CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

2,2'-iminodiethanol;

diethanolamine

EC Number: 203-868-0

CAS Number: 111-42-2

Index Number: 603-071-00-1

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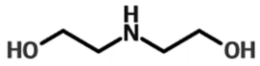
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2'-Iminodiethanol
Other names (usual name, trade name, abbreviation)	Diethanolamine DEA N,N-Diethanolamine Bis(2-hydroxyethyl)amine Bis(β-hydroxylethyl)amine 2,2'-Dihydroxydiethylamine Ethanol, 2,2'-iminobis- Di(2-hydroxyethyl)amine Di(β-hydroxyethyl)amine Diolamine Iminodiethanol
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	203-868-0
EC name (if available and appropriate)	2,2'-iminodiethanol
CAS number (if available)	111-42-2
Other identity code (if available)	-
Molecular formula	$C_4H_{11}NO_2$
Structural formula	
SMILES notation (if available)	OCCNCCO
Molecular weight or molecular weight range	105.14 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
2,2'-Iminodiethanol (CAS No. 111-42-2, EC No. 203-868-0)	100		

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
-				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-071-00-1	2,2'-iminodiethanol; diethanolamine	203-868-0	111-42-2	Acute Tox. 4 * Skin Irrit. 2 Eye Dam. 1 STOT RE 2 *	H302 H315 H318 H373 **	GHS08 GHS05 GHS07 Dgr	H302 H315 H318 H373 **	-	-	-
Dossier submitters proposal					**Add** Acute Tox. 4 Carc. 2 Repr. 1B **Modify** Acute Tox. 4 STOT RE 2	**Add** H332 H351 H360FD **Modify** H302 H373 (haematopoetic system, kidney, nervous system)	**Retain** GHS08 GHS05 GHS07 Dgr	**Add** H332 H351 H360FD **Modify** H302 H373 (haematopoetic system, kidney, nervous system)	-	**Add** oral: ATE = 1100 mg/kg bw inhalation: ATE = 2.8 mg/L (dusts or mists)	-
Resulting Annex VI entry if agreed by RAC and COM					Acute Tox. 4 Acute Tox. 4 Skin Irrit. 2 Eye Dam. 1 Carc. 2 Repr. 1B STOT RE 2	H302 H332 H315 H318 H351 H360FD H373 (haematopoetic system, kidney, nervous system)	GHS08 GHS05 GHS07 Dgr	H302 H332 H315 H318 H351 H360FD H373 (haematopoetic system, kidney, nervous system)	-	oral: ATE = 1100 mg/kg bw inhalation: ATE = 2.8 mg/L (dusts or mists)	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)		
Oxidising gases		
Gases under pressure		
Flammable liquids		
Flammable solids		
Self-reactive substances		
Pyrophoric liquids		
Pyrophoric solids		
Self-heating substances		
Substances which in contact with water emit flammable gases		
Oxidising liquids		
Oxidising solids		
Organic peroxides		
Corrosive to metals		
Acute toxicity via oral route	harmonised classification proposed	Yes
Acute toxicity via dermal route	data not sufficient for classification	
Acute toxicity via inhalation route	harmonised classification proposed	
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation		
Respiratory sensitisation		
Skin sensitisation		
Germ cell mutagenicity	data conclusive, no classification warranted	Yes
Carcinogenicity	harmonised classification proposed	
Reproductive toxicity		
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	harmonised classification proposed	Yes
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment		
Hazardous to the ozone layer		

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The current classification of 2,2-iminodiethanol (DEA) was published on the Adaptation to Technical Progress 00 (ATP 00) from the previous European Directive 67/548/CEE:

Acute Tox 4* - H332

Skin Irrit 2 - H315

Eye Dam. 1 - H318

STOT RE 2* - H373

The asterisks* results from the transfer of entries in previous legislation to the current CLP Regulation and indicates that manufacturers/importers must apply at least this minimum classification, but must apply more severe hazard category if there is available information which shows that the hazard(s) meet the criteria for classification in a more severe category.

In 2012 Substance Evaluation (SEv) of DEA was performed by the German Competent Authority. DEA was originally selected for substance evaluation in order to clarify concerns about: Human health (potential formation of CMR transformation products (suspected CMR)) and exposure (wide dispersive use, high aggregated tonnage). During the evaluation, reproductive toxicity of DEA was identified as an additional concern.

As a consequence, ECHA demanded further testing (EOGRTS) from the registrant (decision February 2014). The EOGRT study was submitted by the registrant in 2018.

Thereafter, SEv was updated based on the existing and newly generated information on DEA. The evaluating member state competent authority (German Competent Authority) considered that an update of the harmonised classification of the substance is necessary. This concerns the need to classify DEA as carcinogenic and as a reproductive toxicant, hazard classes for which DEA currently neither possesses a harmonised classification nor for which it is self-classified by the majority of C&L notifiers.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Germ Cell mutagenicity, carcinogenicity, reproductive toxicity:

There is no requirement for justification that action is needed at Community level for CMR substances.

Acute Toxicity and STOT RE

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to changes in the criteria
Change in existing entry due to new interpretation/evaluation of existing data

Further detail on need of action at Community level

DEA has a “minimum” harmonised classification for STOT RE and Acute Toxicity resulting from the transfer of the classification from Directive 67/548/EEC to the CLP Regulation (EC No 1272/2008), which is indicated in the current Annex VI entry by the asterisks at the corresponding hazard classes. The available data was thus assessed as well for these hazard classes to review appropriate hazard class categories and to remove the asterisks.

In addition, there is a new EOGRT study available delivering new data for STOT RE evaluation.

5 IDENTIFIED USES

Based on the information provided on the dissemination page[1], as of May 2021 the substance is registered at the tonnage band >1000 tpa, with an annual manufacture / or import volume in the EEA between 100 000 and 1 million tonnes. The substance has consumer uses in a range of products, such as fuels, washing and cleaning products, disinfectants, cosmetics and personal care products. It has as well a widespread use by the professional workers in building and construction work, in scientific research as a laboratory chemical,

The release in the environment can occur from outdoor use in long-life materials with high release rate such as tyres, treated textile and wooden products, brake pads in trucks or cars, sanding of buildings, bridges, facades or vehicles including ships. Release can as well occur at industrial sites due to production of the substance, formulation of its mixtures or due to use as a processing aid in the production of articles. Professional use of the substance can likely lead to the environmental release from uses of machine wash liquids and detergents, paints, coatings or adhesives, automotive care products, fragrances and air fresheners.

6 DATA SOURCES

A literature search was conducted on 8 July 2019 and on 15 March 2023 in Pubmed, Scopus, Web of Science, EMBASE, Toxnet, Science Direct and Wiley Online Library. The search string was designed to cover all toxicological endpoints and contained several known synonyms of the substance name:

Diethanolami OR Dihydroxydiethylamin* OR "2,2'-Iminodiethanol* OR "2,2'-Iminobisethanol* OR Aminodiethanol* OR Bishydroxyethylamin* OR DOLA OR Alkanolamin* OR Aminoalcohol* (CAS-Nr. 111-42-2) AND (Toxicity OR Toxicology)*

The IUCLID dossier (Dossier UUID: 2a9ef00a-fd34-4fbf-8079-14b8d1aeca20) was used to source the information on unpublished studies.

[1] https://echa.europa.eu/de/substance-information/-/substanceinfo/100.003.517 accessed on 17.05.2021

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid (waxy solid at 20°C and a syrupy liquid above 30°C)	REACH registration data	experimental result (visual inspection)
Melting/freezing point	27°C at 1013.25 hPa	REACH registration data	experimental result
Boiling point	269.9°C at 1013.25 hPa (decomposes at >200°C)	REACH registration data	experimental result
Relative density	1.1 at 20°C	REACH registration data	experimental result
Vapour pressure	0.00008553 hPa at 20°C	REACH registration data	experimental result
Surface tension	Based on chemical structure, no surface activity is predicted.	REACH registration data	estimated based on chemical structure
Water solubility	completely miscible (1000 g/L at 20°C)	REACH registration data	experimental result
Partition coefficient n-octanol/water	Log Kow: -2.46 (at 25°C; pH 6.8 - 7.3)	REACH registration data	experimental result
Dissociation constant	8.99 at 25 °C	REACH registration data	experimental result
Viscosity	dynamic viscosity: 390.9 mPa.s at 30°C 102.7 mPa.s at 50°C	REACH registration data	experimental result

The information in this table marked with „REACH registration data“ is based on information taken from the REACH registration dossier and ECHA’s public registration information as accessed on 16-03-2020.

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results/Remarks	Reference
Basic toxicokinetics Non guideline study DEA Purity: 97.4%, [^{14}C]-labelled Pharmacokinetics of DEA at high and low dose (10 and 100 mg/kg bw/d) Route: intravenous injection, single exposure Species: rat, Sprague-Dawley, female Urine, faeces sampled in 12 hour intervals up to 96 h; blood samples collected at 5, 10, 15, 30 min and 1, 2, 4, 6, 12, 24, 36, 48, 60, 72, 84 h post-dosing Tissues collected at 96 h: liver, kidneys, heart, brain, stomach, samples of peri-renal fat, skin	**Blood:** Concentrations of radiolabel in red blood cells 2-fold higher than those in plasma 6 h post-administration; rapid decline 6-96 h after administration accumulation of radioactivity in red blood cells; Elimination of radioactivity from plasma and red blood cells biphasic with plasma half-lifes of 0.2/270 and 0.3/113 h at the low- and high-dose and red blood cell half lives of 0.1/169 and of 0.6/154 h, respectively; Calculated clearance from blood: 84 mL/h/kg bw and 242 mL/h/kg bw for the low- and high-dose, respectively. **Tissues:** Analysis of radiolabel 96 h after injection: carcass (35 and 28%), liver (21 and 17%), kidneys (7 and 5%), skin (5%), other organs less than 1%; liver and kidneys with highest concentrations; Excretion: primary route via urine as parent compound; within 96 h 25% of low dose and 35% of high dose excreted in the urine, 1-2% via the faeces.	(Mendrala et al., 2001)
Absorption, distribution and metabolism (extraction of metabolites from tissues using an organic and an aqueous phase) Non-guideline study [^{14}C]-labelled DEA Species: rat, Fischer 344, male and human liver slices Route: gavage Exposure: single and repeated (8 weeks, 5 days/week)	**Distribution:** Highest concentrations of DEA equivalents measured in **liver, kidney, spleen, and brain**, mainly as parent compound in aqueous phase. **Single dose:** < 30% eliminated within 48 h in urine and faeces. **Repeated dose**: distribution of the DEA-derived radioactivity in the liver: 97% in the aqueous phase and2 % in the chloroform phase (phosphatidylcholine and phosphatidylethanolamine containing *N*-methyl 2,2'-Iminodiethanol and *N*,*N*-dimethyl 2,2'-Iminodiethanol), while in brain the aqueous phase contained 77 % of the radioactivity and the chloroform phase 21%; in blood	(Mathews et al., 1995)

Method	Results/Remarks	Reference
Dose: 7 mg/kg bw	20% of radioactivity in phospholipids. **Metabolism:** 30% of DEA-derived phospholipids were ceramides, 70% phosphoglycerides: HPLC separation of **organic extract from rat livers** produced peaks co-eluting with phosphatidyl ethanolamine and phosphatidyl choline, 30% ceramides and the remaining 70% as phosphoglycerides; incubation of **human liver slices** demonstrated **similarly incorporation into ceramides**, followed by methylation: DEA was absorbed by human liver slices (after 4 h: 11 %; after 12 h: 29%) and incorporated into phospholipids, at 4 h, > 80% and at 12 h, ≥ 95% into ceramides. HPLC analysis of **aqueous extract of rat livers** collected at 48 h revealed a large peak of non-metabolised DEA and smaller peaks identified as N-methyl-2,2'-Iminodiethanol, N,N-dimethyl-2,2'-Iminodiethanol, and phosphates of DEA.	
Absorption, distribution and metabolism Non-guideline study [^{14}C]-labelled DEA, purity: > 97% Species: rat, Fischer 344, male housed in individual glass metabolic chambers Route: oral (gavage), dermal and intravenous injection Vehicle: water, ethanol and PBS, respectively Exposure: single and repeated (5 days/week for 2, 4 or 8 weeks) of 7 mg/kg [^{14}C]-labelled DEA Tissues collected at 48 h: adipose, blood, brain, heart, kidney, liver, lung, muscle, skin, spleen	Similar patterns of tissue distribution and retention observed for different routes of application and after single and repeated dosing; Maximum concentrations of DEA equivalents in tissues were reached after 4-8 weeks of repeated exposure. Percentage of dose in tissues after 48 h: 54% after i.v. and 57% after oral administration. Concentrations in tissues: liver (27.3%), muscle (16.3%) and kidney 5%; steady state for bioaccumulation in tissues after about 4 weeks (dose proportionality for bioaccumulation occurred between 0.7 and 7.0 mg/kg/d, but saturation was evident at 200 mg/kg/d); no steady state reached in the blood after 8 weeks. Total percentage of dose recovered in excreta (urine, faeces, carbon dioxide, volatiles) 29% after i.v. and 24% after oral administration, primary route via urine predominantly as parent compound of single dose but < 30% excreted within 48 h; repeated oral doses leading to excretion of more cationic	(Mathews et al., 1997)

Method	Results/Remarks	Reference
	molecules identified as O-phosphorylated DEA, N-methylated DEA, and a product resulting from oxidation of dimethylated DEA. Mean % of dose excreted mainly as a parent compound in the urine in the last week of each dosing: 40.5, 68.9, 79.1 and 92.1 % after 1, 2, 4 and 8 weeks of dosing; Profile of metabolites in the urine changed after several weeks of repeated oral exposure, significant amounts of *N*-methyl-2,2'-Iminodiethanol, *N*,*N*-dimethyl-2,2'-Iminodiethanol and their corresponding *O*-phosphates incorporated into phospholipids, accounting for the retention and bioaccumulation. Half-life of approximately 1 week was calculated for liver, brain, and spleen and excretion in general, half-life of DEA in blood was much longer (around 54 days).	
In vivo and *in vitro* study on phospholipids (incorporation of labelled DEA, choline, and ethanolamine into phospholipids) Species: rat, Sprague-Dawley, male Route: oral (drinking water) Dose: 42, 160, 490 mg/kg bw Exposure: 5 weeks	Competitive mechanism: inhibition of phosphatidyl choline and phosphatidyl ethanolamine synthesis by DEA Incorporation of DEA into phospholipids at constant rate, with longer half-lives favouring accumulation during chronic exposure. Significant inhibition of the synthesis of hepatic and renal phospholipid derivatives of choline and ethanolamine Alteration in hepatic mitochondria observed after 2 weeks of daily administration (42 mg/kg bw), but not after single administration of 250 mg/kg bw; elevation of Mg^{2+}-dependent ATPase activity.	(Barbee and Hartung, 1979b)

Dermal absorption		
Method	**Results/Remarks**	**Reference**
Single-dose skin penetration study *in vivo* Non-guideline study [^{14}C]-labelled DEA, vehicle: ethanol Purity: 99% Species: rat, F344 and mice, B6C3F1, male (4-5/group) Route: dermal and intravenous (rats only) Dose: single dose, semiocclusive, rats: 2-27.5 mg/kg bw and mice: 8-81 mg/kg bw	Rats: 3-16% absorption; mice: 25-58% absorption after 48 h (radioactivity found in tissues, urine, faeces and skin at the dose application site). Dose-dependent increase in absorption. Distribution pattern: liver, kidney as well as in heart, lung, brain and spleen. At comparable dose levels percentage absorbed in mice (30-40%) about 2.5 times higher than in rats (10-20%).	(Mathews et al., 1997)
Single-dose or repeated dose percutaneous absorption study *in vivo* Radiolabelled DEA (1500 mg/kg bw; about 19.5 mg/cm^2, vehicle not reported) Route: dermal, under occlusion for 6 h, single dose or repeated dose with non-labelled compound for 3 or 6 days followed by application of same dose of radiolabelled material left on the skin for 48 h Analysis of urine, faeces and tissues (excluding skin), 48 h after application; repeated dose: analysis of excreta, carcass, internal organs and tissues (excluding skin)	**Single dose:** Absorption 1.4 and 0.64% in unwashed and washed animals, corresponding to skin absorption rates of 45.0 µg/cm^2/h (unwashed skin) and 21.0 µg/cm^2/h (washed skin). **Repeated dose:** Increased absorption after repeated dose: 21% and 41%, corresponding to skin absorption rates of 70 and 137 µg/cm^2/h in animals exposed for 3 and 6 days, respectively.	(Knaak et al., 1997)
Skin penetration studies *in vitro* Non-guideline study Primary skin discs from rats (CD), mice (CD-1), rabbits (New Zealand white) and humans (mammoplasty patients), female [^{14}C]-labelled DEA Purity: 99% Exposure: 6 h Penetration of an undiluted and a 37% (w/w) aqueous solution of [^{14}C]-labelled DEA (20 mg/cm^2) through the skin during a 6 hour sampling period	**Undiluted DEA:** Steady-state phase ranged from 2.5 to 4.0, 3.0 to 5.0, 2.5 to 6.0, and 4.5 to 6.0 h for rat, mouse, rabbit, and human skin preparations, respectively Lag time to reach a steady-state penetration rate was calculated to be 0.6, 0.9, 1.3, and 3.2 h for rat, mouse, rabbit, and human skin, respectively. Steady-state penetration rates of 1.8, 46.3, 0.9, and 5.7 µg/cm^2/h were calculated for rat, mouse, rabbit, and human skin preparations, respectively, corresponding to percentages of 0.56, 6.68, 2.81, and 0.23 for rat, mouse, rabbit, and human skin preparations, respectively. Permeability constants were similar for rats, rabbits, and humans	(Sun et al., 1996)

	37% (w/w) aqueous solution: Steady-state penetration rates of 23.0, 294.4, 132.2, and 12.7 μg/cm^2/h for rat, mouse, rabbit, and human skin preparations, respectively. Permeability rate constant for an aqueous solution of DEA through mouse skin approximately 10 times higher than through rat skin and about 20 times higher than through human skin, suggesting a rank order of **skin penetration of mouse > rabbit > rat > human skin**.	
Percutaneous absorption *in vitro* in human skin samples Non-guideline study Viable and non-viable human skin samples [^{14}C]-labelled DEA added to products from cosmetic formulations (shampoos, hair dyes and body lotions) usually containing DEA condensates with unreacted DEA Purity: 95-99% Exposure: 24 or 72 h	**24 h:** approximately 0.1% of the applied dose in shampoo and hair dye formulations absorbed into the receptor fluid after 5–30 minutes; DEA absorption 2.8% from shampoos, 2.9% from hair dyes and 10.0% from body lotions; no significant difference in absorption through viable and non-viable skin. **72-hour repeated-dose study:** 30% of applied DEA accumulated in skin and approximately 1% absorbed into the receptor fluid from body lotion formulation.	(Kraeling et al., 2004)

Oral absorption

Following oral administration 57% of DEA is absorbed in rats. After absorption, DEA is taken up by several tissues, especially the liver (27%) and the kidneys (5%), with lower amounts (< 1.0%) being found in blood, brain, spleen and heart, mainly via incorporation into phospholipids, thereby competing with ethanolamine and choline as substrates (Mathews et al., 1997).

Dermal absorption

Dermal absorption of DEA appeared to be species- and concentration-dependent based on the available *in vivo* and *in vitro* studies.

In skin penetration studies, the percentages absorbed varied with increasing dose, between 3-16% in rats and 27-58% in mice. Absorption rates from *in vivo* studies in male F344 rats using different experimental conditions ranged from 0.113 μg/cm^2/h (single dose, for 48 h) to 137 μg/cm^2/h (multiple doses, 6 days). DEA appears to facilitate its own dermal absorption, as higher doses were more completely absorbed than lower doses.

In vitro studies using skin preparations have demonstrated that there are considerable interspecies differences in the rate of transdermal penetration of DEA. When the penetration rate is determined for a 37% aqueous solution of DEA, a marked decrease is noted from the mouse (294 μg/cm^2/h) via the rabbit (132 μg/cm^2/h) and rat (23 μg/cm^2/h) to humans (12.7 μg/cm^2/h). That means that for an aqueous solution of DEA the permeability rate through mouse skin was approximately 10 times higher than that through rat skin and about 20 times higher than that through human skin (Sun et al., 1996).

In human skin samples, studies of the penetration of [^{14}C]-DEA from cosmetic formulations (shampoos, hair dyes and body lotions) indicated that approximately 0.1% of the applied dose of shampoo and hair dye formulations was absorbed into the receptor fluid after 5–30 minutes. DEA absorption was found to be 2.8% from shampoos, 2.9% from hair dyes and 10.0% from body lotions after application for 24 h. In a 72-hour repeated-dose study with a body lotion formulation, nearly 30% of applied DEA accumulated in the skin and approximately 1 % was absorbed into the receptor fluid (Kraeling et al., 2004).

Distribution

The tissue distribution of DEA was similar after oral, intravenous and dermal administration. The major portion of DEA that remained in tissues was largely structurally unchanged. The highest concentrations were seen in the liver, kidney, spleen, and brain.

Excretion

DEA is excreted primarily in urine as the parent molecule (25-36% after 48 h) and < 3% in faeces. Analysis of the compounds excreted in urine after single oral administration of DEA revealed non-metabolised DEA and smaller proportions of *O*-phosphorylated and *N*-methylated metabolites. Following treatment for 8 weeks, N-methylation products of DEA also appear in significant amounts in urine. There was minimal conversion to CO_2 or volatile metabolites in breath (Mathews et al., 1995).

Accumulation

Accumulation of DEA may occur in several tissues and in red blood cells. Accumulation of DEA at high levels in liver and kidney is explained by its incorporation into membrane phospholipids (Artom et al., 1949; Barbee and Hartung, 1979b; IARC, 2000). Following repeated exposure of rats to DEA, the extent of methylation and accumulation of aberrant sphingomylenoid lipids in tissues increased. In rats, half-life of DEA for liver, brain, and spleen are approximately one week after repeated administration, while blood half-life is much longer (54 days).

Metabolism

DEA is structurally similar to ethanolamine and choline. Choline is an essential nutrient in all mammals with wide-ranging roles in the body as it is a major source of methyl-groups and is used as a constituent of cell membranes and the neurotransmitter acetylcholine.

Perturbation of choline homeostasis by DEA can be explained by the following mechanisms: DEA competitively inhibits the cellular uptake of choline *in vitro* and causes hepatic changes in choline homeostasis, consistent with apparent choline deficiency, *in vivo* (Lehman-McKeeman and Gamsky, 1999; Lehman-McKeeman et al., 2002). Competitive inhibition of tissue organic cation transporters that usually transport choline is consistent with the accumulation of DEA in mouse liver and kidney tissue (Mathews et al., 1997; Mendrala et al., 2001).

The observation in healthy adults given a choline-deficient diet developed signs of organ dysfunction (fatty liver, liver or muscle cell damage), which was reversed by consumption of a high-choline diet, contributed to the recognition of choline as an essential nutrient (da Costa et al., 2006). The current recommended adequate intake for choline by the US Food and Nutrition Board has been set at 425 mg/d for women and 550 mg/d for men (Zeisel and Da Costa, 2009). The recommended dietary intake for choline in humans should be adjusted for specific vulnerable human populations, including the growing infant, the pregnant or lactating women, and patients with liver cirrhosis, and the patients fed intravenously. Significant variation in the dietary requirements for choline are found in populations with a common genetic polymorphism in genes involved in choline metabolism making this population more susceptible to choline deficiency. During pregnancy and lactation there is a higher demand for choline as choline is delivered to the fetus across the placenta and human milk is rich in choline depleting maternal choline stores. There is some indication that choline, similar to folic acid, is associated with the risk of neural tube defects (Zeisel and Da Costa, 2009). Metabolisms of choline and folic acid intersect at the pathway for methyl-group donation.

DEA can compete with the endogenous precursor ethanolamine in the synthesis of phospholipids (Chojnacki and Krozybski, 1963; Pelech and Vance, 1984). Incorporation into phospholipid head groups by the same biosynthetic pathways as ethanolamine leads to the formation of DEA-containing phospholipid, particularly ceramides, an important component of intracellular signal transduction and differentiation (Mathews et al., 1995). On the other hand, cleavage of phosphatidyl choline to choline and diacylglycerol is the only pathway yielding new molecules of choline in mammals. However, the resulting phospholipids are not chemically identical. DEA is a larger molecule than ethanolamine and has an additional hydroxyl group, increasing the potential for hydrogen bonding. Therefore, molecules of DEA-containing phospholipid can alter the structure and properties of membranes containing these aberrant phospholipids. DEA treatment has been shown to inhibit microsomal enzyme activity and to alter mitochrondrial membrane integrity, possibly resulting in perturbation of intercellular communication and intracellular signal transduction (Barbee and Hartung, 1979a). These adverse effects do not occur *in vitro*, suggesting that the *in vivo* incorporation into the lipid bilayer of membranes is a prerequisite to these changes. Furthermore, the mitochondrial and microsomal effects were time-dependent, as these changes were not observed with acute treatment and increased in severity with repeated dosing (Barbee and Hartung, 1979a).

DEA can also be metabolised via oxidation and methylation and thereby participating in important biosynthetic pathways, including choline oxidation to betaine and methylation reactions involving *S*-adenosyl-methionine (SAM) as methyl donor (Zeisel, 2008). Depletion of intracellular choline leads to a reduced availability of SAM and can result in hypomethylation of DNA and altered expression of genes regulating growth (Newberne et al., 1982). When choline supplies are low choline can be recycled in the liver and redistributed from the kidney, lung and intestine to liver and brain (Zeisel and Da Costa, 2009).

In situ formation of the nitrosamine, 2,2'-(nitrosoimino)bisethanol

As a secondary amine, in principle DEA can be converted to a carcinogenic nitrosamine under favourable conditions (e.g., low pH and heat), in this case N-nitrosodiethanolamine (2,2'-(nitrosoimino)bisethanol; NDELA; CAS 1116-54-7). *N*-nitrosamines are organic nitrogen compounds which can be generated from nitrosating agents and primary, secondary or tertiary amines under certain reaction conditions (Douglass et al., 1978; Loeppky, 1994; Preussmann, 1983). The secondary amines in general are the most reactive compounds towards nitrosating agents generating nitrosamines. *In vivo* nitrosamine formation is thought to occur as a result of a non-enzymatic reaction between an amine and nitrous acid, formed from nitrate in the acid environment of the stomach. The corresponding nitrosamine of DEA, NDELA is mutagenic *in vitro* and causes hepatocellular carcinomas and kidney adenomas in rats (Berger et al., 1987; ECETOC, 1990; IARC, 2000; Lijinsky and Kovatch, 1985; Lijinsky and Reuber, 1984; Preussmann et al., 1982). It has a harmonised

classification as Carc 1B, listed in Annex VI Table 3.1 of CLP-Regulation. However, NDELA was not detected in the urine, blood or gastric contents of B6C3F1 mice that were administered 160 mg/kg per day DEA by dermal application or oral gavage with or without 140 ppm (≈40 mg/kg bw/d) sodium nitrite in their drinking water for 2 weeks (Stott et al., 2000b).

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

DEA is well absorbed after oral administration and distributed to all tissues, mainly to liver and kidneys (Mathews et al., 1997). After dermal administration DEA is well absorbed in mice and rats. In humans it is absorbed in a lesser extend due to the thickness of stratum corneum. Also, there is a dose-dependency of absorbed fractions of DEA, increasing with higher doses. Tissue distribution was similar following administration by all routes examined.

DEA is metabolised via methylation and oxidation or phosphorylation and incorporation into phospholipids. DEA is excreted primarily in urine as the parent molecule, with smaller amounts of *O*-phosphorylated and *N*-methylated metabolites. Once absorbed, DEA displays a biphasic clearance from the blood, with a rapid initial phase (half-life of ~0.1 hour) followed by a prolonged phase (half-life of ~7 days) consistent with its accumulation in the liver and kidney (Mendrala et al., 2001). As DEA can be metabolised by biosynthetic routes common to endogenous alkanolamines (ethanolamine and choline) with the long elimination half-life attributed to incorporation of DEA into phospholipids (Mathews et al., 1995). This may lead to bioaccumulation with chronic dosing.

Species differences were reported for transport from plasma to the liver, oxidation of choline to betaine as well as altered DNA methylation and gene expression. These differences are summarised in Table 9. However, these species differences were mainly associated with the development of liver tumours. Less is known about the mode of action for kidney tumours. For reproductive toxicity an involvement of choline deficiency and a metabolite of choline, platelet activating factor (PAF), is assumed.

Table 9: Species differences for metabolism of DEA adapted from Kirman et al. (2016), mainly discussing the MoA for mouse liver tumours.

