# 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-methoxyethoxy)ethanol;
# diethylene glycol monomethyl ether

**EC Number:** 203-906-6

**CAS Number:** 111-77-3

**Index Number:** 603-107-00-6

**Contact details for dossier submitter:**

**RIVM, The Netherlands**

**National Institute for Public Health and the Environment**

**Centre for Safety of Substances and Products**

**Bilthoven, The Netherlands**

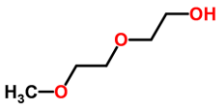
**Version number: 2.0** **Date: 2019-04-04**

# CONTENTS

# 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-methoxyethoxy)ethanol |
| **Other names (usual name, trade name, abbreviation)** | 2-(2-methoxyethoxy)ethan-1-ol, diethylene glycol monomethyl ether, DEGME, 3,6-Dioxa-1-heptanol, Diethylene glycol methyl ether, Diglycol monomethyl ether, Ethanol, 2,2'-oxybis-, monomethyl ether, Ethanol, 2-(2-methoxyethoxy)- (6CI, 8CI, 9CI), Methyl Carbitol, methyl diglycol ether, Methyl Dioxitol |
| **ISO common name (if available and appropriate)** | - |
| **EC number (if available and appropriate)** | 203-906-6 |
| **EC name (if available and appropriate)** | 2-(2-methoxyethoxy)ethanol;<br>diethylene glycol monomethyl ether |
| **CAS number (if available)** | 111-77-3 |
| **Other identity code (if available)** | - |
| **Molecular formula** | C5H12O3 |
| **Structural formula** |  |
| **SMILES notation (if available)** | COCCOCCO |
| **Molecular weight or molecular weight range** | 120.148 g/mol |
| **Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)** | No optical activity |
| **Description of the manufacturing process and identity of the source (for UVCB substances only)** | Not an UVCB |
| **Degree of purity (%) (if relevant for the entry in Annex VI)** | >99% |

## 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-methoxyethoxy)ethanol (DEGME)<br>EC no: 203-906-6 | **99-100%** | **Repr. 2** | **Eye Irrit. 2**<br>**Acute Tox. 4** |

**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 |
|---|---|---|---|---|
| ethane-1,2-diol<br>EC no: 203-473-3 | **0-0.5%** | Acute Tox. 4 | Acute Tox. 4<br>STOT RE 2 | **No** |
| Water, EC no: 231-791-2 | **0-0.1%** | | | **No** |
| 2-metyhoxyethanol<br>EC no: 203-713-7 | **0-0.4%** | Acute Tox. 4 (all routes)<br>Repr. 1B H360FD<br>Flam. Liq. 3 H226 | | **Yes** |
| 2(2-(2-methoxyethoxy)ethoxy)ethanol<br>EC no: 203-962-1 | **0-0.2%** | | Skin irrit. 2, H315<br>Eye Irrit. 2 H319 | **No** |

**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 |
|---|---|---|---|---|---|
| 2,6-di-tert-butyl-p-cresol<br>EC no: 204-881-4 | **Anti-oxidant** | **0.005-0.015%** | | Aquatic Acute 1 H400<br>Aquatic Chronic 1 H410 | **no** |

**Table 5: Other test substances (non-confidential information)**

| Identification of test substance | Purity | Impurities and additives (identity, %, classification if available) | Other information | The study(ies) in which the test substance is used |
|---|---|---|---|---|
| 2-ethoxyethanol (EGEE)<br>EC. 203-804-1 | unknown | unknown | Study on kinetics, follows the same metabolic pathway as DEGME | (Groeseneken, Veulemans, Masschelein, & Van Vlem, 1988) |

All information on purity, impurities and additives was taken from the Risk Assessment Report (RAR, 1999).

# 2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

## 2.1 Proposed harmonised classification and labelling according to the CLP criteria

**Table 6:**

| | 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 | 603-107-00-6 | 2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether | 203-906-6 | 111-77-3 | Repr. 2 | H361d*** | GHS08 Wng | H361d*** | - | | |
| Dossier submitters proposal | 603-107-00-6 | 2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether | 203-906-6 | 111-77-3 | Modify Repr. 1B | Modify H360D | GHS08 Dgr | Modify H360D | - | | |
| Resulting Annex VI entry if agreed by RAC and COM | 603-107-00-6 | 2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether | 203-906-6 | 111-77-3 | Repr. 1B | H360D | GHS08 Dgr | H360D | - | | |

**Table 7: 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)** | 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, H360D | 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 |
| **Hazardous to the aquatic environment** | 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 was previously discussed and/or agreed by the TC C&L (Dir. 67/548/EEC) during meetings in the period of September 1996 to July 1997 (ECBI/34/96, ECBI/21/97 and ECBI/32/97) based on the information present in the Risk Assessment Report (ECB, 2000) prepared by the Netherlands.

# 4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The Health Council of the Netherlands recently re-evaluated the reprotoxic potential of DEGME and concluded that based on the data available, DEGME should be classified as Repr. 1B. Since this differs from the current CLP classification, an update is proposed to change the CLP classification of DEGME to Repr. 1B.

Further detail on need of action at Community level

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on April 1, 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. An evaluation of DEGME was first published in 2003, in a report on diethyleneglycol (mono)alkylethers (Netherlands, 2003) where the classification as reproductive toxicant category 2, T; R61 was proposed in line with Directive 93/21/EEC. This proposed classification was more stringent in comparison to the classification under TC C&L (Dir. 67/548/EEC) which was category 3 Xn; R63. This classification has been transformed to Repr. 2 H361d when CLP came into force under the REACH regulation. Because The Health Council of the Netherlands proposed a more stringent classification under the previous directive, they re-evaluated the data on reproductive effects for DEGME in respect with the current CLP criteria. In the resulting report, published on November 21, 2017, The Health Council of the Netherlands concluded again that the European classification, now under CLP as Repr. 2 H361d, is not strict enough and should be modified to Repr. 1B, H360D (Netherlands, 2017). Although the dossier submitter was unable to retrieve the original classification proposal and associated discussions, in this re-evaluation, all information dating after 1997 can be considered new as the initial evaluation by the commission TC C&L (Dir. 67/548/EEC) was carried out before this date. Notable new information includes more details on the formation and half-life of the reproductive toxicant methoxyethoxyacetic acid (MAA) in both rats and humans after oral exposure (Aasmoe, Mathiesen, & Sager, 1999; Groeseneken et al., 1988; Groeseneken, Veulemans, Masschelein, & Van Vlem, 1989; Triskelion, 2017) and information on the reprotoxic potency of MAA (ECETOC, 2005). The evaluation by ECETOC included some new information dating after 1997 and it seems likely MAA was not considered in the original classification proposal as this was also not the case in the Risk Assessment Report from 1999 (RAR, 1999), which was used as basis for the original classification proposal.

# 5 IDENTIFIED USES

DEGME is primarily used as an intermediate or industrial processing aid and an additive in aviation fuels. Previous use in coatings has declined substantially, remaining only in specialist industrial markets and in professional uses such as specialist printing inks and textile dyes. These uses are small in volume. The use of hydraulic fluid has also been reported. As DEGME is relatively non-volatile, human exposure most likely occurs via the dermal route.

# 6 DATA SOURCES

This CLH report is based on a recent report of the Health Council of the Netherlands, "2-(2-methoxyethoxy)ethanol (DEGME) - Evaluation of the effects on reproduction, recommendation for classification", No. 2017/21, The Hague, November 21, 2017 (Netherlands, 2017). Starting point of

their report were the registration dossier at the European Chemicals Agency (ECHA) and literature databases XTOXLINE, MEDLINE and CAPLUS, up to May 2017.

Sources as cited in the text and tables are mentioned in the reference list.

# 7 PHYSICOCHEMICAL PROPERTIES

## Table 8: Summary of physicochemical properties

| Property | Value | Reference | Comment (e.g. measured or estimated) |
|---|---|---|---|
| **Physical state at 20°C and 101,3 kPa** | Colourless liquid | (Netherlands, 2017), registration dossier | |
| **Melting/freezing point** | -70 °C | | |
| **Boiling point** | 190 – 196 °C | | |
| **Relative density** | 1.02 kg/dm$^3$ | | |
| **Vapour pressure** | ≤0.24 hPa at 25 196 °C | | |
| **Surface tension** | 34.8 mN/m | | |
| **Water solubility** | miscible | | |
| **Partition coefficient n-octanol/water** | -0.682 | | Unclear if experimental or estimated |
| **Flash point** | 91 °C | | |
| **Flammability** | 215 °C | | Self-ignition temperature |
| **Explosive properties** | - | | |
| **Self-ignition temperature** | 215 °C | | |
| **Oxidising properties** | - | | |
| **Granulometry** | - | | |
| **Stability in organic solvents and identity of relevant degradation products** | - | | |
| **Dissociation constant** | 15 (pKa) | | |
| **Viscosity** | | | |

# 8 EVALUATION OF PHYSICAL HAZARDS

Hazard class not assessed in this dossier.

# 9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

## Table 9: Summary table of toxicokinetic studies

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Characterisation of urinary profile<br>Sprague Dawley rats, age 9-10 weeks<br>4 males/dose group<br>Oral exposure to 500, 1000, 2000 mg/kg bw DEGME (purity: 99.8%) by gavage.<br>Non-guideline, GLP | 95% of dose was recovered in 0-48 h urine, mostly as metabolites. The majority of the excreted parent and its metabolites was excreted within the 0-24 h time interval. <5% was excreted as the parent DEGME, ± 90% was excreted as the main metabolite Methoxyethoxyacetic acid (MEAA) and ± 1% was excreted as methoxyacetic acid (MAA), the known reprotoxic metabolite. | 1, reliable with restriction, key study | (Triskelion, 2017) |
| Dermal uptake *in vitro* human abdominal whole skin (abdominal epidermis) model<br>DEGME (purity 98%)<br>Non-guideline, non-GLP | Readily absorbed through the skin, 0.21 ± 0.16 mg/cm$^2$/h | 2, reliable with restriction | (Dugard, Walker, Mawdsley, & Scott, 1984) |
| Dermal uptake *in vitro* rat skin model.<br>DEGME (purity unknown) in Jet-8 fuel.<br>Non-guideline, GLP unknown | 0.05 ± 0.02 mg/cm$^2$/h, but concentration in jet fuel rather small (0.08%). | 3, supporting study | (McDougal, Pollard, Weisman, Garrett, & Miller, 2000) |
| Toxicology review of Glycol Ethers | Two main oxidative pathways, either via ADH or microsomal CYP mixed function oxidase (MFO, O-demethylation or O-dealkylation). | 4, review glycol ethers as a group | (ECETOC, 2005) |
| Wistar Rats, age unknown<br>5 males/dose group<br>Oral exposure to 0.5, 1, 5, 10, 50, 100 mg/kg bw ethylene glycol monoethyl ether (EGEE) by gavage.<br>Purity unknown.<br>Non guideline, non-GLP | Wistar rats appeared to have a smaller metabolic clearance (15 vs 30%), but higher overall excretion (body clearance, 80 vs 13 ml/kg) in comparison to humans. Half-life of EAA was calculated to be 7.2h based on urinary excretion. | 2, reliable with restriction | (Groeseneken et al., 1988) |
| 7 healthy male volunteers<br>Inhalation exposure to 16 mg/m$^3$ EGME (purity unknown) for 4x 50min (4h with 10min breaks) corresponding to a dose of 0.25 mg/kg bw.<br>Measurements EGME and MAA from exhaled air every 10min during exposure and urine samples taken before exposure + every hour during exposure + every day at least twice after exposure (at 2-12h | Retention of EGME high (almost all EGME entered the blood from alveolar space). Half-life of MAA was 77.1h +/- 9.5h based on urinary excretion. | 2, reliable with restriction | (Groeseneken et al., 1989) |

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| intervals) for 5 days | | | |
| 9 SD rats (12 in control group) were given 250 mg/kg bw 2-methoxy 14C ethanol (purity 98%) dissolved in corn oil via ip.<br>Three animals were placed in metabolism cages for urine collection. The remaining six were used for tail vein blood sampling (3-4x <0.5ml in total in up to 48h). 3 animals were killed after 24h instead of 48 to examine testes, seminal vesicles, prostate and liver. Non-guideline, non-GLP. | Rapid metabolism of 2-methoxy ethanol ($t_{1/2}$: 0.6h) to predominantly MAA and to lesser extend methoxyacetylglycine. Clearance half-life of MAA from plasma was estimated to be 19.7 +/- 2.3 h based on clearance of the radioactive labelled substance). At 250 mg/kg bw, tubular lesions and primary spermatocyte degeneration and necrosis was seen. Pre-treatment with pyrazole inhibited metabolism to MAA and protected against the toxic effects. | 2, reliable with restriction | (Moss et al., 1985) |
| Groups of ten female and male Wistar rats were given 100 mg/kg bw MAA (purity >97%) or EAA (purity >98%) via an i.v. bolus administration. Blood samples (tail vein, max 0.25ml) were taken after 2, 5, 7, 10, 24 30, 48 and d 72h. Urine samples were collected at 0-2, 2-5, 5-7, 7-10, 10-24, 24-30, 30-48 and 48-72h. Non guideline, non-GLP | Clearance half-lives of MAA from plasma were 16.6h - 20.6h in female rats and 12.8h - 13.6h in male rats. Based on urinary clearance the half-lives were 21.8h and 21.4h respectively in females and males. No sex difference found based on half-lives estimated from urine samples. The percentage of renal clearance was low (16 - 25%) considered to indicate metabolic clearance. EAA clearance was faster ($T_{1/2}$ was 5.7h – 13.1h). | 2, reliable with restriction | (Aasmoe et al., 1999) |

## 9.1 Summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

*Absorption*

*Oral.* In an assessment of the urinary profile in Sprague Dawley rats, 95% of the oral administered doses (500, 1000, 2000 mg/kg bw) was recovered in 48h, of which the majority was recovered in 24h (Triskelion, 2017).

*Inhalation.* No information is available on the inhalatory uptake of DEGME.

*Dermal*. Dugard et al. (1984) measured the dermal penetration of DEGME through heat-separated human epidermal membranes. They reported a flux of 0.21 mg/cm$^2$/h from the pure chemical. This is about 4-times higher than the flux in rat skin measured for DEGME in jet-8 fuel (McDougal et al., 2000). However, the concentration of DEGME in jet-8 fuel (0.08%) is many fold smaller and the study with rat skin contained an additional skin layer in the form of some dermis that may have reduced the flux (McDougal et al., 2000).

*Metabolism & Excretion*

Data on the group of glycol ethers indicate that two main pathways of metabolism exist, one involving alcohol dehydrogenase and one involving microsomal P450 mixed function oxidation (ECETOC, 2005). The first pathway results in alkoxy acetic acids, whereas the second results in the formation of carbon dioxide via ethylene glycol or propylene glycol. The proposed metabolic pathway of diethylene glycol mono-alkyl ethers (alkoxy-ethoxy-ethanols)[a] and diethylene glycol di-alkyl ethers[b] is illustrated in figure 1 (ECETOC, 2005):

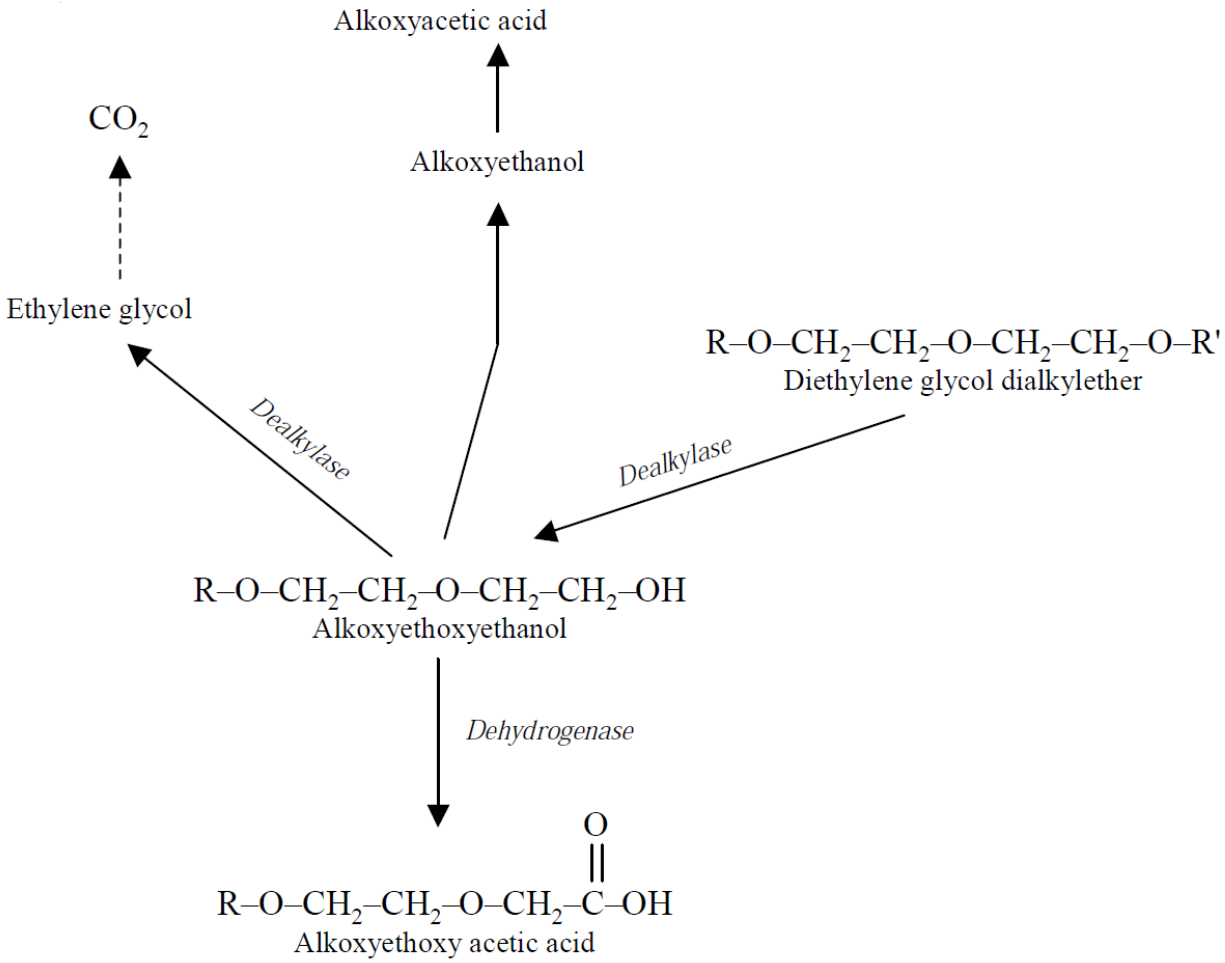



Figure 1. Proposed metabolic pathway for alkoxyethoxyethanols (ECETOC, 2005)

An excretion and metabolism study was conducted with DEGME where Male Sprague-Dawley rats were administered a single dose of 500, 1000 or 2000 mg/kg bw by gavage. Urine was sampled during the periods 0-24h and 24-48h. Analysis was done using liquid chromatography-tandem mass spectrometry (LC-MS). The following metabolites were detected:

Table 10. Recovery of DEGME in urine (% of dosed) in the metabolism study by Triskelion (2017)

| Dose | 500 mg/kg | 1000 mg/kg | 2000 mg/kg |
|---|---|---|---|
| | % (sd) | % (sd) | % (sd) |
| Methoxyethoxyacetic acid (MEAA) | 87.2 - 94.5 (7.5) | 90.9 (8.5) | 87.2 (4.5) |
| Methoxyacetic acid (MAA) | 0.8 – 1.4 (0.1) | 1.1 (0.1) | 0.8 (0.1) |

| | | | |
|---|---|---|---|
| Diethylene glycol (DEG) | 1.6 – 2.4 (0.4) | 2.3 (0.8) | 2.2 (0.4) |
| DEGME-Glucoronide | 1.0 (0.1) | 0.8 (0.1) | 0.7 (0.1) |
| DEGME | 3.4 – 4.9 (0.4) | 3.6 (0.7) | 4.9 (0.7) |

2-Methoxyethanol was not detected. In addition, DEGME-sulfate, HEAA and 2 unidentified metabolites were detected at very low percentages (≤ 0.3%). The total recovery ranged from 95.7% to 103.2% within 48 hours. Based on the detected metabolites and the general knowledge on the metabolism of glycol ethers the following metabolism scheme is proposed:

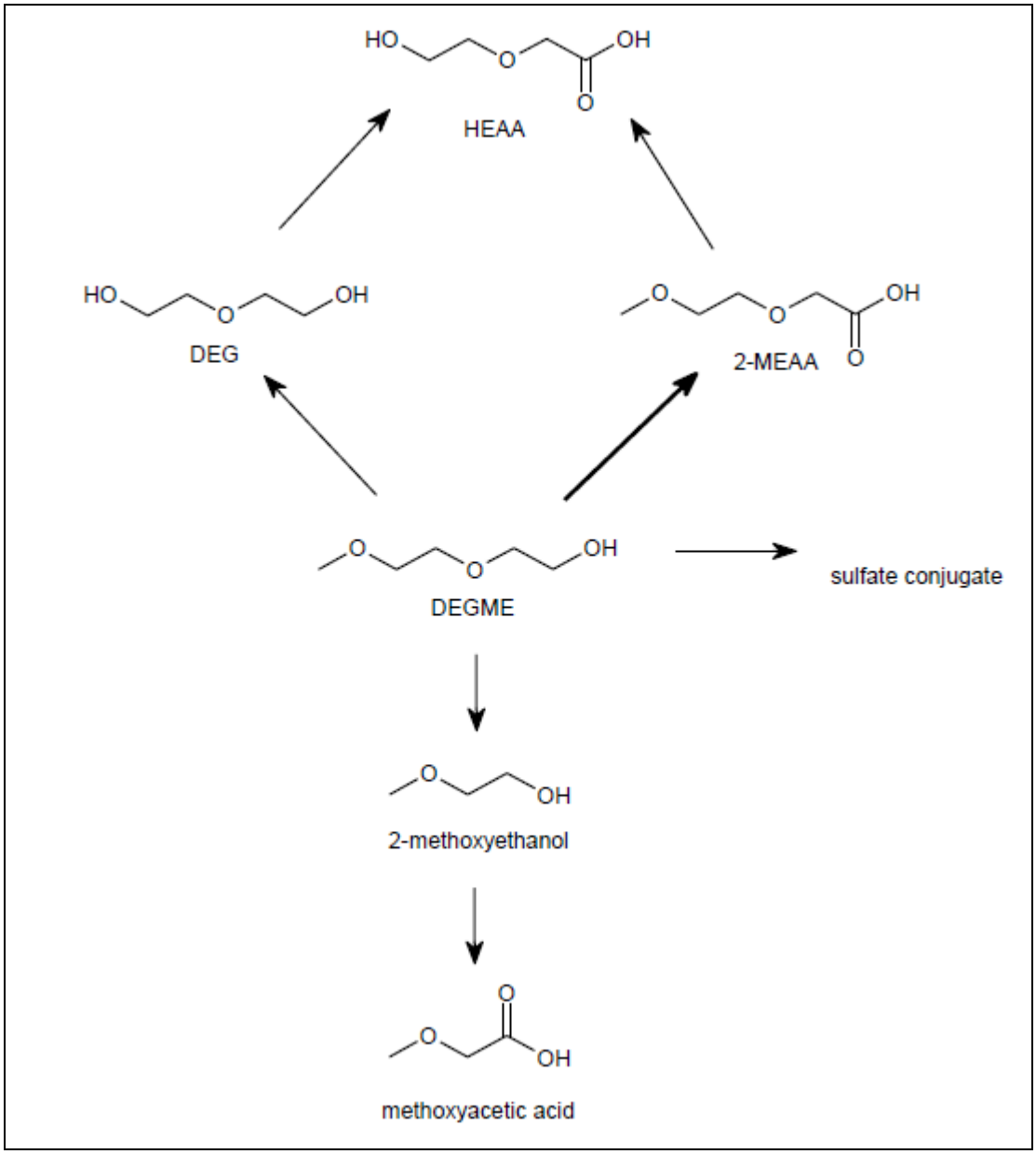



Figure 2. Proposed metabolic pathway of DEGME (Triskelion, 2017).

The metabolite 2-Methoxyacetic acid belongs to the group of alkoxyacetic acids, which are reported to be eliminated slowly and also more slowly in humans as compared to rodents (ECETOC, 2005). In the recent metabolism study of DEGME by Triskelion (2017), the ratio of MAA found between 24h/48h clearance was smaller in comparison to the other metabolites, indicating slower clearance. In another study, Groeseneken et al. (1989) reported the half-life of MAA in seven healthy male volunteers to be 77 hours based on urinary excretion after exposure to EGME, which is considerably longer compared to SD rats (19.7h) based on plasma concentrations after exposure to ME (Moss et al., 1985) or male/female Wistar rats (13.2h/18.6h respectively) based on plasma and urinary excretion after exposure to MAA (Aasmoe et al., 1999). The urinary half-life of EAA, a similar substance as MAA produced via the same metabolic pathway from EGEE, was estimated to be 7.2h in Wistar rats and 42h in humans by Groeseneken et al. (1988) or between 5.7/13.1h in males/females by Aasmoe et al. (1999). These studies illustrate the relatively slow clearance of the toxic alkoxyacetic acid metabolites in comparison to the other metabolites from DEGME and close analogues. Additionally the clearance in humans is slower than in rats which suggest there is a higher internal human concentration and a possible higher potency in humans as compared to rodents.

*Conclusion*

DEGME appears to be readily absorbed through the skin, with an estimated absorption rate of 0.05-0.21 $mg/cm^2/h$. A metabolism study in rats showed that orally administered DEGME is readily absorbed and primarily metabolised to and excreted via the urine as MEAA, and to a limited extent as MAA and DEG. The toxic metabolite MAA is eliminated more slowly in comparison to other metabolites and the clearance is also likely more slowly in humans as compared to rats.

# 10 EVALUATION OF HEALTH HAZARDS

## 10.1 Acute toxicity

Hazard class not assessed in this dossier

## 10.2 Skin corrosion/irritation

Hazard class not assessed in this dossier

## 10.3 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

## 10.4 Respiratory sensitisation

Hazard class not assessed in this dossier

## 10.5 Skin sensitisation

Hazard class not assessed in this dossier

## 10.6 Germ cell mutagenicity

Hazard class not assessed in this dossier

## 10.7 Carcinogenicity

Hazard class not assessed in this dossier

## 10.8 Reproductive toxicity

### 10.8.1 Adverse effects on sexual function and fertility

**Table 11: Summary table of animal studies relevant for toxicity on sexual function and fertility**

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Fischer rats, age: 6-8 weeks<br>10/sex/dose group<br>Exposure by inhalation of 0, 150, 500, 1080 mg/m$^3$ DEGME (purity >99.5%) for 6 hours per day, 5 days/week for 13 weeks.<br>Non guideline, GLP unknown | No effects on reproductive organs seen in gross pathological and histopathological examination (including testis, epididymis, seminal vesicle, prostate, coagulation gland, ovary, oviduct, uterus, cervix and vagina. No general toxicity. | 2, reliable with restriction. | (Miller, Eisenbrandt, Gushow, & Weiss, 1985) |
| Sprague-Dawley Rats, age unknown.<br>50 males/dese group<br>Orally exposure to 613 mg/kg bw/day DEGME (purity 99.5%) by gavage 3-21 days.<br>At 2-day intervals starting at day 3, 5 rats were sacrificed and testis histopathologically examined.<br>Non-guideline, non-GLP | No gross or microscopic abnormalities of the testes were detected, no general toxicity. | 2, reliable with restriction. | (Cheever et al., 1988) |
| Wistar Rats, age unknown<br>4-8 males/dose group<br>Oral exposure to 0 and 2000 mg/kg bw/day DEGME (purity >98%) for 1, 2, 5 and 20 days.<br>Non-guideline, non-GLP | At 2000 mg/kg the animals had lower body weight compared to control animals (± -10% at day 20), lower relative thymus weight (day 5: -37% and day 20: -40%), lower relative liver weight (day 5: -9%, day 20: -10%) and lower relative testis weight (day 5: -16%,day 20: -19%). All noted decreases were statistically significantly different from control. Determination of macroscopic and microscopic effects on the testis were not reported. | 2, reliable with restriction. | (Kawamoto et al., 1990) |
| Wistar Rats, age unknown<br>4-8 males/dose group<br>Oral exposure to 0, 500, 1000 and 2000 mg/kg bw/day DEGME (purity >98%) by gavage for 20 days.<br>Non-guideline, non-GLP | Statistically significant reduction of body weight and relative testis weight after >5 days at 2000 mg/kg bw/day. | 2, reliable with restriction. | (Kawamoto et al., 1990) |
| Hartley Guinea pigs, age 6-8 weeks<br>6 males/dose group<br>Dermal exposure to 0, 40, 200 and 1000 mg/kg bw/day DEGME for 6 hours/day, 5 days/week for 90 days.<br>Non-guideline, non-GLP | No effects seen on examined reproductive organs including the testis. Spleen weight decreased at >200 mg/kg bw/day and mild fatty change in livers in all treated animals | 2, reliable with restriction. | (Hobson, D'Addario, Bruner, & Uddin, 1986) |
| Albino COBS, CD, BR rats, age | All mentioned changes were | 2, reliable with | (Krasavage & |

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| unknown<br>10 males/dose group<br>Oral exposure to 0, 900, 1800, 3600 mg/kg bw/day DEGME by gavage for 6 weeks (5 days/week).<br>Similar to OECD 407, but not all endpoints examined longer time period and males only.<br>Non-GLP, but well reported. | statistically significant:<br>At 1800 and 3600 mg/kg bw/day, there was an increased heart weight and reduced absolute liver weight.<br>At 3600 mg/kg bw/day, body, absolute spleen and brain weight were reduced whilerelative kidney weight was increased and the relative testis weight was reduced.<br>At 1800 mg/kg bw/day, therelative testis weight was increased. | restriction | Vlaovic, 1982) |

### 10.8.2 Summary and overall relevance of the animal studies on adverse effects on sexual function and fertility

No mating studies are available, but a number of non-guideline repeated dose toxicity studies are. These are summarized in Table 11 and shortly described below.

Male and female Fischer rats (10/sex/group) were sub-chronically exposed by inhalation to DEGME vapour at concentrations of 0, 150, 500 or 1,080 mg/m$^3$ (0, 30, 10 or 216 ppm) 6 hours per day, 5 days/week, for 13 weeks (Miller et al., 1985). Following exposure, all animals were weighed, sacrificed and subjected to a complete gross pathological and histopathological examination, including testis, epididymis, seminal vesicles, prostate, coagulating gland, ovary, oviduct, uterus, cervix and vagina. No exposure related mortality occurred during the course of the study. Furthermore, no apparent differences in body weights and in absolute and relative organ weights were observed between control and treated groups of animals. No effects of the treatment were seen in haematology, clinical chemistry analyses, urinalyses and the gross pathological and histopathological examinations.

In a study by Cheever et al. (1988), male Sprague-Dawley rats were treated daily with oral doses of 5.1 mmol/kg bw DEGME (613 mg/kg bw) for up to 20 days. No early deaths or overt signs of toxicity were observed. Selected animals (5 rats per time point) were killed at 2-day intervals on days 3 through 21. Following gross and histopathological examination, it was concluded that no degenerative changes were observed in the testes of any of the treated animals when compared to controls.

In a time course study, male Wistar rats (4-8/group) were daily administered oral doses of 2,000 mg/kg bw/day DEGME by gavage for 1, 2, 5 and 20 days (Kawamoto et al., 1990). After sacrifice, the weights of the liver, kidney, spleen, thymus, heart, lung and testis were determined. After one day, the relative thymus weight was decreased (-27%) whereas the relative kidney weight was increased after 2 days (+5%). Decreases in relative weights of the liver (-9%), spleen (-26%), thymus (-37%) and testis (-16%) were reported after 5 days of dosing. The decrease in thymus (-40%) and testis weights (-19%) was more pronounced after 20 days of treatment. Determination of macroscopic and microscopic effects on the testis were not reported.
In an accompanying dose-response study with oral doses of 0, 500, 1,000 and 2,000 mg/kg bw/day (4-8/group) during 20 days, the weights of the testis and the thymus only were reported (Kawamoto et al., 1990). DEGME was shown to reduce body weight gain at 2,000 mg/kg bw/day compared to the control group from day 10 onwards. No effect on testis weight was observed at doses of 500 and 1,000 mg/kg, but at 2,000 mg/kg the testis weight was reduced relative to the body weight. The relative thymus weight was decreased at 1,000 and 2,000 mg/kg bw/day.

In a repeated dose DEGME toxicity study, male rats (10/group) were administered 0, 900, 1,800 or 3,600 mg/kg bw/day by gavage for 6 weeks (Krasavage & Vlaovic, 1982)). Signs of systemic toxicity, appearance and behaviour were monitored. After sacrifice, haematology, clinical chemistry, gross pathology and histopathology were performed. At the highest dose, the relative testis weight was decreased and atrophy, accompanied by evidence of degenerated spermatozoa in the epididymis and hypospermia, was observed in 50% of the rats. At this dose, clear signs of systemic toxicity were reported.

In a sub-chronical study by Hobson et al. (1986), male Hartley guinea pigs (n=6) were dermally (occlusive) exposed to DEGME at doses of 0, 40, 200 and 1,000 mg/kg bw/day during 90 days (5 days/week, 6 hour/day). Average body weight of exposed animals decreased in a dose-related manner, but it was not statistically significantly different from control animals. At the medium and high doses, the spleen weights (both relative and absolute) were decreased. At the highest dose level, an increase in serum lactate dehydrogenase (LDH) was observed. A mild change in liver fat was observed in all test treatment groups but not in the controls. DEGME exposure did not result in testicular lesions, nor were body weight and the relative and absolute weights of the testes, seminal vesicles and prostate affected.

### 10.8.3 Human data

No studies are available regarding the effects of DEGME on human fertility.

### 10.8.4 Other relevant data

A limited amount of DEGME is metabolised to MAA, which is classified for effects on fertility (Repr. Cat. 1B).

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

Effects on reproductive organs were investigated in four studies with rats (three with males only), and in one study with male guinea pigs. No effects were found in the rat study by Miller et al. (1985), in which rats (both males and females) were exposed via inhalation up to 1,080 mg/m$^3$ for 13 weeks. In another study, oral exposure to 613 mg/kg bw/day up to 20 days did not lead to effects on the rat testes (Cheever et al., 1988). In a rat study reported by Kawamoto et al. (1990), decreases in relative weights of the testes were only seen at the highest dose level of 2,000 mg/kg bw/day for 20 days. At this dose, relative body weight gain, relative thymus weight and relative liver weight were reduced. In a rat study by Krasavage and Vlaovic (1982), a reduced testis weight, testicular atrophy and sperm abnormalities were noted at 3,600 mg/kg bw/day. At this dose, body weight was decreased, relative kidney and heart weights were increased and absolute spleen, liver and brain weights were reduced. Guinea pigs were exposed dermally to up to 1,000 mg/kg bw/day, but this did not result in effects on the testes (Hobson et al., 1986).
Notably, a metabolism study with DEGME shows that limited amounts of 2-methoxyacetic acid is formed in rats (Triskelion, 2017). 2-methoxyacetic acid has been reported to cause testicular damage in rats and mice (at single oral or i.p. doses of 118 mg/kg and higher), and to reduce fertility in mice (at doses of 140 mg/kg bw/day and higher) and is therefore classified for effects on fertility (Repr. Cat. 1B) (ECETOC, 2005). Because fertility was affected even at the lowest tested concentrations, no NOAEL could be determined (ECETOC, 2005).
No further studies are available on possible effects on fertility by DEGME. Taken together, it can be concluded that DEGME may have an effect on testes weight and sperm in rats at oral doses 1,800 mg/kg/d and higher. In the other studies, the applied doses of 1,000 mg/kg bw/day and lower, did not affect the testes.

### 10.8.6 Comparison with the CLP criteria

In rats, male animals showed reduced testis weights (from 1,800 mg/kg bw/day and upwards), testicular atrophy and degenerated spermatozoa (at 3,600 mg/kg bw/day). These effects were accompanied by a reduction in bodyweight and organ weights. The effects on testis weight at these levels are not considered relevant, since it can be a secondary effect due to general toxicity. No data are available on functional reproduction parameters.
The metabolism study with DEGME shows that limited amounts of 2-methoxyacetic acid is formed in rats.
2-Methoxyacetic acid has been reported to cause testicular damage in rats and mice (at single oral or i.p. doses of 118 mg/kg and higher), and to reduce fertility in mice (at doses of 140 mg/kg bw/day and higher) and is therefore classified for effects on fertility (Repr. Cat. 1B). No NOAEL or critical dose levels for effects on fertility in animals has been established for MAA, because effects were already observed at the lowest administered doses. However, as only a limited amount of MAA is formed (around 1%), it is uncertain whether sufficient MAA is formed at dose levels of DEGME relevant for classification. 1% of 1000 mg/kg bw/day DEGME would result in exposure to approximately 10 mg/kg bw/day MAA, which is similar to the lowest effective dose of 8 mg/kg bw/day MAA after repeated dosing in hamsters. However this study is non-standard and it assessed fertility by determining the fertilisation capacity of the spermatozoa *in vitro* after extraction from the animals. No other effects of MAA were determined at or below 10 mg/kg bw/day. It therefore remains uncertain whether DEGME is capable of causing effects on fertility in humans at dose levels relevant for classification.
One of the impurities in substance compositions of DEGME is 2-methoxyethanol. 2-Methoxyethanol is classified as Repr. 1b for fertility and development. In a mixture, a substance has to be classified when the concentration of the reproductive toxicant is above 0.3%. The presence of this impurity has a range between 0-0.4%. It is unclear when and how often the concentration of 2-methoxyethanol is above 0.3%. Therefore, it is considered not relevant for classification of DEGME itself. The substance should rather be classified based on the concentration of 2-methoxyethanol in the batch produced.
In view of the absence of human data and relevant data on functional fertility in animals, a lack of appropriate data precludes the assessment of effect on fertility and therefore classification is not warranted.

### 10.8.7 Developmental effects

**Table 12: Summary table of animal studies on adverse effects on development**

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Sprague-Dawley rats, age unknown<br>9 females/group<br>Oral exposure to 0, 1000, 1495, 2235, 3345, 5175 mg/kg bw/day DEGME (purity unknown) by gavage on GD7-16.<br>Sacrifice on GD 21.<br>Dose finding study.<br>Non-guideline, non-GLP | Gestational body weight was reduced and 2/9 dams died in the highest dose group. At 3345 and 5175 mg/kg bw/day, litter size reduced to 0 and 10% respectively vs 91.2% in the control. At 2235 and 3345 mg/kg bw/day: foetal weight was decreased. Total number of skeletal variations, visceral and cardiovascular malformations was increased at 2235 mg/kg bw/day. | 2, reliable with restriction. | (Hardin, Goad, & Burg, 1986) |
| Sprague-Dawley rats, age unknown<br>12-13 females/group<br>Oral exposure to 0, 720, 2165 mg/kg bw/day DEGME (purity unknown) by gavage on GD7-16. Sacrifice on GD 20. | At 2165 mg/kg bw/day decreased maternal weight, decreased live births per litter (60.5% vs 90.7% in the control), reduced foetal weight, skeletal variations (ribs), visceral cardiovascular malformations, several variations including reduced ossification of | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include slightly fewer animals than recommended, dosing started on GD7, | (Hardin et al., 1986) |

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Non-guideline, non-GLP | various skeletal parts and urinary variations. At 720 mg/kg bw/day: skeletal variations (ribs) and reduced ossification of the appendicular skeleton and dilated renal pelvis. | not 5. Rats shipped from CRL on day 4 after gestation, received on day GD5 and dosed on GD7 | |
| Dose finding study.<br>Wistar Rats, >3 months old<br>4-6 females/dose group<br>Oral exposure to 0, 125, 250, 500, 1000, 2000, 3000 and 4000 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at GD20.<br>Non-guideline, non-GLP | Maternal body weight gain was reduced at ≥2000 mg/kg bw/day. At ≥3000 mg/kg bw/day reduced food consumption by maternal animals. There was a dose-dependent decrease in number of live foetuses. No live foetuses at 3000 and 4000 mg/kg bw/day. | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include fewer animals than recommended, dosing started on GD7, not 5. | (Yamano, Noda, Shimizu, Morita, & Nagahama, 1993) |
| Teratology study.<br>Wistar Rats, >3 months old<br>14 females/dose group<br>Oral exposure to 0, 200, 600 and 1800 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at GD20.<br>Non-guideline, non-GLP | Parental toxicity: At 1800 mg/kg bw/day decreased maternal body weight gain, food consumption and thymus weight. Foetal toxicity: decrease in foetal body weight at 600 and 1800 mg/kg bw/day. At 1800 mg/kg bw/day: external malformations, visceral malformations of the cardiovascular system, increased skeletal variations. At 600 mg/kg bw/day: variations in ossification of sternebrae and vertebrae | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include slightly fewer animals than recommended, dosing started on GD7, not 5. | (Yamano et al., 1993) |
| Postnatal study.<br>Wistar Rats, >3 months old<br>8 females/dose group<br>Oral exposure to 0, 200, 600 and 1800 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at 21 postpartum.<br>Non-guideline, non-GLP | Prolonged gestational period and reduced number of live pups at 1800 mg/kg bw/day. Reduced viability of pups at 600 and 1800 mg/kg bw/day. | 2, reliable with restriction. | (Yamano et al., 1993) |
| Postnatal development test<br>Alpk/AP (Wistar derived) rats, 11-13 weeks old<br>15 females/dose group.<br>Subcutaneously exposed to 0, 255, 510, 1020 ml/kg bw/day DEGME (purity unknown) on GD6-20. Pups examined on PND2 and 5, no gross histopathology performed.<br>Non-guideline, non-GLP | Non-statistical significant reduction in survival at PND5. | 2, reliable with restriction. | (Doe, 1984) |
| CD-1 mice, 6-8 weeks<br>50 females/group<br>Oral exposed to 0, 4000 mg/kg bw/day DEGME (purity 99%) by gavage for 8 days starting on GD7.<br>Non-guideline, non-GLP | At 4000 mg/kg bw/day 5/50 maternal animals died.<br><br>Reduced percentage of viable litters (litters with one or more live-born pups/number of pregnant survivors). Reduced number of live pups per litter (3 vs 10 in the control), and pup survival over days 1-3 postpartum (31% vs | 2, reliable with restriction. | (Schuler, Hardin, & Niemeier, 1984) |

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| | 100% in the control group). | | |
| New Zealand White Rabbits, age not specified<br>25 females/dose group<br>Dermal exposure to 0, 50, 250, 750 mg/kg bw/day DEGME (purity 99.2%) on GD6-18.<br>Foetuses examined on GD29<br>Non-guideline, non-GLP | At 750 mg/kg bw/day 2/25 died and 1/25 at 50 mg/kg bw/day.<br>At >250 mg/kg bw/day, delayed ossification of the hyoid bone and cervical spur of the vertebrae<br>At 750 mg/kg bw/day, decreased weight gain at GD9-11 and haematological changes in parental animals, developmental variations (mild forelimb flexure, slight to moderate dilation of renal pelvis, retrocaval ureter, cervical spurs and delayed ossification of sternebrae. | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include, dosing ended on GD18, not until caesarean section. | (Scortichini, John-Greene, Quast, & Rao, 1986) |

### 10.8.8 Summary and overall relevance of the provided animal studies on adverse effects on development

The developmental effects of DEGME on pregnant Sprague-Dawley rats (Crl:CD (SD) BR) were studied by Hardin et al. (1986). Time-mated females were dosed by gavage with DEGME in distilled water on gestational days 7-16. Doses of 0, 1,000, 1,495, 2,235, 3,345, and 5,175 mg/kg bw/day were used in a preliminary dose-finding study with nine rats per group. Maternal weight was reduced in the highest dose group on days 16 and 21, and at 3,345 mg/kg bw/day on day 21. Extra gestational weight gain was reduced at the highest dose and two rats in this group died. Food consumption in the two highest dose groups was reduced during the first 5 days of exposure. There were no live litters (0/5) in pregnant surviving animals at the highest dose, and only 3 live litters/9 pregnancies at 3,345 mg/kg bw/day. The percentage of live foetuses declined with increasing dose, and was reduced statistically significantly compared to the control group at 3,345 mg/kg bw/day. Similarly, foetal body weight consistently fell with increasing dose. An increased incidence of skeletal variations included rudimentary cervical or wavy/fused ribs at 2,235 mg/kg bw/day. Skeletal ossification was delayed at 1,495 mg/kg bw/day and higher doses. The number of cardiovascular malformations was increased at 2,235 mg/kg bw/day.
Subsequently, pregnant rats were similarly dosed with 0, 720, or 2,165 mg/kg bw/day (12-13 rats per group). Maternal food consumption was reduced in the first 5 days of dosing and gross maternal weight was reduced on day 21 (93% of control) at 2,165 mg/kg bw/day. However, extra gestational weight gain was not influenced by DEGME treatment. Foetal body weights and the number of live implantations were reduced at 2,165 mg/kg bw/day and two of 23 litters were completely resorbed atthat dose. There was no gross evidence of foetotoxicity at 720 mg/kg bw/day. Skeletal variations included rudimentary cervical ribs and bilateral wavy/ fused ribs, with the numbers of both of these being increased at 2,165 mg/kg bw/day. At 720 mg/kg bw/day, the incidence of combined rib variations was elevated. Retarded ossification was apparent in litters receiving the higher dose, and less markedly but still statistically significantly delayed ossification was also present in the 720 mg/kg bw/day dose group. Visceral malformations were predominantly seen in the cardiovascular system, and were statistically significantly increased at 2,165 mg/kg bw/day. One malformation of the heart was reported at 720 mg/kg bw/day. More details about the studies by Hardin et al. (1986), including tables with results can be found in ANNEX I (13.1.1).

In a dose-finding study by Yamano et al. (1993), Wistar rats were exposed by gavage to daily doses of 0, 125, 250, 500, 1,000, 2,000, 3,000 and 4,000 mg/kg bw/day DEGME.[16] The non-pregnant rats (5 rats/group) were treated for 11 consecutive days and pregnant rats (4-6/group) on days 7-17 of gestation. The non-pregnant rats showed a decrease in body weight gain and food consumption at doses above 3,000 mg/kg

bw/day. Haematological measurements in the non-pregnant rats revealed decreased white and red blood cell counts, haemoglobin concentrations and haematocrit levels in a dose dependent way from 1,000 mg/kg bw/day upward. There were no signs of hepatotoxicity, but at the highest dose, relative kidney weight and plasma blood urea nitrogen levels were slightly increased, indicating weak nephrotoxicity. Furthermore, dose-dependent decreases in weights of pituitary glands and thymus were observed. In pregnant rats, maternal body weight gain and food consumption were decreased above dose levels of 2,000 and 3,000 mg/kg bw/day, respectively. The number and body weight of live foetuses decreased in a dose- dependent way, with no live foetuses being seen at 3,000 and 4,000 mg/kg bw/day (total resorption of litters). Acidic urine was measurable at all doses.
Following this dose-finding study, female Wistar rats (14/group) were administered DEGME doses of 0, 200, 600 and 1,800 mg/kg bw/day by gavage from days 7 through 17 of gestation. On day 20 of gestation, dams were sacrificed. At 600 mg/kg, dams were not affected, but foetal body weights were decreased, and the incidence of foetuses with variations was increased. Among these variations, the incidence of thymic remnants in the neck was statistically significantly increased. At 1,800 mg/kg, maternal body weight gain, food consumption and maternal thymus weight were decreased, and visceral malformations of the cardiovascular system were observed in 28% of the foetuses. External malformations (mostly anasarca and anury) were observed in 14% of the foetuses at 1,800 mg/kg bw/day, but not at lower doses. Dilated renal pelvis was noted in 53% of the foetuses at the highest dose. The degree of ossification was considerably affected at 600 and 1800 mg/kg bw/day. In the same experiment, eight dams per group were administered doses of 0, 200, 600 and 1,800 mg DEGME/kg bw/day by gavage from days 7 through 17 of gestation. The duration of gestation was determined and litters were examined immediately after delivery (for litter size, stillborn and live born, sex and external anomalies). On day 4 after birth, culling was performed to leave eight pups per litter. Pups were nursed by their own mothers for 21 days and thereupon, pups and dams were sacrificed. In the highest dose group, the duration of gestation was prolonged by approximately 1.7 days and the number of pups was decreased. The viability of the neonates was markedly affected by the treatment with DEGME and the numbers of live pups on day 4 after birth divided by the numbers of live born pups were 92/100, 95/101, 58/93, and 2/37 for doses of 0, 200, 600 and 1,800 mg/kg, respectively. According to the authors, viability was only statistically significantly reduced at the highest dose. Body weight gain of pups during 21 days after birth was unaffected at 200 mg/kg bw/day, but slightly decreased at a dose of 600 mg/kg bw/day in each sex. No statistically significant effects of DEGME on the pups were found in the skeletal observations on day 21 postnatal either. More details about the studies by Yamano et al. (1993), including tables and figures presenting the data, can be found in ANNEX I (13.1.2).

The effect of DEGME on foetal development when administered subcutaneously was investigated by Doe (1984). Pregnant Alpk/AP (Wistar- derived) rats were injected subcutaneously with 250, 500 or 1,000 µl/kg bw/day DEGME (255, 510 or 1,020 mg/kg bw/day) or a control solution, on days 6 to 20 of gestation. Rats were allowed to litter and the offspring was weighed and the number of dead and live pups recorded on days 1 and 4 postpartum. No maternal toxicity was observed and little or no effect on the development of the pups at 250 µl/kg and 500 µl/kg. There was a slight, but not statistically significant decrease in survival of the offspring of the rats treated with 1,000 µl/kg. No gross pathology or histopathology on the offspring was performed. More details about the studies by Yamano et al. (1993), including tables with results can be found in ANNEX I (13.1.2).

Schuler et al. (1984), studied the effect of DEGME given by gavage during organogenesis in mice (CD-1 mice, n=50) in an in vivo screening test. DEGME was given at 0 or 4,000 mg/kg bw in distilled water (determined to be the $LD_{10}$) once daily for 8 consecutive days starting on gestational day 7. Females were allowed to deliver litters, and the number of live-born pups, their birth weight, growth and survival up to 2-3 days of age were recorded. At this dose, 5/50 mice died. The percentage of viable litters was reduced to 16% (versus 97% in the control group). Furthermore, DEGME treatment was found to reduce the number of live pups per litter (3 versus 10 in the control group) and pup survival over days 1 to 3 postpartum (31% versus 100% survival in the control group). Pup birth weight was reduced to 88% of that of the controls and pup weight gain over days 1-3 was increased to 120% of that of the controls (not statistically significant).

Based on the results of a dose range finding study, female New Zealand White rabbits (25/group) were dermally (occlusive) exposed to undiluted DEGME at doses of 0, 50, 250, and 750 mg/kg bw/day from gestational days 6 to 18 (Scortichini et al., 1986). On gestational day 29, caesarean section was performed, followed by examination of the foetuses for external, visceral, and skeletal alterations. In the highest dose group, maternal toxicity was observed, characterized by decreased weight gain (during pregnancy day 9-11) and slight hematologic changes (i.e. decrease in red blood cells and packed cell volume values). Two of the 25 animals in the highest dose group died. At 250 and 50 mg/kg bw/day, no clinical signs of treatment-related maternal toxicity were observed. An increase in embryonic resorptions was noted at 750 mg/kg bw/day (not statistically significant). Foetal body weights were slightly lower in both the 250 and 750 mg/kg bw/day dose groups than in the controls. An increased prevalence of developmental variations was observed in foetuses in the two highest dose groups. These foetal alterations were mild forelimb flexure, slight-to-moderate dilation of the renal pelvis, retrocaval ureter, cervical spurs (at 750 mg/kg bw/day only) and delayed ossification of the skull and sternebral bones. The hyoid, delayed ossification of the skull and cervical spur of the vertebrae were also increased compared to the control group at 250 mg/ kg bw/day. No adverse developmental effects were observed at the lowest dose (50 mg/kg bw/day).

### 10.8.9 Human data

One case report is available, in which a case of retrocaval ureter, with anomalies in both the cardiovascular and skeletal system is described (Karaman, Gürdal, Oztürk, & Kanberoğlu, 2002). Since the mother was a worker in the textile industry, maternal exposure to DEGME was expected. As the report did not include blood concentration measurements or work place monitoring, however, no conclusions can be drawn about a possible correlation between the observed anomaly and exposure to DEGME.

### 10.8.10 Other relevant information

Scofield et al. performed an in vitro study to determine the effect of DEGME on the development of in vitro cultured forelimb cells isolated from chick embryo's (Scofield, Henderson, Funk, Anderson, & Smith, 2006). After incubation for 5 days, only at the highest tested concentration (0.85 M) a stop in cell proliferation was detected, accompanied by total loss of proteoglycan. This effect was already seen after 24 hours, whereas the lower concentrations did not exhibit this effect. It is of note that DEGME's assumed metabolite methoxyacetic acid (MAA) was active two orders of magnitude lower. For MAA the effect was shown to be related to apoptosis, as seen by DNA fragmentation and upregulation of caspase activity.
In rats, a limited amount of DEGME is metabolised to MAA, which is classified for effects on development (Repr. Cat. 1B). MAA exposure also results in the formation of cardiovascular malformation as observed with DEGME. See Annex 2.

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

Only one report is available on possible human DEGME exposure to the mother of a child with congenital anomalies (retrocaval ureter), but no conclusions can be drawn since actual exposure to DEGME was not demonstrated (Karaman et al., 2002).
Developmental toxicity was investigated in three rat studies, one study with mice and one with rabbits. In two oral rat studies developmental effects were seen after exposure to DEGME (Hardin et al., 1986; Yamano et al., 1993). Hardin et al. (1986) reported a reduction in live litters, malformations of the cardiovascular system (aortic arch and ventricular septum), variations of ribs (rudimentary and wavy/ fused) and reduced foetal weight at relatively high doses (>2,000 mg/kg bw/day) that induced maternal weight loss, possibly due to loss of foetuses. In the main study, at 720 mg/kg bw/day (and not significantly in the dose finding study at 1,000 mg/kg bw/day which may be related to the smaller number of animals), an increase of skeletal

variations (total number of rudimentary and wavy/fused ribs) was observed in the absence of maternal toxicity. In the other rat study, by Yamano et al. (1993), no live litters were observed in a dose finding study at doses (3,000 and 4,000 mg/kg bw/day) that also resulted in a reduced maternal body weight gain. In the main study at 1,800 mg/kg bw/day in the absence of general toxicity, the incidence of resorptions was increased, malformations of the cardiovascular system (aortic arch; ventricular septum) and skeletal variations were found and foetal weight was reduced. At 600 mg/kg bw/day, a decrease in foetal weight and an increase in variations (both skeletal and, statistically not significant, visceral) were reported. In the postnatal study in rats by Yamano et al. (1993), the number of pups delivered was significantly smaller at 1800 mg/kg bw/day and duration of gestation was significantly increased. On PND4, the percentage of live pups was non-significantly reduced at 600 mg/kg bw/day and significantly reduced at 1800 mg/kg bw/day. Body weight gain of pups was slightly reduced at 600 mg/kg bw/day at PND 21 and strongly decreased for the single litter surviving at PND21 from the high dose group.
A non-statistically significant reduction in survival of the offspring at PND5 was seen in rats after subcutaneous exposure to 1,000 µL/kg bw/day (1,020 mg/kg bw/day). In the mouse study, mice were orally exposed to 4,000 mg/kg bw/day (corresponding to the $LD_{10}$) (Doe, 1984). Effects were seen in the percentage of viable litters, the number of live pups per litter and pup survival over the first 3 days post-partum. At this high dose, 10% of the exposed dams died during exposure. Rabbits were dermally exposed to DEGME up to 750 mg/kg bw/day (Scortichini et al., 1986). At the highest dose, at which maternal toxicity was observed, a non-significant increase in embryonic resorptions and developmental variations was found. At 250 mg/kg bw/day, cranial variations were noted in the offspring in the absence of maternal toxicity.
DEGME is metabolised in rats to a small amount of MAA (approximately 1%), which induces comparable cardiovascular malformations as DEGME but at much lower concentrations.

### 10.8.12 Comparison with the CLP criteria

There is insufficient human data to classify the substance in category 1A.

Category 1B:.according to the CLP regulation (EC 1272/2008):

*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 or 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.*

Category 2 may be more appropriate when:
*There is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or 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. Such effects shall have been observed 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 the other toxic effects.*

In several species, severe developmental effects were reported (i.e. reduced foetal viability in rats, mice and rabbits; increased visceral malformations in rats). The severe effects observed in the mouse and the rabbit were observed at a dose level also inducing maternal lethality (approximately 10%). Therefore, these effects may be secondary to the maternal toxicity and should not be considered for classification in category 1B (CLP criteria 3.7.2.4.3).
In two studies with rats, specific and severe developmental effects have been reported, that show a dose-response relationship (Hardin et al., 1986; Yamano et al., 1993). These responses were observed at relatively high doses (≥1,800 mg/kg bw/day), at which also effects on maternal body weight occurred. Notably, it is likely the reduction in maternal body weight at 1800 mg/kg bw/day (Yamano et al., 1993) is a consequence

of the observed reduction in foetal viability. In the study by Hardin et al. (1986) the gestational body weight gain (body weight minus uterus content) was not decreased and the minor decrease in maternal body weight was therefore considered not to have affected the developmental effects. Specific developmental effects, also at relatively high doses, should not be ignored based on limit dose considerations, especially because the studies were not based on test guidelines and have a more limited power (fewer animals for example). Therefore, the developmental effects from these studies should be considered for classification (CLP criteria 3.7.2.5.7 and 3.7.2.5.9). Further, the slightly acidic urine observed at all doses seems irrelevant for the classification of developmental toxicity. The occurrence of the cardiac malformations (malformations of the aortic arch; ventricular septal defects) in rats are not causally related with the maternal toxicity reported. Notably, the cardiac malformations have also been observed in rats at doses below 1,000 mg/kg bw/day. One malformation of the aortic arch (Hardin et al., 1986) and one ventricular septal defect were observed at 720 (Hardin et al., 1986) and 600 mg/kg bw/day, respectively (Yamano et al., 1993). Although not statistically significant at these dose levels, these effects are suggestive of a dose-response relationship. Additionally, an apparent but statistically non-significant reduction in postnatal viability was reported in rats at 600 mg/kg bw/day in the absence of maternal toxicity while this effect was observed to be significant at the high dose of 1800 mg/kg bw/day suggesting dose dependence (Yamano et al., 1993).
Recently, 2-methoxyacetic acid has been confirmed as a metabolite of DEGME in rats (Triskelion, 2017). 2-Methoxyacetic acid is classified in Category 1B for developmental toxicity and causes malformations of the heart (dilated ductus arteriosus and dilated aortic arch) at a single dose of 186 mg/kg bw/day and higher on GD12 (Ritter, Scott, Randall, & Ritter, 1985). Additionally, 2-methoxyacetic acid has been identified as SVHC based on clear endocrine properties. ECETOC (2005) considers it likely MAA is responsible for the effects observed as a consequence of exposure to EGME or DEGME. Finally, 2-methoxyacetic acid belongs to the group of alkoxyacetic acids, which are reported to be eliminated slowly (ECETOC, 2005), and slower in humans than in rats (Aasmoe et al., 1999; Groeseneken et al., 1988, 1989; Moss et al., 1985). The half-life of MAA in humans is approximately 3.1-6.8 times longer than in rats indicating much higher internal exposure to MAA at comparable external exposure to DEGME. This would imply that in humans, developmental effects might occur at lower external exposure levels than in rats. The lowest dose of MAA tested in the evaluation by ECETOC was 39 mg/kg bw/day (between GD 7-18) with SD rats in a teratogenicity study (ECETOC, 2005). Clear teratogenicity was reported at the lowest dose group, including cardiovascular malformations, in 15% of the animals. Because the (plasma/urinary) half-life of MAA in rats is estimated to be in the range of 12.8–21.8 h, MAA will accumulate following repeated exposure of DEGME. This in combination with the fact that in humans the half-life of MAA is higher (77.1h ±9.5h) compared to rats, reprotoxic effects of DEGME through the metabolite MAA cannot be excluded.

Overall, the criteria for classification as developmental toxicant in Category 1B, H360D (presumed human reproductive toxicant) is warranted, based on:

- Increased visceral malformations and postnatal mortality starting at concentrations below the limit dose and reaching statistical significance at concentrations above the limit-dose in the rat in the absence of maternal toxicity.
- Formation of 2-methoxyacetic acid in potentially teratogenic amounts (1% is 10 mg/kg bw/day at the limit dose while it causes malformations from 39 mg/kg bw/day in rats, but lower concentrations have not been tested).
- Furthermore, MAA has a 3.1-6.8 fold longer half-life in humans compared to rats, suggesting that developmental effects may occur in humans at lower external dose levels compared to rats.

### 10.8.13 Adverse effects on or via lactation

### 10.8.14 Animal data

No studies were found regarding the effects of DEGME after lactational exposure in animals.

### 10.8.15 Human data

No studies were found regarding the effects of DEGME after exposure via lactation in humans.

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

No data are available on effects of DEGME exposure on or via lactation

### 10.8.17 Comparison with the CLP criteria

No data are available for comparison with the CLP criteria.

### 10.8.18 Conclusion on classification and labelling for reproductive toxicity

Based on the effects on development found in rats and rabbits, DEGME should be classified in category 1B (suspected human reproductive toxicant) and labelled with H360D (may damage the unborn child) according to Regulation (EC) 1272/2008. Derivation of an SCL was considered seen the limited effects of DEGME at dose levels close to the limit dose of 1000 mg/kg bw/day. Based on the available data the ED10 is estimated to be above 400 mg/kg b/day (no calculation needed) resulting in an SCL of 3% (CLP guidance). However, the available oral data is limited to a number of oral studies in rats with exposure starting relatively late during gestation (day 7) and ending earlier (day 17) than recommended for a normal OECD TG414. Further, no oral developmental study in rabbits is available and no generation study. Further, taking into account the difference in half-life between rats and humans which would affect the external dose at which developmental effects can be expected to some degree. Therefore, it cannot be excluded that effects can be observed at lower dose levels and no SCL is proposed.

In view of the absence of human data and relevant data on functional fertility in animals, no classification is proposed for DEGMEfor effects on fertility.

No human or animal data were available for effects of DEGME through lactation. Therefore, no classification for effects on or via lactation is proposed.

## 10.9 Specific target organ toxicity-single exposure

Hazard class not assessed in this dossier

## 10.10 Specific target organ toxicity-repeated exposure

Hazard class not assessed in this dossier

# 11 EVALUATION OF ENVIRONMENTAL HAZARDS

Environmental hazards are not assessed in this dossier

## 12 REFERENCES


Aasmoe, L., Mathiesen, M., & Sager, G. (1999). Elimination of methoxyacetic acid and ethoxyacetic acid in rat. *Xenobiotica, 29*(4), 417-424. doi:10.1080/004982599238597

Cheever, K. L., Richards, D. E., Weigel, W. W., Lal, J. B., Dinsmore, A. M., & Daniel, F. B. (1988). Metabolism of bis(2-methoxyethyl) ether in the adult male rat: Evaluation of the principal metabolite as a testicular toxicant. *Toxicology and Applied Pharmacology, 94*(1), 150-159. doi:10.1016/0041-008X(88)90345-6

Doe, J. E. (1984). Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment. *Environmental Health Perspectives, VOL. 57*, 199-206.

Dugard, P. H., Walker, M., Mawdsley, S. J., & Scott, R. C. (1984). Absorption of some glycol ethers through human skin in vitro. *Environmental Health Perspectives, VOL. 57*, 193-197.

ECETOC. (2005). *The toxicology of glycol ethers and its relevance to man* (95). Retrieved from http://www.ecetoc.org/wp-content/uploads/2014/08/ECETOC-TR-095-Vol-I.pdf

Groeseneken, D., Veulemans, H., Masschelein, R., & Van Vlem, E. (1988). Comparative urinary excretion of ethoxyacetic acid in man and rat after single low doses of ethylene glycol monoethyl ether. *Toxicology Letters, 41*(1), 57-68. doi:10.1016/0378-4274(88)90008-2

Groeseneken, D., Veulemans, H., Masschelein, R., & Van Vlem, E. (1989). Experimental human exposure to ethylene glycol monomethyl ether. *International Archives of Occupational and Environmental Health, 61*(4), 243-247. doi:10.1007/BF00381421

Hardin, B. D., Goad, P. T., & Burg, J. R. (1986). Developmental toxicity of diethylene glycol monomethyl ether (diEGME). *Fundamental and Applied Toxicology, 6*(3), 430-439. doi:10.1016/0272-0590(86)90216-2

Hobson, D. W., D'Addario, A. P., Bruner, R. H., & Uddin, D. E. (1986). A subchronic dermal exposure study of diethylene glycol monomethyl ether and ethylene glycol monomethyl ether in the male guinea pig. *Fundamental and Applied Toxicology, 6*(2), 339-348. doi:10.1016/0272-0590(86)90249-6

Karaman, M. I., Gürdal, M., Oztürk, M., & Kanberoğlu, H. (2002). Maternal exposure to diethylene glycol monomethyl ether: a possible role in the etiology of retrocaval ureter. *Journal of pediatric surgery, 37*(8).

Kawamoto, T., Matsuno, K., Kayama, F., Hirai, M., Arashidani, K., Yoshikawa, M., & Kodama, Y. (1990). Acute oral toxicity of ethylene glycol monomethyl ether and diethylene glycol monomethyl ether. *Bulletin of Environmental Contamination and Toxicology, 44*(4), 602-608. doi:10.1007/BF01700883

Krasavage, W., & Vlaovic, M. (1982). *Comparative Toxicity of Nine Glycol Ethers: III. Six Weeks Repeated Dose Study*. Retrieved from

McDougal, J. N., Pollard, D. L., Weisman, W., Garrett, C. M., & Miller, T. E. (2000). Assessment of skin absorption and penetration of JP-8 jet fuel and its components. *Toxicological Sciences, 55*(2), 247-255.

Miller, R. R., Eisenbrandt, D. L., Gushow, T. S., & Weiss, S. K. (1985). Diethylene glycol monomethyl ether 13-week vapor inhalation toxicity study in rats. *Fundamental and Applied Toxicology, 5*(6 PART 1), 1174-1179. doi:10.1016/0272-0590(85)90154-X

Moss, E. J., Thomas, L. V., Cook, M. W., Walters, D. G., Foster, P. M. D., Creasy, D. M., & Gray, T. J. B. (1985). The role of metabolism in 2-methoxyethanol-induced testicular toxicity. *Toxicology and Applied Pharmacology, 79*(3), 480-489. doi:10.1016/0041-008X(85)90145-0

Netherlands, H. C. o. t. (2003). *Diethyleneglycol (mono)alkylethers* (2003/10OSH). Retrieved from https://www.gezondheidsraad.nl/nl/taak-werkwijze/werkterrein/gezonde-arbeidsomstandigheden/diethyleneglycol-monoalkylethers-evaluation

Netherlands, H. C. o. t. (2017). *2-(2-methoxyethoxy)ethanol (DEGME)* (2017/21). Retrieved from The Hague: https://www.gezondheidsraad.nl/nl/taak-werkwijze/werkterrein/gezonde-arbeidsomstandigheden/diethyleenglycol-monomethylether-degme

RAR. (1999). *European Union Risk Assessment Report: 2-(2-methoxyethoxy)ethanol CAS-No.:111-77-3, EINECS-No.: 203-906-6, Risk assessment.* Retrieved from

Ritter, E. J., Scott, W. J., Randall, J. L., & Ritter, J. M. (1985). Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxyethanol and methoxyacetic acid in the rat. *Teratology, 32*(1), 25-31. doi:10.1002/tera.1420320105

Schuler, R. L., Hardin, B. D., & Niemeier, R. W. (1984). Results of testing fifteen glycol ethers in a short-term in vivo reproductive toxicity assay. *Environmental Health Perspectives, VOL. 57*, 141-146.

Scofield, E. H., Henderson, W. M., Funk, A. B., Anderson, G. L., & Smith, M. A. (2006). Diethylene glycol monomethyl ether, ethylene glycol monomethyl ether and the metabolite, 2-methoxyacetic acid affect in vitro chondrogenesis. *Reproductive Toxicology, 22*(4), 718-724. doi:10.1016/j.reprotox.2006.05.005

Scortichini, B. H., John-Greene, J. A., Quast, J. F., & Rao, K. S. (1986). Teratologic evaluation of dermally applied diethylene glycol monomethyl ether in rabbits. *Fundamental and Applied Toxicology, 7*(1), 68-75. doi:10.1016/0272-0590(86)90198-3

Triskelion. (2017). *Assessment of the Urinary metabolic profile of diethylene glycol monomethyl ether (DEGME) following oral dosing to male Sprague-Dawley rats* (V20955). Retrieved from

Yamano, T., Noda, T., Shimizu, M., Morita, S., & Nagahama, M. (1993). Effects of diethylene glycol monomethyl ether on pregnancy and postnatal development in rats. *Archives of Environmental Contamination and Toxicology, 24*(2), 228-235. doi:10.1007/BF01141352

# 13 ANNEXES

## 13.1 ANNEX 1, Study summaries

### 13.1.1 Summary of the developmental toxicity study by Hardin et al. (1986)

**Table A1.1. Summary table on methodology and effects**

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Sprague-Dawley rats, age unknown<br>9 females/group<br>Oral exposure to 0, 1000, 1495, 2235, 3345, 5175 mg/kg bw/day DEGME (purity unknown) by gavage on GD7-16.<br>Sacrifice on GD 21.<br>Dose finding study.<br>Non-guideline, non-GLP | Gestational body weight was reduced and 2/9 dams died in the highest dose group. At 3345 and 5175 mg/kg, litter size reduced to 0 and 10% respectively vs 91.2% in the control. At 2235 and 3345 mg/kg: foetal weight was decreased. Total number of skeletal variations, visceral and cardiovascular malformations was increased at 2235 mg/kg bw/day. | 2, reliable with restriction. | (Hardin et al., 1986) |
| Sprague-Dawley rats, age unknown<br>12-13 females/group<br>Oral exposure to 0, 720, 2165 mg/kg bw/day DEGME (purity unknown) by gavage on GD7-16. Sacrifice on GD 20.<br>Non-guideline, non-GLP | At 2165 mg/kg bw/day decreased maternal weight, decreased live births per litter (60.5% vs 90.7% in the control), reduced foetal weight, skeletal variations (ribs), visceral cardiovascular malformations, several variations including reduced ossification of various skeletal parts and urinary variations. At 720 mg/kg bw/day: skeletal variations (ribs) and reduced | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include slightly fewer animals than recommended, dosing started on GD7, not 5, endpoints unclear. Rats shipped from CRL on day 4 after gestation, | (Hardin et al., 1986) |

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| | ossification of the appendicular skeleton and dilated renal pelvis. | received on day GD5 and dosed on GD7 | |

### 13.1.1.1 Introduction and overview study design (methods & endpoints)

First a dose range finding study was performed with fewer animals (9 females/group) but more dose groups (0-5175 mg/kg bw/day). Based on the results, a second study was performed using 12-13 females/group at dose levels of 0, 720 and 2165 mg/kg bw/day where all litters and pups were investigated for gross malformations. The study design is similar to OECD TG414 but with some important deficiencies that do reduce the quality of the study. These deviations include fewer animals than recommended (12-13 instead of the recommended minimum of 16 pregnant females), significant shorter dosing time (GD 7-16 instead of the currently recommended GD5-GD20), very short acclimatisation time for the pregnant rats (received GD5 and dosed 2 days after while at least 5 days of acclimatisation is recommended). The number of dose groups is also smaller (2 + control instead of 3 plus control) although this is mitigated by the presence of the dose range finding study. No clear description is given what endpoints were considered other than general effects such as weight, food consumption and "weight, gross external defects and internal examinations". From the described observations, skeletal and visceral malformations were examined.

### 13.1.1.2 Results

At the highest dose group, 2/9 maternal animals died (Table A1.2). Additionally, food consumption, corrected body weight gain and body weight was significantly reduced in particular at GD16-21. In the second-highest dose group of 3345 mg/kg bw/day body weight (but not corrected body weight gain) was significantly reduced as well at GD21.

**Table A1.2 Dose finding study maternal general data**

DOSE-FINDING STUDY MATERNAL DATA (MEAN ± SD)

| | Dose (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 1495 | 2235 | 3345 | 5175 |
| No. survivors/treated | 9/9 | 9/9 | 9/9 | 9/9 | 9/9 | 7/9 |
| No. live litters/preg. | 9/9 | 8/8 | 4/4 | 8/8 | 3/9* | 0/5* |
| Maternal body weight (g) | | | | | | |
| Day 7 | 207 ± 14 | 216 ± 12 | 215 ± 14 | 211 ± 10 | 210 ± 15 | 208 ± 14 |
| 12 | 246 ± 17 | 251 ± 11 | 254 ± 15 | 247 ± 16 | 240 ± 22 | 228 ± 36 |
| 16 | 275 ± 23 | 276 ± 16 | 282 ± 17 | 278 ± 16 | 261 ± 23 | 244 ± 29* |
| 21 | 337 ± 34 | 327 ± 30 | 337 ± 30 | 325 ± 19 | 279 ± 28* | 239 ± 23* |
| Extra gestational weight gain (g)[a] | 77 ± 18 | 76 ± 22 | 77 ± 17 | 72 ± 10 | 70 ± 18 | 54 ± 19* |
| Food consumption (g) | | | | | | |
| Days 7–12 | 123 ± 18 | 119 ± 11 | 123 ± 12 | 116 ± 11 | 96 ± 20* | 79 ± 21* |
| Days 12–17 | 133 ± 21 | 134 ± 13 | 134 ± 15 | 134 ± 13 | 120 ± 14 | 109 ± 14 |
| Days 17–21 | 107 ± 10 | 111 ± 14 | 114 ± 18 | 116 ± 9 | 109 ± 14 | 92 ± 16 |

[a] (Day 21 body weight)—(gravid uterus weight)—(Day 6 body weight).
* Significantly reduced relative to control group ($p < 0.05$).

**Table A1.3 Dose finding study litter general data**

DOSE-FINDING STUDY LITTER DATA (MEAN ± SD)

| | Dose (mg/kg/day) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1000 | 1495 | 2235 | 3345 | 5175 |
| Implants/litter | 13.2 ± 2.4 | 10.5 ± 3.6 | 13.0 ± 4.8 | 12.4 ± 1.6 | 13.1 ± 2.4 | 14.2 ± 1.3 |
| Live/litter | | | | | | |
| Number | 12.1 ± 3.0 | 9.5 ± 3.3 | 11.5 ± 4.4 | 10.8 ± 1.7 | 3.3 ± 0.6* | 0* |
| Percent | 91.2 ± 11.9 | 90.8 ± 7.9 | 89.7 ± 12.6 | 87.1 ± 9.8 | 9.2 ± 13.8* | 0* |
| Fetal weight (g) | | | | | | |
| Male | 4.0 ± 0.6 | 3.8 ± 0.8 | 3.6 ± 0.6 | 3.5 ± 0.8 | 2.3 ± 1.3* | NA |
| Female | 3.8 ± 0.5 | 3.5 ± 0.8 | 3.3 ± 0.7 | 3.2 ± 0.6* | 2.4 ± 0.5* | NA |
| Gross malformations: litters (fetuses)/mean of percent fetuses per litter | | | | | | |
| No. examined | 9 (109) | 8 (76) | 4 (46) | 8 (86) | 3 (11)[a] | NA |
| Malformations | 1 (1)/1[b] | 0 | 1 (1)/1[c] | 2 (3)/4[d] | 0 | NA |

[a] Includes one dead fetus.
[b] Exencephaly.
[c] Short tail (about half normal length).
[d] One litter had one fetus with acaudia and imperforate anus, and one edematous fetus. A second litter had one fetus with acaudia and imperforate anus.
* Significantly reduced relative to control group ($p < 0.05$).

The number of pregnant animals was reduced in the highest dose group (5/7) and did not produce any live foetuses. At 3345 mg/kg bw/day only 3/9 litters had some live foetuses (table A1.2). Maternal toxicity was considered by Hardin et al. (1986) to be minimal at 3345 based on the approximately 22% observed reduction in body weight. In addition, extra gestational weight gain was not affected at this dose level. Maternal toxicity was not considered relevant at lower doses.
Live foetuses as a percentage of implants and foetal body weight declined with increasing dose (table A1.3) and was significant at 3345 mg/kg bw/day. Foetal body weight of female foetuses was also significantly reduced already at 2235 mg/kg bw/day.
Skeletal malformations were observed in foetuses from all dose groups (table A1.4) but was significant only at doses from 2235 mg/kg bw/day. A very limited number of litters and foetuses in the mid dose group (1496 mg/kg bw/day) were examined. Two foetuses from two different litters out of the 4 litters examined had skeletal malformation (non-significant). The majority of skeletal malformations were bilateral wavy ribs sometimes accompanied with fused ribs, but none of these individual malformations were statistically significant increased. Statistical significant increases of skeletal ossifications were observed at 1495 mg/kg bw/day and higher doses.
An overall increase of dose-related, but mostly not statistically significant, visceral malformations was observed (table A1.5). Only some statistically significant cardiovascular effects were seen at 2235 mg/kg bw/day but not at the higher 3345 mg/kg bw/day dose. Combining skeletal and visceral malformations, the percentage of litters with at least one malformed foetus were 22.3%, 37.5%, 100%, 100% and 100% at 0, 1000, 1495, 2235 and 3345 mg/kg bw/day respectively.

***Main teratology study***

Doses of 720 mg/kg bw/day and 2165 mg/kg bw/day were given via gavage based on the "dose related patterns of embryo/foetal toxicity" seen in the previous dose range finding study. Limited reduced food consumption in the first 5 days and maternal body weight at day 21 was observed in maternal animals at the highest dose (table A1.6). These effects were not considered to have affected reproductive toxicity, in part because the extra gestational weight gain was not reduced. At 2165 mg/kg bw/day litter size and foetal

weight were significantly reduced (table A1.7) and 2/23 litters were completely resorbed. No gross evidence for foetal toxicity observed at 720 mg/kg bw/day although the average body weight was slightly (non-significant) lower compared to the control group.
Many statistically significant skeletal malformations/variations were observed at the highest dose group including wavy/fused ribs and reduced ossification in various bone tissues (table A1.8). At 720 mg/kg bw/day, fewer, but statistically significant rudimentary cervical and wavy bilateral rib malformations (combined) and reduced cranial/appendicular skeleton ossifications were found. These effects were increased more in the high dose suggesting a dose-dependent relationship.
Visceral malformations include predominantly cardiovascular effects in the high dose group and a single case (1/115) in the low dose group and 0/129 cases in the control group suggesting a dose-dependent relationship (table A1.9). Additionally, the incidence of dilated renal pelvis was statistically increased in both dose groups with a treatment related effect. Combining gross, skeletal and visceral effects, then the percentage of litters with at least one malformation were statistically increased in both treatment groups (22.7% of control litters, 52.4% of litters at 720 mg/kg bw/day and 90.5% of litters at the high dose group were affected).

**Table A1.4 Dose finding study skeletal malformations**

DOSE-FINDING STUDY SKELETAL MALFORMATIONS

| | Affected litters (fetuses)/percent[a] dose (mg/kg/day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | | 1000 | | 1495 | | 2235 | | 3345 | |
| Number examined | 9 (55) | | 8 (38) | | 4 (23) | | 8 (42) | | 3 (6) | |
| Malformations | | | | | | | | | | |
| Vertebrae | | | | | | | | | | |
| Missing thoracic | 0 | | 0 | | 1 (1)/3 | | 0 | | 0 | |
| Abnormal cervical arch | 1 (1)/3 | | 0 | | 1 (1)/3 | | 0 | | 0 | |
| Total | 1 (1)/3 | | 0 | | 1 (2)/6 | | 0 | | 0 | |
| Ribs | | | | | | | | | | |
| Rudimentary cervical | 1 (1)/3 | | 0 | | 0 | | 4 | (6)/16 | 1 | (1)/17 |
| Missing | 0 | | 0 | | 1 (1)/3 | | 0 | | 0 | |
| Wavy/fused: unilateral | 0 | | 0 | | 0 | | 1 | (1)/3 | 1 | (1)/17 |
| bilateral | 0 | | 1 (2)/5 | | 2 (2)/7 | | 4 | (6)/15 | 0 | |
| Total | 1 (1)/3 | | 1 (2)/5 | | 2 (3)/10 | | 5 | (12)/31 | 1 | (1)/17 |
| Cleft sternebrae | 0 | | 0 | | 0 | | 1 | (1)/3 | 0 | |
| Total malformations | 1 (1)/3 | | 1 (2)/5 | | 2 (4)/13 | | 6* | (13)/34 | 1 | (1)/17 |
| Variations | | | | | | | | | | |
| Reduced cranial ossification | 2 (2)/3 | | 3 (8)/23 | | 4* (13)/60 | | 6* | (15)/36 | 3* | (5)/83 |
| Sternebrae | | | | | | | | | | |
| Misaligned | 2 (4)/6 | | 4 (5)/14 | | 2 (3)/13 | | 6 | (8)/18 | 2 | (2)/33 |
| Reduced ossification | 1 (1)/2 | | 0 | | 2 (5)/16 | | 2 | (2)/5 | 3 | (4)/67 |
| Total | 3 (5)/7 | | 4 (5)/14 | | 3 (8)/29 | | 6 | (10)/24 | 3 | (5)/83 |
| Vertebrae | | | | | | | | | | |
| Misaligned | 0 | | 2 (2)/5 | | 1 (1)/8 | | 8*** | (15)/36 | 3** | (4)/67 |
| Reduced ossification | 2 (2)/3 | | 1 (1)/4 | | 2 (5)/31 | | 6* | (15)/36 | 3* | (6)/100 |
| Total | 2 (2)/3 | | 3 (3)/9 | | 2 (5)/31 | | 8** | (20)/48 | 3* | (6)/100 |
| Thoraco-lumbar ribs | 4 (4)/8 | | 1 (1)/2 | | 2 (4)/24 | | 6 | (8)/17 | 0 | |
| Appendicular skeleton | | | | | | | | | | |
| Reduced ossification | 2 (2)/3 | | 1 (1)/2 | | 3 (10)/38 | | 5 | (13)/30 | 2 | (3)/50 |
| Total variations | 6 (11)/18 | | 5 (12)/33 | | 4 (18)/80 | | 8 | (32)/76 | 3 | (6)/100 |

[a] Mean of percent fetuses per litter.
Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

**Table A1.5 Dose finding study visceral malformations**

DOSE-FINDING STUDY VISCERAL MALFORMATIONS

| | Affected litters (fetuses)/percent[a] dose (mg/kg/day) | | | | |
|---|---|---|---|---|---|
| | 0 | 1000 | 1495 | 2235 | 3345 |
| Number examined | 9 (54) | 8 (38) | 4 (23) | 8 (44) | 3 (5) |
| Malformations | | | | | |
| Cardiovascular | | | | | |
| Double aortic arch[b] | 0 | 0 | 0 | 3 (4)/10 | 0 |
| Right aortic arch | 0 | 1 (1)/2 | 0 | 1 (1)/3 | 2 (2)/33 |
| Right ductus arteriosis | 0 | 1 (1)/2 | 0 | 1 (1)/3 | 2 (2)/33 |
| Ventricular septal defect | 0 | 1 (1)/2 | 0 | 2 (2)/5 | 2 (2)/33 |
| Total | 0 | 1 (1)/2 | 0 | 4* (7)/17 | 2 (3)/50 |
| Brain | | | | | |
| Hydrocephalus | 0 | 1 (2)/8 | 0 | 3 (8)/17 | 2 (2)/50 |
| Eye | | | | | |
| Folded retina | 0 | 0 | 0 | 1 (1)/3 | 2 (2)/50 |
| Anophthalmia | 1 (1)/2 | 0 | 0 | 0 | 0 |
| Urinary | | | | | |
| Hydroureter | 1 (1)/2 | 0 | 0 | 0 | 0 |
| Hydronephrosis | 1 (1)/2 | 1 (3)/6 | 3 (4)/25 | 1 (1)/3 | 1 (1)/33 |
| Ectopic kidney | 0 | 0 | 0 | 1 (1)/2 | 0 |
| Fused kidneys | 0 | 0 | 0 | 1 (1)/2 | 0 |
| Total | 2 (2)/3 | 1 (3)/6 | 3 (4)/25 | 2 (3)/7 | 1 (1)/33 |
| Miscellaneous | | | | | |
| Tracheo–esophageal transposition | 0 | 0 | 0 | 1 (1)/2 | 0 |
| Ectopic stomach | 0 | 0 | 0 | 1 (1)/2 | 0 |
| Total Malformations | 2 (2)/3 | 3 (6)/16 | 3 (4)/25 | 6 (15)/23 | 3 (5)/100 |
| Variations | | | | | |
| Cardiovascular | | | | | |
| Missing innominate | 0 | 0 | 0 | 4* (6)/15 | 0 |
| Brain | | | | | |
| Hemorrhage | 0 | 0 | 1 (1)/5 | 0 | 0 |
| Urinary | | | | | |
| Dilated renal pelvis | 2 (2)/3 | 4 (4)/11 | 4 (6)/30 | 4 (6)/14 | 1 (1)/33 |
| Dilated ureter | 2 (2)/3 | 3 (3)/10 | 1 (1)/8 | 1 (1)/2 | 0 |
| Total | 4 (4)/6 | 5 (6)/17 | 4 (7)/38 | 4 (6)/14 | 1 (1)/33 |
| Total variations | 4 (4)/6 | 5 (6)/17 | 4 (8)/43 | 7 (11)/26 | 1 (1)/33 |

[a] Mean of percent fetuses per litter.

[b] Ascending aorta bifurcated to form a vascular ring around the trachea and esophagus, then reformed as a single descending aorta.

* Differs significantly from corresponding control group, $p < 0.05$.

[04.01-MF-003.01]

**Table A1.6 Main study maternal data**

MATERNAL DATA (MEAN ± SD)

| | Dose (mg/kg/day) | | |
|---|---|---|---|
| | 0 | 720 | 2165 |
| No. survivors/treated | 25/25 | 25/25 | 25/25 |
| No. live litters/preg. | 22/22 | 21/21 | 21/23 |
| Maternal body weight (g) | | | |
| Day 7 | 213 ± 7 | 213 ± 9 | 212 ± 11 |
| 12 | 248 ± 10 | 251 ± 13 | 245 ± 13 |
| 16 | 278 ± 14 | 279 ± 17 | 273 ± 17 |
| 21 | 332 ± 18 | 332 ± 24 | 308 ± 29* |
| Extra gestational weight gain (g)[a] | 47 ± 13 | 53 ± 14 | 46 ± 11 |
| Food consumption (g) | | | |
| Days 7–12 | 120 ± 13 | 122 ± 14 | 111 ± 13* |
| Days 12–17 | 128 ± 14 | 127 ± 25 | 128 ± 12 |
| Days 17–21 | 105 ± 10 | 109 ± 12 | 106 ± 23 |

[a] (Day 21 body weight)—(gravid uterus weight)—(Day 6 body weight).
* Significantly reduced relative to the control group ($p < 0.05$).

**Table A1.7 Main study litter data**

LITTER DATA (MEAN ± SD)

| | Dose (mg/kg/day) | | |
|---|---|---|---|
| | 0 | 720 | 2165 |
| Implants/litter | 12.6 ± 1.9 | 11.8 ± 2.5 | 12.0 ± 2.7 |
| Live/litter | | | |
| Number | 11.4 ± 2.0 | 10.8 ± 2.8 | 7.4 ± 3.9* |
| Percent | 90.7 ± 8.8 | 90.5 ± 10.0 | 60.5 ± 31.5* |
| Fetal weight (g) | | | |
| Male | 4.6 ± 0.8 | 4.5 ± 0.8 | 3.5 ± 0.8* |
| Female | 4.4 ± 0.7 | 4.2 ± 0.7 | 3.2 ± 0.9* |
| Gross malformations: litters (fetuses)/mean of percent fetuses per litter | | | |
| No. Examined | 22 (252) | 21 (226) | 21 (171) |
| Malformations[a] | 1 (1)/0.4[b] | 0 | 5 (5)/3[c] |

[a] Litters with gross malformations differed significantly across groups ($\chi^2 = 7.93$, $df = 2$, $p < 0.05$), but control and 2165 mg/kg/day groups did not differ significantly by Fischer's exact test ($p < 0.10$).
[b] Acaudia, imperforate anus.
[c] Acaudia, imperforate anus (four fetuses); gross edema (one fetus)
* Significantly reduced relative to control group ($p < 0.05$).

**Table A1.8 Main study skeletal malformations**

SKELETAL MALFORMATIONS

| | Affected litters (fetuses)/percent[a] dose (mg/kg/day) | | |
|---|---|---|---|
| | 0 | 720 | 2165 |
| Number examined | 22 (123) | 21 (111) | 20 (89) |
| Malformations | | | |
| Vertebral | | | |
| Abnormal thoracic arch | 0 | 0 | 1 (1)/2 |
| Missing sacrococcygeal | 0 | 0 | 2 (2)/2 |
| Total | 0 | 0 | 3 (3)/4 |
| Ribs | | | |
| Rudimentary cervical | 1 (2)/2 | 5 (9)/8 | 11*** (16)/18 |
| Wavy/fused: unilateral | 0 | 0 | 3 (3)/3 |
| bilateral | 1 (4)/3 | 4 (6)/6 | 13*** (32)/36 |
| Total | 2 (6)/4 | 9* (15)/15 | 16*** (43)/48 |
| Total malformations | 2 (6)/4 | 9* (15)/15 | 16*** (45)/51 |
| Variations | | | |
| Reduced cranial ossification | 4 (6)/4 | 10* (17)/16 | 16*** (51)/56 |
| Sternebrae | | | |
| Reduced ossification | 1 (1)/1 | 1 (1)/1 | 11*** (22)/28 |
| Misaligned | 9 (13)/10 | 11 (13)/12 | 14 (23)/25 |
| Total | 9 (13)/10 | 12 (14)/13 | 17** (40)/47 |
| Vertebrae | | | |
| Reduced ossification | 0 | 0 | 15*** (44)/58 |
| Misaligned centra | 0 | 2 (4)/4 | 19*** (61)/74 |
| Extra | 1 (1)/1 | 0 | 10** (15)/21 |
| Total | 1 (1)/1 | 2 (4)/4 | 19*** (68)/81 |
| Ribs | | | |
| Reduced ossification | 0 | 0 | 3 (4)/5 |
| Thoraco–lumbar | 8 (10)/7 | 3 (3)/4 | 15* (35)/42 |
| Total | 8 (10)/7 | 3 (3)/4 | 16** (38)/45 |
| Appendicular skeleton | | | |
| Reduced ossification | 1 (1)/1 | 6* (13)/12 | 15*** (41)/53 |
| Total variations | 14 (24)/18 | 15 (33)/30 | 20** (82)/94 |

[a] Mean of percent fetuses per litter.
Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

**Table A1.9 Main study visceral malformations**

VISCERAL MALFORMATIONS

| | Affected litters (fetuses)/percent[a] dose (mg/kg/day) | | |
|---|---|---|---|
| | 0 | 720 | 2165 |
| Number examined | 22 (129) | 21 (115) | 21 (82) |
| Malformations | | | |
| Cardiovascular | | | |
| Double aortic arch[b] | 0 | 0 | 7** (9)/12 |
| Right aortic arch | 0 | 1 (1)/1 | 6** (6)/10 |
| Right ductus arteriosis | 0 | 0 | 1 (1)/5 |
| Ventricular septal defect | 0 | 0 | 14*** (27)/39 |
| Total | 0 | 1 (1)/1 | 15*** (33)/46 |
| Brain | | | |
| Hydrocephalus | 1 (1)/1 | 0 | 0 |
| Eye | | | |
| Folded retina | 0 | 0 | 1 (1)/1 |
| Anophthalmia | 0 | 1 (1)/1 | 0 |
| Microphthalmia | 1 (1)/2 | 0 | 0 |
| Urinary | | | |
| Hydroureter | 1 (1)/1 | 1 (1)/1 | 0 |
| Hydronephrosis | 0 | 2 (2)/2 | 5 (6)/7 |
| Total | 1 (1)/1 | 2 (2)/2 | 5 (6)/7 |
| Total malformations | 3 (3)/3 | 4 (4)/3 | 16*** (37)/50 |
| Variations | | | |
| Cardiovascular | | | |
| Missing innominate | 0 | 1 (1)/1 | 1 (1)/1 |
| Urinary | | | |
| Dilated renal pelvis | 2 (4)/3 | 8* (11)/14 | 12** (17)/23 |
| Dilated ureter | 4 (4)/3 | 3 (5)/5 | 1 (1)/1 |
| Total | 5 (7)/6 | 9 (13)/16 | 12* (18)/24 |
| Total variations | 5 (7)/6 | 10 (14)/17 | 12* (19)/25 |

[a] Mean of percent fetuses per litter.

[b] Ascending aorta bifurcated to form a vascular ring around the trachea and esophagus, then reformed as a single descending aorta.

Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

#### 13.1.1.3 Discussion & Summary

The malformations observed in this study were according to Hardin et al. (1986) very similar as seen with EGEE, EGME and EGEEA likely due to their common metabolism. However, DEGME is not as potent, possibly because the amount of MAA (suspected of being the active toxic metabolite causing the developmental effects) produced is much smaller. In summary, DEGME causes a reduction of litter size and

foetal body weight with two litters being completely resorbed at 2165 mg/kg bw/day in the absence of maternal toxicity. Additionally, clear skeletal and visceral malformations were observed. Minor skeletal and visceral malformations in the low dose group of 720 mg/kg bw/day suggest a dose-dependent relationship. The study seems decently performed and described but lacks histopathological examinations.

### 13.1.2 Summary of the developmental toxicity study by Yamano et al. (1993)

**Table A2.1. Summary table on methodology and effects**

| Method | Results | Klimisch score, Remarks | Reference |
|---|---|---|---|
| Dose finding study.<br>Wistar Rats, >3 months old<br>4-6 females/dose group<br>Oral exposure to 0, 125, 250, 500, 1000, 2000, 3000 and 4000 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at GD20.<br>Similar to OECD TG 414 with deviations (fewer animals than recommended, dosing started on GD7, not 5, individual housing), non-guideline, non-GLP | Maternal body weight gain was reduced at ≥2000 mg/kg bw/day. At ≥3000 mg/kg bw/day reduced food consumption by maternal animals. There was a dose-dependent decrease in number of live foetuses. No live foetuses at 3000 and 4000 mg/kg bw/day. | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include fewer animals than recommended; dosing started on GD7 and ended at GD17, not 5-20, endpoints unclear. | (Yamano et al., 1993) |
| Teratology study.<br>Wistar Rats, >3 months old<br>14 females/dose group<br>Oral exposure to 0, 200, 600 and 1800 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at GD20.<br>Non-guideline, non-GLP | Parental toxicity: At 1800 mg/kg bw/day decreased maternal body weight gain, food consumption and thymus weight. