pups bw PND21 in F1and F2 No effect on relative liver weight in F1 adults, at 226 mg/kg: histopathological lesions 6/10 m and 10/10 f liver "pleomorphismus". Relative kidney weight: ↑ in F1 f: +6% at 118 mg/kg, +14% at 226 mg/kg Absolute liver weight: ↓ in F1 m: -9% at 226 mg/kg	(the paper stated that appr. 50 tissues were fixated) for evaluation of the endocrine, cardiovascular, respiratory, GI, reproductive, nervous and musculoskeletal and hematopoietic systems. Histopathologic changes in vagina, uterus (+ cervix), ovaries, testis, epididymis, prostate, seminal vesicles and coagulating glands lacking. Sperm numbers (testicular homogenisation resistant spermatids and cauda epididymides sperm reserve), sperm motility, sperm morphology not investigated or reported. No functional investigations performed.

In a GLP compliant reproduction/developmental toxicity screening test (OECD TG 421) according to the 1995 protocol the NOAEL of the Substance for fertility and systemic effects for females Wistar was >200 mg/kg bw/d (unpublished study report, 1999). Systemic toxicity in the F0 parental animals were confined to males at the high dose group of 200 mg/kg bw/d. At this dose level lower, but not statistically significant, body weight changes compared to control occurred. Body weight gain was statistically significantly lowered (-35%). Clinical signs consistent of piloerection and respiratory sounds occurred in two males. Gross lesions were noted in the forestomach (erosion/ulcer and thickening of wall in 1/10), lungs (atelectasis, diffuse red discoloration or large hematoma in 3/10) and kidneys (clay coloured discoloration or focal unilateral contraction in the cortex in 2/10 representing cortical scars). No general signs of toxicity in parental females were reported.

There was no difference in the mean number of implantation sites, post implantation losses or delivered pups/dam. No substance related effects on pups viability and mortality, sex ratio or pups bw. An unilateral microphthalmia was the only clinical/necropsy observations in one high dose pup. Based on historical control data (HCD) of the rat strain this finding was considered to be spontaneous in nature (unpublished study report, 1996), however no HCD were presented in the study report.

Based on the lack of indications of systemic toxicity in dams (contrary to the 28-day study, cf. section 7.9.4) and absence of HCD in the report as well as the limitations of the test design (providing only limited means of detecting post-natal manifestations of pre-natal exposure, or effects that may be induced during post-natal exposure according to the OECD TG) the results of this study cannot be interpreted as an absence of reproductive/developmental effects. This is also supported by the following statement from the OECD TG 421 itself: "This Guideline is designed to generate limited information concerning the effects of a test chemical on male and female reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It is not an alternative to, nor does it replace the existing Test Guidelines 414, 416 or 443."

In a two-generation reproduction study (protocol prior 2001, no GLP), Wistar rats were fed 0, 50, 150 or 500 mg/kg bw/d of EDA-dihydrochlorid in the diet (Yang et al., 1984). The dosing expressed as EDA was 0, 23, 118 and 226 mg/kg bw/d and started 100 days prior to cohabitation of F0 rats until weaning of F2 rats. Reproductive indexes and parameters were not affected by treatment. The only histopathological findings described were liver lesions (liver "pleomorphismus" as described in the 90-day RDT, Yang et al. 1983) at the high dose groups with F1 adults females more affected compared to male. Absolute liver weight was decreased by 9% in males, but relative weights remained in the normal range. Body weight gain was lower at the highest dose group in F0 and F1 adults, in F0 female rats slightly (-6%) reduced also already at 118 mg/kg.

Therefore, it is debatable from the limited results presented to base the NOAEL F0 on the slight reduced body weight gain in females characterised by the study authors as "minor", especially because no organ weight changes or histopathology were reported in the publication in the parental F0 generation (Yang et al., 1984). In F1 females absolute and relative kidney weights were increased by 6% at 118 and 226 mg/kg bw/d. WHO JMPR (2015) recommends as a general guidance based on coefficients of variation a rough estimate for the threshold of adversity of a toxicological effect on relative organ weights for kidneys of 15%. Full necropsies were performed but results were poorly reported, therefore it remains unclear which parameters have been investigated. Also endocrine related parameters from the most recent OECD test guideline are lacking. This adds to the considerable uncertainty of the study results and the derived NOAELs. With this caveat no effects on fertility or development have been observed in this two-generation study up to a dose of 226 mg/kg bw/d for EDA.

According to ECHA (2017a) an old existing non-guideline two-generation study may fulfil the standard information requirement or can be used in an weight of evidence assessment.

The Substance is included in the U.S. FDA inventory of food contact substances in the list of indirect additives for paper and paperboard components[12] used in food contact substances at U.S. FDA[13]. Neal-Kluever et al. (2018) assessed a number of Gen-DART (multigenerational developmental and reproductive toxicology) studies submitted to the agency for the pre-market safety assessment. The Substance was included in the assessment tested in a Gen-Dart study as number 23 in the analysis. While the publication focused on the safety assessment of food contact material for infants and did not provide individual results of the tested chemicals, a footnote in the publication indicated that the Substance did no elicit adverse effect in the Gen-DART study (Neal-Kluever et al., 2018). Also in the COSMOS database (version 2017) a few details of the multigeneration reproductive toxicity study were mentioned (dietary exposure of 0, 200, 600, 6000 ppm, test species rat, 2 generation with 2 litters each generation). No critical effect is reported. From these fragments of information no firm conclusion can be drawn, however the information is used for support of the weight of evidence.

[12] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=176.170

[13] https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives&id=DIMETHYLPROPANEDIAMINE

Development

For developmental toxicity no experimental evidence for the Substance, but two studies with the source substance DEAPA according to OECD TG 414 were available in rats and rabbits and are summarized in Table 7.9.7-2.

Table 7.9.7-2: Studies on effects for development

Study / Method	Results	Remarks / Reference
OECD TG 414 (2001) rat (Sprague-Dawley, RjHan: SD) female Test substance: DEAPA, purity >99% 24/dose Dose levels 0, 50, 250, 750 mg/kg bw/d Administration route: gavage, pH-neutralized Vehicle: water Study duration: GD6-GD20	Mortality: day 21 one pregnant female at 750 mg/kg Clinical signs: 250 and 750 mg/kg - 4/24 and 19/23 dams, respectively, showed round back, emaciated appearance, piloerection, loud breathing and/or reddish vaginal discharge (considered to be adverse at 750 mg/kg); ≥250 mg/kg: ptyalism, sneezing, chromorhinorrhea and/or dacryhorrhea **250 mg/kg**: ↓ bw gain (+5 g vs. +15 g in controls on d 6-9, stat. signif.), but returned towards control values from d 9 onwards. ↓ food consumption (d 6-9); **750 mg/kg**: ↓ bw gain (+97 g vs. +149 g in controls), ↓ bw (-12% compared to controls), all stat. signif., ↓ food consumption; ↑ enlargement and raised focus on the spleen in f; coloured focus on the stomach wall **250 and 750 mg/kg**: ↓ gravid uterus weights (-9% and -18% lower than controls). ↓ live foetuses (11.4 and 11.2 vs. 13.2 in HCD), total resorption in 1 f, ↑ mean post-implantation loss (15.5 and 20.0 vs. 3.8 in HCD); **750 mg/kg:** ↓ foetal bw, ↑ number of early resorptions (2.1 vs. 0.6 in controls, stat. sig.) **Foetal examination:** External examination: 750 mg/kg relation to test item could not be ruled out: cleft palate, short trunk, short tail and/or anal atresia together with acaudia in 2 fetus from 2 litters. Soft tissue: ↑ foetal and litter incidences of absent innominate artery at 750 mg/kg; no malformations Cartilage and skeletal examinations: 750 mg/kg: higher litter and foetal incidences of foetuses with incomplete ossification of cervical vertebra(e) centrum Skeletal malformations: absent rib(s): in foetuses of all dose groups, supernumerary lumbar vertebra(e): only in mid-dose animals	Unpublished study report (2016b) Klimisch 1 GLP Parameters investigated: numbers of corpora lutea, implantation sites, uterine scars, early and late resorptions, live and dead foetuses, bw and sex of foetuses external, soft tissue and skeletal abnormalities

Study / Method	Results	Remarks / Reference
	absent lumbar vertebra(e): in control, low-, mid-dose absent thoracic vertebra(e): at mid- and high-dose absent cervical vertebra(e): at high-dose, fused sternebra and metacarpal bone (at high dose) $NOAEL_{maternal}$: 50 mg/kg bw/d (based on clinical signs, bw, gravid uterus weight) $NOAEL_{developmental}$ 50 mg/kg bw/d Expressed as DMAPA equivalents: 39 mg/kg bw/d	Remarks: study authors set the NOAEL for embryo-foetal toxicity and teratogenicity at 50 and 250 mg/kg bw/d in relation of the marked to severe maternal toxicity at 250 and 750 mg/kg bw/d.
OECD TG 414 (2018) rabbit (KBL New Zealand White) female Test substance: DEAPA, purity >99% 24/dose Dose levels 0, 15, 50, 130 mg/kg bw/d Administration route: gavage, pH-neutralized Vehicle: water Study duration: GD6-GD28	No effect on gravid uterus weight, sex ratio, external examination. Body weight and diet: 50 and 130 mg/kg: ↓ bw gain (-12% and -19%, in overall not stat. signif.), no effect on terminal bw, slightly lower food consumption at 130 mg/kg No effects on mean number of corpora lutea, implantation sites, pre-implantation loss, live foetuses. post-implantation loss: ↑increase 2.6%, 4.2%, 6.9%, 9.3% at 0, 15, 50 and 130 mg/kg; HCD (5 studies 7.3-13.2%): not stat, signif. within HCD. cf. see Table below Mean foetal bw ↓ (-7%) at 130 mg/kg External malformations: at 130 mg/kg: 1 fetus with omphalocele At 50 mg/kg: 1 fetus with proboscis, cyclopia and mingocele (correlate with visceral malformation); 1 fetus with open eye, ectrodactylyl and umbilical hernia, 1 fetus from the same litter with umbilical hernia. Soft tissue variations: 50 and 130 mg/kg: absent brachiocephalic trunk with litter/foetal incidences of 77.3%/35.7% and 100%/39.9%, respectively (stat. signif., treatment related). Control 87%/24% and HCD 94.4%/36%. No treatment related soft tissue or skeletal malformations. Several skeletal variations based on delayed ossification and presence of cartilage. Hysterectomy see table below:	Unpublished study report (2021) Klimisch 2 GLP Rational for dose selection: Pre-test: 8/NZW/dose 0, 100, 300 or 750 mg/kg bw/d, GD 6-28 Results: 4/8 and 8/8 were euthanized for ethical reasons at 300 and 750 mg/kg bw/d At 300 mg/kg: ↓ bw gain, ↓ bw -10%, ↓ food intake, ↓gravid uterus weight -22% ↓foetal weight (-11%) Macroscopic lesions in the stomach ≥100 mg/kg, kidneys ≥ 300 mg/kg and in intestines and liver at 750 mg/kg 100, 300 mg/kg: no effect on hysterectomy, no external variations or malformations. Missing parameters of the main test: Dams: weight of the thyroid gland and histopathological assessment lacking

Study / Method	Results	Remarks / Reference
	$NOAEL_{maternal}$: > 130 mg/kg $NOAEL_{developmental}$: 130 mg/kg bw/day	Fetus: AGD lacking Maternal toxicity in the main test at 130 mg/kg was only slight as evidenced by reduced but not statistically significantly different bw gain compared to control, but no changes in bw or clinical signs.

Hysterectomy data on Day 29:

Dose level mg/kg bw/d	**0**	**15**	**50**	**130**	**HCD**
Pregnant females at hysterectomy	23	21	22	23	103
Total number of live foetuses	219	189	182*	213**	949
Mean live foetuses per female	9.5	9	8.3	9.3	8.9-9.9
Total number of resorptions+scars	6	8	16*	22**	--
Mean of resorptions+scars per female	0.3	0.4	0.7	1	--
Total number of early resorptions	4	3	10	13*	--
Mean number of early resorptions per female	0.2	0.1	0.5	0.6	--
Animals with post-implantation loss	**6**	**5**	**10**	**10**	--
Mean % post-implantation loss	**2.6**	**4.2**	**6.9**	**9.3**	**7.3-13.2**

* $p<0.05$, ** $p<0.01$

In a reliable good quality GLP study according to OECD TG 414 DEAPA was administered by gavage to SD rats at doses of 0, 50, 250 and 750 mg/kg bw/d (unpublished study report, 2016b). While adverse maternal effects (clinical signs, reduced body weights and food consumption) occurred at the highest dose, body weight gain and clinical signs were only modestly affected at 250 mg/kg bw/d. At this dose level post-implantation loss values (15.5% vs 4.6% compared to control) and lower mean number of foetuses (11.4 vs 13.5) indicate signs of developmental toxicity not considered secondary to maternal toxicity. In addition these values are outside historical control values. The post-implantation losses at the highest dose of 750 mg/kg bw/d was 20% and lower mean number of foetuses were 11.2 (which are also outside the concurrent control and HCD range).

Concerning external malformations two foetuses from 2 different litters were affected that showed also lower body weights at 750 mg/kg bw/d. Malformations include anasarca, cleft palate, short trunk (absent cervical vertebra(e)) and short tail as well as anal atresia and acaudia. Concurrent control and HCD data of 2402 foetuses of nine studies had an incidence of zero for these findings.

For soft tissue variations the incidences of absent innominate artery (3.9% and 16.7%, foetal and litter incidences respectively) were higher than the upper limit of the HCD (0.8% and 5.0%, respectively) at the 750 mg/kg bw/d. No foetal soft tissue malformations were found.

Concerning skeletal variations higher litter and foetal incidences of foetuses with incomplete ossification of the cervical vertebra(e) centrum (test-item related at 750 mg/kg/ bw/d) occurred. Skeletal malformations for various vertebra(e) and rib(s) were found. Only absence of lumbar vertebra(e) occurred in controls. The skeletal findings comprise of absent rib(s) (found in all treated foetuses), supernumerary lumbar vertebra(e) (only in mid-dose animals), absent lumbar vertebra(e) (in control, low-, mid-dose), absent thoracic vertebra(e) (at 250 and 750 mg/kg) and absent cervical vertebra(e) (only in high-dose foetuses). An overview of malformations is compiled in Table 7.9.7-3.

Table 7.9.7-3: Distribution of foetal malformations (according to unpublished study report, 2016b) Foetal (F) and litter (L) incidences (%)

Dose-level (mg/kg/day)	0	50	250	750	HCD
External					
Litters affected, n (%)	0 (0)	0 (0)	0 (0)	2 (11.1)	1 (0.5)[a]
Foetuses affected, n (%)	0 (0)	0 (0)	0 (0)	2 (0.9)	1 (0.0)[a]
Soft tissue					
Litters affected, n (%)	0 (0)	2 (9.5)	0 (0)	0 (0)	0 (0.0)[a]
Foetuses affected, n (%)	0 (0)	2 (1.6)	0 (0)	0 (0)	0 (0.0)[a]
Skeletal					
Litters affected, n (%)	1 (4.2)	1 (4.8)	3 (13)	2 (11.1)	1 (0.5)[a,b]
Foetuses affected, n (%)	1 (0.6)	5 (3.6)	3 (2.1)	2 (1.8)	1 (0.1) [a,b]
Cervical vertebra(e): absent, F(L)	*0 (0)*	*0 (0)*	*0 (0)*	*0.9 (5.6)*	*0 (0)*
Thoracic vertebra(e): absent, F(L)	*0 (0)*	*0 (0)*	*0.7 (4.3)*	*0.9 (5.6)*	*0 (0)*
Lumbar vertebra(e): absent, F(L)	*0.6 (4.2)*	*3.6 (4.8)*	*0.7 (4.3)*	*0 (0)*	*0 (0)*
Lumbar vertebra(e): supernumerary, F(L)	*0 (0)*	*0 (0)*	*0.7 (4.3)*	*0.9 (5.6)*	*0 (0)*
Sternebra(e): fused, F(L)	*0 (0)*	*0 (0)*	*0 (0)*	*0.9 (5.6)*	*0 (0)*
Rib(s) :absent, F(L)	*0 (0)*	*1.4 (4.8)*	*0.7 (4.3)*	*1.8 (11.1)*	*0 (0)*
Metacarpal bone: fused, F(L)	*0 (0)*	*0 (0)*	*0 (0)*	*0.9 (5.6)*	*0 (0)*
Total					
Litters affected/evaluated (%)	1/24 (4.2)	2/21 (9.5)	3/22 (13.6)	3/18 (16.7)	nr
Foetuses affected/ evaluated (%)	1/157 (0.6)	6/126 (4.8)	3/132 (2.1)	3/102 (2.9)	nr

HCD: Historical Control Data (control data collected from nine studies covering a period ranging from March 2013 to June 2014), (a): mean incidences; (b) fused arch of thoracic vertebra(e) and fused rib(s); nr: not reported in HCD.

Based on the increased post-implantation losses, lower mean number of live foetuses and several skeletal malformations a NOAEL development of 50 mg/kg bw/d is considered justified. While some maternal toxicity was observed at 250 mg/kg bw/d a treatment-

related effect cannot be ruled out, as maternal toxicity was not severe or adverse at this dose level (unpublished study report, 2016b).

In a recent GLP compliant prenatal developmental toxicity study according to OECD TG 414 in rabbits, testing DEAPA, (Klimisch 1), no effects on mean number of corpora lutea, implantation sites, pre-implantation loss, mean number of live foetuses or early or late resorptions were seen (unpublished study report, 2021). A slight increase in the mean post-implantation loss was recorded from 50 mg/kg bw/d (not statistically significant and within the range of the HCD).

Concerning external malformations one fetus from one litter at 130 mg/kg bw/d showed omphalocele. At 50 mg/kg bw/d two litters had malformed foetuses: one fetus with proboscis, cyclopia and mingocele (correlated with visceral malformation); one fetus with open eye, ectrodactylyl and umbilical hernia, one fetus from the same litter with umbilical hernia. The study authors considered these malformations unrelated to the test item based on lack of a dose response relationship, isolated incidence and/or within the range of the HCD. However, as omphalocele and umbilical hernia can be combined as they have embryologically the same origin, the foetal (but not litter) incidences would be slightly higher (0.7%) compared to the HCD data of 0.6% (cf. Table 7.9.7-4). As this malformation is seen in the mid and high dose group in three foetuses, eMSCA is of the opinion that this abdominal wall defect might be treatment related. While omphalocele and umbilical hernia are most common malformations observed in rabbits, an analysis of 2,794 NZW rabbit litters and 20,071 foetuses revealed a litter incidence of 1.10% and a foetal incidence of 0.16% (Daston and Beekhuijzen, 2018). The presented HCD data form the current study collected from five studies between 2019 and 2020 showed a higher litter index for omphalocele, but absence of umbilical hernia. (cf. Table 7.9.7-4). Nevertheless, as the foetal index is above the HCD data and abdominal wall malformations were present in the mid and high dose group but absent in the concurrent control it could be treatment related. External malformations of the abdominal wall were not observed in rats (unpublished study report, 2016b).

Table 7.9.7-4: Distribution of external foetal malformations (according to unpublished study report, 2021) mean litter (L) and foetal indices (F) in %

Dose-level (mg/kg/day)	**0**	**15**	**50**	**130**	**HCD**
Dams with live foetuses, n	23	21	22	23	100
Live foetuses, n	219	189	182	231	949
Nose – proboscis, L (F)	0 (0)	0 (0)	4.5 (0.5)	0 (0)	0 (0)
Eyes – open eye, L (F)	0 (0)	0 (0)	4.5 (0.5)	0 (0)	4.8 (0.5)
Eye - cyclopia, L (F)	0 (0)	0 (0)	4.5 (0.5)	0 (0)	0 (0)
Paw an digit – ectrodactylyl, L (F)	0 (0)	0 (0)	4.5 (0.5)	0 (0)	0 (0)
Trunk – umbilical hernia, L (F)	0 (0)	0 (0)	**4.5 (1.1)**	**0 (0)**	**0 (0)**
Trunk- omphalocele, L (F)	0 (0)	0 (0)	**0 (0)**	**4.3 (0.5)**	**5.6 (0.6)**
Cranium - meningocele, L (F)	0 (0)	0 (0)	4.5 (0.5)	0 (0)	0 (0)
Litters affected, n (%)	0 (0)	0 (0)	2 (9.1)	1 (4.3)	7 (7)
Foetuses affected, n (%)	0 (0)	0 (0)	3 (1.6)	1 (0.5)	8 (0.8)

Concerning soft tissue variations a treatment related effect at 50 mg/kg bw/d and 130 mg/kg bw/d occurred in terms of an absent brachiocephalic trunk with litter/foetal incidences of 77.3%/35.7% and 100%/39.9%, respectively compared to control 87%/24% and HCD 94.4%/36%. The increase was statistically significant and treatment-related, but as it is a common abnormal branching variation it was not considered as adverse by the study authors. The brachiocephalic trunk (innominate artery) missing or shortened was also described for DEAPA in rats (cf. Table 7.9.7-2) as well as for EDA in rats (OECD, 2001).

Skeletal variations associated with delayed ossification occurred at the mid and high dose groups (incomplete or unossified 6th sternebra, incomplete or unossified of 1st metacarpal, incomplete or unossified 5th median phalanx, incomplete ossification of the pubis). Higher litter and/or foetal incidences of foetuses with metacarpal bone cartilage present from 50 mg/kg bw/d, median phalanx and/or pelvic girdle bone(s) cartilage present at 130 mg/kg bw/d were found. Higher litter and foetal incidences of foetuses with cartilage of cervical vertebra(e) present was also detected in rat with DEAPA (unpublished study report, 2016b).

Skeletal malformations in rabbits occurred at 15, 50 or 130 mg/kg bw/day but were of isolated occurrence like absent lumbar vertebra(e), not dose-related (nasal split, skullcap hole, branched rib, fused rib, fused sternebra(e)) or observed in controls with a similar or low incidence (parietal split, absent thoracic hemivertebra(e), misaligned caudal vertebra(e)) and/or within the range of the HCD (all malformations). The $NOAEL_{maternal}$ was set at 130 mg/kg bw/d and the $NOAEL_{developmental}$ at 130 mg/kg bw/d by the study authors (unpublished study report. 2021). However, post-implantation losses were slightly increased (not statistically significant) compared to control (but within HCD). Abdominal wall defects regarded as malformation occurred in the mid (2 foetuses) and high dose group (1 fetus) resulting in a higher foetal incidence compared to HCD and absence of this effect in the concurrent control. Therefore the $NOAEL_{developmental}$ could be lower.

Conclusion: The OECD screening test with the Substance did not reveal effects on mating and fertility, but full spermatogenesis and folliculogenesis are not covered by the test design. Moreover, dose selection was not according to the guideline as no systemic toxicity in dams were observable. In an old two-generation reproduction study with the source substance EDA-dihydrochlorid and a pre-mating period of 100 days no effects on fertility index, gestation index, pub survival index, live pups per litter or gestation length were observed. However, sperm parameters were not investigated as well as many other parameters compared to the current OECD TG 416. Published information in Neal-Kluever et al. (2018) indicate the availability of a multigenerational study with the Substance submitted to another regulatory framework in the U.S. that indicate no critical effects, however the reliability of this information could not be assessed and the study was not available.

While the lines of evidence analysed so far indicate no effects on fertility there are considerable uncertainties with regard to the lack of investigated parameters. An EOGRTS according to OECD TG 443 was undertaken recently with the second source substance DEAPA. A detailed evaluation of this study is outstanding based on the submission of the study after closure of the evaluation in July 2022.

For developmental effects two recent GLP conform PNDT studies in rats and rabbits are available with the source substance DEAPA. After gavage administration with DEAPA post-implantation losses and a lower mean number of live foetuses were evident in rats, and a slight increase in post-implantation losses could also be detected in rabbits (not statistically significant, within HCD). Skeletal treatment related malformations and the overall higher incidence of malformations (compared to controls) in rats in absence of pronounced maternal toxicity at the mid dose at 250 mg/kg bw/d indicate adverse effects on reproduction and development.

Some soft tissue variations including vessels (missing or shortened innominate artery) and skeletal variations like missing or incomplete ossification occurred consistently in rats and rabbits. Maternal toxicity in rabbits at the highest dose was only slight as evidenced by reduced but not statistically significant different body weight gain compared to control, but no changes in body weight or clinical signs were apparent.

Based on the increased post-implantation losses, lower mean number of live foetuses and several malformations concerning the skeleton a $NOAEL_{development}$ of 50 mg/kg bw/d in rat was considered. Maternal toxicity at the next higher dose of 250 mg/kg bw/d consists of a statistical significant lower body weight gain between gestation day 6 to 9 (but body weight was not affected) and some clinical signs. However, the observed developmental effects are unlikely to be solely secondary to this slight maternal toxicity.

The second PNDT study in rabbits resulted in a NOAEL of 130 mg/kg bw/d, however maternal toxicity was minimal. Also abdominal wall defects in the mid and high dose group were higher than concurrent control and HCD on a foetal, but not litter basis, when omphalocele and umbilical hernia were considered together. Thus there is some uncertainty if this malformation is treatment related and not spontaneous. A lower NOAEL would also cover soft tissue variations concerning the absence of the brachiocephalic trunk.

According to Regulation (EC) No 1272/2008, Table 3.7.1(a) substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies.

Reproductive toxicity has been reported in a reliable Klimisch 1 rated OECD TG 414 study with the source substance DEAPA. No mechanistic information is available that qualifies the observed adverse effects as non relevant to humans. Based on the increased post-implantation losses, lower mean number of live foetuses and several malformations concerning the skeleton a $NOAEL_{development}$ of 50 mg/kg bw/d for DEAPA (or 39 mg/kg bw/d expressed as the Substance) in rats was determined. Based on these results classification of DMAPA as Repr. 1B, H360D is warranted.

7.9.8. Hazard assessment of physico-chemical properties

Flash point: 30.5 °C at 1013.25 hPa.

No relevant information on explosivity, flammability and oxidising potential are available.

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

The process for deriving DNEL follows the procedure given in the REACH Guidance on Information Requirements and Chemical Safety Assessment, Chapter R.8: Characterisation of dose [concentration]–response for human health (ECHA, 2012).

According to this Guideline a DNEL for the leading health effect needs to be derived for every relevant human population and every relevant route, duration and frequency of exposure, if feasible. The derivation of DNEL for workers is based on data from studies with experimental animals.

A review of available dose descriptors for toxicity endpoint indicates that the major concern associated with **short-term exposure** to the Substance are acute oral, dermal toxicity, skin corrosion, irreversible effects on the eye, respiratory irritation and skin sensitisation.

Based on the results of the skin and eye corrosion/irritation testing in rabbits, calculation of a DNEL for short-term/acute local effects is not possible because no dose-response data are available for these effects.

The skin sensitisation potential of the Substance was evaluated by *in vitro*, GPMT, LLNA, and a Buehler assay, which have shown a high sensitising potential. In addition a robust dataset of human diagnostic patch tests that showed a relatively high and substantial incidence of reactions further supports the classification and sub-categorization as Skin Sens. 1A.

Based on the available data it is difficult to identify a DNEL for those acute effects. Therefore, a quantitative risk assessment is neither possible for skin corrosion nor for skin sensitisation. Route-to-route extrapolation might be an alternative, however only for systemic effects, not for local effects (ECHA, 2012). Thus the available data trigger a qualitative risk characterisation.

The general approach when no DNEL for these endpoints is available aims at reducing/avoiding contact with the substance. Skin corrosion and strong skin sensitizers are allocated to the high hazard category band on the basis that any measure to eliminate exposure should be considered.

Repeated exposure with the Substance or its structural analogue DEAPA induced local effects as well as alterations in the nervous, immune, renal and (neuro)endocrine system with some effects also seen in liver and the hematopoietic system. In developmental toxicity studies effects on the developing fetus were observed that warranted classification as Repr. 1B.

Overall, two effect types appear potentially relevant for determining the starting points, i.e. those dose descriptors (NOAEL/Cs, LOAEL/Cs) most relevant for setting DNEL/Cs for the Substance:

- Toxicity after repeated exposure
- Developmental toxicity

As it can be depicted from Table 7.9.9-1 all corrected human NOAEC values for the inhalation route are in the same range. Data with the Substance yielded the lowest DNEL

of 1.2 mg/m^3 for worker. Based that the 28-day study was performed with the Substance and in a non-neutralized form the DNEL could also be indicative to cover local irritant effects, however based on route-to route extrapolation this value should be interpreted with caution.

This is also supported by occupational studies performed prior to 1980 (cf. section 7.9.2). An average Substance concentration of below 0.2 ppm showed no decreased lung function changes over the work shift, while 0.9 ppm (mean exposure concentration of 0.9 ppm converted to 3.76 mg/m^3, range 0.55 -1.38 ppm) clearly indicate significant decreases in pulmonary functional tests. A concentration of 0.2 ppm (associated without effects on lung function in this study) would result in 0.84 mg/m^3, which is lower than the proposed DNEL (cf. Table 7.9.9-1).

No OEL value for the Substance is available, however for Ontario (U.S.)[14] the current Occupational Exposure Limits (OELs) under their regulations was set to 2 mg/m^3 or 0.5 ppm (time-weighted average limit).

Therefore, there are considerable uncertainties associated with the derivation of a DNEL for local effects as outlined above.

Table 7.9.9-1: DNEL derivation for DMAPA

CRITICAL DNELS					
Endpoint of concern	**Type of effect**	**Critical study(ies)**	**Corrected dose descriptor(s) (NOAEC)**	**Worker DNEL long-term**	**Remarks**
Repeated dose toxicity	Cardiovascular failure, lungs, forestomach effects	28-day study with DMAPA NOAEL of 50 mg/kg bw/d	88.2 mg/m^3 (human)	1.2 mg/m^3	AF 75
Repeated dose toxicity	Alteration of motor activity, biochemistry and histopathology in brain	90-day study with DEAPA NOAEL of 50 mg/kg bw/d $NOAEL_{DMAPA}$ 39 mg/kg bw/d	68.8 mg/m^3 (human)	2.8 mg/m^3	AF 25
Developmental toxicity	Lower live foetuses, higher post-implantation losses, incomplete ossification, absent brachiocephalic trunk, (skeletal) malformations	PNDT study with DEAPA NOAEL of 50 mg/kg bw/d $NOAEL_{DMAPA}$ 39 mg/kg bw/d	68.8 mg/m^3 (human)	1.8 mg/m^3	AF 37.5

[14] Available at: https://www.ontario.ca/page/current-occupational-exposure-limits-ontario-workplaces-under-regulation-833, 2023-01-30

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

The Substance is classifiable with regard to acute oral toxicity category 4 (H302), acute dermal toxicity category 3 (H311), irritation to the respiratory tract STOT SE 3 (H335), skin corrosion/irritation category 1 (H314), serious eye damage/irritation category 1 (H318), skin sensitisation sub-category 1A (H317) and reproductive toxicity category 1B (H360D).

7.10. Assessment of endocrine disrupting (ED) properties

7.10.1. Endocrine disruption – Environment

Not assessed.

7.10.2. Endocrine disruption - Human health

Not assessed.

7.10.3. Conclusion on endocrine disrupting properties (combined / separate)

Not assessed.

7.11. PBT and VPVB assessment

It is assumed that the substance does not fulfil the PBT or vPvB criteria according to Annex XIII of REACH as the substance is assumed to be readily biodegradable and the bioaccumulation potential is low with a log D value of -3.03 at pH 7. The T criterion is considered to be fulfilled as the substance fulfils the criteria for classification as reproductive toxicant Cat.1B according to CLP Regulation.

7.12. Exposure assessment

7.12.1. Human exposure

The Substance is used at a high tonnage per annum in the European Economic area. It is manufactured in and / or imported at ≥ 10 000 tonnes per year and used by industrial workers, professionals and consumers. The uses are considered to be widespread based on the high tonnage, the high number of uses and the wide range of different users. The uses are also expected to be wide dispersive revealing a relevant potential for human exposure. Whereas industrial workers and professionals might have better and more options for exposure/risk reduction, the same measures cannot be expected or are not available for consumer uses.

Regarding consumer use, use of fuels, lubricants and greases are registered only. Nevertheless, the Substance might be also present in lower concentrations or at residual and impurity levels in products (e.g. when the Substance is used as intermediate) leading to an even broader exposure of professionals and general public than expected based on the registered uses. The Substance is used in the preparation of some surfactants for example - such as cocamidopropyl betaine which is an ingredient in many personal care products including soaps, shampoos, and cosmetics. The presence of the Substance as an impurity in cocamidopropyl betaine is thought to be the cause of irritation experienced by

some individuals like professionals and consumers handling these products. This stresses the wide dispersive character of the substance's uses.

7.12.1.1. Exposure of workers and professionals

Regarding the self-classifications and proposed classification of the Substance, several hazards of the substance reveal no thresholds like skin corrosion, skin sensitization or respiratory irritation. The industrial and professional use concentrations of the Substance are above the concentration limits for skin corrosion and skin sensitization, irreversible eye effects and irritation of the respiratory tract in many processes. In accordance with the REACH guidance part E, table E 3.1 a qualitative assessment is required for suitable risk management measures for limiting human exposure in these cases.

Based on the qualitative assessment, the following risk management measures are introduced by the registrants.

- Use of chemically resistant gloves in combination with specific activity training is required, if dermal exposure cannot be excluded by other technical measures.
- Suitable eye protection like goggles, face shields or full face masks are required to be worn at the workplace to prevent eye exposure.
- Suitable respiratory protection like masks should be worn at the workplace whenever inhalation exposure appears likely.
- Workers are trained to prevent exposure, use risk management measures correctly and to follow the operational conditions.

In addition full skin coverage with appropriate barrier material is considered to be required based on the high hazard category identified by the eMSCA (ECHA, 2016). As the registrants consider the substance to be only skin sens 1B and not skin sens 1A, the hazard category applied in the registration dossiers is moderate.

Quantitative exposure and risk assessment

Based on the skin corrosive properties of the Substance and the use concentrations (given in the registration data) applied by industrial workers and professionals, dermal contact needs to be avoided fully by risk management measures. This might be achieved to a high degree via enclosing processes, high degree of automation, use of appropriate personal protective equipment, training and supervision. Under these circumstances, it is justified to assume no exposure via the dermal route for the quantitative assessment.
Regarding inhalation exposure, the enclosure of processes, high degree of automation, use of appropriate personal protective equipment, training and supervision are expected to result in acceptable concentrations of the Substance in air considering concentration limit for irritation of the respiratory tract. However, the lowest long-term DNEL inhalation for the worker was derived from the oral 28-day study with non-neutralized DMAPA resulting in 1.2 mg/m^3. This DNEL does not cover local effects because for these endpoints (skin corrosion, skin sensitization) and the high uncertainty for route-to-route extrapolation no quantitative estimates could be derived. Occupational studies dated back to the 1970 indicate that even concentrations below 1.2 mg/m^3 can decrease pulmonary functions and adversely affect workers health.
The registrants calculated exposure levels for the individual worker and professional uses (exposure scenarios). The predicted risk is calculated to be acceptable using the DNEL derived by the registrants and the one derived by the eMSCA based on the operational conditions and risk management measures given in the registration data.

As outlined in the ECHA (2016) guidance sensitisation is essentially systemic in nature and it is important for the purposes of risk management to acknowledge that skin sensitisation may be acquired by other routes of exposure than dermal.

As the registrants consider the substance to be only skin sens 1B and not skin sens 1A, the hazard category applied in the registration dossiers is moderate revealing the

corresponding RMM like PPE proposed. Based on the classification proposed by the eMSCA, more advanced RMM than communicated in the current registrations (ECHA, 2016) is required.

7.12.1.2. Exposure of professionals and consumers via products containing lower concentrations of the Substance

The Substance might be also present in lower concentrations or at residual and impurity levels in products (e.g. when used as intermediate). The Substance is used in the preparation of some surfactants for example, such as cocamidopropyl betaine which is an ingredient in many personal care products including soaps, shampoos, and cosmetics. The presence of the Substance as an impurity in cocamidopropyl betaine is thought to be the cause of irritation experienced by some individuals like professionals and consumers handling these products.

7.12.2. Environment

Not assessed.

7.12.3. Combined exposure assessment

Not assessed.

7.13. Risk characterisation

Not assessed.

7.14. References

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7.15. Abbreviations

AA	amidoamine
AD	atopic dermatitis
AOO	acetone: olive oil 4.1
Aq.	aqueous
CAPB	cocamidopropylbetaine
CLP	classification, labelling and packaging
CSR	Chemical Safety Report
DEAPA	3-aminopropyldiethylamine
DMAPA	3-aminopropyldimethylamine
DMSO	dimethyl sulphoxide
DMF	dimethyl formamide
DPRA	direct peptide reactivity assay
EAS	estrogen, androgen, and steroidogenic
EC	European Comission
EDA	ethylene diamine
eMSCA	evaluating Member State Competent Authority
EOGRTS	Extended One-Generation Reproductive Toxicity Study
EtOH	ethanol:water

F	foetal
f	female
FCA	Freund's Complete Adjuvant
FEV	forced respiratory volume
FOP	Functional Observational Battery
GALT	gut-associated lymphoid tissue
GD	gestation day
GEN-DART	multigenerational developmental and reproductive toxicology
GMPT	guinea pig maximisation test
h-CLAT	human Cell Line Activation Test
ISPE	Institute of Skin and Products Evaluation
ITS	integrated testing strategy
LLNA	Local lymph node assay
MAK	maximum workplace concentration
MEK	methylethylketone
MTD	maximum tolerated dose
m	male
NZW	New Zealand White
OECD	Organisation for Economic Co-operation and Development
OEL	Occupational Exposure Limit
OPD	oleamidoproypl dimethylamine
Pet.	Petrolatum
PG	polyethylene glycol
RDT	repeated dose toxicity
RTI	respiratory tract irritation
SLS	sodium lauryl sulfate
SIDAPA	SIDAPA-1000 | Italian (SIDAPA) Baseline Series
TG	Test guideline
TSCA	Toxic Substances Control Act
U.S. FDA	U.S. Food and Drug Administration
wk	week
WoE	weight of evidence

8. ANNEX: Read-across justification

In the following section the read-across has been described according to the guidance for the analogue approach (ECHA, 2008) as well as ECHA (2017b).

In the present SEV read-across using 3-aminopropyldiethylamine (DEAPA) and ethylenediamine (EDA) as source substances has been applied for the endpoints listed in the following table:

Table 8-1: Studies used for read-across

Endpoint	Source Substance	Study type and reference
Repeated dose toxicity	3-aminopropyldiethylamine (DEAPA)	90-d RDT, key study Unpublished study report (2016a). Diethylamino-propylamine. 13-week toxicity study by the oral route (gavage) in rats followed by a 6-week treatment-free period.
	ethylenediamine (EDA)	90-d RDT, supportive study Yang et al. (1983). Acute and subchronic toxicity of ethylenediamine in laboratory animals. Fundam. Appl. Toxicol. 3:512-520.
		90-d RDT, supportive study Unpublished study report (1982b). Report on prechronic studies of ethylenediamine acute, repeated dose and subchronic in rats. Battelle Contract N01 CP 95653-02 to National Toxicology Program.
Reproductive toxicity - fertility	Ethylenediamine (EDA)	Two-generation reproduction study, key study Yang et al. (1984): Two-generation reproduction study of ethylenediamine in Fischer 344 rats (publication), Fundam. Appl. Toxicol. 4:539-546.
Reproductive toxicity - development	3-aminopropyldiethylamine (DEAPA)	PNDT in rats, key study Unpublished study report (2016b). Diethylaminopropyl-amine. Prenatal developmental toxicity study By the oral route (gavage) in rats.

Endpoint	Source Substance	Study type and reference
		PNDT in rabbits, key study Unpublished study report (2021). Diethylaminopropyl-amine - Prenatal Developmental Toxicity Study by Oral Route (Gavage) in Rabbits, August 2021.

Reliability and adequacy of studies used for read-across

According to the ECHA (2008) "Guidance on information requirements and chemical safety assessment", Chapter R.6: QSARs and grouping of chemicals, the used data needs to be assessed for its adequacy. Therefore, the available experimental data have been evaluated for adequacy according to Chapter R.4 ("Evaluation of available information").

For a detailed evaluation of the available data depicted in Table 8-1 please refer to the respective endpoint(s) in this document (chapter 7.9.4 and chapter 7.9.7). The experimental studies for the analogue approach have been analysed for adequacy and reliability and are classified with Klimisch score 1 or 2.

Identity and characterisation of the source substances

The identity of the source substances is compiled in the following Table 8-2.

Table 8-2: Chemical identity of the source substances

SUBSTANCE IDENTITY		
Public name:	ethylenediamine	3-aminopropyldiethylamine
IUPAC name	ethane-1,2-diamine	N,N-diethylpropane-1,3-diamine
EC number:	203-468-6	203-236-4
CAS number:	107-15-3	104-78-9
Molecular formula:	$C_2H_8N_2$	$C_7H_{18}N_2$
Molecular weight range [g/mol]:	60.0983 g/mol	130.2312 g/mol
Synonyms:	EDA	DEAPA, 1,3-propanediamine, 3-diethylaminopropylamine
Chemical structure	 **(source:** European Chemicals Agency, http://echa.europa.eu/)	 **(source:** European Chemicals Agency, http://echa.europa.eu/)

Link of structural similarities and differences with the proposed prediction (analogue approach):

In accordance with the ECHA Guidance (Chapter R.6), substances whose physico-chemical and/or toxicological and/or ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity, may be considered as a group or "category„ of substances. The similarities may be due to a number of factors (ECHA, 2008) e.g.

- Common functional group
- Common precursor or breakdown products
- Constant pattern in changing potency
- Common constituents or chemical classes

In the present read-across the Substance and the source substances EDA and DEAPA share a common functional group: a primary or secondary amino group. DEAPA and the Substance are closely related with the difference that DEAPA has two ethyl groups and the Substance two methyl groups. The Tanimoto similarity index for DEAPA compared to the Substance is 0.93 according to the Comptox dashboard[15]. A registered substance which is more closely related with an index of 1 is N,N,N',N'-tetramethyltrimethylenediamine (CAS No. 110-95-2). However, no RDT or developmental toxicity study with this substance is available in the ECHA database (registration dossier). EDA has a Tanimoto Index of less than 0.8 and thus is structurally less similar to the target compound. Nevertheless it shares a functional group, a primary amine, with the target substance.

A stepwise approach for applying read-across is set out in Chapter R.6 section 6.2.3 "Guidance on a stepwise procedure to perform the analogue approach" (ECHA, 2008). The outcome of this stepwise approach to perform the read-across from DEAPA and EDA to the Substance for the endpoints repeated dose toxicity and reproductive toxicity is provided in this Annex.

For the standard information requirement for sub-chronic toxicity (90-day-study) a key study with DEAPA and supporting studies with EDA were used for read-across to the Substance. For reproduction and fertility a two-generation reproductive toxicity study initiated before 13 March 2015 with the source substance of EDA was used to fulfil the standard information requirement with regard to Annex X of the REACH Regulation (EC) No 1907/2006. For the endpoint development toxicity, studies in two species with DEAPA were used for read-across. Only a reproduction/developmental toxicity screening test according to OECD TG 421 (cf. chapter 7.9.7) is available with the target substance.