Event	Evidence	Species differences	References
Transport from plasma to liver	**Animals:** DEA accumulates in liver (rat and mouse) **Humans:** DEA taken up by human liver slices *in vitro*	Clear difference in hepatocyte DNA synthesis observed *in vitro* (mouse/rat > human), in part due to differential uptake Species differences in organic cation transporters reported in general but not for DEA specifically Accumulation in liver likely driven by membrane transporters which appear to be saturable in rodents. DEA uptake might be increased when choline deficiency is manifested	(Mathews et al., 1995) (Mathews et al., 1997) (Mendrala et al., 2001) (Mathews et al., 1995) (Mendrala et al., 2001) (Kamendulis and Klaunig, 2005) Dresser, 2000 as cited Kirman et al. (2016)
Perturbation of choline homeostasis and decreased SAM concentrations	**Animals:** Inhibiting choline uptake into cells *in vitro* and decreased hepatic choline, betaine, SAM levels in mice when exposed to DEA Additional stress on methyl pool: SAM utilised during DEA metabolism; as betaine	Clear differences in oxidation of choline to betaine (significant pathway for rodents, minor role in humans). Humans rely more on tetrahydrofolate for maintenance of SAM Mice appear more sensitive than rats to effects of SAM: short-term exposure had no effect on	(Lehman-McKeeman and Gamsky, 1999) (Lehman-McKeeman and Gamsky, 2000) (Stott et al., 2000a) (Craciunescu et al., 2009) (Lehman-McKeeman and Gamsky, 2000)

	analogue, methylated DEA metabolites serve as methyl sink **Humans:** Methylation of DEA in human liver slices and human plasma	SAM levels in rats, significant decrease in mice	(Lehman-McKeeman, 2002) (Mathews et al., 1995) Lieber and Packer, 2002 as cited Kirman et al. (2016) (Zeisel and Blusztajn, 1994)
Altered gene expression, DNA synthesis, apoptosis, gap junction intercellular communication (GJIC), cell transformation	**Animals:** Following exposure to DEA: Increased DNA synthesis, increased liver and kidney weights in mice Altered gene expression in mouse hepatocytes *in vitro* Increased apoptosis in mouse neuronal cells GJIC reduced in mouse hepatocytes Concentration dependent transformations in CHO cells *in vitro*, ameliorated by choline supplementation **Humans:** DNA synthesis and GJIC not affected by DEA in human hepatocytes *in vitro*	Clear species difference (mouse-rat > human) reported for DNA synthesis and decreased GJIC in hepatocytes exposed to DEA. Human hepatocytes not responsive when exposed to DEA	(Mellert et al., 2004) (Mellert and Bahnemann, 2001) (Craciunescu et al., 2009) (Lehman-McKeeman and Gamsky, 2000) (Kamendulis et al., 2004; Kamendulis and Klaunig, 2005)

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 10: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Value LD_{50} (mg/kg bw)	Reference
Acute oral toxicity Similar to OECD TG 401, no GLP compliance - no information on purity of test substance Key study Reliable with restrictions	Rat, strain not specified, 10 animals (5 female, 5 male) /group; at 2000 mg/kg and 2500 mg/kg only 5 males	200, 800, 1000, 1250, 1600, 2000, 2500, 3200 mg/kg bw Oral gavage (dissolved in water) Observation daily for 14 d after dosing	**1100 mg/kg bw (female)** 2500 mg/kg bw (male) 1600 mg/kg bw (male+female)	(BASF AG, 1966a)
Acute oral toxicity, no guideline followed - no information on purity Supporting study Reliable with restrictions as only study summary is available	Rat, strain not specified 5 animals/group	**Doses:** no data available Oral gavage of pure substance Observation period following exposure: 14 days	1820 mg/kg bw (95% CL: 1660-2000)	(Smyth et al., 1969)
Acute oral toxicity, no guideline followed - no information on purity Supporting study Reliable with restrictions as only study summary is available	Rat, Wistar rats, female 5 animals/group	**Doses:** no data available Oral gavage of pure substances Observation period following exposure: 14 days	710 mg/kg bw	(Smyth et al., 1970)
Experimental study, no guideline followed - no information on purity - short observation period (18h) Supporting study Reliable with restrictions due to short observation period	Male Sprague-Dawley rats Animals per group: 0, 100 mg/kg: 9 200, 400 mg/kg: 7 800-6400 mg/kg: 8	0, 100, 200, 400, 800, 1600, 3200, 6400 mg/kg bw, single oral dose Dissolved in water Observation till **18 h after exposure**	At 6400 mg/kg: 7/8 animals died Below 6400 mg/kg bw: No mortality, but dose-related increase of liver and kidney weights	(Korsrud et al., 1973)
Review on toxicology of mono-, di- and triethanolamine	Rat Strain not	Doses**:** no data available Oral gavage	700-1700 mg/kg bw (female)	(Knaak et al., 1997) (Review)

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Value LD_{50} (mg/kg bw)	Reference
Study details not available Supporting information Reliability not assignable	specified		1700-2800 mg/kg bw (male)	
Acute oral toxicity Study details not available Supporting information Reliability not assignable	Rat, mouse, rabbit, guinea pig Strains not specified	Doses: no data available Oral gavage	Rat: 3460 mg/kg bw Mice: 3300 mg/kg bw Rabbit: 2200 mg/kg bw Guinea pig: 2200 mg/kg bw	(Izmerov et al., 1982)
Acute oral toxicity Study details not available Supporting information Reliability not assignable	Rat, mouse Strains not specified	Doses: no data available Oral	Rat: ~2300 mg/kg Mice: 4570 mg/kg	(EPA-US, 1989)

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Various studies are available in rats and mice, as well as one each in rabbit and guinea pigs. Only one study is performed similar to OECD TG 401 and indicates a moderate oral toxicity of DEA with a LD_{50} value of 1600 mg/kg bw (rat, both sexes) and a slightly lower LD_{50} value of 1100 mg/kg bw for female subgroups (BASF AG, 1966a). No deaths occurred in dose group up to 1000 mg/kg bw. Reported clinical signs were tumbling, staggering gait, twitches, convulsions, dyspnoea, abdominal lateral position and scrubby coat. Gross pathology revealed hydrothorax, local adhesions of the gut and signs of irritation on the gastro-intestinal tract.

All other studies are less reliable as essential study details are missing.

The studies by Smyth et al. (1969) and (1970) state LD_{50} values in rats of ~1820 and 710 mg/kg bw, respectively. The tested dose range is not reported, but numbers of animals per group and observation period are concordant with 'typical' study design of acute toxicity testing of the OECD TG 401.

Korsrud and coworkers investigated the correlation between serum enzyme activities and liver damage induced by DEA in male Sprague-Dawley rats after a single oral administration of aqueous DEA solutions in the range of 100–6400 mg/kg bw. They reported a mortality only at the top dose (7/8 rats died) while at lower doses liver and kidney weights were increased in a dose-dependent pattern (Korsrud et al., 1973). However, the observation period was limited to 18 h after oral gavage, so this study cannot be used to derive an oral acute toxicity estimate.

Other studies report LD_{50} values in the range of 1700-3460 mg/kg bw for rats, 2300-3300 mg/kg bw for mice, and 2200 mg/kg for rabbits and guinea pigs (EPA-US, 1989; Izmerov et al., 1982; Knaak et al., 1997). There are no details on methodology available for these studies. However, these reports may indicate that rats (in particular female rats) are the most sensitive species with regards to acute toxicity.

10.1.2 Comparison with the CLP criteria

As described above, the lowest available LD_{50} value from a study performed similar to OECD TG 401, is 1600 mg/kg bw for rats, while female rats were more sensitive than males in the study with an LD_{50} value of 1100 mg/kg bw for female rats.

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the oral route. In general, classification is based on the lowest acute toxicity estimate (ATE) value available i.e. the lowest ATE in the most sensitive appropriate species tested. The ATE for the classification is derived using LD_{50} values if available.

The four toxicity categories are defined as follows:

Acute oral toxicity - Category 1: 5 mg/kg bw ≤ ATE

Acute oral toxicity - Category 2: 5 < ATE ≤ 50 mg/kg bw

Acute oral toxicity - Category 3: 50 < ATE ≤ 300 mg/kg bw

Acute oral toxicity - Category 4: 300 < ATE ≤ 2000 mg/kg bw

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the results shown above, it is proposed to classify 2,2-Iminodiethanol as:

Acute Tox. 4 after oral exposure (H302 –Harmful if swallowed).

An ATE value of 1100 mg/kg bw is proposed based on the lowest LD_{50} value derived in the most sensitive species and the most sensitive sex (female rat) from the study performed similar to OECD TG 401 (BASF AG, 1966a). As the LD_{50} values for female rats are markedly lower than those for male rats it is suggested to derive the ATE values based on female animals.

10.2 Acute toxicity - dermal route

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

For the dermal route, no data are provided in the registration dossier. Knaak et al. (1997) reported very high LD_{50} values in the range 8100-12200 mg/kg bw for rabbits, however no study details are provided.

10.2.2 Comparison with the CLP criteria

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by the dermal route. Category 4 would apply if effects are observed at an ATE lower than 2000 mg/kg and greater than 1000 mg/kg.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

There is no data available justifying classification of DEA for dermal acute toxicity. The reported value by Knaak et al. (1997) indicate that DEA does not induce acute dermal toxicity at concentrations requiring classification.

10.3 Acute toxicity - inhalation route

Table 11: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Value LC_{50}	Reference
Inhalation-risk test (IRT) Similar to OECD TG 403, no GLP compliance Reliable with restrictions	Rat Strain not specified 6 animals (3 males, 3 females)	Vapour* inhalation No analytical verification of the test atmosphere concentration Duration of exposure: 8 h Mean substance concentration: 0.2 mg/l air Observation period: 7 days after exposure *vapour generated by bubbling 200 l/h air through a substance column of about 5 cm above a fritted glass disc in a glass cylinder at 24 °C	No mortality occurred	(BASF AG, 1956) Dossier
Inhalation-risk test (IRT) Similar to OECD TG 403, no GLP compliance Reliable with restrictions	Rat Strain not specified 12 animals (6 males, 6 females)	Atmosphere enriched with DEA vapour* No analytical verification of test atmosphere concentration Duration of exposure: 8 h Mean substance concentration: 1.9 mg/m^3 (0.44 ppm) Observation period: 7 days after exposure *vapour generated by bubbling 200 l/h air through a substance column of about 5 cm above a fritted glass disc in a glass cylinder at 20 °C	No mortality occurred	(BASF AG, 1966b) Dossier
Experimental study No guideline followed, no GLP compliance Reliable with restrictions (original publication (thesis) is not available), summary available in Dossier	Rat, Sprague-Dawley 8 animals /group (4 males, 4 females)	Whole body exposure Doses and duration: 1) Exposure for 4h: 0.13 mg/L (30 ppm), 0.55 mg/L (126 ppm), 1.18 mg/L (270 ppm), 3.35 mg/L (768 ppm); aerosol generated from unheated compound 2) Exposure for 80 min and 105 min (6.4 mg/l, ~1470 ppm); vapour and mist generated from	LC_0 3.35 mg/L (4h, aerosol from unheated compound) **At 6.4 mg/L** (vapour and aerosol, heated compound) in total 5/8 rats died: 3/4 after 80 min exposure and 2/4 after 105 min	(Foster, 1971) Dossier

Method, guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Value LC_{50}	Reference
		heated (110°C) compound Observation period not specified. DEA concentration was determined by a colorimetric method	exposure	
Experimental Study Not reliable	No information	Short-term inhalation of vapour (200 ppm) or aerosols (1400 ppm)	"some deaths" occurred, no further details	(Hartung, 1970)

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Two acute toxicity tests (so-called 'inhalation-risk tests') performed similar to OECD TG 403 are available, both reported no mortality induced by inhalation of DEA enriched atmosphere generated from unheated compound at 24°C, respectively 20°C (BASF AG, 1956; BASF AG, 1966b). However, tested DEA concentrations in both tests were very low which can be explained by low vapour pressure of DEA yielding in low vapour concentrations. In addition, DEA concentrations in the chamber atmosphere were not measured and it was not determined whether vapour or aerosols was present.

In acute toxicity studies (without guideline-compliance), DEA (mix of vapour and aerosol, concentration determined by a colorimetric method) at concentrations between 0.13 and 6.4 mg/L (30-1476 ppm) with exposure for 80 minutes up to 4 h were tested in Sprague-Dawley rats (Foster, 1971). After exposure to 6.4 mg/L of DEA (vapour and aerosol generated from heated compound) for 80 minutes, 3/4 rats died 2-4 h post exposure. After an exposure time of 105 minutes, 2/4 rats died 80-90 minutes post-treatment with the same concentration. No mortality occurred in rats after exposure up to 3.35 mg/L (768 ppm, aerosol generated from unheated compound) for 4 h. Clinical signs at this dose were increased respiration rate and increased systolic blood pressure. Furthermore, congestion in lung, liver, and spleen as well as discoloured kidneys and thymus were seen in gross pathology, and pulmonary oedema was noted in histopathology.

Hartung (1970) reports "some deaths" after short-term inhalative exposure to 200 ppm vapour or 1400 ppm aerosol of DEA, however, there are no further details on the study reported. Thus, the study cannot be assessed.

Overall, there is no LD_{50} reported for DEA. However, the studies by Foster (1971) and Hartung (1970) pointed out that DEA induced adverse effects following inhalation exposure to a single dose.

Extrapolating the doses at which 5/8 animals died after 105 minutes (Foster, 1971) to a 4 h exposure time would result in an LD_{50} of 2.8 mg/L (105/140*6.4 mg/L). However, this is contradictive to the result of 4 h exposure to a higher dose of 3.35 mg/L. It is to note that both experiments in this study differ in the production of the vapour/aerosol – in the 4 h durating experiment the sample was not heated, while in the other experiments DEA was heated to 110°C.

10.3.2 Comparison with the CLP criteria

As described above, there is no LD_{50} value reported for DEA.

According to the criteria shown in the Table 3.1.1 of Annex I, Part 3 of CLP, substances can be allocated to one of four toxicity categories based on acute toxicity by inhalation. Thereby, it is important to differentiate between vapour and mist. The guidance document on application of CLP criteria (ECHA, 2017) states that 'differentiation between vapour and mist will be made on the basis of the saturated vapour concentration (SVC)' and 'an LC_{50} well below the SVC will be considered for classification according to the criteria for

vapours; whereas an LC_{50} close to or above the SVC will be considered for classification according to the criteria for mists'.

The tested concentrations of DEA in the screening studies (Foster, 1971) are above DEAs SVC, thus classification according to criteria for **mist** would apply (4 h exposure duration):

Acute oral toxicity - Category 1: ATE ≤ 0.05 mg/L

Acute oral toxicity - Category 2: 0.05 mg/L < ATE ≤ 0.5 mg/L

Acute oral toxicity - Category 3: 0.5 mg/L < ATE ≤ 1.0 mg/L

Acute oral toxicity - Category 4: 1.0 mg/L < ATE ≤ 5.0 mg/L

There are limitations in the available information and no guideline followed, nevertheless the study is seen as sufficient for classification. The indicated LD_{50} of 2.8 mg/L (extrapolated from 6.4 mg/L, 105 minutes exposure) points to a Category 4 classification.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the results shown above, it is proposed to classify 2,2-Iminodiethanol as:

Acute Tox. 4 after inhalative exposure (H332 –Harmful if inhaled).

An ATE value of 2.8 mg/L is proposed based on the screening studies by Foster (1971).

10.4 Skin corrosion/irritation

Not assessed.

10.5 Respiratory sensitisation

Not assessed.

10.6 Skin sensitisation

Not assessed.

10.7 Germ cell mutagenicity

For information only.

Table 12: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation assay Similar to OECD TG 471 Deviation: • Only up to 4000 µg/plate GLP: no Plate incorporation, 30 min preincubation	Diethanolamine Purity: 99.7%	**Key study** **Reliable with restrictions** Bacterial strains: S. typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100, E. coli WP2 and WP2uvrA Test concentrations (with and without metabolic activation (S9 mix)): 0, 125, 250, 500, 1000, 2000, 4000 µg/plate, with and without rat liver S9 (Aroclor 1254 induced) Justification for top concentration: not specified Vehicle: water Negative control: yes Positive control: yes Benzo(a)pyrene, ethylmethanesulphonate, methylmethanesulfonate, cyclophosphamide	**Negative** (with and without metabolic activation) Cytotoxicity: not specified Controls: valid	(Dean et al., 1985)
***In vitro* gene mutation study in bacteria** Similar to OECD TG 471 Deviations: • Only 4 strains • Max. concentration 3333 µg/plate GLP: not specified Preincubation	Diethanolamine Purity: > 99%	**Supporting study** **Reliable with restrictions** Bacterial strains: S. typhimurium TA 1535, TA 1537, TA 98, TA 100 Test concentrations (with and without metabolic activation (S9 mix)): 0, 33, 100, 333, 1000, 3333 µg/plate, with and without rat and hamster S9 (Aroclor 1254-induced) Justification for top concentration: cytotoxicity Vehicle: water Negative control: yes Positive control: yes -S9: sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), 4 -nitro-o-phenylenediamine (TA98); +S9 with all strains: 2-aminoanthracene	**Negative** (with and without metabolic activation) Cytotoxicity: yes, > 3333 µg/plate Controls: valid	(Haworth et al., 1983)
***In vitro* cytogenicity/ chromosome aberration study in mammalian cells**	Diethanolamine Purity: 99.7%	**Supporting study** **Reliable with restrictions** Cell culture: rat liver cell lines RL1 and RL4	**Negative** (without metabolic activation) Cytotoxicity: not specified Precipitations: not specified	(Dean et al., 1985)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Non-guideline study Deviations: • Number of cells evaluated 100 • Individual data missing GLP: no		Test concentrations (with and without metabolic activation (S9 mix)): 0, 0.125, 0.25, 0.5 of GI50 (50% growth inhibition), without metabolic activation Treatment time: 24 h Negative control: not specified Positive control: yes Benzo(a)pyrene, ethylmethanesulphonate, methylmethanesulfonate, cyclophosphamide	Controls: validity not specified	
***In vitro* cytogenicity/ chromosome aberration study in mammalian cells** Similar to OECD TG 473 GLP: not specified	Diethanolamine Purity: not specified, source: Fisher	**Key study** **Reliable without restrictions** Cell culture: Chinese hamster ovary (CHO) cells Test concentrations (with and without metabolic activation (S9 mix)): rat liver S9 (Aroclor 1254-induced) -S9: 0, 101, 505, 2010 µg/ml; +S9: 0, 303, 1010, 3010 µg/ml Justification for top concentration: cytotoxicity Vehicle: McCoy's 5A medium Negative control: yes Positive control: yes mitomycin C (-S9) and cyclophosphamide (+S9)	**Negative** (with and without metabolic activation) Cytotoxicity: yes Controls: valid	(Loveday et al., 1989)
***In vitro* gene mutation in mammalian cells** Similar to OECD TG 476 GLP: yes	Diethanolamine Purity: > 99%	**Key study** **Reliable without restrictions** Cell culture: L5178Y mouse lymphoma cells Test concentrations (with and without metabolic activation (S9 mix)): 0, 25, 50, 100, 200, 300, 400, 600 µg/ml with and without rat liver S9 (Aroclor 1254-induced) Justification for top concentration: cytotoxicity Vehicle: ethanol Negative control: yes Positive control: yes Methylmethanesulfonate	**Negative** (with and without metabolic activation) Cytotoxicity: yes, > 400 µg/ml Precipitations: yes/no Controls: validity not specified	(Myhr et al., 1986)
***In vitro* DNA damage and/or repair study (in vitro sister**	Diethanolamine Purity: not specified,	**Supporting study** **Reliable with restrictions** Cell culture: Chines hamster ovary	**Negative** (with and without metabolic activation)	(Loveday et al., 1989)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
chromatid exchange in mammalian cells) Similar to OECD TG 479 GLP: not specified	source: Fisher	(CHO) cells Test concentrations (with and without metabolic activation (S9 mix)): 0, 150, 500, 1500 µg/ml, with and without rat liver S9 (Aroclor 1254-induced) Justification for top concentration: cytotoxicity Vehicle: water Negative control: yes Positive control: yes cyclophosphamide (with S9); mitomycin C (without S9)	Cytotoxicity: yes Controls: valid	

Table 13: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
***In vivo* mammalian somatic cell study: cytogenicity/ erythrocyte micronucleus** Similar to OECD TG 474 Deviation: • Sign of toxicity already in lowest dose tested GLP: no	Diethanolamine Purity: > 99%	**Supporting study** **Reliable with restrictions** Species: mouse, B6C3F1 Number of animals per group: 10 male, 10 female Target organ(s): peripheral blood samples Administration route: dermal Dose levels: 0, 80, 160, 320, 630, 1250 mg/kg bw Treatment time(s): 13 weeks, 5 days/week Vehicle: ethanol Positive control: yes, urethane (0.2% drinking water) Negative control: yes, concurrent vehicle	**Negative** Toxicity (clinic/cytotoxicity): • Local and systemic signs of toxicity down to the lowest dose level tested Controls: valid	(Witt et al., 2000)

10.7.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

DEA did not induce reverse mutations in Salmonella typhimurium or Escherichia coli (Dean et al., 1985; Haworth et al., 1983). In mammalian *in vitro* systems, DEA did not induce gene mutation in mouse lymphoma cells, chromosomal aberrations in rat hepatocytes, sister chromatid exchange or chromosomal aberrations in cultured Chinese hamster ovary cells (Dean et al., 1985; Loveday et al., 1989; Myhr et al., 1986).

In vivo DEA did not induce micronuclei in peripheral blood erythrocytes of B6C3F1 mice after repeated unoccluded dermal application for 13 weeks at doses of 80–1250 mg/kg showing systemic availability (Witt et al., 2000).

10.7.2 Comparison with the CLP criteria

The data available for germ cell mutagenicity are conclusive and due to the negative results in *in vitro* and *in vivo* testing do not warrant a classification according CLP Regulation and Directive 67/548/EEC.

10.7.3 Conclusion on classification and labelling for germ cell mutagenicity

DEA is not proposed to be classified as germ cell mutagen as there was no evidence of genotoxicity in a battery of standard *in vitro* and *in vivo* tests.

10.8 Carcinogenicity

Two 2-year dermal carcinogenicity studies similar to OECD TG 451 in F344/N rats and B6C3F1 mice (NTP, 1999b; US DHHS, 2002), a short-term test for carcinogenicity in a transgenic mouse model (Spalding et al., 2000) as well as mechanistic studies providing evidence of the possible mode of tumourigenesis of DEA in rodents are available (BASF AG, 2001; BASF AG, 2002; BASF AG, 2003; Lehman-McKeeman et al., 2002; Mellert et al., 2004; Stott et al., 2000a). There were no data available for the oral or inhalation routes.

Table 14: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Relevant information about the study	Results	Reference
Carcinogenicity, dermal Similar to OECD TG 451 GLP: yes	Diethanolamine Purity: > 99% Species: mouse, B6C3F1 Number of animals per group: 50/sex/group Administration route: Dermal Dose levels: 0, 40, 80, 160 mg/kg bw/d Vehicle: ethanol Treatment time: 103 weeks, 5 days/week Post exposure period: 10 days	**Key study** **Reliable without restrictions** **Conclusion:** Clear evidence of carcinogenic activity of DEA in male and female B6C3F1 mice based on increased incidences of liver neoplasms in males and females and increased incidences of renal tubule neoplasms in males according to NTP report LOAEL(carcinogenic effect): 40 mg/kg bw/d **Results:** **General toxicity** **Survival:** not affected in males, significantly reduced in dosed female groups (44/50, 33/50, 33/50; 23/50) **Body weight** in 80 and 160 mg/kg bw male groups lower than in control group after weeks 88 and 77, respectively; terminal mean body weight 96% and 89% respectively. Dosed groups of females showed generally less body weight than vehicle control group during second year of the study, terminal mean body weight 91%, 85% and 76% in dosed female groups. **Non-neoplastic lesions** Dermal administration associated with increased incidences of cytoplasmic alteration (males only) and syncytial alterations of the liver, renal tubule hyperplasia (males only), thyroid gland follicular cell hyperplasia and hyperkeratosis of the skin. **Male mice:** **Liver**: cytoplasmic alterations (1/50, 17/50, 17/50, 12/50), syncytial alteration (0/50, 28/50, 38/50, 23/50) **Kidney**: renal tubule hyperplasia (standard and extended evaluation combined (3/50, 7/50, 7/50, 10/50)) **Thyroid gland**: follicular cell hyperplasia (18/50, 22/49, 30/50, 42/50) **Skin**: hyperkeratosis (0/50, 13/50, 10/50, 17/50) **Female mice:** **Liver**: syncytial alteration (0/50, 2/50, 17/50, 18/50) **Thyroid gland**: follicular cell hyperplasia (18/50, 28/49, 32/50, 39/50)	(NTP, 1999b)

Method, guideline, deviations if any	Relevant information about the study	Results	Reference
		Skin: hyperkeratosis (1/50, 3/50, 8/50, 16/50) **Neoplastic effects** **Male mice:** **Liver:** hepatocellular adenoma 31/50 (62%), 42/50 (84%), 49/50 (98%), 45/50 (90%), historical incidence 118/249 (47.4%±8.9%) hepatocellular carcinoma 12/50 (24%), 17/50 (34%), 33/50 (66%), 34/50 (68%), historical incidence 54/249 (21.7%±2.5%) hepatoblastoma 0/50, 2/50 (4%), 8/50 (16%), 5/50 (10%), historical incidence 1/249 (0.4%±0.9%) combined hepatocellular adenoma, hepatocellular carcinoma or hepatoblastoma 39/50 (78%), 47/50 (94%), 50/50 (100%), 49/50(98%), historical incidence 154/249 (61.8%±9.1%) **Kidney:** adenoma (standard evaluation 1/50 (2%), 4/50 (8%), 6/50 (12%), 6/50 (12%); historical incidence 2/299 (0.7%±1.0%) standard and extended evaluation combined (1/50, 6/50, 8/50, 7/50), adenoma or carcinoma (combined) (standard evaluation 3/50 (6%), 5/50 (10%), 6/50 (12%), 8/50 (16%), historical incidence 54/249 (21.7%±2.5%) standard and extended evaluation combined 3/50, 7/50, 8/50, 9/50) **Female mice:** **Liver:** hepatocellular adenoma 32/50 (64%), 50/50 (100%), 48/50 (96%), 48/50 (96%), historical incidence 133/252 (52.8%±11.4%) hepatocellular carcinoma 5/50 (10%), 19/50 (38%), 38/50 (76%), 42/50 (84%), historical incidence 35/252 (13.9%±7.3%) hepatocellular adenoma or carcinoma 33/50 (66%), 50/50 (100%), 50/50 (100%), 50/50 100%), historical incidence 149/252 (59.1%±6.4%) **Kidney**: no neoplastic findings	
Carcinogenicity, dermal Similar to OECD TG 451 GLP: yes	Diethanolamine Purity: > 99% Species: rat, Fischer 344 Number of animals per group: 50/sex/group Administration route: Dermal Dose levels:	**Key study** **Reliable without restrictions** **Conclusion:** No evidence of carcinogenic activity of DEA in male and female rats NOAEL (carcinogenic effects): 64 mg/kg bw/d (male); 32 mg/kg bw (female) **Results:** **Body weight** in 64 mg/kg bw group of males lower than in control group (-7 %) **Survival:** similar to control	(NTP, 1999b)

Method, guideline, deviations if any	Relevant information about the study	Results	Reference
	Males: 0, 16, 32, 64 mg/kg bw/d Females: 0, 8, 16, 32 mg/kg bw/d Vehicle: ethanol Treatment time: 103 weeks, 5 days/week Post exposure period: 10 days	**Clinical findings:** irritation of the skin at application site **Non-neoplastic lesions** **Male rats:** **Skin:** acanthosis (0/50, 2/50, 4/50, 10/50), hyperkeratosis (0/50, 3/50, 5/50, 11/50), exudate (0/50, 3/50, 2/50, 7/50) **Female rats:** **Kidney:** nephropathy (incidences: 40/50, 47/50, 48/50, 48/50, severity 1.2, 1.5, 1.9, 2.7) **Skin:** hyperkeratosis (3/50, 13/50, 23/50, 23/50), exudate (1/50, 7/50, 7/50, 7/50)	
Short term test for carcinogenicity: Transgenic mouse model (homozygous female Tg.Ac transgenic mice) Non guideline study, but well documented GLP: not specified	Diethanolamine Purity: > 99% Species: mouse, homozygous Tg.AC transgenic mice, female Number of animals per group: 15-20 Administration route: dermal Dose levels: 0, 5, 10, 20 mg DEA/mouse/ application Vehicle: 95% ethanol, positive control: 12-O-Tetradecanoyl-phorbol-13acetate (TPA, approximately 99% pure) Treatment time: 20 weeks, 5 days/week Post exposure period: 10 days	**Supporting study /mechanistic study** **Reliable with restrictions (no guideline study)** **Conclusion:** Inactive in Tg.AC mice NOAEL 20 mg DEA/mouse/application **Results:** Incidences of skin tumours: 1/19, 1/15, 0/15, 1/15, positive control 18/20	(Spalding et al., 2000)

Two-year carcinogenicity studies with topical application in rats and mice were performed by NTP equivalent to OECD TG 451 (NTP, 1999b):

Mice

Groups of 50 male and female B6C3F1 mice received dermal doses of 0, 40, 80 or 160 mg/kg bw/d DEA for 5 days a week for 103 weeks. Survival of dosed females was reduced (44/50, 33/50, 33/50; 23/50). Study authors attributed this to the liver neoplasms observed. There was no effect on survival in males. Terminal mean body weights of 80 and 160 mg/kg bw/d dosed males was lower than controls. Mean body weight of dosed females was lower than controls during the second year of the study. Dermal administration of DEA to mice was associated with increased incidences of cytoplasmic alteration (males only) and syncytial alteration of the liver, renal tubule hyperplasia (males only), thyroid gland follicular cell hyperplasia and hyperkeratosis of the skin. Incidences of hepatocellular adenoma and carcinoma observed in all male and female treated groups of mice were increased over vehicle controls.

In male mice, the incidences of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in all dosed groups and of hepatocellular carcinoma and hepatoblastoma in 80 and 160 mg/kg bw males were significantly increased compared to the vehicle controls (hepatocellular adenoma: 31/50, 42/50, 49/50, 45/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 12/50, 17/50, 33/50, 34/50 ($p < 0.001$, Poly-3 trend test); hepatoblastoma: 0/50, 2/50, 8/50, 5/50 ($p < 0.05$, Poly-3 trend test)). Hepatocellular carcinomas and hepatoblastomas metastasised to the lung in 3, 4, 9, and 7 males and in 0, 3, 6, and 1 females. The size and number of liver neoplasms per animal were greater in DEA-exposed mice than in vehicle controls. The incidences of hepatocellular neoplasms were significantly greater in dosed groups of female mice than in the vehicle control group (hepatocellular adenoma: 32/50, 50/50, 48/50, 48/50 ($p < 0.001$, Poly-3 trend test); hepatocellular carcinoma: 5/50, 19/50, 38/50, 42/50 ($p < 0.001$, Poly-3 trend test)).

The incidence of renal tubule adenoma in males showed a significant positive dose-related trend, however, the increased incidences were not statistically significant for any of the exposed groups (1/50, 4/50, 6/50 and 6/50). Incidences of renal tubule hyperplasia or carcinoma alone did not show a positive dose-related trend. An extended evaluation of the kidneys by step sections, and an extended analysis of proliferative lesions revealed additional adenomas in all DEA-exposed groups, but not in the controls. The combined analysis of single and step sections indicated a significant dose-related trend and significantly increased incidences of renal tubule adenoma in the two highest dose groups (80 and 160 mg/kg bw/d). However, the number of malignant kidney tumours is comparable in the high dose group and the control group. The following table gives an overview of DEA-induced kidney tumours in male mice.

Table 15: DEA -induced kidney tumours in male B6C3F1 mice (NTP, 1999b)

Neoplastic changes in kidneys	**0 (control)**	**40***	**80***	**160***
Adenomas (standard evaluation[1])	1/50	4/50	6/50	6/50
Carcinomas (standard evaluation)	2/50	1/50	0/50	2/50
Adenomas and carcinomas (standard evaluation)	3/50	5/50	6/50	8/50
Adenomas (combined evaluation[2])	1/50	6/50	8/50	7/50
Carcinomas (combined evaluation)	2/50	1/50	0/50	2/50
Adenomas and carcinomas (combined evaluation)	3/50	7/50	8/50	9/50

* Doses in mg/kg bw/d; [1] histopathological evaluation as laid out in OECD TG 451; [2] Summary of findings in standard evaluation and results of extended serial sections

Rats

Groups of 50 male and female F344/N rats were administered dermal doses of 0, 16, 32 or 64 mg/kg bw/d DEA for 5 days a week for 103 weeks. Survival of dosed animals was similar to controls. Mean body weight of males in the 64 mg/kg bw group and of females in the 32 mg/kg bw group was lower compared to controls. Clinical findings attributed to DEA administration were minimal to mild skin lesions at the site of application in the epidermis, which were dose-related and more common in females than in males. An increase in the incidences of acanthosis in 64 mg/kg bw/d males, the incidences of hyperkeratosis in 32 and 64 mg/kg bw/d males and in all dosed female groups, and the incidences of focal accumulations of exudate on the epidermal surface in 64 mg/kg bw males and in all dosed female groups was observed. The incidences and severities of observed nephropathy were significantly and dose-dependently increased in dosed female rats compared to the

vehicle controls. Overall, there was no evidence of carcinogenic activity of DEA in male and female F344/N rats (NTP, 1999b; US DHHS, 2002).

Short term test for carcinogenicity using transgenic mice

In a study using a transgenic mouse model, groups of 15-20 homozygous female Tg.AC mice carrying a zeta-globulin promoted v-Ha-ras gene on an FVB background were administered 200 µl volumes of DEA by dermal application 5 times a week for 20 weeks, corresponding to 0, 5 or 20 mg of DEA per mouse per application. Six weeks after the last application mice were killed. No evidence of chronic irritation or ulceration at the site of application was identified. There was no increased incidence of skin tumours in treated mice in this mouse model (Spalding et al., 2000).

Furthermore, numerous epidemiological studies have investigated exposure to metalworking fluids and the risk of cancer in workers who were probably exposed to DEA and other agents. However, no study results were identified that examined the risk of cancer among persons exposed exclusively to DEA (IARC, 2013).

10.8.1 Short summary and overall relevance of the provided information on carcinogenicity

Overall, under the conditions of a two-year dermal study by NTP, there was clear evidence of carcinogenic activity of DEA in mice (B6C3F1) based on increased incidences of benign and malignant liver neoplasms in males and females, multiple types of liver tumours (adenomas, carcinomas and blastomas) in males and increased incidences of renal tubule neoplasms (mainly adenomas, 1/50, 4/50, 6/50 and 6/50 at 0, 40, 80, and 160 mg/kg bw/d, respectively ($p = 0.05$, Poly-3 trend test)) in males. Carcinogenic effects in the liver were noted at all dose levels tested with significant treatment related tumour increases in both sexes ($\geq$ 40 mg/kg bw/d) indicating that a plateau-effect was already seen at low or mid dose groups (NTP, 1999b; US DHHS, 2002).

However, there are some limitations of the NTP mouse study that should be considered:

- high incidence of benign liver tumours in the B6C3F1 mouse strain exceeding the historical controls (62% in concurrent control versus 47% in historical incidence for 2-year dermal studies with vehicle control groups). Although, the concurrent control of malignant liver tumour incidences is within the historical control incidences (24% in concurrent control versus 21.7% in historical control).

- An experimental design that allowed simultaneous dermal and an unknown amount of oral exposure (by licking the application site) and

- possible confounding influence of ethanol as a vehicle. Ethanol is widely regarded as a risk factor for cancer, including liver cancer ((IARC, 1988; Seitz and Stickel, 2007). However, ethanol is a volatile solvent and it is not known if and how much of the substance is absorbed dermally, although DEA was found to induce skin irritation at the site of application which could influence dermal uptake.

In rats, dermal application of DEA up to 64 mg/kg bw/d in males and up to 32 mg/kg bw/d in females did not induce tumours. However, it was reported that dermal absorption of DEA in rats appear to be 25-fold less than in mice (Knaak et al., 1997). On the other hand, kidney and liver are the target organs in both species after oral administration, as evident from degeneration of renal tubular and liver cells after dietary intake of 90 mg/kg bw/d for 13 weeks in study in rats.

The negative outcome in testing in the Tg.Ac transgenic mouse model by Spalding et al. (2000) may not be predictive as this assay uses skin tumours as an endpoint, thus no data on kidney or liver were collected.

There was no evidence of genotoxicity for DEA in a battery of standard *in vitro/in vivo* tests. However, there is weak evidence that a genotoxic mechanism is involved in the induction of liver tumours by DEA. An elevated frequency of mutations in *β*-catenin *Catnb* genes in liver tumours induced by DEA indicate that *in vivo* mutagenesis such as oxidative DNA damage may be involved and that liver tumours associated with exposure to DEA may evolve through a *ras*-independent pathway. This is different from spontaneous liver tumours which often contain H-*ras* mutations (Hayashi et al., 2003). The mutational profile in liver tumours induced in mice fed a choline-deficient diet is not known.

Potential mechanisms of DEA-induced liver carcinogenicity in the mouse discussed in the literature include:

1) the induction of choline deficiency via altered choline homeostasis leading to a chronic progressive choline deficiency,

2) and the displacement of ethanolamine by DEA in phospholipids, an effect which may result in a reduced endogenous production of choline.

DEA is structurally similar to ethanolamine and choline. Therefore, DEA can competitively inhibit the cellular uptake of choline and can compete with ethanolamine in the synthesis of phospholipids.

It was found that repeated dermal exposure to DEA caused hepatic choline deficiency in mice (Lehman-McKeeman et al., 2002) that appears to be involved in the carcinogenic action of DEA (Lehman-McKeeman and Gamsky, 2000; Stott et al., 2000a). Choline-deficient diet has been shown to increase liver tumours in rats despite the absence of any carcinogen treatment (Nakae et al., 1992) and in spontaneous liver tumour-resistant mouse strain (Denda et al., 2002). This provides evidence that choline-deficiency related carcinogenicity is not a species- or strain-specific phenomenon. It is known that primates, including humans, show choline deficiency under conditions of severe fasting or liver disease (Zeisel and Blusztajn, 1994). Therefore, this mechanism is considered relevant to humans, especially for subgroups that are highly susceptible to inadequate choline status, e.g. due to genetic polymorphism in choline biosynthesis pathway or due to the low choline intake via diet, for example, during pregnancy (Brunst et al., 2014).

On a cellular level, depletion of intracellular choline leads to a reduced availability of *S*-adenosyl-methionine (SAM) and can result in hypomethylation of DNA causing altered expression of genes that regulate growth and possible tumour development (Newberne et al., 1982). DNA hypomethylation was observed in primary mouse hepatocytes which have been treated with DEA, similar to the effects caused by choline-deficient medium (Bachman et al., 2006). Furthermore, DNA synthesis was increased in primary cultures of mouse or rat hepatocytes incubated either with DEA or in medium containing reduced choline; whereas choline supplementation prevented DEA-induced DNA synthesis (Bachman et al., 2006; Kamendulis and Klaunig, 2005). DEA can also induce morphological transformation in Syrian hamster embryo (SHE) cells, which was abolished in the presence of excess choline (Lehman-McKeeman and Gamsky, 2000).

Furthermore, it has been hypothesized that DEA may induce tumours also by disrupting phospholipid metabolism as ethanolamine and choline are precursors for phospholipid biosynthesis. This may perturb cell and organelle membrane function, and the synthesis of fatty acid second messengers (NTP, 1999b). In dermally exposed B6C3F1 mice, the reduction of hepatic choline, phosphocholine, phosphatidylcholine, glycerophosphocholine and S-adenosyl-methionine (SAM) levels occurred, while levels of S-adenosyl homocysteine were increased. This pattern of liver changes was similar to those observed in choline-deficient mice (Lehman-McKeeman, 2002; Lehman-McKeeman et al., 2002; Stott et al., 2000a). In studies performed in rats, a reduction in availability of phosphatidylcholine led to accumulation of fat in the liver and eventually to hepatocarcinogenesis in F344 male rats (da Costa et al., 1993; Ghoshal and Farber, 1984; Mikol et al., 1983; Newberne and Rogers, 1986). However, this was not observed in the available dermal NTP carcinogenicity study.

It is assumed that possible potential mechanisms of DEA carcinogenicity are not fully elucidated and the potential mechanisms of DEA-induced carcinogenesis could be more complex (IARC, 2000; IARC, 2013; Leung et al., 2005; US DHHS, 2002).

However, regarding the mode of action for liver carcinogenesis, several species differences in metabolism have been described (see section 9.1, Table 9; Kirman et al. (2016)). The different aspects include:

- differential uptake of DEA into the liver via membrane transporters leading to accumulation of DEA in the liver

- species differences in the oxidation of choline to betaine which is an important pathway for rodents but has a minor role in humans for the maintenance of hepatic SAM levels leading to mice being more sensitive than rats and humans to short term exposure to DEA.

Species differences reported for hepatic DNA synthesis and decreased gap junction intracellular communication (GJIC) in hepatocytes exposed to DEA. Humans appear non-responsive to these two endpoints. No experimental data are available for identification of potential mechanisms on the induction of kidney tumours in male mice by DEA. No indication is given that the DEA-related choline-deficiency is involved in the renal tumour growth. The dose-related increased incidences of renal hyperplasia observed in male mice could be interpreted as preneoplastic lesion preceeding the tumour growth. The increases of incidences in male mice did not gain significancy, however, some weight of evidence is given to the observed increased kidney tumour rates as kidney tumours in mice occur spontaneously at low incidences (historical incidences of renal tubule neoplasms in male mice were given in the NTP study report as 4/299, 1.3% ± 2.4%) Kidney tumours were not seen in the rat study.

10.8.2 Comparison with the CLP criteria

In conclusion, the carcinogenic potential of DEA for the dermal route of exposure was demonstrated in male and female B6C3F1 mice, in which 103-week-long dermal application of DEA led to a statistically significant increase in the incidence of hepatocellular carcinoma and hepatocellular adenoma in males and females, and of hepatoblastoma in males. A treatment-related increased incidence of renal tubule adenoma was also observed in male mice. It is important to note that tumours of the kidney and hepatoblastoma are rare spontaneous neoplasms in experimental animals.

Considering the criteria for classification into hazard categories for carcinogens according to the CLP Regulation, the following needs to be considered: there is no human evidence available, the available reliable evidence is based on experimental animals. The strength of evidence in experimental animals can be evaluated as sufficient (category 1B) or limited (category 2).

According to the CLP regulation sufficient evidence of carcinogenicity in experimental animals is defined as: *"A causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practices, can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites."*

Whereas limited evidence of carcinogenicity in experimental animals is considered as: "*The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs.*"

Table 16: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Mice, (B6C3F1) Males and females	Statistically significant, treatment related increased incidences of neoplastic lesions in the **liver** and **kidney.** Hepatocellular	Neoplastic lesions in liver in males and females and additionally in kidneys in males	Yes, adenoma as well as malignant metastases in the lung were identified	Not reported	Liver tumours in males and females, kidney tumours in males only	Body weight in 80 and 160 mg/kg bw lower than control group, survival in dosed female groups significantly reduced (but considered as due to tumor-	Studies performed with dermal application, mainly systemic effects observed, some local effects (skin lesions) at site	Possible mode(s) of action: induction of choline deficiency and displacement of ethanolamine in

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	adenoma and carcinoma (males and females) with high background incidences, hepatoblastoma (male only) Kidney adenoma and carcinoma (male only)					related deaths)	of application	phospholipids MoAs and target tissues relevant to humans.

Overall, the evidence of carcinogenicity in experimental animals consists of treatment-related tumour induction showing benign and malignant neoplasms in both sexes of a single species in a single study with some limitations in the study design. Although the high spontaneous background incidences in benign liver tumours may weaken the strength of evidence, liver tumours induced by DEA showed elevated frequency of mutations in *β*-catenin *Catnb* genes and differed from spontaneous liver tumours often containing H-ras mutations (Hayashi et al., 2003). Therefore, classification of DEA as Category 2 carcinogen seems appropriate. This is in line with the IARC assessment which was updated in 2011 (IARC, 2013) with the following conclusions: There is *inadequate evidence* in humans for the carcinogenicity of diethanolamine. There is *sufficient evidence* in experimental animals for the carcinogenicity of diethanolamine. Diethanolamine is *possibly carcinogenic to humans (Group 2B)*.

Specific concentration limit for carcinogens:

To decide on setting of a specific concentration limit for DEA, a T25 value was determined according to EC (1999) as a measure for the intrinsic carcinogenic potency of DEA. The T25 value estimates the dose in chronic studies at which particular neoplastic lesions occur in 25% of the animals of a dose group. For calculating the T25 value it is assumed that there is a linear relationship between potency and administered dose. T25 values were calculated for several statistically significant treatment related incidences of neoplastic lesions from the 2-year NTP studies (NTP, 1999b).

The lowest T25 values for DEA are shown in Table 17.

Table 17: Calculation of T25 values for selected neoplastic lesions from the 2-year NTP study (NTP, 1999b)

Species/sex	Mouse, male								Mouse, female							
Lesion	Hepatocellular adenoma				Hepatocellular adenoma, carcinoma				Hepatocellular adenoma				Hepatocellular adenoma, carcinoma			
Dose (mg/kg bw/d)	0	40	80	160	0	40	80	160	0	40	80	160	0	40	80	160
Exposure (days/week)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Number of animals	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Incidences	31	42	49	45	39	47	50	50	32	50	48	48	33	50	50	50
Incidence (%)	62	84	98	90	78	94	100	100	64	100	96	96	66	100	100	100

Species/sex	Mouse, male								Mouse, female							
Lesion	**Hepatocellular adenoma**				**Hepatocellular adenoma, carcinoma**				**Hepatocellular adenoma**				**Hepatocellular adenoma, carcinoma**			
Net incidence* (%)		57.9	94.7	73.7		72.7	100	100		100	88.9	88.9		100	100	100
Average daily dose		28.6	57.1	114.3		28.6	57.1	114.3		28.6	57.1	114.3		28.6	57.1	114.3
T25*		**12.34**	15.08	38.78		**9.82**	14.29	28.57		**7.14**	16.07	32.14		**7.14**	14.29	28.57

*Calculations according to Dybing, 1997:
Net incidence (%) = [(Dose incidence in % - Control incidence in %) / (100 - Control incidence in %)] * 100
T25 (mg/kg bw/day) = Average daily dose * (25 / Net incidence (%))

All calculated T25 values were in the medium potency range between 1 and 100 mg/kg bw/day. Therefore, no special concentration limit (SCL) is required and the general concentration limit (GCL) should be applied.

10.8.3 Conclusion on classification and labelling for carcinogenicity

Based on the data available, DEA should be classified as Category 2 carcinogen. The generic concentration limit (GCL) should be applied, a specific concentration limit is not required, as DEA is within the medium potency range of carcinogens.

10.9 Reproductive toxicity

10.9.1 Adverse effects on sexual function and fertility

Table 18: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Experimental study OECD TG 443 (EOGRTS), GLP Key study (reliable without restriction) **Oral** (drinking water), no vehicle **Rat** (Crl:Wl(Han) Wistar) 30 M/30 F per dose group (P) 75 M/75 F per dose group (F1)	**2,2'-iminodiethanol** (99.9%) (CAS 111-42-2/ EC 203-868-0) 0, 100, 300, 1000 ppm (nominal) (0, 13, 38, 128 mg/kg bw/d in drinking water) Measured dose levels: M: 0, 6.8, 22, 74 mg/ kg bw/d, F: 0, 10.2, 29.4, 103.9 mg/kg bw/d pre-mating 0, 11.5, 34.9, 116.8 mg/kg bw/d gestation 0, 24.0, 66.3, 173.3 mg/kg bw/d lactation F1: 11, 34, 123 mg/ kg bw/d (M); 13, 39, 137 mg/kg bw/d (F) Daily from 16 days premating until sacrifice	**P0 parental animals** **LOAEL (maternal toxicity)**: 100 ppm corresponding to 15.2 mg/kg bw/d (calculated as mean intake during pre-mating, gestation and lactation period) based on effects on kidney and liver (details see Table 25) **Reproduction** No effects on female oestrus cycle during premating and mating No effects on fertility indices in males and females Increased gestation length (22.0, 22.0, 22.1, and 22.5 days at 0, 100, 300, and 1000 ppm; significant at 1000 ppm) Decreased number of implants (12.3, 12.2, 11.4, and 7.8 at 0, 100, 300, and 1000 ppm; significant at 1000 ppm); therefore, decreased litter size (11.9, 11.8, 11.1, and 7.3 at 0, 100, 300, and 1000 ppm; significant at 1000 ppm) **F1 animals** **Reproduction** Feminisation (tubule-alveolar structure, less amount of cytoplasm, increase in basophilia) of the mammary gland (slight to severe) in 4/16 males and diffuse hyperplasia in 1/16 males (cohort 1A) at 1000 ppm only; increased secretion (eosinophilic amorphous material which was secreted into the ducts) of the mammary gland (minimal) in 1/20, 1/20, 0/20, and 6/20 (significant) females at 0, 100, 300, and 1000 ppm (cohort 1A) Prolonged and irregular oestrus cycle in cohort 1A (4.1, 4.0, 4.2, and 5.5 days at 0, 100, 300, and 1000 ppm; significant at 1000 ppm) and cohort 1B (4.2, 4.0, 4.1, and 5.0 days at 0, 100, 300, and 1000 ppm; significant at 1000 ppm) females; no particular cycle phase was prolonged Decreased macroscopical ovary size (at 1000 ppm: 4/25 females in cohort 1B), probably due to body weight effects (significantly reduced bw at termination in cohort 1B females at 1000 ppm: -18.0%) Ovary atrophy at 1000 ppm (1/20 (grade 2) and 4/25 (grade 4-5) females in cohorts 1A and 1B,	Study report (BASF AG, 2018a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		respectively); Luteal cysts at 300 ppm (1/20 and 1/25 females in cohorts 1A and 1B, respectively) and at 1000 ppm (6/20 and 6/25 females in cohorts 1A and 1B, respectively); Absence of corpora lutea at 1000 ppm: 2/25 females in cohort 1B Significantly decreased number of primordial and growing follicles in the ovary at 1000 ppm in cohorts 1A and 1B (97, 93, and 88% vs control at 100, 300, and 1000 ppm, respectively) Decreased size and immature testicle (3/20 and 3/25 males in cohort 1A and 1B, respectively; (grading moderate to extreme); tubular degeneration (3/20 males of 1A, 2/25 males of 1B, grade 2-3; 2 males of 1B, grade 1) at 1000 ppm Macrovesicular vacuolation in the ductus deference in cohort 1A males in 4/20 and 12/20 animals at 300 ppm and at 1000 ppm, respectively (grades 1-3) **NOAEL (fertility: P0, F1):** **300 ppm** corresponds to 37.7 mg/kg bw/d (calculated mean intake over all cohorts and study sections) **LOAEL (fertility: P0, F1):** **1000 ppm (128.35 mg/kg bw/d)** based on lower number of implants, prolonged/irregular oestrous cycles, pathological changes in sexual organs, and mammary gland (M/F)	
Experimental study OECD TG 421 (modified), GLP, range-finding study Key study (reliable without restriction) **Oral** (drinking water), no vehicle **Rat** (Crl:Wl(Han) Wistar) 10 M/10 F per dose group	**2,2'-iminodiethanol** (99.9%) (CAS 111-42-2/ EC 203-868-0) 0, 500, 1000, 1500, and 2000 ppm (nominal) (0, 46, 95, 137, 144 mg/kg bw/d) Males: 14 days premating; 6 days mating; one week post-mating (total: 4 weeks) Females: 14 days premating; 6 days mating; entire gestation; lactation until	**P0 parental animals** **General toxicity** Decreased food/water consumption and lower body weight; liver, kidney, blood (for details see Table 25) **LOAEL (general toxicity) 500 ppm (46 mg/kg bw/d)** **Reproduction** Cystic corpora lutea at 2000 ppm (2/10 females; only control and high dose investigated) Reduced choline content in the uterus at ≥ 1500 ppm Decreased number of implantation sites (12.1, 10.7, 8.3, 5.9, and 4.0 implants/dam at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at ≥ 1000 ppm) Increased gestation length at 1500 ppm (22.2, 22.4, 22.7, 23.1, and 22.0 days at 0, 500, 1000, 1500,	Study report (BASF AG, 2018b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	post-natal day (PND) 4; in total: 8 weeks	2000 ppm DEA, respectively; significant at 1500 ppm; at 2000 ppm there was only one litter born) Decreased fertility index (100%, 100%, 90%, and 30% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively significant at 2000 ppm) Decreased gestation index (100%, 100%, 89%, and 33% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at 2000 ppm) Decreased number of pups delivered (11.6, 9.8, 6.6, 3.9, and 4.0 at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at ≥ 1000 ppm) **NOAEL (fertility): 500 ppm (46 mg/kg bw/d)** **LOAEL (fertility): 1000 ppm (95 mg/kg bw/d)**	
Experimental RDT study Equivalent or similar to OECD TG 408, GLP NTP study: (reliable without restriction) **Oral**: drinking water Vehicle: water **Rat** (Fischer 344) 10 M/10 F per dose group	**2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/ EC 203-868-0) 0, 320, 630, 1250, 2500, 5000 ppm (males); 160, 320, 630, 1250, 2500 ppm (females) 13 weeks (daily)	**Effects on the reproductive system** (for other effects see Table 25) Testicular degeneration in 3/10 males at 2500 ppm (nominal) corresponding to 202 mg/kg bw/d and in all males at 5000 ppm (nominal) corresponding to 436 mg/kg bw/d Findings at 2500 ppm (nominal) correlated with reduced sperm motility and hypospermia in the cauda epididymis, additional observation of atrophy of the seminal vesicles and prostate glands in the higher dose groups No effects on female reproductive organs or on estrous cycle length or oestrous stages **NOAEL (male fertility): 630 ppm (nominal), 48 mg/kg bw/d** **LOAEL (male fertility): 1250 ppm (nominal), 97 mg/kg bw/d** based on decreases in testis and epididymis weights, microscopically associated with degeneration of seminiferous epithelium and hypospermia NOAEL (female fertility): 2500 ppm (nominal), 242 mg/kg bw/d	Study report and publication (Melnick et al., 1994b; NTP, 1992)
Experimental RDT study Equivalent or similar to OECD TG 408, GLP NTP study: (reliable without restriction) **Oral**: drinking water Vehicle: water	**2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/EC 203-868-0) 0, 630, 1250, 2500, 5000, 10,000 ppm 13 weeks (daily)	No effects on the reproductive system; for other toxic effects see Table 25	Study report and publication (Melnick et al., 1994b; NTP, 1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Mouse (B6C3F1) 10 M/10 F per dose group			
Experimental RDT study Equivalent or similar to OECD TG 411; GLP NTP study: (reliable without restriction) **Dermal**: shaved back of each animal (unoccluded), from the mid-back to the interscapular region Vehicle: ethanol (95%) **Rat** (Fischer 344) or **Mouse** (B6C3F1) 10 M/10 F per dose group	**2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/ EC 203-868-0) Rat: 0, 32, 63, 125, 250, 500 mg/kg bw/d Mouse: 0, 80, 160, 320, 630, 1250 mg/kg bw/d (nominal per unit body weight) 13 weeks (once per day, 5 days/week)	No effects on the reproductive system; for other toxic effects see Table 27	Study report and publication (Melnick et al., 1994b; NTP, 1992)
Experimental study OECD TG 413, GLP RDT study (reliable without restriction) **Inhalation:** aerosol (nose/head only), no vehicle **Rat** (Wistar) 13 M/13 F per dose group	**2,2'-iminodiethanol** (99.89%) (CAS 111-42-2/ EC 203-868-0) 0; 15; 152; 410 mg/m³ MMAD 0.6–1.9 µm 90 days (6 h/day, 5 days/week, 65 exposures)	**Effects on the reproductive system** Diffuse testicular atrophy and minimal to slight atrophy of the prostate in single animals at 410 mg/m³ No pathological findings in females **NOAEC (male fertility): 152 mg/m³** LOAEC (male fertility): 410 mg/m³	Study report and publication (BASF AG, 1996; Gamer et al., 2008)
Reproductive and developmental toxicity study **Dermal**: DEA dissolved	2,2'-iminodiethanol (98.5%) 0, 20, 80, 320 mg/kg	**Maternal toxicity (P0 females)** Keratinisation in the beginning of treatment (320 mg/kg) **F1 offspring of exposed P0 females**	Study report (KFDA, 2007) Inofficial translation

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
in ethanol applied to 2 cm^2 area on the back **Mouse** (C57BL/6) Pregnant females (N=10) Males (N=15) Growth, organ weights and developmental and behavioural investigations	Females from GD6 – PND 21 Male exposure for 4 weeks followed by pairing with control females P0 females were terminated on PND21 and P0 males after 4 weeks of exposure F1 offspring from exposed males or females were terminated at PND 21 and PND 70	Reduced absolute uterus weight in F1 females at PND 70 (≥ 80 mg/kg; not significant at PND 21) **Paternal toxicity (P0 males)** Keratinistion in the beginning of treatment (320 mg/kg) Reduced percentage of motile sperm at all dose groups; dose-dependent decrease (not significant) of several sperm motility parameters **F1 offspring of exposed P0 males** Reduced absolute epididymis weight at PND 21 (≥ 80 mg/kg) and PND 70(320 mg/kg) in F1 males Reduced absolute testis weight at PND 70 in F1 males (320 mg/kg) Reduced absolute prostate weight at PND 70 in F1 males (all dose levels, significant only at 80 mg/kg) Increased absolute uterus weight in F1 females at PND 21 and PND 70 (all dose levels, not significant) Reduced percentage of motile sperm in F1 males at PND 70 (significant at 320 mg/kg); dose-dependent decrease (not significant) of several sperm motility parameters Delayed testes descent in F1 males (≥ 80 mg/kg; not significant)	provided to US-EPA
Mechanistic study **Oral** (unclear whether via gavage) Swiss strain male **albino mice** N = 10 per group	**2,2'-iminodiethanol** ("analytical grade") 0, 110, 165, and 330 mg/kg bw/d DEA or 330 mg/kg bw/d DEA + 10, 25, and 50 mg/kg bw/d curcumin (dissolved in olive oil) 45 days	Significantly decreased serum testosterone levels (all dose levels; dose-dependent) Significantly reduced testicular cholesterol and total lipid levels (all dose levels; dose-dependent) Significantly decreased activity of testicular 3β- and 17β-hydroxysteroid dehydrogenase (330 mg/kg bw/d; dose-dependent) Curcumin is reported to dose-dependently ameliorate the effects of DEA	Publication (Panchal and Verma, 2016)

Table 19: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vitro study Sperm parameters (motility, viability, morphology (Gimesa stain)) measured after 0, 15, 30, 45, and 60 min)	**2,2'-iminodiethanol** ("analytical grade") Sperm suspensions in 0.9% NaCl containing 0, 100, 200, 300, 400, or 500 µg/mL DEA	Semen samples from 10 healthy adult donors (23-25 years) Only samples with sperm counts > 50 mio/mL, > 50% viability, and normal morphology were used	Dose and time-dependent reduction in percentage of motile sperm, and viable sperm (≥ 100 µg/mL) Increased morphologically abnormal sperm (≥ 100 µg/mL)	Publication (Panchal and Verma, 2013)

10.9.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Extended one-generation reproductive toxicity study (EOGRTS), rat, oral according to OECD TG 443 (BASF AG, 2018a)

Based on the data from the dose-range finding study, doses of 0 (deionised water), 100, 300, and 1000 ppm DEA were chosen for the subsequent EOGRT study (OECD TG 443; version 2011) including cohorts 2A, B (developmental neurotoxicity; DNT) and cohort 3 (developmental immunotoxicity; DIT) (BASF AG, 2018a). DEA was administered to Crl:WI(Han) Wistar rats via the drinking water continuously throughout the entire study. Premating exposure of parental animals (P0) was at least 16 days. Pups of the F1 litter were selected (F1 rearing animals) and assigned to cohort 1A (reproductive toxicity; 20/sex/group), cohort 1B (reproductive toxicity; 25/sex/group), cohort 2A (developmental neurotoxicity; 10/sex/group), cohort 2B (developmental neurotoxicity; 10/sex/group), and cohort 3 (developmental immunotoxicity; 10/sex/group). Serum platelet activating factor (PAF) was determined in P0 parents.

