CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

1-ethoxy-2-(2-methoxyethoxy)ethane

EC Number: 213-690-5

CAS Number: 1002-67-1

Index Number: -

Contact details for dossier submitter:

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on behalf of the Austrian Competent Authority (Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology. Radetzkystraße 2, 1030 Vienna, Austria)

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CONTENTS

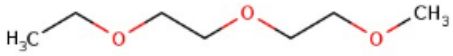
ABBREVIATIONS

AC	Article Category
abs	absolute
AGD	Anogenital Distance
AGI	Anogenital Index (AGD divided by the cubic root of the body weight)
ARN	Assessment of Regulatory Needs
ATE	Acute Toxicity Estimate
bw	body weight
CAS	Chemical Abstract Service
CNS	Central Nervous System
CL	Confidence Limit
d	day
Drg	Danger
EGME	2-Methoxyethanol
GD	Gestation Day
GLP	Good Laboratory Practice
HCD	Historical Control Data
LD	Low Dose
M/F	Male/Female
MAA	Methoxyacetic acid
MD	Mid Dose
NOAEL	No Observed Adverse Effect Level
OECD	Organisation for Economic Co-operation and Development
PC	Product Category
QSAR	Quantitative Structure Activity Relationship
rel	relative
SI	Stimulation Index
SU	Sector of Use
TG	Test Guideline
TD	Top Dose

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)	1-(2-ethoxyethoxy)-2-methoxyethane
Other names (usual name, trade name, abbreviation)	diethylene glycol methyl ethyl ether ethyl diglyme DEGMEE MEDG
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	213-690-5
EC name (if available and appropriate)	1-ethoxy-2-(2-methoxyethoxy)ethane
CAS number (if available)	1002-67-1
Other identity code (if available)	-
Molecular formula	$C_7H_{16}O_3$
Structural formula	 **(source:** European Chemicals Agency, http://echa.europa.eu/**)**
SMILES notation (if available)	CCOCCOCCOC
Molecular weight or molecular weight range	148.20
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)	Not relevant

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 (CLP)	Current self-classification and labelling (CLP)
1-ethoxy-2-(2-methoxyethoxy)ethane EC 213-690-5 CAS 1002-67-1	Conf.	-	Not classified

DEGMEE is a mono-constituent substance. Impurities are not indicated in the registration. According to registrants the substance contains no impurities that would affect the classification of the substance.

Information on the purity of the substance used for testing is given in the study descriptions if available.

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 3: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	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	No current Annex VI entry										
Dossier submitter's proposal	TBD	1-ethoxy-2-(2-methoxyethoxy)ethane	213-690-5	1002-67-1	Repr. 1B	H360FD	GHS08 Dgr	H360FD			

Table 4: Reason for not proposing harmonised classification and status under consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	*hazard class not assessed in this dossier*	No
Acute toxicity via dermal route	*hazard class not assessed in this dossier*	No
Acute toxicity via inhalation route	*hazard class not assessed in this dossier*	No
Skin corrosion/irritation	*hazard class not assessed in this dossier*	No
Serious eye damage/eye irritation	*hazard class not assessed in this dossier*	No
Respiratory sensitisation	*hazard class not assessed in this dossier*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	*hazard class not assessed in this dossier*	No
Carcinogenicity	*hazard class not assessed in this dossier*	No
Reproductive toxicity	Repr. 1B, H360FD	Yes
Specific target organ toxicity-single exposure	*hazard class not assessed in this dossier*	No
Specific target organ toxicity-repeated exposure	*hazard class not assessed in this dossier*	No
Aspiration hazard	*hazard class not assessed in this dossier*	No
Endocrine disruption for HH	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	*hazard class not assessed in this dossier*	No
Endocrine disruption for ENV	*hazard class not assessed in this dossier*	No
PBT/vPVB	*hazard class not assessed in this dossier*	No
PMT / vPvM	*hazard class not assessed in this dossier*	No
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

The substance has no harmonized classification so far.

DEGMEE has 373 C&L notifications. Most of them do not classify the substance. In addition following classifications can be found: Skin Irrit. 2, H315 ; Eye Irrit. 2, H319; Repr. 2, H361; Aquatic Chronic 3, H412; Flam. Liq. 3, H226 [status 12/2023].

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

DEGMEE is registered in the EU in the tonnage band of ≥ 100 to < 1 000 tonnes per year. The substance is used in inks and toners as well as paper chemicals and dyes.

Table 5: Uses as indicated at ECHA dissemination site (summary) [accessed 12/2023].

Categories	Use(s)	Technical function
Manufacture	-	-
Formulation	Formulation Formulation of ink product (PC 18: Ink and toners)	-
Uses at industrial sites	Component of ink prepration in industry (PC 18: Ink and toners, PC 26: Paper and board treatment products, SU 7: Printing and reproduction of recorded media) Industrial use (PC 20: Products such as ph-regulators, flocculants, precipitants, neutralisation agents, PC 21: Laboratory chemicals, SU 9: Manufacture of fine chemicals; SU 24: Scientific research and development) Use of ink bottles, ink cartridges, ink pouch and ink pack (PC 18: Ink and toners, SU 7: Printing and reproduction of recorded media)	-
Uses by professional workers	Widespread use by professional workers (PC 18: Ink and toners, PC 26: Paper and board treatment products, SU 6b: Manufacture of pulp, paper and paper products) Use of ink preparations containing the substance by professional workers (PC 18: Ink and toners, PC 26: Paper and board treatment products, SU 6b: Manufacture of pulp, paper and paper products) Professional use (PC 20: Products such as ph-regulators, flocculants, precipitants, neutralisation agents, PC 21: Laboratory chemicals, SU 20: Health services, SU 24: Scientific research and development) Use of ink bottles, ink cartridges, ink pouch and ink pack (PC 18: Ink and toners, SU 7: Printing and reproduction of recorded media, SU 0: other SU 22)	-
Consumer Uses	Use if ink preparations containing the substance by consumers (PC 18: Ink and toners, PC 26: Paper and board treatment products)	-

Article service life	Ink preparations containing the substance (used by workers, consumers), AC 02: other (intended to be released: ink (printing ink).	-

6 DATA SOURCES

ECHA dissemination site: Registration Dossier - ECHA (europa.eu)

Original study reports on DEGMEE were provided by the registrant(s) for all repeated dose and reproductive toxicity studies available on ECHAs dissemination site.

7 PHYSICOCHEMICAL PROPERTIES

Table 6: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid, clear colourless	ECHA dissemination site	-
Melting/freezing point	<-80°C (at 1028 hPa)	ECHA dissemination site	OECD TG 102
Boiling point	177°C (at 1028 hPa)	ECHA dissemination site	OECD TG 103
Relative density	0.922	ECHA dissemination site	OECD TG 109
Vapour pressure	2.1 Pa (at 25°C)	ECHA dissemination site	OECD TG 104
Surface tension	69.9 mN/m (at 20°C)	ECHA dissemination site	OECD TG 115
Water solubility	>1000 g/L (at 20°C)	ECHA dissemination site	OECD TG 105
Partition coefficient n-octanol/water	-0.1 (at 20°C)	ECHA dissemination site	OECD TG 107
Flash point	69°C (at 1018 hPa)	ECHA dissemination site	EU Method A.9
Flammability	-	ECHA dissemination site	waiving
Explosive properties	Non explosive	ECHA dissemination site	EU Method A.14
Self-ignition temperature	175°C	ECHA dissemination site	EU Method A.15
Oxidising properties	no	ECHA dissemination site	Scientific evaluation based on structure and oxygen balance
Granulometry	-	ECHA dissemination site	-
Stability in organic solvents and identity of relevant degradation products	Stability not considered critical	ECHA dissemination site	waiving
Dissociation constant	-	ECHA dissemination site	OECD TG 112
Viscosity	1.15 mPa s	ECHA dissemination site	QSAR

8 EVALUATION OF PHYSICAL HAZARDS

Not assessed in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

There are no toxicokinetic data available for DEGMEE.

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity - oral route

Not assessed in this dossier.

10.2 Acute toxicity - dermal route

Not assessed in this dossier.

10.3 Acute toxicity - inhalation route

Not assessed in this dossier.

10.4 Skin corrosion/irritation

Not assessed in this dossier.

10.5 Serious eye damage/eye irritation

Not assessed in this dossier.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Not assessed in this dossier.

10.8 Germ cell mutagenicity

Not assessed in this dossier.

10.9 Carcinogenicity

Not assessed in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

DEGMEE was tested in a combined repeated dose toxicity study with reproductive/developmental screening test according to OECD 422 (Anonymous 2014b) as well as three repeated dose studies: a non guideline 14-day study (Anonymous 2014a), a 28-day study according to OECD 407 (Anonymous 2014c) and a 90-day study according to OECD 408 (Anonymous 2022a). All studies were conducted in rat via the oral route, according to GLP and are considered reliable.

Table 7: Summary table of animal studies on adverse effects on sexual function and fertility (for all indicated numbers the difference from control is statistically significant, if not indicated differently)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Combined Repeated Dose Toxicity Study with Reproductive/Developmental Toxicity Screening Test **(OECD 422, March 22, 1996; GLP)** Oral (gavage) Rat (Crl:CD(SD); 9 weeks old) 12/sex/group (including 5/sex/group for the recovery group)	DEGMEE (purity: 99.98%) (vehicle: water); 0 / 50 / 250 / 1000 mg/kg bw/day (10 ml/kg bw); M: 42 days F: 15 days before mating, through gestation and parturition until lactation day 4. Satellite M & F: exposure for 42 days, recovery period until day 57	There were no relevant clinical signs in adult animals (males and females) throughout the study and no relevant effects on body weight or food consumption were reported. **<u>Repeated dose toxicity:</u>** **1000 mg/kg bw/day** Motor activity: There was a dose dependent reduction in motor activity which was statistically significant at 1000 mg/kg bw/day from 20 – 50 minutes after exposure (as low as 1% of the control group). No other relevant finding in the functional observation battery tests. Hematology: low values for platelets, white blood cell count, neutrophils, basophils & large unstained cells in F Blood biochemistry: Lowered ALP values in males, whereas ALP was elevated in two F of this group. In these two F also total bile acid was elevated. Thymus: weight decrease in M (rel: - 30%) Liver: weight increase in M (abs: + 29%, rel: + 24%) & F (abs: + 18%) Histopathology: minimal to mild hypertrophy of centrilobular hepatocytes in 4 M & 3 F *<u>End of recovery period:</u>* Liver: weight increase in M (rel: + 13%) **<u>Reproductive / Developmental toxicity:</u>** **1000 mg/kg bw/day** <u>Dams:</u> Prolonged gestation period: 1 day longer compared to control (23.2 days vs 22.3 days); also at 250 mg/kg bw/day (22.7 days vs 22.3 days, but not statistically significant); it was stated that prolonged gestation might have been caused by lower pup weight (slightly lower pup weight on PND 0, not statistically significant; M: -4%, F: -2%) and lower pup number (-23%). Decreased delivery index: 79.5 ± 12.1 % vs 94.4 ± 7.8 % in the control group Total litter loss in 5 F (5/10) – offspring died mostly due to cannibalism by dams, and did not show any signs of suckling.	Anonymous (2014b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		(total litter loss: 1 on LD 1, 3 on LD 2, 1 on LD 3) No necropsy abnormalities were noted in these 5 F with total litter loss. No abnormality observed in delivery and nursing in any dam In 1 F no mating confirmed – no abnormality at necropsy in the ovary nor in the testis or epididymis of the male counterpart. <u>Offspring:</u> High number of stillborn pups (11 of 99 vs 2 of 128 in control) and % of stillborn (11.13% vs 1.8% in controls) Low birth index: 70.5% vs 90.1% in control (pup number 77% of the control) Viability on PND 4 was low: 48.2% vs 98.8% in controls (pup number 41% of the control) Pup body weights were lower (though not statistically significant): M: PND 0: - 4%, PND 4: - 13%; F: PND 0: - 2%, PND 4: - 10% <u>Males:</u> Epididymis: weight decrease (abs: - 22%, rel: - 24%) Histopathology: Minimal to mild degeneration / necrosis of spermatocyte / spermatid in the testis of 3/12. In one of these males mild decrease of spermatocyte / spermatid numbers was noted. Minimal or mild decrease of sperm number and cell debris in the duct of epididymis were noted in 2/12. <u>*End of recovery period:*</u> Testis & epididymis weight decrease (testis: abs: - 26%, rel: - 23%; epididymis: abs: - 31%, rel: - 29%) Histopathology: Minimal to mild degeneration / necrosis of spermatocyte / spermatid in testis in 4/12. In 3 of these M minimal to mild decrease of spermatocyte / spermatid numbers. Minimal, mild or moderate sperm and cell debris in the duct of epididymis in 4/12. **No relevant effects reported at 50 & 250 mg/kg bw/day.**	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
14-day dose range finding study No guideline, non-GLP (but in GLP environment and with full documentation, but without QA audit) Oral (gavage) Rat (SPF Sprague-Dawley-Crl: OFA (SD); 8 weeks old) 3/sex/group Standard examinations Functional & neurobehavioural tests (before the first dosing, on d7 and on d14 animals were observed according to a standardised observation battery for neurobehavioural, neurovegetative or psychotropic signs or neurotoxic effects. Animals were weighted on the day of randomisation, on d1, d7 & d14. Food consumption was determined weekly. All animals were subjected to gross necropsy.	DEGMEE (Purity: 100%) (vehicle: water); 0 / 500 / 1000 / 2000 mg/kg bw/day (10 ml/kg bw); For 14 days	**2000 mg/kg bw/day:** Mortality & clinical signs: 1/3 F found in moribund condition on d5. From d2 to d5 this animal showed clinical signs (reduced or absence of spontaneous locomotor activity, staggering gait or piloerection 1h or 3-4 h after treatment) → euthanised on d5 for ethical reasons Absence or reduced spontaneous locomotor activity: 5/5 animals 1 h or 3-4 h after treatment from d1 to d14. Staggering gait: 4/6 animals (M+F) from d1 to d5. Piloerection: 1/3 F from d1 to d5. Liver weight, increase: M, abs: + 7%, rel: +32%; F, abs: + 37%, rel: + 40% **1000 mg/kg bw/day:** Reduced spontaneous locomotor activity: 4/6 animals Staggering gait: 2/6 animals Piloerection: 2/6 animals, 1 h or 3-4 h after treatment from d2 to d5. Liver weight, increase: M, abs: + 14%, rel: + 24%; F, abs: + 14%, rel: + 11% **500 mg/kg bw/day:** Liver weight, increase: M, abs: + 4%, rel: + 13%; F, abs: + 11%, rel: + 4%	Anonymous (2014a)
28-day study **(OECD 407; October 16, 2008; GLP)** Evaluation of the male genital tract in accordance with Note for Guidance on the Detection of Toxicity to Reproduction for Medicinal Products, Addendum: Toxicity on Male Fertility (CPMP/ICH/136/95; ICH S5B(M)) Oral (gavage) Rat (SPF Sprague-Dawley – Crl: OFA (SD); 7 weeks old) 5/sex/group (main and	DEGMEE (Purity: 100%) (vehicle: water); 0 / 250 / 500 / 1000 mg/kg bw/day (10 ml/kg bw); For 28 days Recovery group 42 days; control & 1000 mg/kg bw/day	**1000 mg/kg bw/day:** <u>Mortality & clinical signs:</u> 2/5 males were found dead on d24 and d28, respectively. Both showed decreased spontaneous locomotor activity on d9. No other clinical signs or unusual findings at necropsy were noted in these two animals and the cause of death was not established. All males in this group had decreased spontaneous locomotor activity between d7 and d16. Absence of spontaneous locomotor activity was seen in 1/10 males on d9. Decrease in abdominal tone was seen in 1/10 males on d14. No other clinical signs in males or females. <u>Haematology and coagulation:</u> Lower haemoglobin (-8%), red blood cell counts (-4%) & reticulocyte count (-31%) was seen on d29 in males compared to control. These effects were reversed on d43 after the	Anonymous (2014c)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
recovery groups)		recovery period. Blood biochemistry: Lowered alkaline phosphatase activity on d29 in M (-36%) & F (-26%). These effects were reversed on d43 after the recovery period. Cholesterol was markedly elevated in F (+37%). Remained higher on d43 (+21%). Organ weights: Liver: elevated in M (rel to bw: +23%) & F (abs: +28%, rel to bw: +16%), which was not seen on d43 after the recovery period. Adrenals: lowered in M (abs: -35%, rel to bw: -31%), not stat. signif. after recovery Spleen: elevated in M (rel: +31%), which was not seen on d43 after the recovery period. Thymus: lower in M (abs: -61%, rel to bw: -58%) & F (abs: -50%, rel to bw: - 54%), did not reverse during recovery period (M: abs: -67%, rel to bw: -68%; F: abs: -42%, rel to bw: -45%) Testis: lower, but not stat. signif. (abs: -27%, rel to bw: -19%, rel to brain: -24%); after recovery period stat. signif. (abs: -23%, rel to bw: -25%, rel to brain: -20%). Epididymes: lower, but not stat. signif. (abs: -18%, rel to bw: -11%, rel to brain: -16%); after recovery period stat. signif. (abs: -21%, rel to bw: -23%, rel to brain: -19%). Macroscopic findings: 3/4 M showed white areas on the spleen on d29. On d43 (multiple) punctate change was seen on the sub-maxillary lymph nodes in 2/4 M and 2/5 F. Histopathology: Liver: periacinar hepatocytic hypertrophy: 7/9 vs 0/10 in controls Thymus: atrophy in 9/9 vs 1/10 (reversible) Spleen: focal peritonitis: 3/9 vs 0/10 Testes: tubular dilatation: 3/4 vs 0/5; tubular degeneration: 2/4 vs 1/5; intratubular spermatid debris: 4/4 vs 0/5 Epididymes: tubular dilatation: 1/4 vs 0/5; free luminal degenerated spermatids: 2/4 vs 0/5; hypospermia: 3/4 vs 0/5 Testicular and epididymal changes persisted longer than 2 weeks.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Sperm analysis: - Motility: lower motility of spermatozoa, non-statistically significant (47 vs 80 in controls) higher count of immobile spermatozoa, non-statistically significant (37 vs 24) – associated with a lower percentage of mobile spermatozoa (56.8 vs 76.3) - Morphology: A statistically significant lower percentage of normal spermatozoa (i.e. -23.5%) and a statistically significant higher percentage of spermatozoa with head anomaly (more than 5-fold) associated with a non statistically significant higher percentage of isolated head in sample (more than 3-fold) - Numeration in epididymis: A non statistically significant lower number of spermatozoa in the tail of left epididymis (2.34 vs 3.78 in controls) No sperm effects at lower doses. **500 mg/kg bw/day:** Mortality & clinical signs: No mortality. 3/5 males showed a decrease in abdominal tone on d7 and 1/5 males showed a decrease in body tone on d25 Haematology and coagulation: Lower haemoglobin (-10%), red blood cell counts (-11%) & reticulocyte count (-24%) was seen on d29 in males compared to control. Blood biochemistry: Lowered alkaline phosphatase activity on d29 in M (-34%) & F (-31%). Cholesterol was elevated in F (+23%). Organ weights: Liver weight: elevated in M (rel to bw: +18%) Histopathology: Liver: periacinar hepatocytic hypertrophy in 6/10 vs 0/10 in controls Thymus: atrophy in 7/10 vs 1/10 (reversible) Spleen: focal peritonitis in 3/10 vs 0/10	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		250 mg/kg bw/day: No mortality or clinical signs. <u>Haematology and coagulation:</u> Lower reticulocyte count (-17%) was seen on d29 in males compared to control. <u>Blood biochemistry:</u> Lowered alkaline phosphatase activity on d29 in M (-26%) & F (-16%). Cholesterol was elevated in F (+21%). <u>Histopathology:</u> Liver: periacinar hepatocytic hypertrophy in 2/10 vs 0/10 in controls (sex not specified) Thymus: atrophy in 2/9 vs 1/10 (reversible) (sex not specified)	
90-day study **(OECD 408; 27 June 2018; GLP)** Oral (gavage) Rat (SPF; Crl:CD(SD) rats; 6 weeks old) 10/sex/group (main groups) & 5/sex/group (recovery groups)	DEGMEE (purity > 99.9%) (vehicle: water); 0 / 110 / 330 / 1000 mg/kg bw/day; (10 ml/kg bw); For 90 days Recovery group: 118 days; control & 1000 mg/kg bw/day	No mortalities at any dose level and no clinical signs reported. **1000 mg/kg bw/day:** <u>Body weight:</u> M (from d71 onwards): up to -11%; F (from d57 onwards): up to -9% Body weight remained lower in the recovery group (mostly not statistically significant). Food consumption: M (from d15 onwards): up to -14% in the dosing period. <u>Hematology:</u> At the end of dosing period: M: Hemoglobin concentration: -8%; MCH: -6%; MCHC: -5%; Reticulocytes: -52%; F: Hemoglobin concentration: -6%; MCH: -7%; MCHC: -4%, Reticulocytes: -30% Only some of these parameters were still affected after the recovery period. <u>Blood biochemistry:</u> M: GGT: + 69%; HDL- & LDL-cholesterol: + 42% / + 70% (dose dependent decrease in ALP in M & F). <u>Necropsy findings:</u> Smallness of testis and epididymis, bilaterally: 6/10 M Smallness of ovary: 1/10 F	Anonymous (2022a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		After recovery period smallness of testis and epididymis: 3/5 M Organ weights: Liver: M: rel: + 17%; F: rel: + l5% (reversible) Thymus: M: abs -46%; rel: -38% (reversible) Testis: After dosing period: abs: -27%; rel (non stat. signif.): -18% After recovery period: abs: -17%; rel (non stat. signif.): -8% Epididymis: After dosing period: abs: -34%; rel: -26% After recovery period: abs: -30%; rel: -23% Seminal vesicle: After dosing period: abs: -18%; rel (non stat. signif.): -8% Histopathology: Stomach: minimal pancreatic acinar metaplasia of chief cells in the glandular stomach: M: 8/10, F: 2/10 After recovery period: minimal pancreatic acinar metaplasia of chief cells in the glandular stomach: 4/5 M & 1/5 F Liver: minimal centrilobular hypertrophy: M: 10/10, F: 10/10 Testis: Decrease in germ cells (spermatogoniums, spermatocytes, and spermatids) – minimal: 4/10, mild: 2/10, moderate: 2/10; Degeneration/necrosis of germ cells - minimal: 6/10 After recovery period: decrease in germ cells - minimal 3/5, mild 1/5 Epididymis: Cell debris in lumen – minimal: 6/10, mild: 1/10; Decrease in luminal sperm – minmal: 2/10, mild 2/10, moderate: 2/10 After recovery period: Luminal cell debris - minimal 4/5; Decrease in luminal sperm: minimal 1/5 Ovary: Atrophy – minimal: 3/10 **330 mg/kg bw/day:** Hematology: At the end of dosing period: M: MCHC: -2%; Reticulocytes: -22%; F: MCHC: -2%	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		Blood biochemistry: M: HDL- & LDL-cholesterol: + 23% / + 88% Organ weights: Liver: F: rel: + 8% Histopathology: Liver: minimal centrilobular hypertrophy: M: 3/10, F: 5/10 Ovary: Atrophy – minimal: 1/10	