A key question is which differences in chemical structure between the source and target substances affect the toxicokinetic to a degree that would invalidate the read-across. The Substance, DEAPA and EDA share common functional primary and/or tertiary amino groups. Dealkylation is considered to be a prominent pathway for tertiary aliphatic amines with small alkyl groups like methyl or ethyl groups rapidly removed. Primary aliphatic amines can also undergo oxidation to form nitroso and nitro derivatives or oxidative deamination takes place. As the Substance has a tertiary and primary amine group reaction kinetics and intermediates formed qualify DEAPA and EDA as source substances. However, the Substance and DEAPA are more similar in terms of chemical structure and expected degradation pathways involving phase I and phase II reactions with assumed slight differences in the reaction kinetics. Therefore, studies with DEAPA were given more weight in the assessment.

Several common metabolites are predicted by QSAR estimates (cf. section 7.9.1), but experimental proof or robust toxicokinetic data are not available for these two substances.

[15] https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID5025102#similar-molecules

However, common functional groups do indicate also a biological similarity in effects (cf. Table 8-4). Some metabolites of the target and source substances are closely related to intermediates involved in the metabolism of polyamines that are important for many vital cell functions (Pegg, 2016).

Another factor that could influence the toxicity is structurally related and refers to the chelating properties of the compounds, especially EDA and might probably partly explain the adverse effects on the eyes seen in a study with EDA (Unpublished study report, 1982). EDA is a well-known bidentate chelating ligand with two nitrogen atoms donating their lone pairs of electrons when ethylenediamine acts as a ligand. The two nitrogen atoms of the Substance reveal the same donating potential. Independent from its different structure and stereochemistry, complexes are also reported for the Substance (e.g. with platinum, Shahabadi, N. 2009). Although no information is available on DEAPA-complexes, they cannot be excluded based on the structural similarities among these three substances and the presence of two amine groups. Some effects on hematology were observed in the RDT studies with DEAPA and EDA that could be related to Fe deficiency due to metal chelating, however, these findings were only modest (cf. section 7.9.4).

Bias that may influence the prediction

Studies with the source substances were conducted with a neutralized form (dihydrochlorid solution). However, for systemic toxicity the influence is considered to be minor (Yang et al. 1984). The 28-day study and the reproduction/developmental toxicity screening test with the Substance were performed not neutralized form which could have an influence of dose selection and observed local effects.

For EDA it is expected that for toxicokinetic, that assumes that mainly phase II reactions take place, amount and occurrence of intermediates may be more different compared to the Substance and to the second source substance DEAPA. In addition, the 90-day study with the dietary administration route of EDA showed more similar effects than gavage.

Currently endpoints such as immunotoxicity, developmental neurotoxicity or endocrine disrupting properties are not adequately assessed for none of the three substances despite the available data with DEAPA indicating a concern. However, within a compliance check with DEAPA (ECHA 2018) an Extended One-Generation Reproductive Toxicity Study (EOGRTS) has been requested, which may close the gap in the current toxicological assessment.

Hypothesis for the analogue approach

1 DEAPA and EDA used as source substance

Endpoint: Repeated dose toxicity

DEAPA displays a high structural similarity to the Substance (see Figure 8-1).

Figure 8-1: Chemical structures of DEAPA and DMAPA (source: European Chemicals Agency, http://echa.europa.eu/)

The chemicals are aliphatic amines. The structural difference is that DEAPA has two tertiary amines with ethyl groups. In the case of the Substance the alkyl moieties are methyl groups. EDA has only primary amino groups. The compounds have no isomers. The read-across approach is used for repeated dose toxicity (90-day study) (key studies) in a weight of evidence argumentation.

No species-specific mode of action for DEAPA or EDA on target organs was identified. However, pronounced gender differences were observed for all three chemicals in the RDT studies with females being more susceptible to toxic effects. One study with the Substance (OECD TG 421) identified males more sensitive compared to females, however, the pre-mating period was 14 days and dams could have different metabolism so results for females might not be fully comparable to the RDT 25-day study with the Substance.

Concerning estimates of relative toxic hazards the Substance and DEAPA are assigned to Class I (Low) according to the Cramer classification scheme of the OECD Toolbox (V 4.1). For EDA class III, high toxic hazard is predicted. The predicted metabolite 3-aminopropanal is a reactive metabolite that can target and adversely affect neuronal tissue at higher concentrations (Ivanonva et al. 2002).

A comparison of the available RDT studies is hampered by the different study durations (28-d with the target and 90-d with the source substances) as well as administration form (neutralized or not neutralized by gavage). However, all no effect values are in the same range (cf. Table 8-4: Data matrix for the analogue read across: mammalian toxicity). Upon study duration extrapolation the Substance showed a lower NOAEL compared to the structural related DEAPA. Predominant findings in the 28-day study were most likely associated with local irritating/corrosive properties of the Substance. The most relevant 90-day investigation based on a high quality updated study protocol was performed with DEAPA. A multitude of changes in organ systems below the MTD indicate that DEAPA could adversely affect the nervous, immune, renal and (neuro)endocrine system after subchronic exposure. Vacuoles were observed in kidneys, brain (choroid plexus), pars nervosa (pituitary gland), spleen, mesenteric lymph node and GALT starting from a dose level of 250 mg/kg bw/d. The NOAEL was 50 mg/kg bw (corrected for the Substance 39 mg/kg bw/d).

DEAPA and the Substance increased serum levels of one or more blood biochemical parameters indicative of liver injury (ALP, AST and/or ALT, protein) but without pronounced liver toxicity after 28 or 90 days of exposure. DEAPA also reduced red blood cells and haematocrit and prolonged prothrombin time in males. Supportive studies with EDA confirmed targets such as kidney, liver and mild hematopoietic alterations. The lowest effect value obtained for EDA was from the dietary study in rats with a NOAEL of 23 mg/kg bw/d (corrected for the Substance 37 mg/kg bw/d). Adverse eye lesions including several distortion of structures of the eye including the retina, a Fe sensitive tissue, were only seen in a study with EDA. EDA is also known for its metal chelating properties (OECD SIDS, 2001) and from the structures it is assumed that, though to a lower extent, DEAPA and the Substance could act as chelating agents as well. Depression of haematocrit and haemoglobin values was found in rats with EDA (Unpublished study report, 1982b) that could be indicative of iron deficiency (amongst other causes). However, its role in the current toxicological profile of the substances is not sufficiently investigated.

Endpoint: Toxicity to reproduction

Fertility effects

Figure 8-2: Chemical structures of DMAPA and EDA (source: European Chemicals Agency, http://echa.europa.eu/)

EDA displays structural similarity to the Substance (see Figure 8-2). For metabolism of the source chemical EDA and the target DMAPA please refer to the section above. While the studies for this endpoint with the source substance EDA indicate no effects on fertility the read-across is further strengthened by another line of evidence based on literature (Neal-Kluever et al., 2018). A summary of a Gen-DART study with the Substance supports the findings of the available animal studies indicating no effects on fertility.

However, the QSAR Toolbox V4.1 DART scheme v.1.0 gave an alert for EDA (known precedent reproductive and developmental toxic potential based on di-substituted hydrocarbons) but not for the Substance or DEAPA. Some primary amines like butylamine are known for their adverse developmental effects (OECD SIDS, 2011).

No effects on reproductive organs for the Substance, EDA or DEAPA were found in oral repeated dose toxicity studies (cf. section 7.9.4) but a trend towards an increase in mean oestrous cycle length was observed for DEAPA (unpublished study report, 2016a).

Developmental effects

DEAPA displays a high structural similarity to the Substance (see Figure 8-1). Dealkylation is a likely pathway producing metabolites that are common breakdown products of the target and source substance DEAPA.

However, experimental proof was not provided in the available data set. Nevertheless, trimethylenediamine (or 1,3-propanediamine) was estimated as likely common metabolite of DEAPA and the Substance amongst others, and the DART Scheme of the QSAR Toolbox V4.1 gave an alert for this structure (cf. Figure 8-3). Manen et al. (1983) reported that trimethylenediamine inhibits ornithine decarboxylase in pregnant mice leading to foetal growth retardation.

Figure 8-3: Chemical structure of trimethylenediamine or 1, 3-propanediamine (source: European Chemicals Agency, http://echa.europa.eu/)

1,3-propanediamine is also an intrinsic human metabolite and involved in the arginine/proline metabolic pathways and the beta-alanine metabolic pathway[16]. In the PNDT study in rats with DEAPA lower foetal body weights were reported but in presence of severe maternal effects (unpublished study report, 2016b). But also for EDA foetal weight and crown-rump length were significantly reduced at a high dose (DePass et al. 1987). However, main results of the unpublished study report (2016b) with DEAPA indicate adverse developmental effects in the species tested (rats, rabbits) warranting classification for Repr. 1B. Increased post-implantation losses, lower mean number of live foetuses and

[16] http://www.hmdb.ca/metabolites/HMDB0000002

several (skeletal) malformations lead to a $NOAEL_{development}$ of 50 mg/kg bw/d (corrected for the Substance 39 mg/kg bw/d) and are considered supportive for classification as Repr 1B (development).

Based on the available data no single mode of action can be currently established. In addition to reported or potential developmental effects for primary amines in literature such as butylamine (OECD SIDS 2011, NTP, 1993) and the observed effects for DEAPA, a tertiary amine, makes this read-across on the presence (and not absence) of adverse effects for this endpoint acceptable.

Purity / impurities

The Substance, EDA and DEAPA are mono-constituent substances. The reported purities in the relevant studies are high (above 99%).

Chemical property similarity

The Substance, EDA and DEAPA belong to the group of lower primary aliphatic amines. All three members contain two amine groups. Whereas the Substance and DEAPA have one primary and one tertiary amine group, EDA has two primary amine groups instead. The basic structures of the Substance and DEAPA are the same, but DEAPA consists of two ethyl groups instead of two methyl groups as the Substance. EDA represents the dealkylated form in this set.

Based on similar structures, functional groups and narrow molecular weight range, the physicochemical properties of these three substances are similar or range at the same order of magnitude (cf. Table 8-3). Whereas some of the properties are considered to be quite similar (e.g. water solubility, partition coefficient log K_{OW}, presence as liquids at room temperature), others follow a trend depending on the molecular weight of the substance. As all three members reveal no hydrolysable groups in their structures, hydrolysis is not expected to be relevant as degradation pathway. Nevertheless, the primary amine groups are ionisable, as indicated by the given dissociation constants.

Table 8-3: Data matrix for the analogue read-across: physico-chemical properties[1, 2, 3]

Substances	Ehylenediamine[2] (EDA)	3- Aminopropyl-dimethylamine[1] (DMAPA)	3-Aminopropyl-diethylamine[3] (DEAPA)
Read-across	Source chemical	Target chemical	Source chemical
State of the substance at 20°C and 101.3 kPa	liquid	liquid	liquid
Flash point	38-42°C	30.5°C	51.5°C
Melting point	11°C	-70°C	No information
Boiling point	117°C	135°C	170°C

Relative density	0.897 g/cm^3 (20°C)	0.8133 g/cm^3 (20°C)	0.8237 g/cm^3 (20°C)
Vapour pressure	1300 Pa at 20°C	5900 Pa at 20°C	1996 Pa at 20°C
Dissociation constant pKa:	7.23 - 7.44 (25°C) 9.7 - 10. 18 (25°C)	9.33 (25°C)	10 at 25°C (calculated)
Water solubility	1000 g/L (20°C)	1000 g/L (20°C)	1000 g/L (20°C)
Partition coefficient octanol/water	-0.352 (20°C)	-0.16 (20°C)	-0.36 (20°C)
Hydrolysis	No hydrolysable chemical structures	No hydrolysable chemical structures	No hydrolysable chemical structures
Biodegradation	Experimental result: Readily biodegradable (EU Method C.4-E)	Experimental results: Readily biodegradable (OECD 301D)	Experimental results: Readily biodegradable; (OECD 301A)

Information source: [1]https://echa.europa.eu/registration-dossier/-/registered-dossier/14823
[2]https://echa.europa.eu/registration-dossier/-/registered-dossier/15765
[3]https://echa.europa.eu/registration-dossier/-/registered-dossier/5611

Mammalian toxicological data

As depicted in Table 8-4 the Substance, DEAPA and EDA have some similar toxicological patterns with regard to mammalian toxicological endpoints.

Concerning local effects all three substances are corrosive based on a high pH value. They all share skin sensitizing properties with EDA being also harmonized classified for respiratory sensitization.

For acute oral toxicity the compounds meet the thresholds for classification for acute toxicity category 4 (oral route) with effect values being in the same range. EDA and the Substance are classified for dermal toxicity in category 3 with LD50 values around 500 mg/kg bw/d. For the Substance the LD50 values for dermal toxicity were quite variable with the lowest value in rabbits reported at ~820 mg/kg. Thus all three analogues meet the criteria for Acute Tox. 3, H311: Toxic in contact with skin.

The analogue substance EDA und DEAPA are not harmonised classified for acute inhalation toxicity according to the C&L inventory[17]. However, the registrant(s) classified EDA for acute inhalation in category 4. While a study with the Substance does not justify a classification for acute inhalation toxicity (based on study design and dosing), irritating effects on the respiratory tract were observed.

In repeated dose toxicity studies the determined effect values are in the same range for all the analogue substances, however for the Substance only a 28-day study was available indicating a lower NOAEL, if extrapolated to study duration (17 mg/kg bw/d compared to 23 or 50 mg/kg bw/day for EDA or DEAPA, respectively). Also gender differences with females more sensitive was a common finding in these studies. Oral administration of the unneutralized Substance resulted in local effects upon a 28 day dosing regime in the GI tract, lung lesions and kidney effects (OECD TG 407, OECD 421). The results of the OECD 421 screening study with the Substance confirmed the NOAEL from the 28-day study as well as GI and lung effects in male. But in this study females/dams showed no systemic toxicity. The pre-mating period was 14 days and dams could have different metabolism, so results for females might not be fully comparable. Targets of DEAPA in a 90-day rat

[17] https://echa.europa.eu/information-on-chemicals/cl-inventory-database

study are the nervous, immune, renal and (neuro)endocrine system at higher doses. Minor haematological effects were also observed.

All three substances increased serum levels of one or more blood biochemical parameters indicative of liver injury (ALP, AST and / or ALT) but only EDA showed marked liver toxicity with decreased liver weights and histopathological alterations (only after dietary administration). The lowest effect value obtained was from this dietary study with EDA in rats with a NOAEL of 23 mg/kg bw/d based on liver (enzyme) effects and some changes in hematology. Haematological parameters such as RBC, haematocrit or PT alterations were seen after in the RDT study with DEAPA, but not with the Substance, for the later only a 28-day study available. In a second study ophthalmic lesions at a LOAEL of 100 mg/kg bw/d were recorded after gavage exposure to EDA. This effects was not seen in the gavage study with DEAPA nor with the Substance.

Yang et al. (1983) reported on possible neuronal effects of EDA described in literature, however no overt signs of neurotoxicity or microscopic lesions of neuronal tissues were observed in the 90-day dietary rat study with EDA. Markers for neurotoxic effects (functional and histopathological) were detected in the 90-day study with DEAPA, clinical signs that could possibly be interpreted as signs of neurotoxicity were reported in acute toxicity studies with the Substance, however high dose levels prevent a definitive conclusion for these studies.

The renal system is also a target for all three compounds in studies with repeated doses (cf. Table 8-4) with observed changes in urinary parameters or kidney weights and histology. Different rat strains, study duration and administration form (neutralized or not neutralized) and routes might contribute to differences observed in the overall database concerning the RDT studies.

Based on in vivo studies as a follow-up for some positive findings in in vitro mutagenicity studies for DEAPA and EDA, the available experimental evidence indicate no concern for this endpoint for the Substance and its read-across substances.

The available experimental studies on reproduction performed with the Substance and EDA indicate no adverse effects on fertility (cf. Table 8-4). However, for development with DEAPA post-implantation losses and a lower mean number of live foetuses were evident in rats. Also a slight increase in post-implantation losses could be detected in rabbits (not statistical significant, within HCD). Skeletal malformations and the overall higher incidence of malformations (compared to controls) in rats in absence of pronounced maternal toxicity at the mid dose at 250 mg/kg bw/d indicate adverse effects on reproduction and development with a $NOAEL_{development}$ of 50 mg/kg bw/d.

Some soft tissue variations concerning vessels (missing or shorten innominate artery) and skeletal variations on missing or incomplete ossification occurred consistently in rats and rabbits and these effects were observed with DEAPA and EDA. The second PNDT study in rabbits with DEAPA resulted in a $NOAEL_{development}$ of 130 mg/kg bw/d, however maternal toxicity was minimal. Also abdominal wall defects in the mid and high dose group with a foetal incidence higher than concurrent control and HCD raises uncertainty if this malformation was really spontaneous in nature. In support of the above mentioned findings NTP (1993) reviewed the available developmental data on EDA and suggested “that EDA may have the potential to induce developmental toxicity (growth retardation or prenatal mortality), but not teratogenicity, at doses which also cause maternal toxicity. No evidence for developmental toxicity below the maternally toxic range was found in any of the studies...” Reduced foetal body weight was also recorded for DEAPA at adverse maternal dose level. The reported no effect values in Table 8-4 are in the same range.

Table 8-4: Data matrix for the analogue read across: mammalian toxicity

Substances	Ehylenediamine (EDA) [1]	3-aminopropyl-dimethylamine (DMAPA) [2]	3-aminopropyl-diethylamine (DEAPA) [3]
Read-across	Source chemical	Target chemical	Source chemical
Acute Toxicity: Oral	LD50 866 mg /kg (rats, OECD 401, no GLP) Acute Tox. 4, H302	LD50f = 377 mg/kg (female rats, GLP) Local effects Acute Tox. 4, H302	LD50 = ~830 mg/kg (rats, OECD 401) Acute Tox. 4, H302
Acute Toxicity: Inhalation	LC50 14.7 mg/L (vapour, 4-h exposure back calculated from 8-h, rats, no GLP) Acute Tox. 4, H332	LC50 assumed to be higher than 4.31 mg/L/4h (likely <20 mg/L/4h, vapour, rats, in-house protocol, no GLP) STOT SE 3 (lung, inhalation), H335	Inhalation risk test saturated vapour concentration/4h: No mortalities
Acute Toxicity: Dermal	LD50 >1000 mg/kg (rabbit, 16 CFR 1500.40, no GLP) LD50 >1000 mg/kg (rats, method n.r, GLP study) Acute Tox. 3, H311	LD50 >400 mg/kg and <2000 mg/kg (rats, GLP) Severe local skin effects, tremors and sedation at 1000 mg/kg bw LD50 820 mg/kg bw (rabbit, no GLP) Acute Tox. 3, H311	LD50 >1000 mg/kg (rabbit, OECD 402) LD50 = 1848 mg/kg bw (rabbit, EPA OTS 798.1100) Acute Tox. 3, H311
Skin irritation	Skin Corr. 1B, H314	Skin Corr. 1B, H314	Skin Corr. 1B, H314
Eye irritation	corrosive	Eye Dam. 1, H318	corrosive
Skin Sensitization	Skin Sens. 1, H317 Resp. Sens. 1, H334	Skin Sens. 1A, H317	Skin Sens. 1, H317
Repeated Dose Toxicity	1) NOAEL = 23 mg/kg bw/d (dietary, Fischer 344 rat, based on liver and haematological effects) no GLP, OECD TG 408 but many parameters missing **Other targets: liver, lungs (tracheitis);** more **females** showed liver effects and alterations of hematology	NOAEL = 50 mg(kg bw/d (gavage, Wistar rat, OECD 407, based on clinical signs, mortality, **lung** effects probably related to corrosivity, local effects), GLP, no effects on bw; NAOEL =50 mg/kg bw/d (gavage, Wistar rat, OECD 421) based on GI, lung and kidney effects.	NOAEL = 50 mg/kg bw/d (gavage, SD rat), GLP, OECD TG 408 with many parameters investigated, but thyroid hormone measurements lacking; **targets: nervous, renal, immune and (neuro)endocrine system** at 750 mg/kg (functional, organ weight and/or histopathological effects).

Substances	Ehylenediamine (EDA) [1]	3-aminopropyl-dimethylamine (DMAPA) [2]	3-aminopropyl-diethylamine (DEAPA) [3]
	Hematology: 470 mg/kg, m: ↓ RBC, ↑MCV, ↓glucose, ↑AST, ↑ALT (also at 118 mg/kg) ↑ALP; f: ↓ RBC, haematocrit, ↑ MCV (also at 118 mg/kg), ↓glucose, ↑ ALP, ↑ AST, ↑ALT **NOAEL $_{DMPA}$ = 37.4 mg/kg bw/day** (expressed as DMAPA) 2) LOAEL = 100 mg/kg bw/day (**gavage**, Fischer 344 rats, based on **eye effects** in females) similar to OECD TG but many parameters missing, no GLP Other targets: **kidney** (renal tubular degeneration, necrosis, regeneration at 600 mg/kg bw/d)	**Females** more susceptible in OECD 407 (but not OECD 421) 4/10 premature death in highest does group at 250 mg/kg bw/d assumed cause cardiorespiratory failure (1 f spleen and lymphatic atrophy) ⇑AST ⇓ protein at 250 mg/kg bw/d could be related to altered liver function, no effect on hematology **NOAEL extrapolated to 90 days = 17 mg/kg bw/d** (sub-acute to sub-chronic AF of 3)	Minor effects on the hematopoietic system, but PT in males prolonged. Histopathological lesions in the brain and behavioral effects at 250 mg/kg kg/bw in **females** lead to a NOAEL selection of 50 mg/kg bw/d. 750 mg/kg ⇑AST ⇑ALP, uric acid not measured, hematuria, glucosuria In males at 750 mg/kg: ↓RBC, ↓hemoglobin+↓PCV (tendency also at ≥250 mg/kg), ↑reticulocytes, ↑prothrombin time **NOAEL $_{DMAPA}$ = 39 mg/kg bw/d** (expressed as DMAPA)
Gene mutation in bacteria (in vitro)	Borderline positive result in an Ames test (TA100) Also negative results reported in another AMES study.	Negative (+/-S9) in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537. (OECD 471, no GLP, but NTP study) Negative (+/-S9) in *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538. (OECD 471, GLP study)	Negative (+/-S9) in *Salmonella typhimurium* TA 1535, TA 1537, TA 98, TA 100, (TA 1538) (OECD 471, GLP study)
Chromosomal aberration (in-vitro)	Negative human lymphocyte cultures (OECD TG 473, GLP)	Negative human lymphocyte cultures (OECD TG 473, GLP)	Negative human lymphocyte cultures (OECD TG 473, GLP)
Mammalian gene mutation (in vitro)	--	Negative mammalian cell gene mutation assay in mouse lymphoma cells (OECD 476, GLP)	Positive (-S9), small colony formation; mammalian cell gene mutation assay in mouse lymphoma cells (OECD 490, GLP)
Indicator mutagenicity tests	-Negative in unscheduled DNA Synthesis (UDS) test in rat primary hepatocytes (no GLP) - Negative in a sister chromatid exchange assay in mammalian cells (no GLP)	--	--

Substances	Ehylenediamine (EDA) [1]	3-aminopropyl-dimethylamine (DMAPA) [2]	3-aminopropyl-diethylamine (DEAPA) [3]
Genetic Toxicity in vivo	Negative Rodent dominant lethal assay- Negative *Drosophila melanogaster* sex-linked recessive lethal test	--	Negative Rat Alkaline Comet Assay (OECD 489, GLP)
Carcinogenicity	Negative NOAEL 9 mg/kg bw/d (rat, OECD 453, no GLP, diet) Targets: liver, kidney; high dose: rhinitis and tracheitis in male, reduced bw Negative (2 year, mice, dermal, 25µg 3 times/wk, 1%)	--	--
Toxicity to reproduction (oral)	$NOAEL_{fertility}$ >226 mg/kg bw/day (highest dose tested) $NOAEL_{F0}$ = 23 mg/kg bw/day (highest dose tested) Target: ↓bw, at higher doses liver, kidney (Fischer rat, no information on GLP) No fertility or developmental toxicity was reported in a two-generation study (old study) up to dose levels of 226 mg/kg bw/day $NOAEL_{fertility\ DMAPA}$ >384 mg/kg bw/d (expressed as DMAPA)	$NOAEL_{parental,\ male}$ =50 mg/kg bw/d (based on clinical signs and lesions in forestomach, lungs and kidney at 200 mg/kg). $NOAEL_{parental,\ female}$ >200 mg/kg bw/d $NOAEL_{developmental,\ F1}$ >200 mg/kg (OECD TG 421, GLP, Wistar rat)	Supporting: In the 90-day RDT study, trend for increase in estrous cycle length at 250 and 750 mg/kg bw/d (OECD TG 408, GLP, SD rat)

Substances	Ehylenediamine (EDA) [1]	3-aminopropyl-dimethylamine (DMAPA) [2]	3-aminopropyl-diethylamine (DEAPA) [3]
Developmental toxicity	$NOAEL_{maternal}$= 23 mg/kg bw/day (rats, based on bw, diet consumption, no TG) $NOAEL_{development}$ =118 mg/kg bw/d (reduced foetal weight and crown-rump lengths, slight effects on foetal morphology (e.g. shorten or missing absent brachiocephalic trunk), but no indication of teratogenicity (CSR, 2017) $NOAEL_{maternal\ and\ developmental}$: ≥80 mg/kg bw/d in rabbits (NTP 1993)	No data (read-across)	$NOAEL_{maternal}$ =50 mg/kg bw/d $NOAEL_{development}$ =50 mg/kg bw/d (OECD 414, rats, GLP) Lower live foetuses, higher post-implantation reduced weight, incomplete ossification, absent brachiocephalic trunk, (skeletal) malformations including absent rib(s). $NOAEL_{development\ DMAPA}$ =39 mg/kg bw/d (expressed as DMAPA) $NOAEL_{maternal}$: 130 mg/kg (based on transient reduced bw gain) $NOAEL_{developmental}$ 130 mg/kg bw/day but abdominal wall defects at 50 and 130 mg/kg with foetal incidence higher than control (OECD 414, rabbits, GLP)

Information source: [1]https://echa.europa.eu/registration-dossier/-/registered-dossier/15765, [2]https://echa.europa.eu/registration-dossier/-/registered-dossier/14823, CSR (2017)
[3]https://echa.europa.eu/registration-dossier/-/registered-dossier/5611