Fertility effects in P0 females were apparent at 1000 ppm including a significantly decreased number of implantation sites (7.8 vs 12.3 in control) and a lower number of pups per dam (7.3 vs 11.9 in control), both decreasing in a dose-dependent manner (see Table 18). The gestation length was significantly increased at 1000 ppm (22.5 days vs 22.0 days in control). Fertility index, gestation index, and post-implantation losses were not affected by DEA treatment. The rate of live-born pups was not affected by the test substance but the number of litters with stillborn pups (considered for developmental toxicity) was significantly higher at 1000 ppm (8 vs 2 in control) with a dose-dependent increase. Mean body weights in the high dose P0 females were significantly lower compared to controls in different phases of the experiment with the strongest effect seen at the end of pregnancy (-15.1% vs control; for more details on body weight effects see Table 20). Corrected maternal body weight gain[2] was reduced in all dosed groups (-20.5, -18.4, and .37.5% vs. control at 100, 300, and 1000 ppm, respectively) resulting in a 9.8% lower maternal body weight on lactation day 1 (LD 1), corresponding to "corrected body weight". Therefore, the lower body weight at the end of pregnancy is in part a reflection of the lower number of implantations and litter size (note that pup birth weight was unchanged; see Table 21).

In the F1 generation, effects on reproductive parameters were observed in F1 females such as prolonged and irregular oestrus cycles (at 1000 ppm in cohorts 1A and 1B) and a decreased number of primordial and growing follicles in the ovary (at 1000 ppm in cohorts 1A and 1B combined: 88% of control values). The lower number of primordial and growing follicles is considered adverse and as a specific effect on fertility and sexual development. However, it should be kept in mind that the high dose F1 females had significantly lower mean body weights (F1A: -14.7%, F1B: -18% at termination; see Table 21), and it is known that ovarian development is sensitive to general toxicity and body weight effects. Luteal cysts were observed in the mid and high dose F1 females (at 1000 ppm: 6/20 and 6/25 females in cohort 1A and 1B, respectively; at 300 ppm: 1/20 and 1/25 females in cohort 1A and 1B, respectively). Some F1 females showed a decreased macroscopical ovarian size (at 1000 ppm: 4/25 females in cohort 1B). Of these 4 animals, all displayed diffuse ovarian atrophy and two of them did not have any corpora lutea present. These animals had a considerably lower body weight compared to controls (30-50% lower) as well as compared to other animals within this dose-group. Similarly, in cohort 1A, the single female (1/20) which showed diffuse ovarian atrophy had a markedly lower body weight both within its dose-group (the lightest animal there) as well as compared to the control mean (30% lower vs control). Therefore, the decreased macroscopical ovarian size with associated histological findings might be (at least partially) secondary to body weight effects as a reduction of body weight to approximately 70% of control values have been associated to lower ovary weight[3], although the observed effects on the ovary such as ovary atrophy, or absence of corpora lutea are deemed to be linked to the severity of lower ovarian weights. However, in addition to the high dose group, luteal cysts were also observed with low incidence at the mid dose. Because body weight effects were mild and insignificant at the mid dose (-4.7%, and -5.1% vs control

[2] Corrected maternal body weight gain = maternal body weight at LD 1 minus maternal body weight at GD 1

[3] See Chapin, 1993; Seki et al., 1997 as cited in the RAC opinion on dodecylphenol https://echa.europa.eu/documents/10162/e797debd-63b2-3907-2f31-319941901d59

in cohort 1A and 1B, respectively; see Table 21), the increase of luteal cysts at the mid and high dose is considered rather a specific effect of the test compound on female sexual function and fertility. Combined, the effects on oestrous cycle and ovarian morphology/histology in F1 females are considered adverse because they indicate disturbed ovarian development and function. However, an influence of general toxicity and lower body weight on some of the affected parameters might be implicated.

Of the high dose males in cohort 1A and 1B, 3/20 and 3/25 animals, respectively, displayed immature testicles of macroscopically reduced size together with epididymial aspermia (histopathology of epididymis performed only in cohort 1A), and a decreased size of secondary sexual organs. Specifically these animals had severely reduced body weights both within their dose-group as well as compared to the control mean (42- 67% lower vs. control). Therefore, the above described changes are considered secondary to immaturity and marked body weight effects. However, of these immature animals, one in cohort 1A and two in cohort B, in addition showed tubular degeneration that cannot be readily explained by body weight effects. Furthermore, one cohort 1B male at the low dose and two additional high dose cohort 1B males displayed tubular degeneration. The finding in the low dose cohort 1B male is considered incidental due to a lack of dose response, whereas the finding at the high dose is considered treatment related and adverse. As the tubular degeneration also occurred in animals that were not immature and because this finding implicates disturbed spermatogenesis, it is considered as a specific effect on sexual function and fertility. In addition, macrovesicular vacuolation in the ductus deference was reported in the mid and high dose cohort 1A males in 4/20 and 12/20 animals, respectively, with increased severity at the high dose. This finding is considered treatment related and as a specific effect on sexual function and fertility.

Further findings of importance for classification with regard to fertility and sexual function included pathological changes in the mammary glands of both sexes (feminisation in F1 males; increased secretions in F1 females). At 1000 ppm, 1/16 cohort 1A males displayed diffuse hyperplasia of mammary gland tissue and 4/16 males showed feminisation of mammary gland structures. Furthermore, 6/20 females in cohort 1A had increased secretion at 1000 ppm (grade 1). Unspecific side-effects of other toxicities (e.g. body weight, liver, kidney, blood) cannot explain the effects on mammary gland histology. Both, the findings of hyperplasia and feminisation in males, and increased secretions in females might indicate a deregulation of oestrogen and/or prolactin signalling at the systemic and/or tissue level. Although the adversity of mammary gland feminisation in males is not clear, in females, the increased secretions indicate a disturbed development/function of the mammary gland, potentially leading to impaired lactation or other pathological consequences later in live. Therefore, the changes in mammary gland histology are considered as adverse and relevant for classification.

Dose-range finding study (OECD TG 421), rat, oral; for subsequent EOGRTS (BASF AG, 2018b)

A dose range-finding study for a subsequent EOGRTS (OECD TG 443) was performed following the protocol of OECD TG 421 with some modifications (BASF AG, 2018b). DEA was administered to groups of 10 male and 10 female Crl:WI(Han) Wistar rats (P0 parental animals) via the drinking water at doses of 0 (deionised water), 500, 1000, 1500 and 2000 ppm corresponding to 0, 46, 95, 137, and 144 mg/kg bw/d, respectively.

In males, treatment covered two-weeks premating, the mating period (6 days), and one week postmating (P0 males were exposed in total for 4 weeks). In females, treatment covered 2-weeks premating, the mating period (6 days), and the entire pregnancy. Dams were allowed to raise their pups until post-natal day (PND) 4. The P0 females were exposed in total for 8 weeks. Parameters determined in parental animals comprised recordings of mortality and clinical observations, food and water consumption, body weight data, and male and female reproductive data. Organ weights were determined (brain, kidney, liver, testis, epididymis, uterus) and haematology (N = 5 per sex and dose group) as well as histology of selected organs was performed (control and high dose: brain, spinal cord (cervical, thoracic and lumbar cord), testis, epididymis, ovary, and uterus; all dose groups: kidney and liver). In all parental males and females (with litter), choline content was determined in blood plasma (approximately one week before sacrifice), liver, kidney, and uterus by HPLC-MS.

Body weight was significantly lower in males at all dose-groups (up to -11% vs controls at 2000 ppm at termination). In females, body weight was slightly lower at the start of gestation (-2.6%, -4.0%, -5.8%, and -5.3% vs controls at 0, 500, 1000, 1500, and 2000 ppm, respectively; significant only at 1500 ppm). At the end of gestation, body weights were significantly lower from 1000 ppm onwards (-5.3%, -10.7%, -17.5%, and -18.4% vs controls at 500, 1000, 1500, and 2000 ppm, respectively), whereas at the start of lactation, the

difference to the control group was not significant and below 10% in all dose groups (-3.3%, -4.5%, -6.4%, -9.8% vs controls at 500, 1000, 1500, and 2000 ppm, respectively; no significance; see Table 22). Therefore, the lower body weights during gestation are to a good part a reflection of the lower number of implantations and increased post-implantation losses (see below). Reproductive toxicity was observed at ≥ 1000 ppm, including cystic corpora lutea (2/10 females at 2000 ppm; only control and high dose investigated), decreased implantation sites (12.1, 10.7, 8.3, 5.9, and 4.0 at 0, 500, 1000, 1500, and 2000 ppm DEA, respectively; significant at ≥ 1000 ppm), increased post-implantation losses (significant at ≥ 1500 ppm; considered for developmental toxicity), and decreased number of pups/dam (11.6, 9.8, 6.6; 3.9, and 4.0 at 0, 500, 1000, 1500, and 2000 ppm DEA, respectively; significant at ≥ 1000 ppm). Only one litter was born at 2000 ppm. Gestation index (33% compared to control) and fertility index (30% compared to control) were significantly lower at 2000 ppm. Mating behaviour and copulation were normal in all test groups.

Reproductive and developmental toxicity study, mouse, dermal (KFDA, 2007)

A reproductive and developmental toxicity study was performed by the Korea Food & Drug Administration using C57BL/6 mice (KFDA, 2007). An unofficial English translation is available which was initially provided by Dow Chemicals to US-EPA.

Males (N = 15) were exposed by applying 0, 20, 80, or 320 mg/kg DEA (dissolved in ethanol) for 4 weeks to an area of 2 cm2 on the back (unclear whether test area was occluded). Exposed P0 males were mated with unexposed females and terminated after successful mating. In a parallel experiment pregnant females (N=10; mated with unexposed males) were exposed to similar doses of DEA from GD 6 until weaning (PND 21). P0 females were terminated on PND 21. For parental animals, growth, gross lesions, organ weights and sperm motility parameters in males were determined. A caesarean section was performed on a subset of pregnant females on GD 18 and foetuses were evaluated for skeletal and soft tissue malformations (unclear how many dams were sacrificed). F1 offspring from exposed P0 males and P0 females was raised to determine several developmental/behavioural landmarks and to perform behavioural testing until PND 70 (i.e. rota rod test at PND 28, open field test on PND 35, passive avoidance test on PND 42, hot plate test on PND 45, and fear conditioning test on PND 61). Autopsy on a subset of F1 animals was done at PND 21 and the remaining animals were terminated at PND 70. Post autopsy, gross lesions and organ weights of F1 animals were determined.

Male F1 offspring from exposed F0 males showed a dose-dependent reduction of the absolute epididymis weight (significant at ≥ 80 mg/kg at PND 21 and at 320 mg/kg at PND 70), and absolute testis weight was significantly lower at PND 70 in the high dose group (320 mg/kg). Absolute prostate weight at PND 70 was reduced in all dose groups but statistical significance was attained only at 80 mg/kg. The minimal, non-significant changes in body weight observed (-1.1% and -4% vs controls at PND 21 and PND 70, respectively) cannot explain the lower absolute organ weights. In addition, sperm analysis showed a reduced percentage of motile sperm in F1 males at PND 70 (significant at 320 mg/kg), and a dose-dependent decrease (not significant) of several sperm motility parameters. Female F1 offspring showed an increase in absolute uterus weight at PND 21 and PND 70 at all dose levels but this was not significant.

Repeated dose toxicity study, oral, rats (Melnick et al., 1994b; NTP, 1992)

For study details and general toxicity effects, see chapter 10.12.

When DEA was orally applied to F344 rats via drinking water at dose levels of 0, 320, 630, 1250, 2500, or 5000 ppm for males, or 0, 160, 320, 630, 1250 and 2500 ppm for females for 13 weeks, decreases in testis and epididymis weights were noted in males at ≥ 1250 ppm (corresponding to ≥ 97 mg/kg bw/d). Testicular degeneration was observed in all high dose males (5000 ppm, corresponding to 436 mg/kg bw/d) and in 3/10 males at 2500 ppm (corresponding to 202 mg/kg bw/d). Significant body weight effects were observed in males from 630 ppm onwards in a dose-dependent fashion (terminal body weights were -5.0, -11.0, -18.0, -28.7, and -44.2% lower compared to controls at 320, 630, 1250, 2500, and 5000 ppm, respectively). Atrophy of the seminal vesicles and prostate glands in male rats of the higher dose groups were additionally observed. The decrease in testis and epididymis weights at ≥ 1250 ppm (corresponding to ≥ 97 mg/kg bw/d) was associated microscopically with degeneration of seminiferous epithelium as well as hypospermia and reduced sperm motility and hypospermia in the cauda epididymis at ≥ 2500 ppm (corresponding to ≥ 202 mg/kg bw/d).

No effect on the reproductive organs of the females was noted up to 2500 ppm (corresponding to 242 mg/kg bw/d), and no effects on oestrus length or oestrous stages were seen, respectively. The LOAEL for reproductive effects in males (decreases in testis and epididymis weights) was established at 1250 ppm (corresponding to 97 mg/kg bw/d). The NOAEL for reproductive effects in males was 630 ppm (corresponding to 48 mg/kg bw/d) and in females 2500 ppm (corresponding to 242 mg/kg bw/d), respectively (Melnick et al., 1994b; NTP, 1992).

Repeated dose toxicity study, inhalation, rats (BASF AG, 1996; Gamer et al., 2008)

For study details and general toxicity effects, see chapter 10.12.

DEA as a liquid aerosol was tested according to OECD TG 413 using head-nose exposure in each 13 male and 13 female Wistar rats. The animals were exposed to 0, 15, 152, and 410 mg/m³ for 6 h daily, 5 times/week for 3 months (65 exposures). There was an influence on the male reproductive system in form of diffuse testicular atrophy and minimal atrophy of the prostate in single animals at the high concentration only (0.4 mg/L) (no further details). No weight changes were noted in the reproductive organs of males. In females, neither weight changes nor pathological findings (uterus, mammary gland) were observed. The NOAEC for toxic effects on the reproductive organs was 0.15 mg/L in males (atrophy of testes and prostate) and for females at 0.4 mg/L (BASF AG, 1996; Gamer et al., 2008).

Reproductive toxicity study, mouse, oral (Panchal and Verma, 2016)

The effect of DEA alone or in combination with curcumin on the activity of testicular steroidogenic enzymes (3β- and 17β-hydroxysteroid dehydrogenases), testicular lipid and cholesterol content, and plasma testosterone levels was investigated in Swiss strain male albino mice after 45 days of exposure (Panchal and Verma, 2016). Male mice (N = 10) were orally exposed to 0, 110, 165, and 330 mg/kg bw/d DEA ("analytical grade"), or to 330 mg/kg bw/d DEA + 10, 25, and 50 mg/kg bw/d curcumin (dissolved in olive oil). A vehicle control group (olive oil, 0.2 mL/animal/day) and a group receiving 50 mg/kg bw/day curcumin only were also included in the experiment. It is not reported how the administration of the test compounds was performed (gavage/diet/drinking water). The control group however "was maintained without any treatment".

DEA treatment resulted in a significant and dose-dependent decrease of serum testosterone levels (significant at ≥ 110 mg/kg bw/d), testicular cholesterol as well as total lipid levels (significant at ≥ 110 mg/kg bw/d), and activity of testicular 3β- and 17β-hydroxysteroid dehydrogenase (significant at 330 mg/kg bw/d). Co-treatment with curcumin dose-dependently ameliorated the effects of DEA on above mentioned parameters and the study authors suggest that this is due to the antioxidant properties of curcumin.

***In vitro* study on human sperm samples (Panchal and Verma, 2013)**

Semen samples from 10 healthy adult donors (23-25 years of age) were used to prepare sperm suspensions in 0.9% NaCl containing 0, 100, 200, 300, 400, or 500 µg/mL DEA (Panchal and Verma, 2013). Only samples with sperm counts > 50 mio/mL, > 50% viability, and normal morphology were used. Sperm parameters (motility, viability (trypan blue), morphology (Gimesa stain)) were measured after 0, 15, 30, 45, and 60 min. DEA treatment (≥ 100 µg/mL) resulted in a significant dose- and time-dependent decrease in motile and viable sperm. Furthermore, the percentage of morphologically abnormal sperm was significantly dose- and time-dependently increased (≥ 100 µg/mL).

10.9.3 Comparison with the CLP criteria

The evidence of reproductive toxicity of DEA was obtained from animal testing. Three GLP compliant rat studies are available for assessment of effects on fertility for DEA: an Extended One-Generation Reproductive Toxicity Study (EOGRTS) according to OECD TG 443 (BASF AG, 2018a) including a preceding dose-range finding study according to a modified protocol of OECD TG 421 (BASF AG, 2018b), a three-month nose-only inhalation study to DEA aerosols according to OECD TG 413 (BASF AG, 1996; Gamer et al., 2008) and a sub-chronic oral treatment study via drinking water (protocol similar to OECD TG 408; (Melnick et al., 1994b; NTP, 1992)).

Under the conditions of an EOGRTS including cohorts 2A, 2B (developmental neurotoxicity), and 3 (developmental immunotoxicity) in Wistar rats receiving 0, 100, 300, and 1000 ppm in drinking water (BASF

AG, 2018a), consumption of water and food as well as body weight gain were reduced in P0 females at ≥ 300 ppm during gestation and lactation. In P0 males, food consumption was lower than in controls at 1000 ppm during premating, and lower body weight gain (average 23-25%) and body weight was seen at ≥ 300 ppm (see Table 20).

Table 20: Body weight effects in P0 animals in the EOGRTS

	P0 males				**P0 females**			
Dose [ppm]	0	100	300	1000	0	100	300	1000
Start pre-mating N	--- 30	+0.1 30	0.0 30	0.0 30	--- 30	+0.3 30	0.0 30	-0.1 30
Start mating N	--- 30	-1.1 30	-1.9 30	**-5.1*** 30	--- 30	+2.6 30	-0.1 30	**-4.1*** 30
Start gestation N	---	---	---	---	--- 30	+2.5 30	-0.7 30	**-5.1*** 30
End gestation N	---	---	---	---	--- 30	-0.6 30	-3.0 27	**-15.1*** 30
Start lactation N	---	---	---	---	--- 30	-1.1 30	-3.5 27	**-9.8*** 29
End lactation N	---	---	---	---	--- 30	-0.5 30	-1.6 27	**-8.5*** 29
Termination N	--- 30	-2.2 30	**-5.3*** 30	**-10.0*** 30	--- 30	0.0 30	**-2.5*** 30	**-8.1*** 30

Change vs. ctrl [%], * $p \leq 0.05$

The number of implants in P0 dams was significantly decreased (7.8 vs 12.3 in controls) at 1000 ppm, accompanied by a lower litter size (7.3 vs 11.9 in controls). Gestation length was significantly increased at 1000 ppm (22.5 vs 22 days in controls). No effects on fertility were observed in P0 males. Although body weights in the high dose P0 females were significantly lower compared to controls, the difference vs control did not exceed 10% except at the end of gestation (see Table 20) which can be explained by the lower number of implantations. Therefore, the magnitude of body weight effects is considered not pronounced enough to explain the reduced implantations and litter size. Also other toxicity (e.g. on liver, kidney, blood) was observed at the same dose-level (see section 10.12). However, the dossier submitter does not consider these findings as "*marked systemic toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma)*" in accordance with section 3.7.2.2.1.1 of the Guidance on the Application of the CLP criteria (ECHA, 2017), and reproductive toxicity cannot directly be associated to these effects. Therefore, the effect of DEA on gestation length, implantations and litter size is considered a specific effect on sexual function and fertility.

Fertility effects in F1 females included prolonged/irregular oestrous cycles, and decreased primordial/growing ovarian follicles (1000 ppm). Although it should be kept in mind that the high dose F1 females had significantly lower body weights (F1A: -14.7%, F1B: -18% vs controls at termination; see Table 21), the body weight effects are not pronounced enough to simply explain these findings, and the effects on oestrous cycle and ovarian follicle counts are, therefore, considered relevant for classification. In addition, histology revealed luteal cysts, absence of corpora lutea, diffuse ovarian atrophy, and reduced macroscopical ovarian size in a number of F1 females. Whereas, the decreased macroscopical ovarian size with associated histological findings might be secondary to the pronounced body weight effects in these particular animals, the finding of luteal cysts at the mid and high dose is considered rather a specific effect of the test compound on female sexual function and fertility and is therefore relevant for classification.

In F1 males, treatment-related effects included degeneration of testicular tubules (at 1000 ppm: 1/20 and 3/25 animals of cohorts 1A and 1B, respectively), and macrovesicular vacuolisation of the ductus deferens (in cohort 1A: 12/20 and 4/20 animals at 1000 ppm and 300 ppm, respectively). Furthermore, testicular immaturity (at 1000 ppm: 3/20 and 3/25 animals in cohort 1A and 1B, respectively) accompanied by epididymal aspermia and a decreased macroscopical size of prostate, epididymides, and seminal vesicles was observed. These findings occurred particularly, however not exclusively, in animals with conspicuously lower body weight. As

the tubular degeneration also occurred in animals that were not immature and because this finding implicates disturbed spermatogenesis, it is considered adverse and being a rather specific effect on sexual function and fertility. Similarly, the finding of vacuolation of the ductus deference in cohort 1A is considered adverse and cannot be explained by body weight effects or unspecific toxicity. In addition, the pathological changes in the mammary glands of both sexes (feminisation in F1 males; increased secretions in F1 females) at the high dose are considered relevant for classification for sexual function and fertility.

Table 21: Body weight effects in F1 animals in the EOGRTS

	F1 males				**F1 females**			
Dose [ppm]	0	100	300	1000	0	100	300	1000
PND 0 birth weight	---	+1.5	+1.5	0.0	---	+1.5	+1.5	-3.1
N	30	30	27	29	30	30	27	28
PND 4 after culling	---	0.0	-1.9	**-5.8***	---	0.0	0.0	**-7.0***
N	30	30	27	27	30	30	27	26
PND 21 weaning	---	-2.7	**-6.7***	**-13.2***	---	-2.1	**-5.7***	**-13.4***
N	30	30	27	27	30	30	27	26
F1A start dosing	---	-3.7	**-9.4***	**-21.7***	---	-3.3	-5.4	**-17.1***
N	20	20	20	19	20	20	20	20
F1A termination	---	-1.1	-6.8*	**-25.7***	---	-0.8	-4.7	**-14.7***
N	20	20	20	19	20	20	20	20
F1B start dosing	---	-6.2	**-8.6***	**-21.7***	---	-3.2	**-7.6***	**-18.4***
N	25	25	25	25	25	25	25	25
F1B termination	---	-1.8	-4.3	**-19.9***	---	-0.7	-5.1	**-18.0***
N	25	25	25	24	25	25	25	24
F1-2A start dosing	---	-4.4	-5.5	**-18.1***	---	-0.3	-5.1	**-20.5***
N	10	10	10	10	10	10	10	10
F1-2A termination	---	-1.2	-5.9	**-15.2***	---	-0.7	-5.7	**-16.0***
N	10	10	10	10	10	10	10	10
F1-2B termination[#]	---	-5.8	-4.1	**-16.5***	---	-2.4	**-8.3***	**-15.5***
N	10	10	10	10	10	10	10	10
F1-3 start dosing	---	-0.4	-7.2	**-19.7***	---	-1.7	-5.7	**-20.5***
N	10	10	10	10	10	10	10	10
F1-3 termination	---	+0.8	-5.5	**-19.7***	---	+0.5	-1.6	**-14.3***
N	10	10	10	10	10	10	10	10

Change vs. ctrl [%], * $p \leq 0.05$, [#] termination on PND 22

The dose-range finding study (BASF AG, 2018b) for the beforehand-mentioned EOGRTS, based on a modified OECD TG 421, similarly showed significant effects on female fertility (see Table 18). Reproductive toxicity was observed at ≥ 1000 ppm including reduced implantation sites, decreased litter size, and lower gestation and fertility indexes (at 2000 ppm). Except for an increased incidence of cystic corpora lutea at 2000 ppm (2/10 vs 0/10 in controls; only high dose and control P0 animals were evaluated), no histological findings were observed in reproductive organs. Lower body weight in treated P0 females was seen during different phases of the study (see Table 22), and differences in body weight compared to controls were highest at the end of pregnancy (-18.7% vs. control at the high dose). Corrected maternal body weight gain[4] was decreased in all dosed groups (-8.9, -8.5, -11.4, and -46.9% vs. control at 500, 1000, 1500, and 20000 ppm, respectively). However, on LD 1, effects on body weight were not statistically significant and of lower magnitude (maximum -9.9% vs. control at the high dose) than at the end of gestation, reflecting the decreased number of implantations and litter size (note that weight determination was done only for dams that produced a litter). Therefore, the body weight effects are considered not pronounced enough to explain the reduced implantations and litter size.

[4] Corrected maternal body weight gain = maternal body weight at LD 1 minus maternal body weight at GD 1

Similarly, the other toxic effects seen on e.g. liver and haematology (see section 10.12) cannot directly be associated with the observed reproductive toxicity. In conclusion, the effect of DEA on fertility index, implantations, and litter size is considered relevant for classification.

Table 22: Body weight effects in P0 animals in the dose-range finding study for the EOGRTS

	Males					**Females**				
Dose [ppm]	0	500	1000	1500	2000	0	500	1000	1500	2000
Start pre-mating N	--- 10	+0.4 10	+0.4 10	+0.7 10	-0.3 100	--- 10	+2.0 10	+0.7 10	+0.7 10	+2.3 10
Start mating N	--- 10	-1.7 10	-2.8 10	**-4.3*** 10	**-7.0*** 10	--- 10	-2.2 10	**-5.1*** 10	**-4.5*** 10	-2.5 10
Start gestation N	---	---	---	---	---	--- 10	-2.6 10	-4.0 10	**-5.8*** 9	-5.3 3
End gestation N	---	---	---	---	---	--- 10	-5.25 10	**-10.7*** 10	**-17.5*** 9	**-18.4*** 3
Start lactation N	---	---	---		---	--- 10	-3.3 10	-4.5 10	-6.4 9	-9.9 1
Termination N	--- 10	-3.7 10	**-6.7*** 10	**-7.9*** 10	**-11.0*** 10	--- 10	-4.1 10	**-7.1*** 10	**-11.1*** 9	**-12.0*** 10

Change vs. ctrl [%], * $p \leq 0.05$

In sub-chronic repeated dose toxicity studies in rats, toxic effects of DEA on male fertility were observed by the oral (Melnick et al., 1994b; NTP, 1992) and inhalation (BASF AG, 1996; Gamer et al., 2008) routes. A decrease in absolute/relative weights of testis and epididymis, testicular degeneration, atrophy of the seminal vesicles and prostate glands, and associated effects on spermatology were observed in the oral study from 1250 ppm (97 mg/kg bw/d) onwards corresponding to a NOAEL of 630 ppm (48 mg/kg bw/d) for reproductive effects in males. Via inhalation, DEA induced diffuse testicular atrophy and minimal atrophy of the prostate at 0.4 mg/L, corresponding to a NOAEC of 152 mg/m^3. In none of the subchronic repeated dose studies, histopathological effects were observed in female reproductive organs.

Combined, these data from standard repeated dose tests give reason for concern that DEA may induce toxicity to the male reproductive system. The effects occurred at dose levels causing other systemic effects (e.g. body weight, blood, kidney, liver, and brain) and its severity and biological plausibility to cause the observed reproductive effects need consideration. In particular in the oral study, there was pronounced systemic toxicity at the high doses (terminal body weight: -5.0, -11.0, -18.0, -28.7, and -44.2% vs control at 320, 630, 1250, 2500, and 5000 ppm, respectively). Nonetheless, it seems that the effects on testes and epididymis at 1250 and 2500 ppm are rather indicating a specific effect of DEA on male fertility. The findings of testicular and prostate atrophy seen at the high dose in the inhalation study support this notion as body weight effects were less pronounced (-13.2% vs controls). Therefore, the observed testicular effects provide supporting evidence for classification.

A study performed by the Korean Food and Drug Administration investigated the reproductive and developmental toxicity of DEA in mice offspring after dermal exposure of either paternal or maternal animals (KFDA, 2007). In P0 males, a significant decrease in motile sperm ($\geq$ 20 mg/kg bw/d) accompanied by reduced sperm motility (non-significant) was detected. Male offspring from exposed fathers similarly showed a significant decrease in motile sperm (320 mg/kg bw/d). Furthermore, in F1 males at 320 mg/kg bw/d, weight of epididymis and testis was significantly lower compared to controls and this effect could not be explained by the minimal body weight effects (-4% vs controls) observed. No fertility-related parameters were changed in P0 females due to DEA exposure. Offspring from exposed dams showed no significant fertility effects except for a significantly reduced absolute uterus weight in F1 females on PND 70 (320 mg/kg bw/d).

An *in vitro* study using human sperm samples revealed a significant and dose-dependent decrease in motile and viable sperm, and the percentage of morphologically normal sperm was reduced (Panchal and Verma, 2013). An oral reproductive toxicity study with DEA performed in mice by the same authors (Panchal and Verma, 2016) reported a significant and dose-dependent decrease of serum testosterone levels (significant at

≥ 110 mg/kg bw/d), testicular cholesterol as well as total lipid levels (significant at ≥ 110 mg/kg bw/d), and activity of testicular 3β- and 17β-hydroxysteroid dehydrogenase (significant at 330 mg/kg bw/d).

Further evidence of toxic effects on fertility is provided by a study investigating the neurotoxicity of DEA in mice (Craciunescu et al., 2006); details see Table 23. Dermal exposure from GD 7-17 resulted in a dose-dependent and significant reduction of viable foetuses per litter (≥ 160 mg/kg bw/d) and a lower total number of viable foetuses (not significant, effect considered for developmental toxicity). However, there was no reporting of maternal toxicity except for mentioning one dead dam in the high dose group (640 mg/kg bw/d), and it is unclear whether the decrease of viable litters is due to lower implantation sites or due to developmental toxicity (postimplantation losses/stillbirth).

In summary, DEA induced significant effects on fertility parameters in several studies. There were pronounced effects on the number of implants and on litter size of P0 animals in the EOGRTS (and in particular the preceding dose-range finder study) as well as further effects on reproductive organs in F1 animals (ovary, testis, mammary gland). In addition to the EOGRTS, there are supporting *in vivo* and *in vitro* studies demonstrating toxic effects on male sexual organs, sperm parameters, and steroidogenesis. A variety of other toxic effects (see 10.12) occurred at similar or lower doses than those causing reproductive toxicity in several studies. Nonetheless, the effects of DEA on fertility and sexual function are considered as specific and not just a non-specific side-effect of other toxicity.

Moore et al. (2018) hypothesised that reduced synthesis of platelet-activating factor (PAF) and/or formation of a functionally impaired PAF analogue is the predominant mode of action underlying the effects on fertility of alcohol amines. In fact, in the EOGRTS (BASF AG, 2018a), DEA treatment reduced plasma and tissue choline, and plasma PAF levels in P0 females. The adverse effects of DEA on fertility and sexual function are probably related to its interference with choline uptake and metabolism but are, nonetheless, a specific intrinsic property of the substance and not just unspecific side-effects of other toxicity.

The following DEA-induced effects on fertility and sexual function shall be considered for classification:

- Decreased number of implantations and litter size, and prolonged gestation of P0 females in the EOGRTS; decreased number of implantations and litter size in the preceding dose-range-finder study; dose-dependent and significantly lower number of viable foetuses per litter in the study by Craciunescu et al. (2006) (supporting evidence)

- In F1 females in the EOGRTS: Prolonged/irregular oestrous cycles; decreased number of primordial and growing follicles; histopathological changes in the ovary (luteal cysts) and mammary gland (increased secretion into the ducts)

- Histopathological changes in reproductive organs in F1 males (tubular degeneration and vacuolation of the ductus deference) in the EOGRTS; effects on weight of testes and epididymides, testicular and prostate atrophy, and spermatology in repeated dose toxicity studies (BASF AG, 1996; Gamer et al., 2008; Melnick et al., 1994b; NTP, 1992)

- Supporting evidence: significant decrease of motile sperm in P0 and F1 males observed in the study by KFDA (2007); significant negative impact on human sperm motility, viability, and morphology *in vitro* (Panchal and Verma, 2013), and decreased serum testosterone levels in mice (Panchal and Verma, 2016)

Considering the criteria for classification into hazard categories for reproductive toxicants according to the CLP Regulation (Annex I, Table 3.7.1 (a)), the following needs to be considered: there is no human evidence available, the available reliable evidence is based on experimental animals, and the adverse effects on reproduction are considered not to be a secondary non-specific consequence of other toxic effects and thus fulfilling the criteria for Category 1B.

10.10 Adverse effects on development

Table 23: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Experimental study OECD TG 443 (EOGRTS), GLP Key study (reliable without restriction) **Oral** (drinking water), no vehicle **Rat**(Crl:Wl(Han) Wistar) 30 M/30 F per dose group (P) 75 M/75 F per dose group (F1)	**2,2'-iminodiethanol** (99.9%) (CAS 111-42-2/ EC 203-868-0) 0, 100, 300, 1000 ppm (nominal) (0, 12.75, 37.68, 128.35 mg/kg bw/d) 16 days premating daily until sacrifice	**Parental animals (P0)** Decreased food/water consumption and lower body weight; effects on liver, kidney, blood (for details see Table 18 and Table 25) **F1 animals** Significantly lower pup viability index between PND 0-4 at 1000 ppm (increased number of dead or cannibalised pups; two litters completely lost) Lower pup body weight gain and body weights at ≥ 300 ppm until weaning (at 1000 ppm: max -13.4% in PND 21 females) Decreased water consumption at ≥ 300 ppm (max -13% to -16% at 1000 ppm in cohort 1B) Decreased food consumption at ≥ 300 ppm in males (max -17.2% at 1000 ppm in cohort 1A) and females at 1000 ppm (max -17% in cohort 1B) Decreased body weight gain and body weight throughout the study (postweaning) (Cohorts 1A, 1B, 2A, 3) at ≥ 300 ppm in males (max -9.4% after weaning in cohort 1A) and males/females at 1000 ppm (max -25.7% in males in cohort 1A, and -18% in females in cohort 1B at termination) Piloerection in several males and females at 1000 ppm (not observed in P0) Mortality at 1000 ppm (1/40, 2/50, 1/20 animals in cohorts 1A, 1B, and 2A, respectively) Eosinophilic cysts in the pars distalis of the pituitary at all dose levels in cohorts 1A and 2A, and at 1000 ppm in cohort 2B (not observed in P0 animals) **Developmental neurotoxicity** High stepping gait in several males and females (2/40, 5/25, 3/50, 1/20, and 2/20 animals in cohort 1A, 1B, 2A and 3, respectively) at 1000 ppm (not observed in P0) Significantly increased widths of hippocampus (+7% vs control) and cerebellum (+12% vs control)	Study report (BASF AG, 2018a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		in males, and parietal cortex (+20% vs control) in females at 1000 ppm (only high dose and controls of cohort 2A investigated) Degeneration of nerve fibers (medulla oblongata, and spinal cord) in males and females at PND 77 (cohort 2A; mostly at 1000 ppm); not observed at PND 22 in cohort 2B; not observed in P0 Decreased maximum amplitude in the auditory startle response test in males and females at 1000 ppm (cohort 2A at PND 22) No habituation to test environment of males and females in the auditory startle response test at 1000 ppm (cohort 2A at PND 22) **Developmental immunotoxicity** Significantly lower percentage of CD4 lymphocytes (-10.8% vs control) and increased percentage of CD8 lymphocytes (+17,8% vs controls) at 1000 ppm; therefore, decreased CD4/CD8 ratio (-22.9% vs control)	
Experimental study OECD TG 421 (modified), GLP, range-finding study Key study (reliable without restriction) **Oral** (drinking water), no vehicle **Rat**(Crl:Wl(Han) Wistar) 10 M/10 F per dose group	**2,2'-iminodiethanol** (99.9%) (CAS 111-42-2/ EC 203-868-0) 0, 500, 1000, 1500, and 2000 ppm (nominal) (0, 46, 95, 137, 144 mg/kg bw/d) Males: 14 days premating; 6 days mating; one week post-mating (total: 4 weeks) Females: 14 days premating; 6 days mating; entire gestation; lactation until PND 4 (total: 8 weeks)	**P0 parental animals** Decreased food/water consumption and lower body weight; effects on liver, kidney, blood (for details see Table 18 and Table 25) **Developmental toxicity** Increased post-implantation loss (4.1%, 9.9%, 22.4%, 31.3%, and 81.0% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at ≥ 1500 ppm) Reduced pup survival at 1500 ppm (viability index: 99%, 100%, 92%, 32% and 100% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at 1500 ppm; note that at 2000 ppm there was only one litter born)	Study report (BASF AG, 2018b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Experimental study Reproductive and developmental toxicity study **Dermal**: DEA dissolved in ethanol applied to 2 cm^2 area on the back **Mouse** (C57BL/6) Pregnant females (N=10) Males (N=15) Growth, organ weights and developmental and behavioural investigations	**2,2'-iminodiethanol** (98.5%) 0, 20, 80, 320 mg/kg Females from GD 6 – PND 21 Males for 4 weeks followed by pairing with control females P0 females were terminated on PND 21 and P0 males after 4 weeks of exposure F1 offspring from exposed males or females were terminated at PND 21 and PND 70	**F1 offspring of exposed P0 females** Reduced body weight gain and body weight until PND 28 in F1 males and females (320 mg/kg) Increased latency time in hot plate test in F1 males and females (at all dose levels, not significant) Decreased entry latency in passive avoidance test in F1 males and females (all dose levels, not significant) Decreased performance in rota-rod test in F1 females (at all dose levels; not significant in males) Decreased fear conditioning in F1 males and females (all dose levels, not significant) **F1 offspring of exposed P0 males** Delayed fur-appearance in (320 mg/kg; not significant) Delayed testes descent in F1 males (≥ 80 mg/kg; not significant) Significantly increased latency time in hot plate test (at all dose levels in males, not dose-dependent; at 320 mg/kg in females, dose-dependent) Decreased performance in rota-rod test in F1 females (all dose levels, not dose-dependent; not significant) Decreased entry latency in passive avoidance test in F1 males (all dose levels, dose-dependent; not significant)	Study report (KFDA, 2007) Unofficial translation provided to US-EPA
Experimental study No guideline; GLP Range-finding study for subsequent OECD TG 414 (see BASF AG (1993)) **Inhalation:** aerosol (head/nose only), no vehicle **Rats** (Wistar) 10 pregnant females per group	**2,2'-iminodiethanol** (> 98.7%) (CAS 111-42-2/ EC 203-868-0) 0; 100; 200; 400 mg/m^3 MMAD: 0.6-1.2 µm 6 h/day from GD 6-15	**P0 parental animals** Increased absolute (400 mg/m^3) and relative liver weight (≥ 200 mg/m^3) Increased AST (400 mg/m^3) Decreased serum cholesterol and triglyceride (≥ 200 mg/m^3) Increased serum sodium and creatinine (≥ 200 mg/m^3) **Reproductive/developmental toxicity** Decreased mean placental weight (≥ 200 mg/m^3); unclear: summary says one time “decreased” another time “increased” No effects on foetal weights or external findings	Study report (BASF AG, 1991)
Experimental study OECD TG 414, GLP	**2,2'-iminodiethanol** (> 98.7%)	**P0 parental animals** Vaginal haemorrhages in 8 of the 21 pregnant rats on GD (200 mg/m^3)	Study report (BASF AG,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Key study (reliable without restriction) **Inhalation:** aerosol (head/nose only), no vehicle **Rats** (Wistar; Chbb:THOM) 25 pregnant females per group	(CAS 111-42-2/ EC 203-868-0) 0; 10; 50; 200 mg/m^3 MMAD: 0.6-1.2 µm 6 h/day from day 6 through day 15 post coitum (p.c.)	**Reproductive/developmental toxicity** Increased number of foetuses with skeletal variations (mainly rudimentary cervical ribs) at 200 mg/m^3	1993)
Experimental study Equivalent to OECD TG 414 Key study (reliable without restriction) **Dermal:** Diluted DEA or water **Rats**: 25 pregnant Crj:CD (SD) females per group	**2,2'-iminodiethanol** (purity ≥ 99.4%) 0, 150, 500 and 1500 mg/kg bw/d Due to a dosing error, the mid-dose group received only 380 instead of 500 mg/kg bw/d GD 6–15 (daily for 6 h to the clipped backs)	**P0 parental animals** Moderate to severe skin irritation (≥ 380 mg/kg bw/d) Reduced maternal body weight gain and corrected final body weight (-4.5% at 1500 mg/kg bw/d) Increased absolute and relative kidney weights (≥ 380 mg/kg bw/d) Decreased HCT, MCV, and MCH (≥ 150 mg/kg bw/d); reduced erythrocyte counts (≥ 380 mg/kg bw/d), decreased HGB and decreased platelet counts (1500 mg/kg bw/d). Increased lymphocytes and total leukocytes (1500 mg/kg bw/d) **Reproductive/developmental toxicity** Increased incidences of skeletal variations (delays in ossification; e.g. proximal hindlimb phalanges, forelimb metacarpals) at 1500 mg/kg bw/d) **LOAEL (maternal toxicity): 150 mg/kg bw/d** **NOAEL (developmental toxicity): 380 mg/kg bw/d**	Study report and publication (Marty et al., 1999; Neeper-Bradley, 1992)
Experimental study Equivalent to OECD TG 414 Key study (reliable without restriction) **Dermal:** Diluted DEA or water **Rabbits**: 15 pregnant New Zealand white rabbits per group	**2,2'-iminodiethanol** (purity ≥ 99.4%) 0, 35, 100, and 350 mg/kg/day GD 6–18 (daily for 6 h to the clipped backs)	**P0 parental animals** Skin lesions and irritation (350 mg/kg bw/d) Reduced food consumption in the posttreatment period (350 mg/kg bw/d) Reduced body weight gain during GD 6-18 (≥ 100 mg/kg bw/d; not significant) Increased absolute and relative liver weight (350 mg/kg bw/d; not significant) Increased relative kidney weight (350 mg/kg bw/d; not significant) Discoloration of the kidneys (350 mg/kg bw/d) **Reproductive/developmental toxicity**	Study report and publication (Marty et al., 1999; Neeper-Bradley, 1992)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No effects observed **NOAEL (maternal toxicity): 35 mg/kg bw/d** **NOAEL (developmental toxicity): 350 mg/kg bw/d**	
Experimental study No guideline; no GLP **Oral**: gavage; vehicle: distilled (Pico) water **Rats** (Sprague-Dawley-derived (CD®)); 12 pregnant females per dose	**2,2'-iminodiethanol** (> 98.7%) (CAS 111-42-2/ EC 203-868-0) 0, 50, 125, 200, 250, 300 mg/kg bw/d GD 6–19; necropsy at PND 21	**Maternal toxicity** Two females at 300 mg/kg bw/d were terminated in extremis on GD 11 Due to excessive toxicity (26% lower body weight compared to controls on GD12), remaining females of the 300 mg/kg bw/d group were terminated before scheduled necropsy and excluded from further summary of results One female at 200 mg/kg bw/d was terminated in extremis on GD 22 (attempting to deliver a litter of 15 pups, all of which were found dead *in utero* at necropsy At 250 mg/kg bw/d, one female was found dead on GD 15 and one was terminated in extremis on GD 21 with 12 dead pups *in utero* (the calculated LD10 was 218 mg/kg bw/d under the conditions of this study) Decreased water consumption during early pregnancy (GD 9-12: +3.5%, -15.3%, -14.6%, and -34.7% at 0, 50, 125, 200, and 250 mg/kg bw/d, respectively; significant at 125 and 250 mg/kg bw/d); no significant changes later in pregnancy or overall from GD 6-19 Reduced feed intake [g/d] (+2.5%, -1.7%, -13.1%, and -39.7% at 0, 50, 125, 200, and 250 mg/kg bw/d, respectively; significant at ≥ 200 mg/kg bw/d) Reduced body weight gain and body weight (at 200 mg/kg bw/d: -10% vs control at GD 12 and -7% on PND 4; at 250 mg/kg bw/d: -16% to -20% vs control at GD 12-20, -6% at PND 4; significant at ≥ 200 mg/kg bw/d); higher body weight at sacrifice at PND 21 (not significant) Increased absolute kidney weight (up to +14% vs control at 250 mg/kg bw/d; significant at ≥ 125 mg/kg bw/d) **Reproductive/developmental toxicity** Increased post-implantation losses (2.5%, 5.8%, 3.4%, 17.3%, and 51% at 0, 50, 125, 200, and 250 mg/kg bw/d, respectively; significant at ≥200 mg/kg bw/d) Increased postnatal mortality on PND 0-4 (0%, 0.6%, 1.8%, 2.8%, and 13.4% at 0, 50, 125, 200, and 250, respectively; significant at ≥ 125 mg/kg bw/d); no significant effect at PND 7-14 (0%, 0%, 2.1%, 1.1%, and 5.0% at 0, 50, 125, 200, and 250, respectively) Significantly lower pup birth weight at 250 mg/kg bw/d (+3.9%, +3.8%, -7.4%, and -13.9% at 0,	Study report and publication (NTP, 1999a; Price et al., 2005)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		50, 125, 200, and 250 mg/kg bw/d, respectively) Reduced post-natal pub body weight gain and body weight (on PND21: +4.3%, +5.2%, -9.6%, and -10.4% at 0, 50, 125, 200, and 250 mg/kg bw/d; significant at ≥ 200 mg/kg bw/d)	
Experimental study No guideline (post-natal mouse screening test/Chernoff and Kavlock test); GLP **Oral**: gavage; vehicle: distilled water **Mice** (Swiss Albino) 48-49 mice per group	**2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0) 0 and 450 mg/kg bw/d GD 6-15 Pups maintained till PND3	**Maternal toxicity** No maternal toxicity was observed during the exposure period Body weight significantly higher on PND 0 (+6.3% vs control), and significantly lower on PND 3 (-6.1% vs control) **Reproductive/developmental toxicity** Slightly but significantly increased gestation length (18.2 days in controls vs 18.5 days at 450 mg/kg bw/d) Live litters: 89% of control vs 79% at 450 mg/kg bw/d (not significant), and full litter loss in 11% of controls vs 21% at 450 mg/kg bw/d Birth weight unchanged; decreased pup weight gain (PND 0-3): body weight -23.8% vs control at 450 mg/kg bw/d Reduced neonatal survival: 95% in control vs 77% at 450 mg/kg bw/d (significant)	Study report (NTP, 1987)
Neuro-developmental toxicity study (reliable with restrictions, no guideline; no GLP) **Dermal**: DEA dissolved in 95% ethanol **Mouse** (c57BL/6) 6 pregnant females per dose group	**2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0) 0, 20, 80, 160, 320, and 640 mg/kg bw/d GD 7-17	**Maternal toxicity:** One animal of the high dose group died Lower liver content of choline and its metabolites at 80 mg/kg bw/d (no other dose groups studied) No other information on maternal toxicity **Reproductive toxicity:** Significantly and dose-dependently lower number of viable foetuses/litter (8.2, 8.0, 6.3, 4.8, 3.3, and 2.7 viable foetuses at 0, 20, 80, 160, 320, and 640 mg/kg bw/d, respectively; significant at ≥ 160 mg/kg bw/d) Decreased mitosis (56±14% based on phospho-histone 3) and increased apoptosis (170±10% and 178±7% based on TUNEL and activated caspase 3, respectively) in the hippocampus at 80 mg/kg bw/d (no other dose studied)	Publication (Craciunescu et al., 2006)
Mechanistic *in vivo/in vitro* study (reliable with restrictions, no guideline; no GLP)	**2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0)	**In vivo** Two pups from two dams:	Publication (Niculescu et al., 2007)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
In vivo Dermal Pregnant **Mice** (c57BL/6) Analysis of DEA-metabolites (LC-ESI-IDMS) from 2 pups of different dams	80 mg/kg bw/d from E3 – E17 DEA dissolved in 95% ethanol	DEA concentration in fetal brain: 0.023 and 0.026 mM. Concentration of phospho-DEA in fetal brain: 1.6 mM and 1.3 mM	
Neuro-developmental toxicity study (reliable with restrictions, no guideline; no GLP) **Dermal** **Mouse** (c57BL/6) 3 groups of 7 pregnant females were treated with the vehicle (1.78 µL acetone per g bw), and one group of 7 pregnant females per dose group 5 non pregnant mice treated with either vehicle or 80 mg/kg bw/d DEA for 11 days **Parameters:** Mitosis (phospho-histone 3) Apoptosis (activated caspase-3) Choline and DEA-metabolites (LC-ESI-MS) in liver and plasma of non-pregnant females	**2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0) 0, 5, 40, 60, and 80 mg/kg bw/d DEA dissolved in acetone E3 – E17	**Mitosis (phospho-histone 3):** Significantly decreased mitosis (approximately 50% of controls) at 80 mg/kg bw/d **Apoptosis (activated caspase 3):** Significantly increased apoptosis (approximately 175% of controls) in the hippocampus at 80 mg/kg bw/d **Choline and DEA-metabolites in liver and plasma of non-pregnant females (LC-ESI-MS):** Increased concentrations of DEA and DEA metabolites in plasma and liver, and decreased concentrations of choline and choline metabolites in liver	Publication (Craciunescu et al., 2009)

Table 24: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic *in vivo/in vitro* study (reliable with restrictions, no guideline; no GLP)	**2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0) **In vitro** DEA:70 µM choline-chloride + 3 mM DEA DEA + choline (CS-DEA): 210 µM choline chloride + 3 mM DEA Control: 70 µM choline chloride Choline supplemented (CS): 210 µM choline chloride	***In vitro* study part** Mouse (C57BL/6J) cortical neuronal precursor cells (at E14) **Parameters:** Mitosis (BrdU) Apoptosis (TUNEL) Choline-uptake (radiolabelled choline-chloride) Choline and DEA-metabolites (LC-ESI-IDMS) Choline kinase activity (yeast origin)	***In vitro* study part** **BrdU incorporation:** Significantly decreased mitosis after treatment with DEA (3 mM) after 48 h (3.8% vs 16% in controls) and 72 h (2.6% vs 15.4% in controls) Choline supplementation (CS-DEA: 3 mM DEA + 210 µM choline chloride) restored mitosis after 48 h (11.7% vs 16% in controls) and 72 h (14.0% vs 15.4% in controls) No difference in mitosis between control and CS group (210 µM choline chloride) after 48 h and 72 h **TUNEL** After 48 h, CS (2.7%) and CS-DEA (3.5%) treatment resulted in decreased apoptosis compared to control (6.1%) and DEA (5.5%) treatment After 72 h, DEA increased apoptosis (14.8% vs 4.8% in controls). Choline supplementation (CS-DEA: 3 mM DEA + 210 µM choline chloride) prevented this effect of DEA (5.6% vs 4.8% in controls) **Choline-uptake** Uptake of choline into neural precursor cells was decreased by DEA treatment (59% of controls). Choline-supplementation (CS-DEA) mitigated this effect (about 87% of controls) **Choline metabolites** Choline supplementation (CS) increased the intracellular concentrations of choline and phosphocholine DEA decreased the concentrations of choline and phospho-choline; CS-DEA prevented the decrease of choline but not the decrease of phospho-choline **Choline kinase** Choline kinase has much lower affinity for DEA than for choline. Still, DEA is a substrate for choline kinase, and DEA inhibited the phosphorylation of choline (significant at 40 mM DEA)	Publication (Niculescu et al., 2007)

10.10.1 Short summary and overall relevance of the provided information on adverse effects on development

Extended one-generation reproductive toxicity study (EOGRTS), rat, oral (OECD TG 443) (BASF AG, 2018a)

In the EOGRTS (including cohorts 2A, B for developmental neurotoxicity (DNT) and cohort 3 for developmental immunotoxicity (DIT)) 100, 300, and 1000 ppm DEA was administered to rats via the drinking water (BASF AG, 2018a). The detailed study description is provided in chapter 10.9.2. Relevant effects on development were observed as follows:

A significantly lower viability index due to dead and cannibalised F1 pups was observed at 1000 ppm (14 vs 1 in control) during early lactation between PND 0-4 (viability index 100%, 99%, 99%, and 93% (significant) at 0, 100, 300, and 1000 ppm DEA, respectively). Birth weight was not affected by treatment but lower pup body weight gain and body weights at ≥ 300 ppm until weaning were observed (at 1000 ppm: -13.2% and -13.4% vs controls in males and females at weaning, respectively; see Table 21). Post-weaning, body weight gain and body weight was lower in F1 animals at doses ≥ 300 ppm throughout the study period (terminal body weights at the high dose vs control in cohort 1A: -25.7% in males and -14.7% in females; cohort 1B: -19.9% in males and -18% in females; cohort 2A: -15.2% in males and -16% in females; cohort 2B: -16.5% in males and -15.5% in females; cohort 3: -19.7% in males and -14.3% in females; see Table 21). Average water consumption (max. -16.1% at 1000 ppm in females of cohort 1B) and food consumption (max. -17.2% at 1000 ppm in males of cohort 1A) was significantly decreased in both sexes at 1000 ppm. Piloerection was reported during different study sections at 1000 ppm. Mortality was observed in F1 animals in the high dose group (at 1000 ppm: 1/40, 2/50, and 1/20 animals in cohorts 1A, 1B, and 2A, respectively).

Neurotoxicity was apparent in F1 animals of both sexes. High stepping gait occurred during different study sections at 1000 ppm in the F1 animals (2/20 males in cohort 1A; 2/25 males and 3/25 females in cohort 1B; 1/10 females in cohort 2A; 1/10 males and 1/10 females in cohort 3). In contrast, no behavioural changes were observed in the P0 generation.

In the auditory startle response test (performed with cohort 2A animals at PND 22), the maximum amplitude of males and females at 1000 ppm was reduced compared to the controls during the entire measurement (significant for males of block 1, and for the mean of blocks 1-5, and for females of block 5). Furthermore, there was no habituation to the test environment in males at ≥ 300 ppm.

Significantly increased widths of hippocampus (+7% vs control) and cerebellum (+12% vs control) in males, and parietal cortex (+20% vs control) in females at 1000 ppm (only high dose and controls were investigated) were detected by brain morphometry.

Histology revealed multifocal degeneration of nerve fibers of the medulla oblongata and the spinal cord in cohort 2A animals (PND 77) of both sexes (mostly at 1000 ppm). No such findings were reported in animals of cohort 2B analysed at PND 22, or in P0 animals.

In the pars distalis of the pituitary gland of males and females, eosinophilic cysts were observed in all dosed groups in cohort 1A (0/20, 3/20, 5/20, and 15/20 males, and 0/20, 3/20, 2/20, and 15/20 females at 0, 100, 300, and 1000 ppm, respectively) as well as in cohort 2A (PND 77) (0/10, 1/10, 1/10, and 3/9 males and 0/10, 3/10, 4/10 and 8/10 females at 0, 100, 300, and 1000 ppm, respectively). The same observation was made in animals of cohort 2B (PND 22) at 1000 ppm (4/10 males and 5/10 females) but not in the P0 generation. According to the authors, these cysts were different from the cysts that occur sporadically as background lesion in the pituitary gland. Spontaneous cysts which were also observed in the present study were described with a *ciliated epithelium and a mucinous content*. The treatment-related cysts were multifocally distributed in the pars distalis, had a *non-ciliated, irregular border with an eosinophilic homogenous content with occasionally clear vacuoles at the border*.

Analysis of plasma thyroxin (T4) and thyroid-stimulating hormone (TSH) levels revealed a dose-dependent increase of T4 in females at PND 4 (median +12%, and +27% vs control at 100, and 300 ppm, respectively; significant already at 100 ppm; no plasma was available at the high dose). In PND 4 males, T4 was also slightly increased at 300 ppm (median +11.8% vs control; only 2 males were analysed at 1000 ppm). In females at PND 22, T4 was significantly increased at 1000 ppm (median +24%, +46.4%, and +48.9% vs control at 100,

300, and 1000 ppm, respectively), and there was a trend for higher T4 levels in males (median +16.6%, +14.8%, and +22.6% vs control at 100, 300, and 1000 ppm, respectively). Similarly at PND 92, there was a trend for increased T4 levels with increasing dose in males (median +6.8%, +6.7%, and +18.2% vs control at 100, 300, and 1000 ppm, respectively) and in females (median +5.2%, +21.3%, and +37.3% vs control at 100, 300, and 1000 ppm, respectively) without reaching statistical significance. In contrast, in P0 animals, T4 was increased only in males at the high dose (median +26.7%, significant). No treatment-related changes in TSH levels or histological changes of thyroid follicular cells were observed in any generation or study phase.

Regarding developmental immunotoxicity, no treatment-related effects were observed in the T-cell dependent antibody response (TDAR) in cohort 3 animals. However, a significantly lower percentage of CD4 lymphocytes (-10.8% vs control) and an increased percentage of CD8 lymphocytes (+17.8% vs control) at 1000 ppm and, therefore, a decreased CD4/CD8 ratio (-22.9% vs control) was detected by splenic lymphocyte subpopulation analysis in cohort 1A females.

Dose-range finding study (OECD TG 421), rat, oral; for subsequent EOGRTS (BASF AG, 2018b)

In the dose range-finding study for the subsequent EOGRTS (according to OECD TG 421 with some modifications), DEA was administered to groups of 10 male and 10 female Crl:WI(Han) Wistar rats (P0 parental animals) via the drinking water at doses of 0, 500, 1000, 1500 and 2000 ppm corresponding to 0, 46, 95, 137, and 144 mg/kg bw/d, respectively (BASF AG, 2018b). In males, treatment covered 2-weeks premating, the mating period (6 days), and one week postmating (P0 males in total 4 weeks). In females, treatment covered 2-weeks premating, the mating period (6 days), and entire gestation (total 8 weeks). Dams were allowed to raise their pups until post-natal day 4 (PND 4). Pups were sacrificed at PND 4. For pups, viability, weight data, and sex were determined. At PND 4, macroscopic examinations for skeletal or visceral abnormalities of pups were performed.

Post-implantation losses were dose-dependently increased (4.1%, 9.9%, 22.4%, 31.3%, and 81.0% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; significant at ≥ 1500 ppm), and pup survival (till PND 4) was significantly lower at 1500 ppm (viability index: 99%, 100%, 92%, 32% and 100% at 0, 500, 1000, 1500, 2000 ppm DEA, respectively; note that at 2000 ppm there was only one litter (with 4 pups) born).

Prenatal developmental toxicity study (OECD TG 414) – rat, inhalation (BASF AG, 1993)

DEA was examined according to OECD TG 414 (version 1981) using 25 pregnant female Wistar rats (Chbb:THOM) per group (BASF AG, 1993). The animals were exposed to an aerosol in a head/nose exposure system for 6 h/day on day 6 through day 15 of gestation (GD) at concentrations of 0, 10, 50, and 200 mg/m^3 (MMAD: 0.6-1.2 µm). Maternal toxicity was noted at the highest concentration (200 mg/m^3), as evidenced by adverse clinical symptoms (vaginal haemorrhages) in 8 of the 21 pregnant rats on GD 14. At 200 mg/m^3, a markedly increased number of foetuses with skeletal variations (mainly cervical rib(s)) was recorded. There were no adverse effects on dams or foetuses at the low or mid concentrations (10 or 50 mg/m^3). The NOAEC for maternal and prenatal developmental toxicity was 50 mg/m^3.

In a non-guideline range finding study (BASF AG, 1991) for the main inhalative OECD TG 414 (BASF AG, 1993) study described above, pregnant female Wistar rats were exposed to aerosol concentrations of 0, 100, 200, and 400 mg/m^3 DEA (MMAD: 0.6-1.2 µm) during GD 6- 15. Parameters investigated were: Mortality, clinical signs/findings, body weight/body weight gain, corrected body weight, necropsy findings of the dams, reproduction data of the dams, uterus weight, placenta weight, foetus weight, external examination of the foetuses, and clinical pathology and haematology in the dams. Maternal toxicity was evident at ≥ 200 mg/m^3. Absolut (at 400 mg/m^3) and relative (at ≥ 200 mg/m^3) liver weight was significantly increased, and serum concentrations of cholesterol and triglycerides were decreased (at ≥ 200 mg/m^3). Furthermore, serum concentrations of sodium and creatinine were increased at ≥ 200 mg/m^3. There were significant changes in mean placental weight at ≥ 200 mg/m³. However, it is unclear from the study summary whether placental weight was increased or decreased. Therefore, the biological relevance of this finding cannot be judged.

Prenatal developmental toxicity study (OECD TG 414) – rat, dermal (Marty et al., 1999; Neeper-Bradley, 1992)

The prenatal developmental toxicity of DEA after dermal application was investigated in a study comparable to OECD TG 414 in groups of 25 Crj:CD (SD) rats (Marty et al., 1999; Neeper-Bradley, 1992). Diluted DEA or water was administered daily for 6 h directly to the clipped backs from GD 6-15 at intended concentrations

of 0, 150, 500 and 1500 mg/kg bw/d. The mid dose of 500 mg/kg bw/d had to be reduced to 380 mg/kg bw/d due to a preparation error. At 380 and 1500 mg/kg bw/d moderate to severe skin irritation was noted. Maternal body weight gain and corrected final body weight was lower (-4.5% vs control) at 1500 mg/kg bw/d. Absolute and relative kidney weights were significantly increased at ≥ 380 mg/kg bw/d. Haematological effects were observed in all treatment groups (decreased HCT, MCV, and MCH). Further effects at higher doses included reduced erythrocyte counts (≥ 380 mg/kg bw/d) and HGB (1500 mg/kg bw/d), and decreased platelet counts (1500 mg/kg bw/d). The 1500 mg/kg bw/d group also showed increased lymphocytes and total leukocytes. In the foetuses, there were no effects of treatment on body weight or on incidence of external, visceral, or skeletal malformation/abnormalities. Increased incidences of six skeletal variations involving the axial skeleton and distal appendages were observed in litters from the 1500 mg/kg bw/d group. These alterations consisted primarily of delays in ossification (proximal hindlimb phalanges, forelimb metacarpals). The LOAEL for maternal toxicity was 150 mg/kg bw/d, while the NOAEL for prenatal developmental toxicity was 380 mg/kg bw/d. The NOAEL for teratogenicity was > 1500 mg/kg bw/d (Marty et al., 1999; Neeper-Bradley, 1992).

Prenatal developmental toxicity study (OECD TG 414) – rabbit, dermal (Marty et al., 1999; Neeper-Bradley, 1992)

New Zealand white rabbits (15 per group) were administered cutaneous to aqueous solutions of DEA under an occlusive dressing for 6 h daily (Marty et al., 1999; Neeper-Bradley, 1992). Doses of 0, 35, 100, and 350 mg/kg bw/d were applied from days 6–18 of pregnancy. Dams at 350 mg/kg bw/d showed several signs of marked skin irritation, reduced food consumption, and discoloration in the kidneys but no haematological changes. Body weight gain was reduced at ≥ 100 mg/kg bw/d. There was no impairment of gestational parameters. No evidence of developmental toxicity was observed at any dose level, especially, there were no apparent effects of treatment on the incidences of external, visceral, or skeletal abnormalities. The NOAEL for maternal toxicity was 35 mg/kg bw/d, the NOAEL for prenatal developmental toxicity including teratogenicity was 350 mg/kg bw/d (Marty et al., 1999; Neeper-Bradley, 1992).

Developmental toxicity screening study – rat, oral (NTP, 1999a; Price et al., 2005)

Groups of 12 Sprague-Dawley-derived (CD®) rats were given oral doses (gavage) of 0, 50, 125, 200, 250, 300 mg/kg bw/d DEA from gestation days (GD) 6–19 (screening study, no guideline followed). Maternal toxicity was noted in form of morbidity or mortality at 200 and 250 mg/kg bw/d. All females at 300 mg/kg bw/d had to be killed early due to excessive toxicity. The water intake was transiently affected during early gestation (125 and 250 mg/kg bw/d) but was comparable to controls for all measurement periods after GD 12. Maternal absolute kidney weight was increased on PND 21 from 125 mg/kg bw/d. Significantly reduced maternal body weight gain and body weight, as well as reduced feed intake, were noted at ≥ 200 mg/kg bw/d. Exposure to 50 mg/kg bw/d was not associated with any significant maternal toxicity during or after the treatment period. Developmental toxicity consisted of significantly increased postimplantation losses at ≥ 200 mg/kg bw/d. Early postnatal mortality (PND 0-4) was significantly increased at ≥ 125 mg/kg bw/d. Pup body weight was significantly lower at 250 mg/kg bw/d on PND 0, and at ≥ 200 mg/kg bw/d on PND 21. For both maternal and postnatal developmental toxicity, the NOAEL was 50 mg/kg bw/d, based on decreased water intake and increased absolute kidney weights in dams, and early post-natal mortality in offspring at the next higher dose of 125 mg/kg bw/d (NTP, 1999a; Price et al., 2005).

Developmental toxicity study – mice, oral (NTP, 1987)

Swiss Albino mice (48-49 per group) were treated with oral doses (gavage) of DEA at a dose of 0 or 450 mg/kg bw/d DEA. No adverse maternal toxicity was observed during the treatment period. Among the pregnant rats 11% of controls, and 21% of DEA treated mice had full litter losses. Live litter size remained unchanged but neonatal survival was significantly lower in the DEA group (77% vs 95% in controls). Pup birth weight was not affected but pup body weight gain (PND 0-3) was negatively affected by DEA (body weight -23.8% vs control at PND 3).

Studies on neurogenesis, mouse (Craciunescu et al., 2009; Craciunescu et al., 2006; Niculescu et al., 2007)

A series of *in vitro* and *in vivo* studies have been published investigating the effects of DEA on prenatal neurogenesis in mice (Craciunescu et al., 2009; Craciunescu et al., 2006; Niculescu et al., 2007). Craciunescu et al. (2006) dosed pregnant female C57BL6 mice (N = 6) dermally from GD 7- 17 with DEA at 0, 20, 80, 160, 320 and 640 mg/kg bw/d (vehicle: 95% ethanol). At GD 17, foetuses were removed and counted, and

maternal livers of the 80 mg/kg bw/d group where collected for analysis of choline compounds. Brain (hippocampus) of the 80 mg/kg bw/d foetuses was investigated for mitosis (immunostaining of phospho-histone 3) and apoptosis (TUNEL-staining, and immunostaining of activated caspase 3). At the highest dose (640 mg/kg bw/d), one dam died before GD 17. DEA at 80 mg/kg bw/d (no other dose groups where investigated) significantly reduced the hepatic concentration of choline and its metabolites in dams. No other measures for maternal toxicity were reported. The number of viable foetuses per litter was dose-dependently decreased (significant from 160 mg/kg bw/d; 2.7 at 640 mg/kg bw/d vs 8.2 in controls). Similarly, the total number of viable foetuses was dose dependently-decreased (not significant; 16 at 640 mg/kg bw/d vs 49 in controls). DEA treatment with 80 mg/kg bw/d significantly reduced mitosis (56 ± 14% of controls based on phospho-histone 3) and increased apoptosis (170±10% and 178±7% of controls based on TUNEL and activated caspase 3, respectively) of neuronal progenitor cells at the ventricular surface of the ventricular zone of the hippocampus. No other dose groups were investigated.

In an *in vitro* study (Niculescu et al., 2007), mouse (C57BL/6J) cortical neuronal precursor cells (at GD14) were treated with either control medium (70 µM choline), DEA containing medium (3 mM DEA + 70 µM choline), choline-supplemented medium (CS; 210 µM choline), or choline supplemented medium with DEA (CS-DEA; 3 mM DEA + 210 µM choline). After 48 h and 72 h, mitosis (BrdU labeling) and apoptosis (TUNEL-staining) were determined. DEA significantly decreased mitosis in cortical neuronal precursor cells after 48 h (3.8 vs 16 % in controls) and 72 h (2.6% vs 15.4% in controls), and significantly increased apoptosis after 72 h (14.8% vs 4.8% in controls). Choline supplementation restored mitosis after 48 h (11.7% vs 16% in controls) and 72 h (14.0% vs 15.4% in controls). Furthermore, choline supplementation prevented the increase of DEA-induced apoptosis at 72 h (5.6% vs 4.8% in controls). In addition, this study demonstrated that choline uptake into neural precursor cells was decreased by DEA treatment (59% of controls). This effect was mitigated by choline supplementation (choline uptake reached about 87% of control levels). DEA decreased the concentrations of choline and phospho-choline in neural precursor cells. The decrease of choline but not the decrease of phospho-choline could be prevented by choline supplementation. Experiments with purified choline kinase demonstrated phosphorylation of DEA albeit at a much lower rate than choline. Choline phosphorylation by choline kinase was inhibited in the presence of DEA (significant at 40 mM).

In a further *in vivo* study, the dose-response of neurogenesis upon DEA treatment was investigated in mice (Craciunescu et al., 2009). Pregnant C57BL/6 mice (N = 7) were dosed dermally from GD 7-17 with DEA at 0, 5, 40, 60, and 80 mg/kg bw/d (vehicle: acetone). At GD 17, foetuses were removed and brains (hippocampus) of two male foetuses from each dam were analysed for mitosis (immunostaining for phospho-histone 3) and apoptosis (immunostaining for activated caspase-3). In addition, 5 non-pregnant female mice were treated with either vehicle or 80 mg/kg bw/d DEA for 11 days in order to determine DEA and choline metabolites in plasma and liver. In foetuses, maternal treatment with 80 mg/kg bw/d DEA significantly decreased mitosis (hippocampus: 54.1% of controls; cortex: 58.9% of controls), and significantly increased apoptosis (hippocampus: 177.2% of controls) at the ventricular surface of the ventricular zone. At lower doses, no significant effects on mitosis or apoptosis were detected. Exposure of adult non-pregnant mice to DEA at 80 mg/kg bw/d increased the concentrations of DEA and DEA metabolites in plasma and liver, and decreased the concentrations of choline and choline metabolites in liver.

10.10.2 Comparison with the CLP criteria

In conclusion, exposure of rats and mice to DEA leads to several developmental effects in offspring related to fertility, immunotoxicity, and neurotoxicity.

The key study for evaluating developmental toxicity is an oral EOGRTS (OECD TG 443) in rats including the DNT and DIT cohorts (BASF AG, 2018a). Several toxicological endpoints (e.g. blood, kidney, liver) were similarly affected in parental animals and in offspring. On the other hand, certain effects on fertility parameters in the F1 generation could not be observed in parental animals and, therefore, are of developmental aetiology. These include effects on differential ovarian follicle count, oestrus cycle, histology of male and female sexual organs, as well as effects on the mammary glands. These findings are, however, considered for classification regarding fertility and sexual function (see section 10.9.3).

Although in this study, pup birth weight was not affected by DEA treatment, the viability index of F1 pups was significantly decreased at 1000 ppm during early lactation (PND 0-4), and postnatal body weight gains

were impaired at ≥ 300 ppm. It is unclear whether the effects of DEA during lactation are mediated via impaired lactation, maternal transfer of DEA, or are a result of prenatal exposure (considering the long elimination time of DEA). In any way, maternal toxicity during pregnancy or lactation as judged from body weight effects in dams (at 1000 ppm: -15.1% lower body weight vs control at the end of pregnancy (influenced by the lower number of implantations), and -9.8% and -8.1% at the start and end of lactation, respectively; see Table 20) seems not severe enough to explain early postnatal mortality and impaired growth. Postweaning growth was also compromised by DEA throughout the study period, and body weight effects in F1 adults were clearly more pronounced than in the parental generation (see Table 21), indicating a developmental aetiology. Therefore, the findings of early postnatal mortality and depressed body weight gain in the F1 generation are considered relevant for classification for developmental toxicity.

Developmental neurotoxicity of high relevance for classification was evident in F1 males and females. Behavioural changes (high-stepping gait and piloerection) occurred exclusively in all F1 cohorts at several time-points and were not observed in P0 animals. Whereas piloerection is interpreted as a sign of unspecific toxicity/discomfort, the high-stepping gait is considered as a sign of neurotoxicity and taken forward for classification. Neurobehavioural testing revealed lower maximum amplitudes in the auditory startle response test in males and females and no habituation to the test environment was apparent in males. Histologically, degeneration of nerve fibers in the spinal cord and medulla oblongata were observed in cohort 2B animals on PND 77. Although similar effects have been documented in adult rats in a former repeated dose toxicity study (Melnick et al., 1994b; Neeper-Bradley, 1992), no histological changes were reported in the P0 generation of the present EOGRTS, indicating higher sensitivity of F1 animals under these experimental conditions. Changes in brain morphometry were observed for some parameters (widths of hippocampus and cerebellum in high dose males, and parietal cortex in high dose females; however, only high dose and control animals were investigated). In the light of the histological and behavioural changes mentioned above, and without historical control data, this morphometric findings cannot simply be dismissed (as done by the study authors) and are taken forward as supporting evidence for developmental neurotoxicity.

Eosinophilic cysts were observed in the pars distalis of the pituitary (anterior pituitary), occurring exclusively in F1 animals in cohort 2A and 2B (in cohort 2A even at the lowest dose (100 ppm) tested) but not in the parental generation. Although the functional relevance of these cysts remains unknown, their appearance in the master gland of the endocrine system, with the affected part regulating several hormonal axes via synthesis and secretion of stimulating hormones relevant for growth, reproduction, lactation, thyroid function and others, is of high concern and is considered as an adverse developmental effect relevant for classification.

Regarding changes in plasma T4, significant increases or trends for increases were observed at several ages in the F1 generation. Whereas in males, increased T4 was observed in the P0 animals with a trend for higher values in F1 animals, in females T4 increases were exclusively observed in the F1 generation, indicating a developmental effect on the thyroid hormone system. In humans, increased thyroid hormone levels during (brain) development are considered similarly detrimental as are too low levels; both can result in neurodevelopmental consequences (Korevaar et al., 2016). Because a relationship between the observed neurodevelopmental findings in the present EOGRTS and changes in T4 cannot be excluded, the increased T4 levels are considered adverse even without thyroidal histopathological findings, providing supporting evidence for developmental toxicity.

Immunotoxicity was evident from analysis of spleenic lymphocyte subpopulations which revealed effects on T-helper cells and cytotoxic T-cells in F1 females (decreased CD4/CD8 ratio) at the high dose (1000 ppm). Although it remains unknown whether similar effects occurred in the P0 generation (because not investigated in P0), the changes in splenic lymphocyte subpopulations are considered as supporting evidence for developmental (immuno-) toxicity.

Further evidence for developmental toxicity of DEA is seen in the dose-range finder study for the EOGRTS discussed above. This study showed a clear and dose-dependent increase of post-implantation losses and decreased pup survival (till PND 4). Maternal toxicity during pregnancy or lactation as judged from body weight effects in dams (at 2000 ppm: -18.4% lower body weight vs control at the end of pregnancy (influenced by the lower number of implantations), and -9.9% at the start of lactation, respectively) is considered not severe enough to explain the increased postimplantation losses, and postnatal mortality.

Besides the EOGRTS, several guideline and non-guideline studies with different routes of exposure are available investigating developmental effects of DEA. Prenatal developmental toxicity studies according to OECD TG 414 via inhalation (BASF AG, 1993) or dermal exposure (Marty et al., 1999; Neeper-Bradley, 1992) in rats showed maternal toxicity (body weight, kidney, blood, liver). Developmental effects were restricted to an increase of skeletal variations in foetuses (BASF AG, 1993; Marty et al., 1999; Neeper-Bradley, 1992). Similarly, in rabbits, a dermal study according to OECD TG 414 revealed maternal toxicity (food consumption, body weight, kidney) but no developmental toxicity was evident (Marty et al., 1999; Neeper-Bradley, 1992). On the other hand, an oral non-guideline screening study in rats (NTP, 1999a; Price et al., 2005) reported developmental toxicity after applying DEA by gavage from GD 6-19. Strong maternal toxicity was observed in the high-dose dams (300 mg/kg bw/d) which had to be terminated early. For the remaining dose groups, this study reported an increase in post-implantation losses at ≥200 mg/kg bw/d, increased early post-natal mortality at ≥125 mg/kg bw/d, and reduced pup birth weight and postnatal weight gain at 250 mg/kg bw/d. The maternal toxicity at these dose levels was rather mild and consisted of lower body weight and weight gain in comparison to controls, and increased kidney weight. Although the body weight difference to control seems high at the end of pregnancy (-6.2 and -19.6% vs control at 200 and 250 mg/kg bw/d), this parameter is influenced by significant postimplantation losses. On PND 0, no significant differences vs controls in maternal body weight was recorded, and on PND 4, the difference was only -7% and -6.4% vs control at 200 and 250 mg/kg bw/d, respectively. Therefore, postimplantation and early postnatal survival and growth are considered as specific developmental toxicity relevant for classification. These findings are supported by the study in mice performed by Craciunescu et al. (2006), showing a dose-dependent decrease of viable fetuses, as well as by another developmental/reproductive toxicity study in mice, demonstrating significant effects of a single dose of DEA (450 mg/kg bw/d from GD 6-15) on postnatal survival despite absence of maternal toxicity (NTP, 1987).

Regarding developmental neurotoxicity of DEA, there is supporting evidence from further non-guideline studies. The reproductive and developmental toxicity study performed by KFDA (2007) reported significant effects in behavioural testing in F1 animals after dermal DEA exposure of either paternal or maternal animals. Furthermore, a series of *in vitro* and *in vivo* studies in mice demonstrated effects of DEA on prenatal neurogenesis (Craciunescu et al., 2009; Craciunescu et al., 2006; Niculescu et al., 2007).

In conclusion, exposure to DEA induces developmental toxicity consisting of decreased postimplantation as well as postnatal survival, impaired pup (and adult offspring) growth, immunotoxicity, and neurotoxicity. The finding of eosinophilic cysts in the pituitary and changes in T4 levels in F1 animals in the EOGRTS are of similar concern. The above-mentioned findings in F1 animals are unlikely to occur as unspecific side-effects of general toxicity (e.g. blood, kidney, liver, body weight). Given the disturbance of choline uptake and homeostasis by DEA and the importance of choline and choline-metabolites in cell function and signal transduction, the adverse developmental effects of DEA are considered as a specific intrinsic property of the substance. Therefore, the following DEA-induced developmental effects shall be considered for classification:

- Lower postimplantation and postnatal survival, and decreased offspring growth in the EOGRTS and particularly in the preceding dose-range finder study; increased postimplantation losses, and reduced postnatal survival and growth in further developmental/reproductive toxicity studies (NTP, 1987; NTP, 1999a; Price et al., 2005) (supporting evidence)

- Decreased splenic CD4/CD8 ratio in female offspring in the EOGRTS

- High-stepping gait (F1 animals in the EOGRTS); lower maximum amplitudes, and no habituation to the test environment (F1 males in the EOGRTS) in the auditory startle response test; behavioural changes in offspring reported in the study performed by KFDA (2007) after dermal DEA exposure of either paternal or maternal animals

- Degeneration of nerve fibers in the spinal cord and medulla oblongata, and changes in brain morphometry (supporting evidence) in offspring in the EOGRTS

- Supporting evidence for DNT: effects of DEA on prenatal neurogenesis in *in vivo* and *in vitro* mechanistic studies (Craciunescu et al., 2009; Craciunescu et al., 2006; Niculescu et al., 2007)

- Eosinophilic cysts in the pars distalis of the pituitary (F1 animals in the EOGRTS)

- Increased T4 levels in offspring in the EOGRTS (supporting evidence)

Considering the criteria for classification into hazard categories for reproductive toxicants according to the CLP Regulation (Annex I, Table 3.7.1 (a)), the following needs to be considered: there is no human evidence available, the available reliable evidence is based on experimental animals, and the adverse effects on development are considered not to be a secondary non-specific consequence of other toxic effects and thus fulfilling the criteria for Category 1B.

10.10.3 Adverse effects on or via lactation

DEA showed clear adverse effects on early postnatal survival and growth. To the best of the DS's knowledge, there are no studies regarding lactational transfer of DEA. Direct effects on the mammary gland have not been observed in parental animals in the EOGRTS (only in the F1 generation). Therefore, it is unclear whether the effects of DEA on pups during lactation are due to impaired lactation (direct effects on the mammary gland, or compromised lactational behaviour), or induced by maternal transfer of DEA via the milk, or are a result of prenatal exposure (considering also the long elimination time of DEA).

In conclusion, the available data are not sufficient for classification of DEA for adverse effects on or via lactation

10.10.4 Conclusion on classification and labelling for reproductive toxicity

DEA showed significant effects on fertility parameters in several studies. In particular, there were pronounced effects on the number of implants and the litter size in the EOGRTS and the preceding dose-range finder study as well as further effects on reproductive organs in F1 animals (ovary, mammary gland) in the EOGRTS. In addition to the EOGRTS, there are supporting *in vivo* and *in vitro* studies demonstrating toxic effects on male sexual organs, sperm parameters, and steroidogenesis.

Exposure to DEA leads to several developmental effects in offspring including decreased postimplantation and postnatal survival and decreased growth, immunotoxicity, and neurotoxicity with supporting evidence for disturbed neurogenesis *in vitro* and *in vivo*. The finding of eosinophilic cysts in the pituitary and changes in T4 levels in F1 animals in the EOGRTS are of similar concern.

Consequently, classification of DEA as a reproductive toxicant (Repr. 1B, H360FD) is considered adequate.

10.11 Specific target organ toxicity-single exposure

Not assessed.

10.12 Specific target organ toxicity-repeated exposure

Table 25: Summary table of animal studies on STOT RE via oral route.

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	**Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)**	**Remarks**	**Reference**
OECD TG 443 (EOGRTS), GLP **2,2'-iminodiethanol** (99.9%) (CAS 111-42-2/EC 203-868-0) oral (drinking water), no vehicle 0, 100, 300, 1000 ppm (nominal) (approx. 0, 6.8, 21.5, 73.4 mg/kg bw/d mean dose in parental M; approx. 0, 10.2, 29.4, 103.9 mg/kg bw/d mean pre-mating dose in parental F approx. 0, 11.5, 34.9, 116.8 mg/kg bw/d mean gestation dose in parental F approx. 0, 24.0, 66.3, 173.3 mg/kg bw/d mean lactation dose in parental F) 2 weeks prior to breeding and continuing through mating period (up to two weeks), approximately 4 additional weeks (M) or gestation (three weeks) and lactation (three weeks) for F, daily until sacrifice	Significant effects in **parental** animals occurring from doses ≥ 100, 300, 1000 ppm as follows: **General Toxicity:** ↓ water consumption ≥ 300 ppm in F, at 1000 ppm: max. ↓ 18% during GD 14–18 and max. ↓ 45% during lactation ↓ food consumption ≥ 300 ppm in F (max. ↓ 29% at 1000 ppm during lactation) and at 1000 ppm in M (max. ↓ 9% during premating) ↓ bw at start of mating at 1000 ppm: -5.1% (M) and -4.1% (F) ↓ bw: start gestation at 1000 ppm: -5.1%; end gestation: -15.1%, start lactation: -9.8%, ↓ bw at termination ≥ 300 ppm: -5.3%, -10.0% (M) and -2.5%, -8.1% (F) **Blood (*microcytic anaemia*):** ↓ MCV ≥ 100 ppm: **-2.9**, -4.4, -6.4% (M) and at 1000 ppm -5.4% (F) ↓ RBC at 1000 ppm: -12.9% (M), -10.4% (F) ↓ HGB ≥ 300 ppm: -5.7, **-21.1%** (M) and -3.2, -14.8% (F) ↓ HCT ≥ 300: -3.9%, -15.1% (F) and at 1000 ppm -18.6% (M) ↓ MCH at 300 ppm: -5.6% (M) and at 1000 ppm -4.8% (F) ↓ MCHC at 1000 pmm -2.9% (M) coagulation parameters: ↑ platelet counts ≥ 300 ppm: +14, +30% (M) ↓ prothrombin time ≥ 300 ppm -6.1, -11.1% (M) and -4.7, -7.6% (F)	Key study experimental study (reliable without restriction) RDT study **LOAEL** **(P0 parental animals):** **100 ppm** (6.8 mg/kg bw/d in M, 15.2 mg/kg bw/d in F (mean intake during pre-mating, gestation and lactation period) Effects on blood, kidney, liver based on: ↓ MCV, ↑ abs. (F) + rel. (M/F) kidney/liver weight; **Adverse effect dose levels relevant for classification:**	Study report (BASF AG, 2018a)

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)	Remarks	Reference
Rat (Crl:Wl(Han) Wistar) 30 M/30 F per dose group (P) Samples from 10 M/10 F per group (P) at termination for haematology and clinical chemistry	**Glandular stomach (only F):** Erosion/ulcer **≥ 300 ppm** (2/21, 4/21), increased oedema with inflammatory cell infiltrates ≥ 100 ppm (6/20, 12/21, 10/21 compared to 2/20 in control) **Liver:** ↑ abs. wt. ≥ 100 ppm: +9, +12, +15% (F), ↑ rel. wt. ≥ 100 ppm : +**4**, +7, +2% (M) and +9, +15, +25%(F) centrilobular hypertrophy at 1000 ppm: 4/30 (M), and 10/30 (F) enzyme activities at 300 and 1000 ppm (M): - ALT: - 14%, n.s. - AST: n.s., + 66% - ALP: + 41%, +54% **Kidney:** ↑ abs. wt. ≥100 ppm: + 13, +16, +14% (F), and ≥300 ppm +6, +6% (M) ↑ rel. wt. ≥100 ppm: + **5**, +**12**, +18% (M) and + 13, +19, +24% (F) tubular degeneration/regeneration incidence (severity): **≥ 300 ppm (M): 5/21 (Grade 1)**, 19/21 (9 Grade 1, 10 Grade 2) and ≥ 1000 ppm (F): 20/20 (6 Grade 1, 10 Grade 2, 4 Grade 3) ↑ multifocal mineral depositions (M ≥ 1000 ppm and F ≥ 300 ppm) ↓ urine specific gravity and ↑ urine volume (M ≥ 300 ppm) **Brain:** ↑ abs. wt. (+ 2%, 2%, n.s. (F)), ↑ rel. wt. ≥ 300 ppm (+ 6, 11% (M) and + 5, 9% (F)) **Heart:** ↓ abs. wt. (-3, - 6, -10% (M) and -9 % (F) at 1000 ppm)	Microcytic anaemia (-21.1% decreased HGB in M) at **1000 ppm, and** Nephrotoxicity M/F at **≥ 300/1000 ppm** Note: No histological findings in brain, cervical, thoracic, and lumbar cord (only high dose of 144 mg/kg investigated) No clinical signs of neurotoxicity	
OECD TG 421 (modified), GLP, Range-finding study oral (drinking water), no vehicle 0, 500, 1000, 1500, and 2000 ppm (nominal)	**Food/water consumption and body weight:** ↓ water consumption ≥ 2000 ppm (F) during gestation (GD 10-18, max. ↓ 36%) ↓ food consumption ≥ 500 ppm in M (max. ↓ 18% at 2000 ppm) and ≥ 1000 ppm in F during premating (up to 46%) and lactation (37-52%)	Key study Experimental study (reliable without restriction) Range-finding study	Study report (BASF AG, 2018b)

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)	Remarks	Reference
(0, 46, 95, 137, 144 mg/kg bw/d) males: 14 days premating; 6 days mating; one week post-mating (total: 4 weeks) females: 14 days premating; 6 days mating; entire gestation; lactation until PND 4 (total: 8 weeks) **Rat**(Crl:Wl(Han) Wistar) 10 M/10 F per dose group	↓ bwg and ↓ terminal bw ≥ 500 ppm (-4, -7, -8, -11% (M) and -4, -7, -11, -12% (F) **Blood (*microcytic anaemia*)** Blood parameters measured at day 28 in males, at day 56 in females ↓ RBC ≥ 1500 ppm (M)/500 ppm (F):): -11.4, -17.9% (M) and **-9.6**, -9.2, -**19.3**, -24.6% (F), ↓HGB ≥ 500 ppm (M/F): -5,7, -11,4, -18.2, -22.7% (M) and **13.0**, 15.2, **-22.8**, -28.3% (F) ↓ HCT ≥ 1000 /500 ppm (M/F), ↓ MCV ≥ 500/1000 ppm (M/F), ↓ MCH at 1000 and 1500 ppm (F), ↓ MCHC at 1500 ppm (F), ↓ RET ≥ 1000 ppm (M), ↑ PLT ≥ 500 ppm (M) ↓ $NEUT_{Abs}$ ≥ 500 ppm (M); not significant at 1000 ppm, ↓ $MONO_{Abs}$ ≥ 1000 ppm (M), ↓ EOS_{Abs} ≥ 500 ppm (M), ↓ NEUT ≥ 500/1500 ppm (M/F), ↓ MONO ≥ 1500 ppm (M/F), ↓ EOS ≥ 500/2000 ppm (M/F), ↑ LYMPH ≥ 500/1500 ppm (M/F) Reduced choline content in plasma ≥ 1000/1500 (M/F) **Kidney** ↑ abs. wt. ≥ 500 ppm: +12, +9, +6, +5% (M, not significant at 1500, 2000 ppm) and +18,+13,+15,+19% (F) ↑ rel. wt. ≥ 500 ppm: +16/+17/+15/+18% (M) and +23,+22,+33, +35% (F) Degeneration/regeneration of tubular epithelium (≥ 500 ppm in M/F, more severe in F): M ≥ 500 ppm all 10 animals affected in all dose groups with increasing severity (grade 1/2/3/4): **10/0/0/0**; 0/6/4/0; 1/5/4/0; 0/7/3/0, none in controls F ≥ 500 ppm all 10 animals affected in all dose groups with increasing severity (grade 1/2/3/4): **10/0/0/0**; 1/5/4/0; 0/0/6/4; 0/0/3/7, none in controls Tubular casts (≥ 1000/500 ppm in M/F), mineralisation (≥ 1000 ppm M/F); ↑ cell proliferation (at 2000 ppm/≥ 500 ppm in M/F, based on Ki-67 staining); ↑ KIM-1 staining (M/F; only high-dose group investigated)	**LOAEL: 500 ppm** based on effects on blood and kidney **Adverse effect dose levels relevant for classification: Nephrotoxicity** M/F at **≥ 500 ppm** (46 mg/kg bw) in combination with **Anaemia in F ≥ 500 ppm, (**10% decrease in RBC and HGB) **Anaemia** decreased RBC and HGB with effect size of ≥ 20% at ≥ 1500 ppm (137 mg/kg bw) in F	

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)	Remarks	Reference
	Reduced choline content in kidney (≥ 500 ppm M/F) **Liver** ↑ abs. wt. ≥ 500 ppm: +8, +13, +23, +20% (M) and +18, +23, +24, +22% (F) ↑ rel. wt. ≥ 500 ppm: +13, +21, +33, +34% (M) and +23, +32, +39, +38% (F) Centrilobular (≥ 1500 ppm, F) and diffuse hypertrophy (≥ 1500 ppm, M); peripheral fatty changes (≥ 1000 ppm M/F); discolouration (clay) (≥ 1500 ppm M/F) Reduced choline content in liver (≥ 1000/500 ppm M/F)		
Equivalent or similar to OECD TG 408, NTP-Study: Test procedure in accordance with national standards (NTP), GLP **2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/EC 203-868-0) **Oral**: drinking water Vehicle: water M: 0, 320, 630, 1250, 2500, 5000 ppm (0, 25, 48, 97, 202, 436 mg/kg bw/d) F: 0, 160, 320, 630, 1250, 2500 ppm (0, 14, 32, 57, 124, 242 mg/kg bw/d) 13 weeks (daily) **Rat** (Fischer 344) 10 M/10 F per dose group Effects on male reproductive system are reported in 10.9.1 and Table 18.	**Mortalities:** 2/10 M at 5000 ppm Significant effects ≥ 160, 320, 630, 1250, 2500 ppm: **Clinical examinations:** ↓ bwg ≥10 % (M/F ≥ 630/320 ppm), max. -44% at 5000 ppm (M) and -25 % at 2500ppm (F) ↓ water consumption (M/F ≥ 630/2500 ppm) tremors, emaciation, abnormal posture, rough hair coat (M/F ≥ 2500/1250 ppm) **Blood (*microcytic anaemia*):** decreased MCV: M ≥ 320 ppm: -1.9, -3.7, -7.4, -9.3, -9,3 % F ≥ 160 ppm: -**1.8**, -3.6, -5.4, -8.9, -12.5% decreased RBC from 320 ppm: M: n. s. , -6.71, -16.60, -27.19 -35,0% F: -6.67, -10.00, -**19.29**, -23.45% decreased HGB from 320 ppm: M: -**3.4**, -10.1, -14.9, -33.8, -39,9% F: - **8.6**, -13.9, -**25.2**, -30.5% **Kidney:** ↑ abs. wt. F: + **30**, 27, 26, 32, 39%, ↑ rel. wt. M: n.a., + 11, 13, 12, 2% and F: + **36**, 39, 36, 53, 87% Increased incidence of nephropathy: M, ≥ 320 ppm: 2/10 (1.0), 2/10 (1.0), 3/10 (1.0), 6/10 (1.0), 10/10 (2.4);	Key study experimental study (reliable without restriction) RDT study **LOAEL:** **160 ppm in F** **(14 mg/kg bw/d)** based on ↓ MCV, nephrotoxicity, ↑ kidney rel. and abs. wt. 320 ppm in M (25 mg/kg bw/d) based on ↓ MCV, ↓ HGB, ↑ rel. kidney wt. **Adverse effect dose levels relevant for classification:** **≥ 1250 ppm (124 mg/kg bw)** microcytic anaemia based on ↓ HGB together with ↓ RBC (20% level) and ↓ MCV reached in F at 1250 ppm (in M RBC -16.6%) in	Study report (Melnick et al., 1994b; NTP, 1992)

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)	Remarks	Reference
	control 6/10 (1.0) F, ≥ 160 ppm: 9/10 (1.0), 10/10 (1.5), 10/10 (1.4), 9/10 (1.0), 2/10 (1.0); controls 2/10 (1.0) ↑ incidence and/or severity of tubular necrosis: M, 5000 ppm 10/10 (1.0); F ≥1250 ppm: **1/10 (1.0)**, 3/10 (1.0), no necrosis in control groups ↑ incidence and/or severity of tubular mineralisation (M/F: ≥ 1250/160 ppm): M, ≥ 1250: 1/10 (1.0), 10/10 (1.8), 10/10 (1.7); 0/10 in control group F, ≥ 160: 10/10 (2.0), 10/10 (2.5), 10/10 (3.0), 10/10 (2.4), 10/10 (1.7); but high incidence (10/10 (1.3)) in control group **Brain (medulla) and spinal cord:** demyelination (minimal to mild) M/F, ≥ 2500/1250 ppm: 10/10	combination with increased tubular necrosis in F ≥1250 ppm Demylination in 10/10 females at 124 mg/kg bw/d is an adverse effect (at all severity levels) Although target effects were observed at a dose slightly above the guidance value (100 mg/kg bw/d), these are considered supportive for classification	
Equivalent or similar to OECD TG 408, NTP-Study: Test procedure in accordance with national standards (NTP), GLP **2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/EC 203-868-0) **Oral**: drinking water Vehicle: water 0, 630, 1250, 2500, 5000, 10,000 ppm (0, 104, 178, 442, 807, 1674 mg/kg bw/d in M; 0, 142, 347, 884, 1154, 1128 mg/kg bw/d in F) 13 weeks (daily) **Mouse** (B6C3F1) 10 M/10 F per dose group	**Mortalities:** ≥ 5000 ppm all mice died before the end of the study Most sensitive significant effects ≥ 630, 1250, 2500 ppm: **Kidney:** Significant effects from 1250 ppm: ↑ abs. wt. M: + 10, 14 %, ↑ rel. wt. M: + 20, 26 % and F: +13, 31 % ↑ incidence nephropathy M: 5/10, 8/10 **Liver:** ↑ abs. wt. (M: + **13**, 24, 41 % and F: + 28, 39, 85 %) ↑ rel. wt. (M: + **18**, 29, 56 % and F: + 25, 53, 124 %) From 630 ppm incidence (severity) of cytologic alteration (hypertrophy with ↑ eosinophilia, and disruption of hepatic cords): **9/10 (2.0),** 10/10 (2.8), 10/10 (3.0) in M, 10/10 (1.9), 10/10 (2,8), 10/10 (3.0) in F none in controls ↑ nuclear pleomorphism, multinucleated hepatocytes and necrosis ↑ enzyme activities from 1250 ppm: ALT (M/F): + n.s./28, 196/128 % and Sorbitol-DH (M): n.s., + 84 %	Key study experimental study (reliable without restriction) RDT study **LOAEL:** 630 ppm **Adverse effect dose levels relevant for classification:** ≥ 630 ppm (equal to 104 mg/kg bw/d in males and 142 mg/kg bw/d in females) based on necrotic liver damage	Study report (Melnick et al., 1994a; NTP, 1992)

Method, guideline, deviations if any, test substance, dose levels duration of exposure, species, strain, sex, no/group	Results (only significant effects relevant for STOT RE are listed here: effects relevant for LOAEL are highlighted in bold, and effects relevant for classification in bold and blue)	Remarks	Reference
Samples from all mice at termination for haematology	**Heart:** ↑ abs. wt. (F at 2500 ppm); ↑ rel. wt. (M/F ≥ 2500/1250 ppm) minimal to marked degeneration and necrosis of cardiac myocytes from 2500 ppm (M/F)		

Table 26: Summary table of animal data on STOT RE via inhalative route.

Method	Results	Remarks	Source
According to OECD TG 412, GLP **2,2'-iminodiethanol** (99.5 %) (CAS 111-42-2/EC 203-868-0) **Inhalation:** aerosol (nose/head only), no vehicle 0; 110; 210; 400 mg/m³ MMAD 3.7–4.8 µm 2 weeks (6 h/day, 5 days/week) **Rat** (Wistar) 10 M/10 F per dose group Blood samples at the end of exposure	Effects observed from 400 mg/m³: Body weight and weight gain: slightly decreased body weight and impaired bwg (77% of controls) in M Clinical chemistry: slightly decreased cholesterol values (~15–23% reduction) in M and F **Liver:** increased liver weights (13%) in F No effects on blood and brain parameters.	Supporting Study RDT study (10 exposures) experimental study (reliable without restriction) NOAEC (systemic toxicity): 210 mg/m³ **LOAEC (systemic toxicity):** **400 mg/m³** based on ↓ bwg (M), ↓ cholesterol (M/F), ↑ liver wt. (F)	Study report (BASF AG, 1993)
OECD TG 413, GLP **2,2'-iminodiethanol** (99.89 %) (CAS 111-42-2/EC 203-868-0) **Inhalation:** aerosol (nose/head only), no vehicle 0; 15; 150; 400 mg/m³ (target) 0; 15; 153; 410 mg/m³ (analytically determined) MMAD 0.6–1.9 µm 90 days (6 h/day, 5 days/week) **Rat** (Wistar) 13 M/13 F per dose group Blood samples from 10 animals daily	**Significant systemic effects** **≥ 150; 400 mg/m³** **Blood (*microcytic anaemia*)** at 400 mg/m³: ↓ MCV: -4/-3% (M/F) ↓ RBC: -6.2/-8.5% (M/F) ↓ HGB: -10.2/-13.9% (M/F) **Liver:** ↑ rel. wt. ≥ 150 mg/m³: M: n.s., + 9% and F: +10, +19% ≥ 150 mg/m³: slightly increased ALP (M/F) and decreased ALT (M) **Kidney:** ↑ rel. wt. ≥ 150 mg/m³: M: +10, +13%, and F: +12, +16% minimal/slight tubular hyperplasia in some F and intratubular lithiasis (M) Urinalysis: ≥ 150 mg/m³ (M): ↑ excretion of renal tubular epithelium cells including casts; M/F: ↑ blood in urine at 400 mg/m³	Key study RDT study (65 exposures) experimental study (reliable without restriction) NOAEC (systemic toxicity): 15 mg/m³ **LOAEC (systemic toxicity):** **153 mg/m³** based on ↑ kidney (M/F)/liver (F) wt., renal tubular effects, ↑ ALP (M/F), ↑ erosions in glandular stomach (F) Brain: no histopathological effects	Study report (BASF AG, 1996; Gamer et al., 2008)

Method	Results	Remarks	Source
Effects on male reproduction system reported in 10.9.1.	**Glandular stomach (F):** ↑ erosions (conc.-dependent) **Local effects**: **≥ 15 mg/m³** focal squamous metaplasia of the laryngeal epithelium (severity level 1/2, 10/10 animals) ≥ 150 mg/m³ ↑ laryngeal squamous hyperplasia (conc.-depend.), ↑ incidence and severity of local inflammation of larynx and trachea	**LOAEC (local effects):** **15 mg/m³** based on focal squamous metaplasia of ventral laryngeal epithelium at the base of the epiglottis, severity level 1/2 in all 10 animals No NOAEC for local effects could be established.	
OECD TG 413, GLP **2,2'-iminodiethanol** (99.89%) (CAS 111-42-2/EC 203-868-0) **Inhalation:** aerosol (nose/head only), no vehicle 0; 1.5; 3; 8 mg/m³ (target) 0; 1.57, 3.43, 8.18 mg/m^3 (analytical determined) MMAD 0.6–0.7 µm 90 days (6 h/day, 5 days/week) **Rat** (Wistar) 10 M/10 F per dose group (without recovery period) 10 F per dose group (with 3 month recovery period)	**Local effects:** (F: all lesions reversible after 3 month recovery) ≥ 3 mg/m³ M: 3/10 with focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis (minimal/adaptive) ≥ 8 mg/m³ M + F: 9/10 M and 9/10 F with focal squamous metaplasia of the laryngeal epithelium at the base of the epiglottis M + F: 3/10 M and 3/10 F with submucosal inflammation (adverse) M: 2/10 with squamous metaplasia at the region of ventral pouch and arytenoid cartilages	Key study RDT study (65 exposures) experimental study (reliable without restriction) NOAEC (local effects): 1.5 mg/m^3 **LOAEC** (local effects): **3** mg/m^3 based on squamous metaplasia in the larynx	Study report (BASF AG, 2002)

Table 27: Summary table animal studies relevant for STOT RE via dermal route.

Method	Results	Remarks	Source
Equivalent or similar to OECD TG 411, NTP-Study: Test procedure in accordance with national standards (NTP), GLP **2,2'-iminodiethanol** (> 99%) (CAS 111-42-2/ EC 203-868-0) **Dermal**: shaved back of each animal (unoccluded), from the mid-back to the interscapular region Vehicle: ethanol (95%) 0, 32, 63, 125, 250, 500 mg/kg bw/d (nominal per unit body weight) 13 weeks (once per day, 5 days/week) **Rat** (Fischer 344) 10 M/10 F per dose group	**Significant effects: ≥ 32,** 63, 125, 250, 500 mg/kg bw/d **Blood (*microcytic anaemia*):** decreased MCV: M: **-1.9**, -3.8, -8.0, -10.2, 12.5 % F: **-1.8**, -3.6, -5.5, -9.4, -13.7 % decreased RBC: M: n. s. , n. s. , -3.50, -12.27, -30.44 % F: **-3.81**, -7.92, -14.50, -22.29, -35.63 % decreased HGB: M: n. s. ,-2.6, -8.4, ***-20.2,*** *-40.9%* F: **-4.7**, -9.8, *-17.4*, ***-29.2,*** *-47.6%* **Kidney:** ↑ abs. + rel. wt. (M/F) ↑ incidence (severity) nephropathy (F) ≥ 32 mg/kg bw/d**:** 9/10 (1.3), 10/10 (1.4), 10/10 (1.7), 7/10 (1.1), 4/10 (1.0) in comparison to 3/10 (1.0) in the control group tubular mineralisation (F, high/all doses: 9/10 (1.0), 10/10 (1.6), 10/10 (1.9), 10/10 (1.1), 10/10 (1.0) vs 4/10 (1.0) in the control group, M at 500 mg/kg bw/d: 9/10 (1.9)) tubular necrosis (F, **≥ 250 mg/kg bw/d: 2/10 (1.0),** 10/10 (1.0)) **Brain (medulla oblongata):** minimal demyelination ≥ 250 mg/kg bw/d: M: n.s., 10/10, F: **7/10**, 9/10 **Local effects**: Skin lesions: (M/F: ≥ 63/32 mg/kg bw/d) ↑ severity/incidence hyperkeratosis M/F: acanthosis (≥ 63 mg/kg bw/d) ulceration and inflammation (M/F: ≥ 250/125 mg/kg bw/d)	Key study RDT study experimental study (reliable without restriction) **LOAEL** (local and systemic effects): **32 mg/kg bw/d** based on hyperkeratosis (F), ↓ MCV (M/F), ↓ RBC (F), ↓ HGB (F), nephrotoxicity (F), ↑ kidney wt. (M/F) **Adverse effect dose levels relevant for classification:** **250 mg/kg bw/d** (although slightly above the guidance dose of 200 mg/kg bw/d, but consistent with effects seen in oral studies) based on decreased HGB (M/F), tubular necrosis (F), demyelination (F)	Study report 1992 (Melnick et al., 1994b; NTP, 1992)
Equivalent or similar to OECD TG 411, NTP-Study: Test procedure in accordance with national standards (NTP), GLP **2,2'-iminodiethanol** (CAS 111-42-2/ EC 203-868-0)	Significant effects: ≥ 80, 160, 320, 630, 1250 mg/kg bw/d **Liver:** ↑ abs. wt. (M: n.s., +16, 35, 35, 48 % and F: + **23**, 28, 43, 47, 92 %) ↑ rel. wt. (M: n.s., + 17, 31, 36, 57 % and F: + **11**, 19, 33, 45, 89 %) Hepatocellular necrosis (M) hepatocellular cytological changes: increased nuclear pleomorphism (M/F: ≥ 80/160 mg/kg bw/d) ↑ enzyme activities from 320 mg/kg bw/d:	Key study RDT study experimental study (reliable without restriction) **LOAEL** (local and systemic effects): **80 mg/kg bw/d** based on	Study report (Melnick et al., 1994a; NTP, 1992)

Method	Results	Remarks	Source
Dermal: shaved back of each animal (unoccluded), from the mid-back to the interscapular region Vehicle: ethanol (95 %) 0, 80, 160, 320, 630, 1250 mg/kg bw/d (nominal per unit body weight) 13 weeks (once per day, 5 days/week) **Mouse** (B6C3F1) 10 M/10 F per dose group Blood samples were collected from the retroorbital sinus at the end of study	ALT: + n.s./n.s., 102/n.s., 183/47 % (M/F) and Sorbitol-DH: + **25**, 76, 91 % (M) **Kidney:** ↑ abs. wt. (M: +**10**, 10, 18, 17, 30 % and F: + **7**, 14, 11, 16, 24 %) ↑ rel. wt. (M: +**8**, 11, 15, 18, 36 % and F: n.s., n.s., n.s., +15, 23 %) minimal to mild renal tubular necrosis (M/F: ≥ 1250 mg/kg bw/d) **Heart:** ↑ abs. wt. (M/F at 1250 mg/kg bw/d) cardiac myocyte degeneration **Local effects**: Skin lesions: acanthosis (M/F: ≥ 80 mg/kg bw/d) minimal to mild hyperkeratosis (M/F: ≥ 320/1250 mg/kg bw/d) ulceration and inflammation (M/F: ≥ 630 mg/kg bw/d)	↑ liver wt.(F), hepatocellular necrosis (M), ↑ kidney wt. (M/F), acanthosis (M/F)	
Equivalent or similar to OECD TG 451, GLP **2,2'-iminodiethanol** (> 99 %) (CAS 111-42-2/ EC 203-868-0) **Dermal**: unoccluded Vehicle: ethanol (95 %) 0, 16, 32, 64 mg/kg bw/d (M) 0, 8, 16, 32 mg/kg (F) 103 weeks (once per day, 5 days/week) **Rat** (Fischer 344/N) 50 M/50 F per dose group	Non-neoplastic significant effects: ≥ 8, 16, 32, 64 mg/kg bw/d **Liver:** decrease of incidences of basophilic foci (M/F: ─/31, 5/20, 1/7, 2/─ per 50 animals) **Kidney:** nephropathy incidence (severity): **47 (1.5)**, 48 (1.9), 48 (2.7) out of 50 Fs, in control group 40/50 (1.2) **Mammary Gland:** ↓ incidence of fibroadenoma (5 of 50 F at 32 mg/kg bw/d compared to 14 of 50 F in vehicle control) **Local effects**: Skin lesions (incidence per 50 animals): minimal hyperkeratosis (M/F: ─/13, n.s./23, 5/23, 11/─) and exudate (M/F: ─/7, n.s./7, n.s./7, 7/─) acanthosis (10 of 50 M at 64 mg/kg bw/d)	Key study 2 year study experimental study (reliable without restriction) **LOAEL** (local and systemic effects): **8** mg/kg bw/d based on hyperkeratosis/exudate (F) and nephropathy (F) **Adverse effects relevant for classification:** 8 mg/kg bw/d Nephropathy (F) based on dose-related increases in severity grades	Study report 1999 (NTP, 1999b; US DHHS, 2002)
Equivalent or similar to OECD TG 451, GLP **2,2'-iminodiethanol** (> 99 %)	Non-neoplastic significant effects: ≥ 40, 80, 160 mg/kg bw/d **Liver:** ↑ incidences of hepatocyte changes: cytoplasmic (M: 17/50, 17/50, 12/50; 1/50 in control group) and syncytial alteration (M/F ≥ 80 mg/kg bw/d: 38/17, 23/18 per 50 animals, 0/50 M/F in control)	Key study 2 year study experimental study (reliable without restriction)	Study report (NTP, 1999b; US DHHS, 2002)

Method	Results	Remarks	Source
(CAS 111-42-2/ EC 203-868-0) **Dermal**: unoccluded Vehicle: ethanol (95%) 0, 40, 80, 160 mg/kg bw/d (M/F) 103 weeks (once per day, 5 days/week) **Mouse** (B6C3F1) 50 M/50 F per dose group	**Kidney:** ↑ incidence of renal tubule hyperplasia (M at 160 mg/kg bw/d: 10/ 50, in control group 1/50) **Thyroid Gland:** ↑ incidence of follicular cell hyperplasia (M/F: 22/28, 30/32, 42/39 per 50 animals compared to 18 of 50 M/F in vehicle control) **Local effects**: Skin lesions: minimal hyperkeratosis (M/F: 13/n.s., 10/8, 17/16 per 50 animals)	**LOAEL** (local and systemic effects): **40** mg/kg bw/d based on hyperkeratosis (M), liver lesions (M) and lesions in thyroid gland (M/F) Tumor data and attributed reduced survival see 10.8)	

Oral

Extended one-generation reproductive toxicity study, rat (EOGRTS; OECD TG 443) (BASF AG, 2018a)

Based on the data from the dose-range finding study, 0 (deionised water), 100, 300, and 1000 ppm DEA were chosen for the subsequent EOGRT study (OECD TG 443; version 2011) including cohorts 2A, 2B (developmental neurotoxicity; DNT) and cohort 3 (developmental immunotoxicity; DIT) (BASF AG, 2018a). DEA was administered to Crl:WI(Han) Wistar rats via the drinking water continuously throughout the entire study. Premating exposure of parental animals (P0) was at least 16 days. After mating, females were allowed to deliver and rear their pups (F1 pups) until PND 4 (standardisation) or PND 21 or 22 (depending on the cohort). Serum platelet activating factor (PAF) was determined in P0 parents.

General toxicity in parental animals included reduced water consumption in females from 300 ppm (at 1000 ppm: up to ↓ 18% during GD 14–18, and up to ↓ 45% during lactation). Food consumption was reduced in females from 300 ppm (at 1000 ppm: up to ↓ 29% during lactation). In males, food consumption was significantly lower than in controls at 1000 ppm during premating (up to ↓ 9%). Body weight gain was decreased in females at ≥ 300 ppm during GD 4-7 (up to ↓5%), and at 1000 ppm from premating day 7 throughout gestation and lactation (up to ↓33% during gestation). In males, body weight gain was decreased from 300 ppm (at 300 ppm: average of 23%; at 1000 ppm: average of ↓ 25%). Consequently, significantly lower body weights were detected: at 1000 ppm at start of mating -5.1% in males and -4.1 in females; at termination ≥ 300 ppm: -5.3, -10.0% in males and -2.5, -8.1% in females, respectively. In females, at 1000 ppm body weight was reduced up to -15% during gestation.

Haematology revealed microcytic anemia in both sexes with several parameters affected at ≥ 300 ppm including reduced haemoglobin (HBG) in males and females, haematocrit (HCT) in females, and mean corpuscular haemoglobin content (MCH) in males. Mean corpuscular volume (MCV) in males was already significantly reduced at 100 ppm.

Effects on white blood cell counts were mostly apparent at 1000 ppm (decreased absolute counts of neutrophils (NEUTA) and basophils (BASOA) in both sexes; decreased absolute and relative counts of monocytes (MONOA, MONO) and eosinophils (EOSA, EOS), and relative counts of basophils (BASO) in females). Platelets (PLT) were increased in males at ≥ 300 ppm and prothrombin time was significantly decreased in both sexes from 300 ppm. PAF was significantly decreased in females of the high dose group (↓ 31% based on the median values) but not in males.

In females, erosion/ulcer (≥ 300 ppm), and oedema and inflammatory cell infiltrations (≥ 100 ppm) in the glandular stomach were observed.

At all dose levels, DEA significantly increased the absolute and/or relative weight of the kidney in both sexes. Histology revealed tubular degeneration/regeneration (≥ 300 ppm in males; at 1000 ppm in females), accompanied with decreased urine specific gravity and increased urine volume in males from 300 ppm. Significantly higher absolute (at 1000 ppm in males; ≥ 100 ppm in females) and relative liver weights (≥ 100 ppm in males and females) were apparent at all dose levels. Centrilobular hypertrophy was observed in males and females at 1000 ppm. AST and ALP were significantly increased in males at 1000 ppm. Analysis of plasma hormone levels (T4 and TSH) revealed a dose-dependent increase of T4 levels in males reaching statistical significance at 1000 ppm. No treatment-related effects on TSH levels, thyroid weight or histology were detected.

Dose-range finding study (OECD TG 421), rat, for subsequent EOGRTS (BASF AG, 2018b)

A study following the OECD TG 421 served as a dose range-finding study for a subsequent EOGRTS (OECD TG 443). DEA was administered to groups of 10 male and 10 female Crl:WI(Han) Wistar rats (P0 parental animals) via the drinking water at doses of 0, 500, 1000, 1500 and 2000 ppm corresponding to 0, 46, 95, 137, 144 mg/kg bw/d, respectively (BASF AG, 2018b). In males, treatment covered 2-weeks premating, the mating period (6 days), and one week postmating (in total 4 weeks). The P0 females were exposed in total for 8 weeks. Parameters in parental animals comprised recordings of mortality and clinical observations, food and water consumption, body weight data, and male and female reproductive data. Organ weights were determined (brain, kidney, liver, testis, epididymis, uterus) and haematology (N = 5 per sex and dose group) as well as histology of selected organs was performed (control and high dose: brain, spinal cord (cervical, thoracic and

lumbar cord), testis, epididymis, ovary, and uterus; all dose groups: kidney and liver). Cell proliferation in kidney and liver was determined by Ki-67 immuno-staining in all dose groups, and kidney injury was investigated by KIM-1 (Kidney Injury Molecule-1) staining in the control vs high dose group. In all parental males and females (with litter), choline content was determined in blood plasma (approximately one week before sacrifice), liver, kidney, and uterus (HPLC-MS).

General toxicity in parental animals included reduced water consumption (at 2000 ppm: up to ↓ 36% in females during GD 10–18) and reduced food consumption (≥ 500 ppm in males, ≥ 1000 ppm in females; max. ↓ 18% in males at 2000 ppm), decreased body weight gain and terminal body weight (at 2000 ppm: ↓ 11% in males and ↓ 12% in females). Haematological effects were observed in both sexes indicative of microcytic anaemia with parameters affected already at the lowest dose tested (≥ 500 ppm: dose-dependent decreases in HGB in males and females, exceeding the 20% level at 1500 ppm in F (-17.9% in M at 2000 ppm) and decreases in RBC (at 1500 ppm close to the 20% level in M and F, exceeding 20% in F at 2000 ppm and reduced mean cellular volume in M ≥ 500 and F ≥ 1000 ppm (details see Table 24). Males appear to be less sensitive than females, however, males were dosed for only 4 weeks compared to females treated 8 weeks. Decreased values for absolute and relative values for neutrophilic granulocytes, eosinophilic granulocytes and monocytes indicate an suppressive effect on monocytic cells.

At all dose levels, DEA significantly increased the absolute and/or relative weights of kidney and liver in both sexes. Histologically findings in the kidney included degeneration/regeneration of the tubular epithelium (in males and females at all dose levels), tubular casts (in males from 1000 ppm; in females at all dose levels), mineralisation of the outer stripe of the outer medulla as well as the medulla (in males and females from 1000 ppm), and increased cell proliferation based on Ki-67 staining (in males at 2000 ppm; in females at all dose-levels). Increased KIM-1 staining was observed in the high dose group in males and females (only the high dose was investigated).

In the liver, histopathology revealed centrilobular (in females from 1500 ppm) as well as diffuse hypertrophy (in males from 1500 ppm), and peripheral fatty changes (in males and females from 1000 ppm). Discolouration (clay) of the liver was observed macroscopically in males and females from 1500 ppm. Increased cell proliferation based on Ki-67 staining was noted in the liver of the 2000 ppm males only. Analysis of choline in plasma and tissues (kidney, liver, uterus) revealed reduced choline in all investigated matrices. In some cases, choline content was already reduced from 500 ppm onwards, for example in kidney where no further decrease of choline could be detected with increasing doses.

Repeated dose toxicity study in rats (Melnick et al., 1994b; NTP, 1992)

The 13-week drinking water study in F344 rats (10 per sex per group) showed significant dose-dependent haematological changes following exposure to DEA at all concentrations tested: 320, 630, 1250, 2500, and 5000 ppm (0, 25, 48, 97, 202, 436 mg/kg bw/d) in males, and 160, 320, 630, 1250, and 2500 ppm (0, 14, 32, 57, 124, 242 mg/kg bw/d) in females. The animals developed dose-dependently a moderate, poorly regenerative, normochromatic, microcytic anaemia from the lowest dose level onwards. Females were more sensitive to haematological effects. Decreased HGB was apparent in both sexes from 320 ppm onwards in both sexes (-3.4, -10.1, -14.9, -33.8% (M) and -8.6, -13.9 , -25.2, -30.5% (F)). Decreased MCV (≥ 630/160 ppm (M/F)) and RBC (≥ 630/320 ppm (M/F)) was apparent in females already at lower dose levels than in males, however both decreasing in dose-dependent matter in both sexes. Haematological effects were not associated with microscopic changes in the femoral bone marrow. Toxic responses were also found in the kidney: increased absolute (only females from 160 ppm) and relative weight (≥ 630/320 ppm (M/F)), dose-dependent increase in nephropathy and mineralisation incidence and severity from 160 ppm (F, 14.8 mg/kg bw) and 320 ppm (M, 25 mg/kg bw/d), respectively, and increased incidence of tubular necrosis (5000/≥ 1250 ppm (M/F)). In addition, targets of toxicity are the brain and spinal cord (demyelination; M/F: ≥ 2500 ppm/≥ 1250 ppm), and testis (decreases in testis and epididymis weights (≥ 1250 ppm). The latter is associated microscopically with degeneration of seminiferous epithelium and with reduced sperm motility and hypospermia in the cauda epididymis (≥ 2500 ppm) and degeneration of the seminiferous tubules at 5000 ppm. Furthermore, liver weights were dose-relatedly increased without a histopathological correlate in male and female rats (M/F: ≥ 630 ppm/ ≥ 320 ppm). A no-observed-adverse-effect level (NOAEL) was not achieved for haematological changes, and nephropathy. The LOAEL for both toxic effects on blood and kidney was 320 ppm in males (25 mg/kg bw/d) and 160 ppm in females (14 mg/kg bw/d).

Repeated dose toxicity study in mice (Melnick et al., 1994a; NTP, 1992)

B6C3F1 mice (10 per sex per group) were given 0, 630, 1250, 2500, 5000, and 10,000 ppm DEA (0, 104, 178, 442, 807, 1674 mg/kg bw/d in males; 0, 142, 347, 884, 1154, 1128 mg/kg bw/d in females) in the drinking water for 13 weeks. Dose-dependent hepatotoxicity, nephrotoxicity, and cardiac toxicity was observed. All males and females from 5000 ppm and 3/10 females at 2500 ppm died prematurely. Body weight gains were decreased in males that received 2500 ppm and in females from 1250 ppm. Absolute and relative liver weights were increased in a dose dependent manner in male and female mice, associated with increases enzyme activities as well as with multiple morphological alterations in the liver. These were apparent from the lowest dose onwards (630 ppm) in form of hypertrophy, increased eosinophilia, and disruption of hepatic cords, increased nuclear pleomorphism, multinucleated hepatocytes with increasing severity grades (1.9 to 3.0). In addition, increasing incidence of hepatocellular necrosis (minimal to mild) starting from 2500 ppm (M) or 1250 ppm (F) were observed. Increases in absolute and relative kidney weights in male mice were associated with a dose-dependent increase in incidences of nephropathy among those mice that survived to the end of the study. Relative heart weights increased in males (2500 ppm) and females (≥ 1250 ppm) associated with minimal-to-marked degeneration and necrosis of cardiac myocytes from 2500 ppm. Myocardial degeneration was generally more severe in mice that died early than in those that survived to study termination. The most sensitive effect was necrotic liver damage at all concentrations. A LOAEL of 630 ppm (equal to 104 mg/kg bw/d in males and 142 mg/kg bw/d in females) was achieved in male and female mice.

Inhalation

Repeated dose toxicity study in rats, inhalative, (BASF AG, 1996; Gamer et al., 2008) and (BASF AG, 2002) (OECD 413), and (BASF AG, 1993) (OECD 412)

The two key studies (nose-only 90-day inhalation toxicity studies) were performed with similar test design. Groups of Wistar rats of each sex were exposed to a liquid aerosol of DEA for 6 h/day, 5 times/week for 3 months (65 exposures). In the main study groups of rats (13/sex/group) were exposed to 15, 150, or 400 mg/m^3 DEA. The DEA atmospheres consisted of a large proportion (92–95%) of respirable aerosol particles (MMAD 0.6–1.9 µm). The possibility of a neurotoxic potential was investigated in this study (BASF AG, 1996; Gamer et al., 2008). The second study was aimed at specifically investigating respiratory tract toxicity. Recovery groups of rats (10/sex/group) were exposed to target concentrations of 1.5, 3, 8 mg/m^3 with MMADs ranging from 0.6 to 0.7 µm. Further recovery groups of 10 additional female rats exposed to 0, 3 or 8 mg/m³, which were observed for a post-exposure period of three months. For each study, control groups with the respective number of animals/sex were exposed to clean air.

In the main study no functional or morphological evidence of neurotoxicity was found. Reduction in body weight development was observed in males exposed to 400 mg/m^3. Animals of all exposure concentrations showed a focal squamous metaplasia of the ventral laryngeal epithelium at the base of the epiglottis. Additionally, animals exposed to 150 and 400 mg/m^3 revealed a concentration-dependent increase in laryngeal squamous hyperplasia, as well as incidence and severity of local inflammation of larynx and trachea. Therefore, 15 mg/m^3 was derived as the LOAEC for local effects in the upper respiratory tract in this study. Systemic effects were observed in animals exposed to ≥150 mg/m^3. There were slight increases of liver weights and serum alkaline phosphatase serum levels without histopathological correlate indicative for a concentration dependent adaptive response. In the kidneys minimal or slight tubular hyperplasia was observed in some females and intratubular lithiasis that was slightly more pronounced than in controls in some males. A mild normochromic microcytic anaemia occurred from 150 mg/m^3 and influence on the male reproductive system consisting of diffuse testicular atrophy and minimal atrophy of the prostate in single animals was present at 400 mg/m^3 only. A NOAEC of 15 mg/m^3 was derived for systemic toxicity (e.g. liver and kidney effects).

In the second sub-chronic study similar effects in the larynx as seen in the main study were observed at 8 mg/m³. Male and female rats showed a focal squamous metaplasia of the laryngeal epithelium and submucosal inflammation in a few cases as well. At 3 mg/m³, three of 10 males developed a focal squamous metaplasia but no inflammatory cell reaction was visible. Examination of the upper respiratory tract after the recovery period of three months revealed no histological changes. No DEA related morphological effects were observed in the larynx at 1.5 mg/m³. Such finding of "larynx squamous metaplasia" was evaluated and discussed on 1st international ESTP expert workshop and was assessed as "non-adverse" (Kaufmann et al.,

2009). The experts stated that minimal and focal "laryngeal squamous metaplasia" at the base of the epiglottis in the absence of cilia and flattening of the normally cuboidal, laryngeal epithelium does not fulfil the criteria of a complete "laryngeal squamous metaplasia" (Dungworth et al., 2001). For such focal and minimal lesions at the base of the epiglottis the new term of a "laryngeal epithelial alteration" was introduced. This morphological correlate for only a slight irritation was regarded to be not-adverse in character, as no significant dysfunction of the larynx is to be expected. Because DEA induced laryngeal squamous metaplasia was observed as well-differentiated, reversible and without dysplastic changes it is not regarded to be a pre-neoplastic lesion in this study at concentrations up to 8 mg/m³. In contrast, concentration-related increase in laryngeal squamous hyperplasia and an extension to the region of ventral pouch and arytenoid cartilages have been observed at 150 mg/m³ and above in the main study. The potential for progression with exposures of longer duration should be considered and cannot be ruled out (Kaufmann et al., 2009; Osimitz et al., 2007). Thus, the reversible squamous metaplasia observed at 3 mg/m^3 may be regarded to be of borderline toxicological significance. Because the effect increased in severity and extension and due to its absence in the laboratory control animals it is considered to represent the "lowest observed adverse effect concentration" (LOAEC) for local effects. Neither the nasal cavity, nor trachea or lungs exhibited any substance induced histomorphological changes at any concentration. The No observed effect concentration (NOEC) for local effects was found to be 1.5 mg/m³.

In the third study sub-acute study investigated inhalation toxicity of DEA in male and female Wistar rats using head-nose exposure for 14 days. Ten males and females/group were exposed to a liquid aerosol at concentrations of 0, 0.1, 0.2, or 0.4 mg/L for 6 h daily at 5 times/week. Only at the highest concentration slight signs of systemic effects in form of impaired body weight gain, slightly decreased cholesterol values in males and females, and increased liver weights in females were observed. A NOAEC of 0.2 mg/L was derived (BASF AG, 1993).

Dermal

Repeated dose toxicity study in rats and mice, dermal, Equivalent or similar to OECD TG 411 (Melnick et al., 1994a; Melnick et al., 1994b; NTP, 1992)

Groups of 10 male and 10 female F344 rats were administered DEA in 95% ethanol at dose levels in the range of 0, 32, 63, 125, 250, 500 mg/kg bw/d once per day, 5 days per week for 13 weeks (Melnick et al., 1994b; NTP, 1992). The dosing solution was applied to the shaved back of each animal (unoccluded), from the mid-back to the interscapular region. Mortality was observed in the highest dose groups. Animals showed lesions of the kidney, skin, and brain. Body weight gains were reduced in rats at the upper doses. Skin lesions at the site of application including ulceration and inflammation, hyperkeratosis, and acanthosis were observed at all dose levels. A moderate, poorly regenerative, normochromatic, microcytic anaemia similar to that seen in the drinking water study was also developed in male and female rats exposed by topical application to DEA. Dose-dependent decreases in red blood cell parameters (reduced MCV, RBC, and HGB) were observed even at the lowest dose of 32 mg/kg bw/d. No histological changes in femoral bone marrow were observed. The kidney was also identified as a target organ mainly in females. Increases in absolute and relative kidney weights ($\geq$ 32 mg/kg bw/d) were associated with increased severity or increased incidences of nephropathy, renal tubular cell necrosis or tubular mineralisation. Liver weights were dose-dependently increased in male and female rats corresponding with mild serum biochemical changes but not associated with histopathological findings. Other treatment-related effects included demyelination with vacuolisation in the brain of all males and females at 500 mg/kg bw/d which was also noted in 7/10 females at 250 mg/kg bw/d. The LOAEL for anaemia, nephropathy and hyperkeratosis of the skin in rats was 32 mg/kg bw/d. A NOAEL for local and systemic effects was not derived from this study (Melnick et al., 1994b; NTP, 1992).

In B6C3F1 mice (10/sex/group), doses of 80–1250 mg/kg bw/d DEA in 95% ethanol were applied dermally once daily on five days per week for 13 weeks onto the shaved back of each animal (unoccluded), from the mid-back to the interscapular region, using a calibrated micropipette (Melnick et al., 1994a; NTP, 1992). Two male mice administered 1250 mg/ kg bw/d DEA were killed in a moribund condition during weeks 2 and 9; four top-dose female mice died or were killed in a moribund condition during weeks 2 and 3. The highest dose induced a decrease in body weight in males compared with controls; females exhibited no body weight effects. Skin toxicity was observed at the site of application at all dose levels consisting of acanthosis at the low dose

level and with a dose-dependent increased incidence up to ulcerations, inflammation and hyperkeratosis at higher dose levels. Liver weights were dose-dependently increased from the low dose group onwards, which were associated with multiple hepatocytic changes, but hepatocellular necrosis occurred only in male mice. Kidney toxicity including tubular necrosis as well as cardiac myocyte degeneration were found in both males and females at 1250 mg/bw kg/d. The LOAEL for local effects (skin at the application site seen as acanthosis) in both male and female mice was 80 mg/kg bw/d. The LOAEL (systemic) for increased liver weights with associated morphological findings in the liver in female mice, increased kidney weight in male mice was 80 mg/kg bw/d. A NOAEL for local and systemic effects was not derived in this study (Melnick et al., 1994a; NTP, 1992).

Two-year carcinogenicity studies, dermal, rats and mice, equivalent to OECD TG 451 (NTP, 1999b; US DHHS, 2002)

Detailed study/method descriptions are provided in chapter 10.8. Only nonneoplastic lesions and general toxicity are described below.

Mice

Groups of 50 male and female B6C3F1 mice received dermal doses of 0, 40, 80 or 160 mg/kg bw/d DEA for 5 days a week for 103 weeks. Survival of dosed females was reduced (44/50, 33/50, 33/50; 23/50). Study authors attributed this to liver neoplasms observed (see details in chapter 10.8.). There was no effect on survival in males. Mean body weights of 80 and 160 mg/kg bw/d dosed males and all DEA dosed females were lower than controls.

Dermal administration of DEA to mice was associated with increased incidences of cytoplasmic alteration in males (≥ 40 mg/kg bw/d: 17/50, 17/50, 12/50 vs 1/50 in control) and syncytial alteration of the liver in both sexes (≥ 40 mg/kg bw/d: 28/50, 38/50, 23/50 (M) and 2/50, 17/50, 18/50 (F)).

In males, increased incidences of renal tubule hyperplasia were apparent from doses ≥ 40 mg/kg bw/d (7/50, 7/50, 10/50 vs 3/50 in control). Thyroid gland follicular cell hyperplasia was dose-dependently increased in both sexes: 22/49, 30/50, 42/50 (M) and 28/49, 32/50, 39/50 (F) versus 18/50 (M/F) in control groups.

Hyperkeratosis, acanthosis, and exudate were treatment-related changes in the skin at the site of application. The LOAEL for local and systemic effects of 40 mg/kg bw/d was the lowest dose tested.

Rats

Groups of 50 male and female F344/N rats were administered dermal doses of 0, 16, 32 or 64 mg/kg bw/d DEA for 5 days a week for 103 weeks. Survival of dosed animals was not affected and similar to controls. Mean body weight of males in the 64 mg/kg bw/d group (from week 8 to week 89) and of females in the 32 mg/kg bw/d group (from week 97 onwards) was lower compared to controls. Clinical findings attributed to DEA administration were minimal to mild skin lesions at the site of application in the epidermis, which were dose-related and more common in females than in males. An increase in the incidences of acanthosis in 64 mg/kg bw/d males, the incidences of hyperkeratosis in 32 and 64 mg/kg bw/d males and in all dosed female groups, and the incidences of focal accumulations of exudate on the epidermal surface in 64 mg/kg bw/d males and in all dosed female groups was observed. The incidences and severities of observed nephropathy were significantly and dose-dependently increased in dosed female rats (incidences: 47/50, 48/50, 48/50, severity 1.5, 1.9, 2.7) compared to the vehicle controls (40/50, severity 1.2).

The NOAEL for local effects was 16 mg/kg bw/d and 32 mg/kg bw/d for systemic toxicity in males. In females the LOAEL for local and systemic toxicity was 8 mg/kg bw/d.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

Repeated dose toxicity studies on DEA have been conducted in rats and mice using oral, inhalation and dermal routes of administration. Significant toxic effects of DEA were observed in 90-day repeated dose studies for all three examined application routes. DEA caused toxic effects at multiple organ sites in rats and mice after exposure via drinking water, after topical application or by inhalation. In rats target organs of DEA toxicity included the blood (microcytic anaemia), kidney, nervous system, and skin (site of application). In mice

exposure to DEA caused toxic effects in the liver, kidney, heart (myocard), and skin (site of application). Data suggest that rats are somewhat more sensitive than mice to the toxic effects of DEA.

Oral

From the oral EOGRT study in rats, observed **adverse effects relevant for classification are microcytic anaemia** apparent in significantly decreased HGB in males at 1000 ppm (73.4 mg/kg bw/d) **and nephropathy** starting from 300 ppm (21.5 mg/kg bw/d) in males and 1000 ppm (73.4 mg/kg bw/d) in females (BASF AG, 2018a). Incidence and severity of the tubular degeneration and regeneration in the kidney increased in a dose-dependent manner in males from 300 ppm (5/21, grade 1) to 19/21 (9 grade 1, 10 grade 2) at 1000 ppm and was apparent at 1000 ppm in females 20/20 (6 grade 1, 10 grade 2, 4 grade 3) as well. In addition, reduced specific urine gravity and a higher urine volume (the latter not statistically significant) was found in males. In conjunction with the histopathological alterations in the kidney, this change is regarded as treatment-related and advers.

The LOAEL value obtained from this study is 100 ppm (6.8 or 10.2 mg/kg bw/d M/F, respectively), based on reduced mean erythrocyte volume, changes on absolute (F) and relative (M and F) kidney and liver weight. Although these effects were of low/mild severity at the low dose level, they are considered adverse; severity and/or incidences increased dose-dependently. Microcytic anaemia exceeded the 20% level for HGB in males at 1000 ppm (73.4 mg/kg bw/d) and is together with nephrotoxicity observed in males/females at 300/1000 ppm relevant for classification. It is to noted that similar toxicity (haemolytic anaemia, reduced liver and kidney weight, nephropathy) were observed in the F1 generation of the study. Here, females of F1 of generation were more sensitive regarding to microcytic anaemia with changes being more profound at the lowest dose tested. However, for classification on STOT RE only the parental generation was assessed. Details of the effects apparent in F1 generation are discussed in chapter 10.9.

Similar effects were observed in the OECD TG 421 study (dose-range finder study for the EOGRTS) (BASF AG, 2018b). Severe microcytic anaemia was observed in both sexes (reductions on RBC/HGB at ≥ 20% level was seen in females from 1500 ppm (137 mg/kg bw/d) and in males (at the end of 4 weeks of treatment) at 2000 ppm (144 mg/kg bw/d). Study length was shorter than 90 days (56 days for females), thus these findings support classification. The 10% level was reached in females from 500 ppm (46 mg/kg bw/d) which is considered relevant for classification in combination with observed kidney effects at this dose. From the lowest dose tested all animals showed tubular degeneration and regeneration in the kidney in dose-dependent severity. From 1000 ppm (95 mg/kg bw) onwards severity was grade 2 to 3 in both sexes, and thus considered as relevant for classification.

The results from the EOGRTS and corresponding range-finding study are supported by the oral NTP study (Melnick et al., 1994b; NTP, 1992), where a dose-dependent increase in nephropathy and mineralisation incidence and severity was observed from 160 ppm (F, 14.8 mg/kg bw/d). It is to note that in this study a high incidence of mineralisation was apparent in the control group as well, but of lower severity. Significantly reduced HGB was apparent in both sexes from 320 ppm indicative of microcytic anaemia. From doses ≥ 620 ppm (57 mg/kg bw/d) in female rats the decrease of HGB is > 10%, from 1250 ppm (124 mg/kg bw/d) the decrease is greater than 20% and in combination with tubular necrosis from 1250 ppm in females warranting classification.

In addition, the nervous system was observed to be a target of toxicity with apparent demyelination in the brain (medulla) and spinal cord starting from 1250 ppm in females and 2500 ppm in males. In males, these doses are above limits warranting classification whereas in females 100% of the animals were affected at 1250 ppm (124 mg/kg bw/d) which is rather close to the guidance value of 100 mg/kg bw/d and supports that classification for STOT RE is warranted.

In mice (Melnick et al., 1994a; NTP, 1992), effects on the kidney were less pronounced and above limits for classification. However, liver necrosis und disruption of hepatic cords were apparent at 630 ppm in most animals of both sexes increasing in severity in dose-dependent manner and are considered relevant for classification. Increased ALT as well as Sorbitol-DH values apparent at higher doses support these findings as adverse liver damage. In addition, nuclear pleomorphism und multinucleated hepatocytes indicates abnormal, possibly preneoplastic cell proliferation.

Inhalation

Aerosol exposure of rats to DEA for 90 days resulted in systemic effects such as anaemia, liver dysfunction and kidney lesions as well as local irritating effects on the upper respiratory tract. Squamous metaplasia in combination with inflammatory cell infiltration in the larynx or with wider extension to other larynx area than the base of the epiglottis occurring from 3 mg/m³ was considered adverse (LOAEC). Incidence of squamous hyperplasia was quite high with 9/10 animals affected, however severity was low (grade 1 in all animals). Recovery was noted at the end of the recovery period which was unusually long (3 months instead of 4 weeks recommended in the OECD TG 413). The tracheal mucosa was similarly affected at 150 mg/m³. In contrast, the reversible minimal and focal laryngeal squamous metaplasia at the base of the epiglottis only in the absence of cilia and flattening of the normally cuboidal, laryngeal epithelium (Dungworth et al., 2001; Kaufmann et al., 2009). Respiratory tract effects were also seen in the second 90-day inhalation study (BASF AG, 1996; Gamer et al., 2008) at the lowest dose tested (15 mg/m^3) mild metaplasia was apparent in all animals, with development of additional effects such as hyperplasia and inflammation in dose dependent severity and increasing incidence. Following 14 days of aerosol exposure, no local effects but only systemic effects such as increased liver weights were observed. The only experimental data available on human exposure to airborne DEA were obtained from clinical provocation tests. DEA-induced occupational asthma was diagnosed following specific bronchial provocation tests in an exposure chamber. A positive reaction was observed in a 39-year-old male metal worker after inhalation exposure to aerosols from a warmed cutting fluid containing diethanolamine and triethanolamine (Piipari et al., 1998). Apart from that, three cases of occupational asthma caused by other ethanolamines were reported (Savonius et al., 1994): Two metal workers exposed to a cutting fluid containing triethanolamine, and one cleaner exposed to a detergent containing monoethanolamine.

None of the effects is considered to be severe or serious enough to justify a classification as STOT RE.

Dermal
In a 90-day and a chronic study via the dermal route, local (hyperketarosis) and systemic effects similar to other administration routes were observed.

10.12.2 Comparison with the CLP criteria

The available results from animal studies show that DEA induces adverse effects following subchronic exposure by all routes of exposure. As the data suggest that rats are more sensitive than mice to the toxic effects of DEA, mainly studies in rats were assessed for classification.

The available 90-days (13 weeks) studies and the EOGRTS were performed according to the guideline methods and are reliable without restrictions.

Table 3.9.2 and Table 3.9.3 of the guidance document on the application of CLP criteria state dose/concentration guidance values for distinction between Category 1 (significant toxic effects at generally low exposure concentrations) and Category 2 (significant toxic effects at generally moderate exposure concentrations) repeated dose toxicity for all exposure routes. For studies of shorter or longer exposure duration the effective dose can be extrapolated to a 90-day value using Haber's rule. In Table 28 the extrapolated effective dose as well as classifications supported by each study are summarised.

Via oral route, the extrapolated effective dose obtained from the EOGRT study (BASF AG, 2018a) as well as the 90-day NTP (NTP, 1992) study indicate a classification into Category 2. Via inhalation, effects in both 90-day studies are not severe enough to justify classification. However, effects are similar to the effects observed upon administration via other routes and thus are considered supportive for classification.

The 90-day and chronic dermal studies support the existing classification into Category 2.

In conclusion, the weight of evidence of all data justifies classification into Category 2.

Table 28: Classification supported by available studies using extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days or 13 weeks, respectively, using Haber's rule.

Study reference	Species	Lowest effective dose	Length of exposure	Extrapolated effective dose (90 days/13 weeks)	Classification supported by the study
Oral route					
(BASF AG, 2018a)	rat	21.5 mg/kg bw/d (M) 148,1 mg/kg bw/d (F)	~8 weeks (M) ~10 weeks (F)	13.3 mg/kg bw/d (M) (x 0.62) 114,1 mg/kg bw/d (F) (x 0.77)	Category 2
(BASF AG, 2018b)	rat	46 mg/kg bw/d (F)	8 weeks (F)	10.6 mg/kg bw/d (x 0.66)	Category 2
(Melnick et al., 1994b; NTP, 1992)	rat	124 mg/kg bw/d	13 weeks	124 mg/kg bw/d	Category 2 (slightly above the guidance value but considered supportive for classification)
(Melnick et al., 1994a; NTP, 1992)	mouse	104 mg/kg bw/d (M)	13 weeks	104 mg/kg bw/d (M)	Category 2
Inhalative route					
(BASF AG, 1993)	rat	400 mg/m^3 = 0.4 mg/l (systemic effects)	2 weeks (6h/d, 5d/week)	0.064 mg/l (x 0.16)	-
(BASF AG, 1996; Gamer et al., 2008)	rat	15 mg/m^3 (local effects)	90 days (6h/d, 5d/week)	0.015 mg/l	-
(BASF AG, 1996; Gamer et al., 2008)	rat	8 mg/m^3 (local effects)	90 days (6h/d, 5d/week)	0.008 mg/l	-
Dermal route					
(Melnick et al., 1994b; NTP, 1999b)	rat	250 mg/kg bw/d	13 weeks (1x/day, 5d/week)	250 mg/kg bw/d	Category 2 (although slightly above the guidance dose of 200 mg/kg bw/d, but consistent with effects seen in oral studies)
(NTP, 1999b; US DHHS, 2002)	rat	8 mg/kg bw/d	103 weeks (1x/day, 5d/week)	64 mg/kg bw/d (x 8)	Category 2

10.13 Conclusion on classification and labelling for STOT RE

Available data supports the existing harmonised classification of DEA as STOT RE 2, H373 (haematopoetic system, kidney, nervous system). The liver was also identified as a target organ, but with less coherence across studies.

10.14 Aspiration hazard

Not addressed in this dossier

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not addressed in this dossier

12 EVALUATION OF ADDITIONAL HAZARDS

Not addressed in this dossier

13 ADDITIONAL LABELLING

Not addressed in this dossier

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