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

OECD 422 study, oral (gavage), rat (Anonymous 2014b):

The study was conducted according to guideline (OECD 422, March 22, 1996) and GLP and is considered reliable and adequate (for details on the study and exact numbers see Table 7). Rats were exposed to 0, 50, 250 or 1000 mg/kg bw/day and there were no relevant clinical signs in adult animals (males and females) throughout the study and no relevant effects on body weight or food consumption were reported. The body weight gain during the gestation period (calculated as mean body weigh on GD 0 minus mean body weigh on LD 0) was comparable between the groups, but was by 28% higher in the top dose group compared to the control group (see Table 8). This effect is also visible when subtracting the weight of the litters on LD 0 from body weight of the dams on LD 0 (see Table 9). The higher weight gain in top dose dams might be explained by the lower number of foetuses per dam in this group, however, the toxicological relevance of this observation is uncertain. No toxicity was seen in the low and mid dose groups (50 & 250 mg/kg bw/day), but effects were reported at 1000 mg/kg bw/day.

The main repeated dose effect was increase in absolute and relative liver weight in males and females of the top dose, which was accompanied by centrilobular hepatocellular hypertrophy of minimal to mild nature in 4 males and 3 females. No histopathological or blood biochemical changes indicative of liver injury were reported. After the recovery period the liver effects reversed, except for slightly elevated relative liver weight in males.

Relative thymus weight was only reduced in top dose males (-30%) and not described after recovery. As such, it is not considered to be a finding of toxicological relevance in this study.

Low motor activity was observed in females of the top dose group, but in absence of adverse effects on the moving pattern and histological changes, these effects were not considered severe.

Regarding reproductive organs, there was a decrease in absolute and relative epididymis weight (see Table 11) and mild degeneration/necrosis of spermatocytes/spermatids was observed in testis of three top dose males. In one of these males mild decrease of spermatocyte/spermatid number was noted. Minimal or mild decrease of sperm number and cell debris in the duct of epididymis were reported in two males.

After the recovery period, also absolute and relative testis weights were reduced and absolute and relative epididymis weights were further decreased. Minimal to mild degeneration/necrosis of spermatocyte/spermatid in testis was described in four males. In three of these males minimal to mild decrease of spermatocyte/spermatid number was reported and minimal, mild or moderate sperm and cell debris was seen in the duct of epididymis in four males. This indicates that the effects on the male reproductive organs became more severe after the recovery period.

In the dams of the top dose group the gestation period was prolonged by one day compared to the controls. The underlying reason is unknown, but in the study report it was speculated that it could be related to the lower number of newborns in this group. Mean body weights of newborns were only slightly lower than controls (M: -4%, F: -2%; both not statistically significant).

There were no changes in number of corpora lutea, implantations or implantation index, indicating that implantation was not adversely affected, however, there was a decrease in delivery index and an effect on the maintenance of pregnancy after implantation.

In the offspring of the top dose group there was a decrease in birth index and on PND 4 viability index was reduced. And although there were no abnormalities in the delivery or nursing behaviour of dams, most offspring died after birth, resulting in total litter loss in 5/10 dams. The pups did not suckle and the cause of death was mainly cannibalism by dams. While the pup body weight was comparable among groups on PND 0 (only a slight, non-statistically significant decrease in top dose, see above), there was a clear decline on PND 4 (see Table 12). The study report speculated that as there were no adverse effects on the offspring on PND 0, that there could be an adverse effect of DEGMEE on the offspring via milk. However, although there was hardly any effect on bodyweights on PND 0, the number of pups delivered was clearly lowered, due to post-implantation loss. It can therefore be concluded that the offspring was adversely affected in utero. In addition it cannot be excluded that the adverse effects appearing between PND 0 and 4 were induced during in utero exposure.

Despite the considerable adverse effects observed on the male reproductive organs, copulation and conception were not adversely affected. However, it might be that the effects on male reproductive organs/sperm take longer than two weeks to become manifest, as treatment prior to mating was only for 2 weeks. The lack of adverse effects on fertility parameters might further be explained by the much higher sperm reserve in rats versus humans (Mangelsdorf und Buschmann 2003).

Table 8: Body weight gain in dams during gestation (OECD 422; Anonymous 2014b)

Dose group [mg/kg bw/day]	Mean bw GD 0 [g]	Mean bw LD 0 [g]	Mean bw gain during gestation [g]	% of control
0	259.34	303.16	43.82	-
50	271.93	318.96	47.03	107.3
250	250.56	295.05	44.49	101.5
1000	262.01	317.99	55.98	127.7

Table 9: Body weight of dams on lactation day (LD) 0 corrected for pup body weight on LD 0 (OECD 422, Anonymous 2014b)

Dose group [mg/kg bw/day]	Mean bw LD 0 [g]	Mean litter weight (males & females) LD 0 [g]	Corrected dam bw LD 0 [g]	% of control
0	303.16	86.2	216.9	-
50	318.96	97.3	221.6	102.2
250	295.05	89	206	95
1000	317.99	65.1	252.9	116.6

Table 10: Mean maternal body weight on different time points and % of control (OECD 422, Anonymous 2014b)

Dose group [mg/kg bw/day]	Treatment day 1	End of pre-mating period	GD 0	GD 20	LD 0
Mean maternal body weight [g]					
0	224.66	255.7	259.34	408.51	303.16
50	223.3	263.25	271.93	436.63	318.96
250	221.45	245.6	250.56	405.12	295.05
1000	223.45	255.75	262.01	407.13	317.99
% of control					
50	99.4	103	104.9	106.9	105.2
250	98.7	96.1	96.6	99.2	97.3
1000	99.5	100	101	99.7	104.9

Table 11: Male reproductive organ weights [mean ± SD] at the end of treatment and after two weeks recovery (OECD 422, Anonymous 2014b).

Dose group	0 mg/kg bw/d	50 mg/kg bw/d	250 mg/kg bw/d	1000 mg/kg bw/d
At the end of treatment				
bw [g] n	463.90 ±20.53 12	463.91 ± 31.83 12	469.30 ± 31.83 12	465.3 ± 29.05 12
Testes [g] n	3.220 ± 0.408 7	3.217 ± 0.266 12	3.393 ± 0.242 12	3.013 ± 0.429 7
Testes, relative n	0.755 ± 0.093 7	0.744 ± 0.087 12	0.773 ± 0.051 12	0.680 ± 0.106 7
Epididymides [g] n	1.253 ± 0.093 7	1.277 ± 0.071 12	1.288 ± 0.049 12	0.983 ± 0.124** (-21,5%) 7
Epididymides, relative n	0.293 ± 0.025 7	0.295 ± 0.021 12	0.294 ±0.023 12	0.222 ± 0.033** (-24%) 7
At the end of 2 week recovery period				
bw [g] 	506.76 ± 28.55 5			491.78 ± 16.04 5
Testes [g] n	3.302 ± 0.196 5	-	-	2.444 ± 0.467** (-26%) 5
Testes, relative n	0.704 ± 0.071 5	-	-	0.540 ± 0.099* (-23%) 5
Epididymides [g] n	1.298 ± 0.055 5	-	-	0.982 ± 0.089** (-24%) 5
Epididymides, relative n	0.276 ± 0.013 5	-	-	0.197 ± 0.023** (-29%) 5

*: $p < 0.05$; **: $p < 0.01$

Table 12: Male and female pup body weights [g] on PND 0 and PND 4 (OECD 422, Anonymous 2014b).

Dose group	0 mg/kg bw/d	50 mg/kg bw/d	250 mg/kg bw/d	1000 mg/kg bw/d
Male pups				
Pup number, PND 0	63	82	59	53
Pup number, PND 4	62	81	59	25
Mean pup bw/litter, PND 0; n	6.918 ± 0.629 10	6.956 ± 0.371 12	7.489 ± 0.854 10	6.643 ± 0.651 10
Mean pup bw/litter, PND 4; n	11.366 ±1.148 10	11.262 ± 0.830 12	11.99 ± 2.089 10	9.870 ± 1.366 5

Female pups				
Pup number, PND 0	65	90	68	46
Pup number, PND 4	64	90	67	27
Mean pup bw/litter, PND 0; n	6.576 ± 0.487 10	6.627 ± 0.472 12	6.952 ± 0.693 10	6.440 ± 0.693 9
Mean pup bw/litter, PND 4; n	10.933 ± 0.947 10	10.933 ± 0.998 12	11.216 ± 1.809 10	9.829 ±1.232 5

Non-guideline dose range finding study, 14-days, oral (gavage), rat (Anonymous 2014a):

In a non-guideline study groups of 3 rats per sex and dose were exposed to 0, 500, 1000 or 2000 mg/kg bw/day for fourteen days (for details on the study and exact numbers see Table 7). There was a slight dose dependent decrease in body weight gain in males (+27%, +21%, +19% & +5%, in control, low, mid & top dose group, respectively) which was parallel to a dose dependent decrease in food consumption (week 1: 30, 27, 25 & 20 g/animal per day; week 2: 29, 25, 23 & 18 g/animal per day). No clear effect on body weight was seen in females and also the food consumption in females was only slightly affected in mid and top dose groups and therefore not considered a relevant toxicological effect.

There was a dose dependent increase in absolute and relative liver weight, in both males and females, no other organ weights were affected. No relevant histopathological findings were reported.

Up to a dose of 1000 mg/kg bw/day no mortality or major systemic toxicity was observed. Based on this study 1000 mg/kg bw/day was derived as dose not to be exceeded in a 28-day study.

OECD 407 study, oral (gavage), rat (Anonymous 2014c):

The study was conducted according to guideline (OECD 407, October 16, 2008) and GLP and is considered reliable and adequate (for details on the study and exact numbers see Table 7). Five animals per sex and dose were exposed to 0, 250, 500 or 1000 mg/kg bw/day for 28 days. Two out of ten top dose males died on d24 and d28, respectively. Despite a decrease in spontaneous locomotor activity on d9, no other clinical signs were observed in these animals and no remarkable findings were reported at necropsy (no in depth histopathology assessment was conducted).

The only clinical finding was reduced spontaneous locomotor activity on all top dose males between d7 and d16, and the absence of spontaneous locomotor activity in 1/10 top dose males on d9.

Body weight and food consumption was not affected at any time up to the highest dose.

At the end of the treatment period (d29), there was a slightly lower haemoglobin concentration in males of the mid and top dose groups compared to the controls (see Table 7). This change was associated with lower red blood cell counts. A dose-related lower reticulocyte count was also seen (from the lowest dose tested). None of these changes were present at the end of the two week recovery period.

At the end of the treatment period (d29) there was a lower alkaline phosphatase (ALP) activity in males and females of all dose groups and a higher dose-related cholesterol level in females, which was of marked degree mainly in the top dose group. These changes were not reported after the two week recovery period.

Despite these reversible blood-biochemical changes, the dose dependent increase in liver weight and hepatocellular hypertrophy in males and females, which was also reversible, is considered an adaptive response.

Other organ weights that were affected include an increase in spleen weight in males and a decrease in thymus weight in males and females. The thymus weights remained lower after the two week recovery period (see Table 7), which was accompanied by a dose dependent increase in animals with thymus atrophy, with all animals affected in the top dose group. Thymus atrophy was reported to be reversible after the recovery period.

The most prominent finding was seen in testis and epididymis, as reduced weight and histopathological changes in the top dose remained after the recovery period. There was also a lower sperm count with lower motility, higher count of immobile spermatozoa and lower percentage of normal spermatozoa (higher percentage of head anomaly or isolated head in sample) in the top dose males (for details see Table 7). In low

and mid dose groups no changes in reproductive organs or in morphology and the number of the spermatozoa were reported.

OECD 408 study, oral (gavage), rat (Anonymous 2022a):

The study was conducted according to guideline (OECD 408, June 27, 2018) and GLP and is considered reliable and adequate (for details on the study and exact numbers see see Table 7). Ten animals per sex and dose were exposed to to 0, 110, 110 or 1000 mg/kg bw/day for 90 days.

No mortality was observed at any dose level. No clinical signs were reported and no relevant observations were made in functional observation box testing.

During the dosing period, a statistically significant decrease in the body weight was noted in males at 1000 mg/kg on d71 and thereafter and females at 1000 mg/kg on d57 and thereafter; in addition during the dosing period food consumption was lower in top dose males. During the recovery period, a statistically significant decrease or a decreasing tendency of the body weight was noted in both sexes at 1000 mg/kg, despite no effects on food consumption.

Hemoglobin concentration, MCH, MCHC, reticulocytes and its ratio, and platelet count were statistically significantly lowered in both sexes of the top dose group at the end of the dosing period. Only some of these parameters were still affected after the recovery period. Some other haematological parameters were also affected but these changes were minimal and/or did not show dose dependence.

At the end of the dosing period a statistically significant increase of GGT in top dose males and of HDL- and LDL-cholesterol in mid and top dose males was described. Regarding the remaining blood-biochemical parameters it is noted that several liver related parameters were affected, however, in a rather inconsistent way and changes were mostly within the range of the control group and/or were not dose dependent. No changes were seen at the end of the recovery period.

Statistically significant changes in organ weights were seen after the dosing period for liver (increased absolute and relative weight in top dose males and mid and top dose females), thymus (decreased absolute and relative weight in top dose males) and testis and epididymis (decreased absolute and relative weight in top dose males). Testis (absolute weight) and epididymis (absolute & relative) weight was still lower in the top dose group after the recovery period, while the other organ weight changes were reversible.

Smallness of testes and epididymis was noted bilaterally in 6/10 (60%) males and smallness of ovary in 1/10 females of the top dose group. After the recovery period bilateral smallness of testis and epididymis was noted in 3/5 (60%) top dose males.

Histopathological changes were seen in the stomach of top dose males and females (minimal pancreatic acinar metaplasia of chief cells in the glandular stomach), liver of mid and top dose males and females (minimal centrilobular hepatocellular hypertrophy), testis of top dose males (minimal to moderate decrease in germ cells, minimal degeneration/necrosis of germ cells), epididymis of top dose males (minimal to mild cell debris in lumen, minimal to moderate decrease in luminal sperm) and ovary of mid and top dose females (minimal atrophy in mid and top dose groups). The effects in liver and ovary were not seen after recovery period, the remaining effects were still seen, but mostly with fewer incidences of lesser severity (for details on histopathological changes see Table 7).

At the end of the dosing period, statistically significant decreases in plasma total T_4 concentration in top dose males (-22%) and plasma total T3 concentration in top dose females (-17%) was noted. At the end of the recovery period, a statistically significant decrease in total T3 concentration was reported for top dose females. Changes in T4 in females and T3 in males, as well as changes in TSH in males and females were rather inconsistent (increases and decreases without dose dependency). With no relevant findings on thyroid weight and histopathology, these hormone changes are not considered to be of toxicological significance.

Overall summary of data relevant for sexual function and fertility

Relevant information for the assessment of adverse effects on sexual function and fertility comes from a combined repeated dose toxicity study with reproductive/developmental toxicity screening test, a non-guideline 14-day study, a 28-day study and a 90-day study. All studies were conduced according to guideline (where available) and GLP and are considered reliable; the test species was rat.

Male reproductive organs were considerably affected, but also some indications for adverse effects on female reproductive organs were observed.

Male reproductive organs were consistently affected across all studies, including

- reduced organ weights: testis and epididymis weight were reduced in all studies
- macroscopic findings: small testis and epididymis, bilateral, 60% of the animals at the end of treatment and after recovery (90-day study, Anonymous 2022a)
- histopathological findings:
    - degeneration/necrosis of spematocyte/spermatid in testis and epididymis, reduced number of spermatocytes/spermatids in testis and epididymis, cell debris in duct of epididymis (OECD 422, Anonymous 2014b)
    - degeneration/necrosis of germ cells in testis (spermatogonia, spermatocytes & spermatids, min. to mod. in 8/10), decreased number of germ cells in testis (minimal in 6/10), cell debris in ductal lumen of epididymis (minimal to mild in 7/10), decreased number of luminal sperm (min. to mod. in 6/10) (90-day study, Anonymous 2022a)
- detailed analysis of sperm parameters (28-day study, Anonymous 2014c):
    - Motility: low motility of spermatozoa (47 vs 80 in control, non-statistically significant), increased number of immobile spermatozoa (37 vs 24 in control, non-statistically significant) – associated with a lower percentage of mobile spermatozoa (56.8% vs 76.3% in control)
    - Morphology: decreased percentage of normal spermatozoa (-23.5%), statistically significant, increased percentage of spermatozoa with head anomaly (more than 5-fold) – associated with higher percentage of isolated head in sample (more than 3-fold)
    - Numeration in epididymis: lower number of spermatozoa in the tail of left epididymis (non-statistically significant)

Despite the considerable effects seen on reproductive organs and spermatogenesis no adverse effects on fertility were noted in the OECD 422 study (Anonymous 2014b). However, the exposure only started two weeks before mating, indicating that this time period might be too short for DEGMEE to have an impact on male reproductive organ function and the presence of intact sperm. The lack of adverse effects on fertility parameters might further be explained by the much higher sperm reserve in rats versus humans (Mangelsdorf und Buschmann 2003).

Where investigated these effects were mostly not reversible. Some effects were less severe after the recovery period in the 90-day study (Anonymous 2022a), but in some instances the effects became even more severe after the recovery period (OECD 422, (Anonymous 2014b).

Male reproductive organs and fertility are considered to be the main target of DEGMEE induced reproductive toxicity, but also females were affected. In the 90-day study (Anonymous 2022a) ovary atrophy was reported in 1/10 mid dose females and in 3/10 top dose females, no ovary atrophy was seen after the recovery period. In some studies also the ovary weight was lower, but not statistically significant.

Also the decreased delivery index (79.5 ± 12.1 % vs 94.4 ± 7.8 % in the control group) might be an indication of adverse effects of DEGMEE on fertility. The prolonged gestation period (23.2 days vs 22.3 days in control)

observed in top dose dams of the OECD 422 study (Anonymous 2014b) is not considered to be an adverse effect on reproduction, but rather a consequence of the lower number of offspring in this group.

The effects on male reproductive organs and spermatogenesis were only seen at the top dose which was 1000 mg/kg bw/day in all available studies. No respective findings were seen at lower doses. The findings are still considered relevant as at this dose no relevant general toxicity was reported. In most studies no clinical signs were observed and there were no adverse effects on body weight or food consumption up to the highest dose tested. The only exception is the 28-day study (Anonymous 2014c) in which two of ten animals died of the top dose (two males), but the cause of death could not be established. The only clinical finding in this study was reduced motor activity in top dose males. Reduced motor activity was also described in males and females of the top dose of the OECD 422 study (Anonymous 2014b).

In all available studies there was a dose dependent increase in liver weight and slight increase in hepatocellular hypertrophy, which was mostly reversible and which is considered to be an adaptive response. Another consistent finding was reduced thymus weight and in one study (90-day study, Anonymous 2022a) a dose dependent increase in thymus atrophy was reported from the lowest dose tested, but the thymus effects were fully reversible. There were also some hematological changes and blood biochemical effects, but these findings were mostly not consistent and/or not severe enough to demonstrate severe general toxicity.

In summary, it can be concluded that in the available studies there was no severe general toxicity present and the observed effects male reproductive organs are therefore not considered to be unspecific or secondary to parental toxicity.

10.10.3 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

No human data is available to justify classification in Category 1A.

Upon DEGMEE exposure in rats there were clear indications for adverse effects on male reproductive organs, consisting of reduced testis and epididymis weight across all studies, small testis & epididymis, degeneration/necrosis of spermatocytes/spermatids in testis and epididymis, reduced number of spermatocytes/spermatids, cell debris in epididymal duct as well as adverse effects on sperm parameters (reduced mobility, increased abnormal sperm, low number of spermatozoa in the tail of epididymis). Where investigated these findings were not reversible during the recovery period, but in contrast often deteriorated. These findings were supported by some effects on female reproductive organs, i.e. occasionally reduced ovary weights (though not statistically significant) and dose dependent ovary atrophy (reversible). Also the lower

birth index observed in the OECD 422 study is considered supportive evidence for adverse effects on sexual function and fertility. These findings were seen at doses without severe general toxicity and are considered not to be secondary to parental toxicity (in line with the definitions of maternal/parental toxicity in Annex I, Sections 3.7.2.3.5 and 3.7.2.4.2-4 of the CLP Regulation as well as Section 3.7.2.2.1 of the CLP Guidance document).

According to above classification criteria there was “clear evidence” for adverse effects on sexual function and fertility in animal studies. The relevant studies have no serious deficiencies, thus a classification into Repr. 1B (H360F) is considered appropriate.

10.10.4 Adverse effects on development

DEGMEE was tested in an oral prenatal developmental toxicity study according to OECD 414 in rat (Anonymous 2022b). The study was conducted according to GLP and is considered reliable. Also from the OECD 422 study (Anonymous 2014b) relevant information on DEGMEEs adverse effects on development can be derived. This study is described under section 10.10.1. Adverse effects on sexual function and fertility.

Table 13: Summary table of animal studies on adverse effects on development (for all indicated numbers the difference from control is statistically significant, if not indicated differently)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
PNDT **(OECD 414, 25 June 2018; GLP)** Oral (gavage) Crl:CD(SD) rats (12 weeks old) 20 F / group All females of all groups got pregnant - 20 dams per group.	DEGMEE (Purity: > 99.9%) (vehicle: water); 0 / 110 / 330 / 1000 mg/kg bw/day (10 ml/kg bw); Gestational day (GD) 6 - 19	No death, moribundity, or abortion occurred during the study. No clinical signs were observed in all animals. No relevant findings in any dam upon necropsy. Food consumption was slightly lower on GD 8 (-7% in MD and TD) and on GD 17 (-9.6% in TD). On other occasions the effect on food consumption was not statistically significant and not always dose dependent (including an increase in food consumption in the TD on GD 11, + 2%). No effect on maternal body weight (after correction for gravid uterine weight). **1000 mg /kg bw/day:** Gravid uterus weight: 40.39g ± 22.36 vs 83.6g ± 8,52 in controls (-52%) Post implantation loss index, high: 57.9% ± 38.6% vs 4.1% ± 5.2% in control Early resorption index, high: 39.8% ± 29.1% vs. 4.1% ± 5.2% in control Late resorption index, high: 18.2% ± 17.1% vs 0 in control Male and female foetal body weight lower than in control goup: M: -34%; F: -32% Number of live fetuses lower than in control group: -59% *Further details on observations on cesarean section, number of live fetuses and foetal body weight are included in table Table 15* <u>External examination:</u> In 3 foetuses of 2 litters anal atresia and acaudate were reported.	Anonymous (2022b)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		AGD: significantly decreased in male fetuses: M: -8.1% (F: -2.2%, non stat signif) AGI: significantly increased in male & female fetuses: M: +5.3%; F: +11.2% <u>Skeletal examination:</u> - Skeletal anomalies: Hemicentric thoracic centrum: in 14 foetuses (23.3%) vs 2 foetuses (1.3%) in the control. - Skeletal variations: Total number of variations: 78.6% (51 foetuses) vs. 19.2% (29 foetuses) in control Bipartite ossification of thoracic centrum: 48.2% (33 foetuses) vs. 2.1% (3 foetuses) in control Wavy rib: 3.2% (3 foetuses) (non stat signif) vs. 0% in control Full supernumerary rib: 11.5% (6 foetuses) (non stat signif) vs. 1.7% (3 foetuses) in control Short supernumerary rib: 22.7% (13 foetuses) (non stat signif) vs. 14.9% (22 foetuses) in control Bipartite ossification of sternebra: 17.1% (12 foetuses) vs. 0% in control Bipartite ossification of lumbar centrum: 12.5% (8 foetuses) vs. 0.5% (1 foetus) in control 7 Lumbar vertebrae: 44.6% (24 foetuses) vs. 44.6% (24 foetuses) in control <u>Visceral examination:</u> Malformations: Total number of visceral anomalies: 79.7% (42 foetuses) vs. 6.5% (9 foetuses) Thymic remnant in the neck: 75.6% (40 foetuses) vs. 4.6% (6 foetuses) **330 mg /kg bw/day:** Male and female foetal body weight was statistically significantly lower than in control group: M: -12%, F: -12.3% <u>Skeletal examination:</u> - Skeletal variations: Total number of variations: 39.2% (57 foetuses) vs. 19.2% (29 foetuses) in control Bipartite ossification of thoracic centrum: 9.6% (15 foetuses) vs. 2.1% (3	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		foetuses) in control Wavy rib: 3.2% (3 foetuses) (non stat signif) vs. 0% in control Short supernumerary rib: 18.8% (29 foetuses) (non stat signif) vs.14.9% (22 foetuses in control) Bipartite ossification of sternebrae: 3.5% (4) vs. 0% in control Bipartite ossification of lumbar centrum: 2.7% (4 foetuses) vs. 0.5% (1 foetuses) in control Lumbar vertebrae: 1.3% (2 foetuses) vs. 0% in control **110 mg/kg bw/day:** <u>Skeletal examination:</u> - Skeletal variations: Bipartite ossification of sternebrae: 3.2% (5 foetuses) vs. 0% in control *A detailed overview on the anomalies and variations is included in Table 16, which also includes a comparision with the available HCDs.*	

OECD 414 study, oral (gavage), rat (Anonymous 2022b):

The study was conducted according to guideline (OECD 414, June 25, 2018) and GLP and is considered reliable and adequate (for details on the study and exact numbers see Table 13).

Maternal toxicity:

No death, moribundity, or abortion occurred during the study. No clinical signs were observed in any of the animals. Body weight in top dose dams was statistically significantly lower on GD 20 compared to the control group, but not when corrected for gravid uterine weight (see Table 14). Food consumption was occasionally reduced in mid and top dose group, but reductions were statistically significant only on GD 8 (-7%) and on GD 17 (-9.6%) at 1000 mg/kg bw/day and on GD 8 (-7%) at 330 mg/kg bw/day. Although food consumption in the 330 and 1000 mg/kg bw/day groups was significantly lower on GD 8, this was considered toxicologically insignificant because the values on GD 11 were comparable to those in the control group without a statistically significant difference and not accompanied by body weight loss.

At necropsy no treatment-related changes were observed in any dam. No changes in maternal thyroid weight or histopathology were reported, though when using the corrected maternal body weight (final body weight minus gravid uterine weight) a dose dependent decrease in relative thyroid weight was observed (with -10% in the TD compared to the control group). TSH and T4 levels were statistically significantly increased in top dose females, while no effects on T3 levels were observed. Overall, the findings in thyroid and thyroid hormone levels are not considered to be of toxicological significance.

No treatment related changes were seen in the placenta in any dose group (one case of placental enlargement in the top dose group was not considered relevant as it was said to be within the provided HCDs consisting of 10 studies).

Table 14: Terminal dam body weight, corrected terminal dam body weight and gravid uterine weight (Anonymous 2022b)

Dose group [mg/kg bw/day]	n	Terminal maternal body weight		Gravid uterus weight		Corrected terminal maternal body weight	
		g	% of control	g	% of control	g	% of control
0	20	421.71 ± 20.7		83.6 ± 8.52		388.11	
110	20	425.64 ± 16.82	100.9	81.59 ± 7.91	97.6	344.05	101.8
330	20	411.33 ± 22.28	97.5	75.5 ± 11.68	90.3	335.83	99.3
1000	20	386.69 ± 27.02	91.7	40.39 ± 22.36 **	48.3	346.3	102.4

** … statistically significant, $p < 0.01$ (Steel test)

Effects on offspring:

Gravid uterine weight in the top dose group was statistically significantly lower than in the control group (-52%). Also in low and mid dose gravid uterine weight was lower than in control (-2.5% und –9.7%, respectively), but not statistically significant.

Post implantation loss index and early and late resorption index in the top dose group were significantly higher than in the control group. Post implantation loss index was 57.9% and 7 of 20 dams showed total embryo resorption in the top dose group. Along with these changes, the number of live foetuses was significantly lower in the top dose group (6.1 vs 14.7 in the control group; -59%).

In addition, mean body weights of male and female foetuses of the top dose were also significantly lower than in the control group (males: -34%, females: -32%) which was also observed in mid dose foetuses (males: -12%, females: -12.3%), despite only a slight, non statistically significant effect on number of mid dose foetuses (13.6 vs 14.7 in the control group; -7.5%). Non statistically significant effects were also seen in the low dose (mean body weights: males: -4.5%, females: -6%; number of live foetuses: 14.5 vs 14.7 in the control group; -1.4%), see also Table 15.

Table 15: Details on observation on caesarean section (Anonymous 2022b).

Parameter		Control	100 mg/kg bw/d	330 mg/kg bw/d	1000 mg/kg bw/d
# of animals		20	20	20	20
# of corpora lutea (A)	Total	323	307	306	308
	Mean ±SD	16.2 ± 2.2	15.4 ± 1.4	15.3 ± 1.7	15.4 ± 0.9
# of implants (B)	Total	306	299	287	294
	Mean ±SD	15.3 ± 1.2	15.0 ± 1.3	14.4 ± 1.9	14.7 ± 1.0
Pre-implantation loss (A-B)/(A)	Total	17	8	19	14
	%	4.5 ± 7.1	2.5 ± 3.7	6.1 ± 11.0	4.4 ± 6.4
# of dead implants					
Early resorptions	Total	12	9	14	118
	%	4.1 ± 5.2	3.0 ± 4.1	5.1 ± 6.1	39.8 ± 29.1**
Late resorptions	Total	0	0	1	54
	%	0	0	0.7 ± 3.2	18.2 ± 17.1**
Dead foetuses	Total	0	0	0	0
	%	0	0	0	0
Post-implantation loss (C)	Total	12	9	15	172
(C)/(B)	%	4.1 ± 5.2	3.0 ± 4.1	5.8 ± 7.9	57.9 ± 28.6**
# of live foetuses:	Mean ±SD	14.7 ± 1.6	14.5 ± 1.4	13.6 ± 2.3	6.1 ± 5.5$$
Male		156	170	136	54
Female		138	120	136	68

Total		294	290	272	122
Sex ratio M/(M+F)	(%)	53.1 ± 13.9	58.4 ± 12.5	50.4 ± 15.0	45.3 ± 9.5 (13)
Weight of foetuses (g):					
Male	Mean ±SD	3.84 ± 0.35	3.66 ± 0.29	3.37 ± 0.35##	2.55 ± 0.21##
Female	Mean ±SD	3.63 ± 0.37	3.42 ± 0.24	3.19 ± 0.24$$	2.48 ± 0.18$$

##: p<0.01 (Dunnett test); $$: p<0.01 (Steel test); **: p<0.01 (Wilcoxon test)

External examination:

Anal atresia and acaudate was reported in 3 foetuses of 2 litters of the top dose group.

In top dose male foetuses AGD was significantly lower compared to controls, while AGI, which is the AGD corrected for the possible influence of body weight, was significantly higher in male and female foetuses of the top dose. Foetal body weight was considerably lower in the top dose group (> 30% lower than in the control foetuses) and changes of AGD and AGI were only seen in this top dose group. No other parameters than foetal body weight are available that might help with the interpretation of these effects on AGD, like e.g. reproductive organ weight of the foetuses. However, as the effect on foetal body weight was considerable it is likely that the changes in AGD as well as AGI are a consequence of this effect.

Skeletal examination:

A statistically significant increase in skeletal anomalies was seen in the top dose group with 14 foetuses (23.3%) affected vs 2 foetuses (1.3%) in the control. Incidences at the low and mid dose group were equal to the control group (i.e. 2 foetuses, 1.3%). For details see Table 16.

In the top dose all 14 skeletal anomalies were hemicentric thoracic centrum. One foetus with hemicentric thoracic centrum was seen each at low (0.7%) and mid (0.6%) dose group. The other findings (i.e. 1 foetus with short rib at the low dose (0.6%) and 1 foetus with hemicentric lumbar centrum (0.7%) at the mid dose) were not considered treatment related as the increase was not statistically significant and they were limited to one incidence each. It is noted that according to devtox.org[1] hemicentric thoracic centrum is classified as grey zone effect between malformation and variation, but ECETOC (2002)[2] lists it under malformation.

There was also a dose dependent increase in skeletal variations, reaching statistical significance at mid and top dose, with 78.6% (51 foetuses) and 39.2% (57 foetuses), respectively. In the control group there were 19.2% (29 foetuses) and in the low dose 25.1% (37 foetuses) affected, details are listed in Table 16. It should be noted that according to devtox.org[1] the majority of these findings are not considered variations, but grey zone effects between malformation and variation.

Statistically significant increase in bipartite sternebrae was already seen in the low dose, with a dose dependent increase up to the top dose (12 foetuses, 17.1%), which is according to devtox.org a grey zone effect between malformation and variation.

For some variations the increase was not strictly dose dependent and not always statistically significant, however, in their sum these findings clearly demonstrate an adverse effect on skeletal development (see Table 16)

Table 16: Overview of skeletal malformations and variations (Anonymous 2022b)

	ECETOC (2002)[2]	devtox.org[1]		Control	100 mg/kg bw/d	330 mg/kg bw/d	1000 mg/kg bw/d	HCD§
n, F1 animals				20	20	20	13	
n, fetuses examined				157	155	146	69	
Anomalies (malformations) – according to study report:								
Anomalies, overall			#	2	2	2	14	-

[1] devtox.org: https://www.devtox.org/index_en.php

[2] ECETOC (2002): https://www.ecetoc.org/wp-content/uploads/2014/08/MON-031.pdf

			%	1.3 ± 5.6	1.3 ± 4.1	1.3 ± 4.1	23.2 ±19.0**	-
Hemicentric thoracic centrum	Malf.	Grey-zone	#	0	1	1	14	-
			%	0	0.7 ± 3.2	0.6 ± 2.8	23.2 ±19.0**	-
Short rib	Var.	Grey-zone	#	2	1	0	0	-
			%	1.3 ± 5.6	0.6 ± 2.8	0	0	-
Fused rib	Malf.	Malf.	#	0	1	0	0	-
			%	0	0.7 ± 3.2	0	0	-
Hemicentric lumbar centrum	Malf.	Grey-zone	#	0	0	1	0	-
			%	0	0	0.7 ± 3.2	0	-
Variations – according to study report:								
Skeletal, overall			#	29	37	57	51	8-32
			%	19.2 ± 24.2	25.1 ± 25.6	39.2 ±22.7**	78.6 ±24.9**	6.27 – 20.4
Bipartite ossification thoracic centrum	Var.	Grey-zone	#	3	4	15	33	0-4
			%	2.1 ± 6.8	3.1 ± 7.9	9.6 ± 11.7**	48.2 ±33.7**	0 - 2.9
Wavy rib	Var.	Var.	#	0	0	3	3	-
			%	0	0	3.2 ± 8.5	3.2 ± 8.4	-
Full supernumerary rib	Var.	Malf./ Grey-zone	#	3	5	5	6	0-8
			%	1.7 ± 5.4	3.4 ± 7.5	3.2 ± 7.0	11.5 ± 19.4	0 – 6.03
Short supernumerary rib	Var.	Grey-zone	#	22	11	29	13	-
			%	14.9 ± 21.4	7.1 ± 8.7	18.8 ± 18.3	22.7 ± 28.1	-
Asymmetry of the sternebrae	-	Grey-zone	#	2	13	5	3	-
			%	1.1 ± 3.3	9.3 ± 14.7**	4.1 ± 8.5	4.7 ± 9.2	-
Bipartite ossification of sternebrae	Var.	Grey-zone	#	0	5	4	12	0-4
			%	0	3.2 ± 5.7*	3.5 ± 8.4*	17.1 ±22.5**	0 – 2.77
Bipartite ossification of lumbar centrum	-	Grey-zone	#	1	1	4	8	-
			%	0.5 ± 2.2	0.6 ± 2.8	2.7 ± 5.5	12.5 ± 18.0*	-
Supernumerary lumbar arch	-	Grey-zone	#	0	0	1	0	-
			%	0	0	0.7 ± 3.2	0	-
Supernumerary lumbar centrum	-	Grey-zone	#	0	0	1	0	-
			%	0	0	0.7 ± 3.2	0	-
5 lumbar vertebrae	-	Var.	#	1	0	0	0	-
			%	0.6 ± 2.8	0	0	0	-
7 lumbar vertebrae	-	Grey-zone	#	0	0	2	24	0-2
			%	0	0	1.3 ± 3.8	44.6 ±36.8**	0 – 1.2

*: p<0.05; **p<0.01 (Wolcoxon test); §HCDs: consisted of 10 studies since 2014 – which is outside the 5 year period before the year of the study, i.e. 2021 (for cells marked with "-" there were no data available); % of the number of fetuses examined; Malf.: malformation; Var.: varitation; Grey-zone: Effect between malformation and variation

Another skeletal finding was a dose dependent and statistically significant decrease in ossification (Table 17), which is likely demonstrating developmental delay and matches with the dose dependent lower foetal body weight, which reached statistical significance at mid and top dose.

Table 17: Degree of ossification (Anonymous 2022b).

	Control	100 mg/kg bw/d	330 mg/kg bw/d	1000 mg/kg bw/d
n, F1 animals	20	20	20	13
n, fetuses examined	157	155	146	69
# of sarcocaudal body	8.0 ± 0.3	7.4 ± 0.4**	6.6 ± 0.6**	3.1 ± 1.2**
# of sternebrae	5.9 ± 0.2	5.7 ± 0.3	4.9 ± 0.7**	2.0 ± 1.1**

**p<0.01 (Steel test)

Visceral examination:

Also for visceral malformations a clear dose dependent increase was observed, reaching statistical significance at mid and top dose (see Table 18 below).

The main finding was an increase in thymic remnant in the neck, which was statistically significant in mid and top dose. In addition single incidences of ventricular septum defect, persistent A-V canal or malpositioned aorta origin were seen in the top dose only and are considered supportive findings for developmental toxicity (however, data on HCD are missing).

According to the study report thymic remnant is listed as an anomaly, but in the discussion of the report it is described as a consequence of low foetal body weight, which does not demonstrate teratogenicity. Devtox.org[1] lists the effect as grey-zone effect between malformation and variation, ECETOC (2002)[2] as variation.

In this respect it is noted that in mid and top dose foetal body weight was statistically significantly lowered, which could also be related to developmental delay. It is unclear how the lower foetal body weight or developmental delay could explain the occurrence of thymic remnant in the neck, but in the absence of relevant maternal toxicity lower foetal body weight and developmental delay are considered to be an adverse effect on its own.

Table 18: Overview of visceral malformations (Anonymous 2022b)

		Control	100 mg/kg bw/d	330 mg/kg bw/d	1000 mg/kg bw/d
n, F1 animals		20	20	20	11
n, fetuses examined		137	135	126	53
Visceral malformations, overall	#	9	10	35	42
	%	6.5 ± 13.3	7.5 ± 12.1	30.6 ± 36.2*	78.7 ± 19.4**
Thymic remnant in the neck	#	6	4	32	40
	%	4.6 ± 11.9	3.1 ± 6.4	28.3 ± 34.7**	75.6 ± 24.7**
Ventricular septum defect	#	0	0	0	2
	%	-	-	-	3.6 ± 8.1
Persistent A-V canal	#	0	0	0	1
	%	-	-	-	2.3 ± 7.5
Malpositioned aorta origin	#	0	0	0	1
	%	-	-	-	2.3 ± 7.5
Retroesophageal subclavian	#	0	1	0	1
	%	-		-	1.5 ± 5.0
Dilated renal pelvis	#	0	0	1	0
	%	-	-		-
Dilated ureter	#	4	5	4	1
	%	2.8 ± 5.8	3.7 ± 8.0	4.8 ± 12.9	1.5 ± 5.0

*: $p<0.05$; **$p<0.01$ (Wolcoxon test); No HCDs included in the study report

Thymus development is very similar in rodents and humans. The thymus emerges from the thymus bud, which is located in the third pharyngeal pouch, from which it detaches and migrates to its final location in the chest cavity close to the heart (Gordon und Manley 2011). This process is tightly regulated by different genes and several mouse mutants have been described, in which normal migration is hindered and in which thymic remnants in the neck are observed (Gordon und Manley 2011). As such thymic remnants might not be caused by developmental delay or lower foetal body weight, but could also result from interference with normal thymus development. In this respect it should be noted that the occurrence of thymic remnants is not commonly seen in PNDT studies together with lower foetal body weight or developmental delay.

Thymic remnants are rarely observed in humans and it appears to cause symptoms relatively more common in children than in older patients with over 50% of children cases experiencing respiratory and feeding difficulties. Airway compromise and feeding disturbances are caused by compression of the parapharyngeal space or vagus nerve and by the rapid expansion of the cyst attributed to fluid accumulation or to hemorrhage.

Some patients report small fluctuations in lesion size, whereas others experience rapid enlargement after minor trauma, vaccination or upper respiratory tract infection (Saggese et al., 2002). Cough, respiratory infections and pressure on the phrenic nerve have also been described in relation with ectopic thymus in children (Statham et al., 2008). It can be concluded that this lesion can cause considerable health constraints in humans, although surgical removal is stated to be curative (Saggese et al. 2002), (Statham et al. 2008).

Given that also in adult animals the thymus was a target organ of DEGMEE toxicity (Anonymous 2014c, Anonymous 2022a, Anonymous 2014b) the clear dose dependent and statistically significant increase in thymic remnants could also be a specific effect and not a mere consequence of the lower foetal body weight or developmental delay.

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

Studies relevant for the assessment of adverse effects on development are the OECD 422 study (Anonymous 2014b) described in Chapter 10.10.1 and the pre-natal developmental toxicity according to OECD 414 (Anonymous 2022b).

- OECD 422 (Anonymous 2014b): Despite only slight general maternal toxicity up to the highest dose tested and no abnormalities in delivery and nursing in any dam, the below findings were reported for the top dose group of 1000 mg/kg bw/day (no effects at lower doses, 50 & 250 mg/kg bw/day):

- Decreased delivery index: 79.5 ± 12.1 % vs 94.4 ± 7.8 % in the control group
- Low birth index: 70.5% vs 90.1% in control
- High number of stillborn pups (11 of 99 vs 2 of 128 in control) and % of stillborn (11.13% vs 1.8% in controls)
- PND 0: pup number was statistically significantly lower in the top dose group (-23% compared to control), while pup weight was only slightly lower (M: -4%, F: -2%, non-statistically significant).
- PND 4: pup number -59% compared to control, pup body weight: M: -13%, F: -10%
- Viability on PND 4 was low: 48.2% vs 98.8% in controls (pup number 41% of the control)
- Total litter loss in 5 dams (5/10): offspring died mostly due to cannibalism by dams, and did not show any signs of suckling (total litter loss: 1 on LD 1, 3 on LD 2, 1 on LD 3); necropsy did not show any abnormalities in these 5 dams

- OECD 414 (Anonymous 2022b): There was no relevant maternal toxicity observed at any dose level. No mortalities, moribundity, or abortion occurred during the study. No clinical signs were observed in any of the animals. There were no relevant effects on food consumption or body weight (after correction for gravid uterine weight) and at necropsy no treatment-related changes were observed in any dam. Developmental toxicity was mainly observed in the top dose (1000 mg/kg bw/day) as summarised below. Where these effects were also seen at low and/or mid dose (110 & 330 mg/kg bw/day) this is indicated:

- Gravid uterine weight – statistically significantly lower (-52%), also in low (-2.5%) and mid dose (-9.7%), but not statistically significant.
- Post implantation loss – statistically significantly higher in top dose: 57.9% ± 38.6 vs 4.1% ± 5.2 in control (early resorptions: 57.9% ± 38.6 vs 4.1% ± 5.2 in control; Late resorption index: 18.2% ± 17.1 vs 0 in control)
- Male and female foetal body weight - statistically significantly lower in mid and top dose: M: -34%; F: -32% and M: -12%, F: -12.3%, respectively

- Number of live foetuses – statistically significantly lower in the top dose vs control: -59%
- External examination revealed 3 top dose foetuses of 2 litters with anal atresia and acaudate and there was an increase in AGI in both, top dose males and females (while AGD was reduced in top dose males).
- Skeletal malformations and variations were clearly increased in the top dose, some variations and grey zone effects were already seen at the mid and low dose group like e.g. the dose dependent increase in bipartite ossification of thoracic centrum and bipartite ossification of lumbar centrum (details are listed in Table 16).
- Also visceral changes were observed, with a dose dependent increase in thymic remnant, which was statistically significant in mid and top dose.

Overall, it can be concluded that DEGMEE treatment was foetotoxic and lead to reduced pup viability. In addition a dose dependent reduction in foetal body weight was observed. In the absence of any indication for relevant maternal toxicity in the dams these effects are not considered to be secondary non-specific findings.

DEGMEE also had an adverse impact on skeletal development, including several different malformations and variations and for those effects which were also observed at lower doses, there was a dose dependent increase in foetuses affected.

The 3 top dose foetuses from 2 different litters with anal atresia and acaudate further support that DEGMEE has teratogenic potential.

Also visceral malformation/variations were increased, with thymic remnant being the most prominent effect. Thymic remnants have been described to be a consequence of lower foetal body weight and a sign of developmental delay and it was reported that it should not be considered as malformation but variation. Even if this effect was only a sign of developmental delay, it is considered a relevant finding. It is, however, very likely to be a specific effect on the developing thymus. In humans thymic remnants in the neck is a rare observation which is linked to considerable health constraints.

The single incidences of visceral malformations seen in the top dose group are considered supportive evidence as no such effects were seen at lower doses or in the control group, though no HCDs are available for these findings.

10.10.6 Comparison with the CLP criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). Adverse effects on development

- The classification of a substance in Category 1A is largely based on evidence from humans.
- The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in

the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

No human data is available to justify classification in Category 1A.

In experimental animal studies in rats exposure to DEGMEE during gestation and until PND 4 of rats resulted in adverse effects on development. There was clear evidence of foetotoxicity (dose dependent reduction in foetal body weight, significantly reduced gravid uterine weight, post-implantation loss and resulting lower foetal numbers) and reduced pup viability (low number of pups on PND 0, lower pup weight and viability on PND 4). Upon *in utero* exposure DEGMEE induced skeletal and visceral malformations and variations (mostly with dose dependence and statistically significant) and there was an increase in AGI in both males and females (statistically significant). These findings were not accompanied by maternal toxicity up to the highest dose tested for the pre-natal developmental toxicity and only slight general toxicity in the top dose of the OECD 422 study. As such these observation are not considered to be non-specific secondary findings. These studies have no deficiencies and are fully in line with the study protocols, thus classification as Repr 1B, H360D is considered appropriate.

10.10.7 Adverse effects on or via lactation

No information on presence of DEGMEE or its metabolites in milk of experimental animals or humans is available.

The studies available with DEGMEE do not contain the relevant information needed to assess DEGMEE's potential to induce adverse effects on or via lactation.

Though there were clear adverse effects on pups in the OECD 422 study which deteriorated during the time period from LD 0 to LD 4 it is not possible to assign these effects to adverse effects of DEGMEE on the quality of milk or to exposure of the pups to DEGMEE and/or its metabolites via milk. It is not possible to exclude that in utero exposure to DEGMEE induced these effets, in contrast the findings are rather indicative for developmental effects.

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

Not relevant.

10.10.9 Comparison with the CLP criteria

According to CLP regulation substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the basis of: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

No classification for effects on or via lactation is justified.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

There is clear evidence for adverse effects on sexual function and fertility (testes and epididymis damage, interference with spermatogenesis, ovary atrophy, reduced birth index), which is not an unspecific secondary effect to general toxicity, and on the foetuses exposed in utero (foetotoxicity, reduced number of foetuses,

reduced foetal weight, skeletal and visceral malformations and variation in the absence of relevant maternal toxicity) as well as on pups exposed up until PND 4 (reduced viability on PND 4).

According to above classification CLP criteria there was “clear evidence” for reproductive toxicity. A classification into Repr. 1B; H360FD is proposed.

10.10.11 Read across and mode action information

In this CLH dossier no read across from other closely related substances to assess DEGMEEs properties is included, as the available data on the substance itself are considered sufficient to support a classification as Repr 1B, H360FD. Nevertheless, as there are many closely related substances known to have the same classification and to induce the same or very similar effects, some information on these substances and on international activities, which have acknowledged the close relation and similar properties of these substances, is presented below.

DEGMEE belongs to the chemical group of glycol ethers with the generic structure CH3-O-CH2-CH2-O-R (where R is –H or an organic substituent). Prominent members of this group are EGME (2-methoxyethanol or 2-ME, CAS 109-86-4), MAA (methoxyacetic acid, CAS 625-45-6), tetraglyme (CAS 143-24-8), triglyme (CAS 112-49-2), diglyme (CAS 111-96-6) or monoglyme (CAS 110-71-4) and most of these substances have a harmonized classification as Repr 1B, H360FD. Based on their chemical structure, these glycol ethers show potential to be metabolized to methoxyacetic acid (MAA). MAA is also a member of this group, it has a harmonized classification as Repr 1B, H360FD and is presumed to be responsible for the reproductive toxicity of this group of compounds. On this basis ECHA has placed DEGMEE in the Ethylene Glycol Ether - ARN group and highlighted it for harmonized classification for reproductive toxicity (ARN Ethylene Glycol Ethers, unpublished).

The formation of MAA (via 2-ME, another member of this group with a harmonized classification as Repr 1B, H360FD) has been demonstrated for the closely related substance diglyme (one member of the group) and based on the structural similarity it can be expected that also DEGMEE is metabolized in a comparable manner.

Several modes of action for the adverse effects on male reproductive organs and spermatogenesis as well as for the adverse effects on development have been proposed, including interference with purine nucleotide synthesis (OECD 2019, ECETOC 2005) or interference with androgen receptor signaling (Bagchi et al. 2009, Bagchi et al. 2011). However, no final conclusion can be drawn and it is likely that more than one mode of action contribute to the observed adverse effects.

In ECETOC (2005) the effects of this group of substances are summarized as follows. The pattern of developmental effects in a number of species is characterized by a range of structural anomalies (affecting the development of the cardiovascular system, CNS and urogenital system, as well as skeleton), with foetotoxicity and embryo lethality occurring at higher doses. Effects upon fertility are largely related to testicular atrophy, characterized by selective degeneration of pachytenic spermatocytes in rodents. ECETOC (2005) further states that these effects are related to the amount of MAA formed by the respective group member.

The ECETOC (2005) report also described toxicity to lymphoid organs and tissues for this group of chemicals, including pronounced thymus weight reduction and atrophy upon repeated exposure in rats, mice and rabbits. Also for this toxic effect the metabolite MAA was made responsible. It is noted that also for DEGMEE thymus weight reduction was observed consistently across studies and in the 28-day study also a dose dependent increase in the incidence of thymus atrophy was reported. These effects were reversible after exposure to DEGMEE was ceased.

Reversible neurological effects have been reported in humans upon EGME exposure and in rats inhalation exposure to EGME for 7 days resulting in an inhibition of avoidance-escape response and impairment of hind limb motor function (ECETOC 2005). These effects were described to be different to the transient CNS depression, which is often seen in relation to inhalation exposure with other organic solvents. In this respect it should be noted that for DEGMEE also some behavioral effects were reported in rat, i.e. reduced motor activity in the OECD 422 study as well as in the 14-day and 28-day studies.

10.11 Specific target organ toxicity-single exposure

Not assessed in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Several repeated dose toxicity studies in which DEGMEE was applied via the oral route are available. These studies are described in the section reproductive toxicity as they are relevant for the assessment of DEGMEEs adverse effects on sexual function and fertility and as reproductive toxicity is the only hazard class open for discussion (see Chapter 10.1).

10.13 Aspiration hazard

Not assessed in this dossier.

11 ENDOCRINE DISRUPTION FOR HUMAN HEALTH

Not assessed in this dossier.

12 EVALUATION OF AQUATIC HAZARDS UNDER CLP ANNEX I, 4.1

Not assessed in this dossier.

13 ENDOCRINE DISRUPTION FOR THE ENVIRONMENT

Not assessed in this dossier.

14 PERSISTENT, BIOACCUMULATIVE AND TOXIC (PBT) OR VERY PERSISTENT, VERY BIOACCUMULATIVE (VPVB) PROPERTIES UNDER CLP ANNEX I, 4.3

Not assessed in this dossier.

15 PERSISTENT, MOBILE AND TOXIC (PMT) OR VERY PERSISTENT, VERY MOBILE (VPVM) PROPERTIES UNDER CLP ANNEX I, 4.4

Not assessed in this dossier.

16 EVALUATION OF ADDITIONAL HAZARDS

Not assessed in this dossier.

17 ADDITIONAL LABELLING

Not relevant.

18 REFERENCES

Anonymous (2014a): 14-day dose range finding study in the rat by the oral route. Online available at https://echa.europa.eu/de/registration-dossier/-/registered-dossier/19407/7/6/2/?documentUUID=160737bc-de5a-466d-a73e-083f429db0a2.

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19 ANNEXES

Confidential Annex I and II are attached as a separate document.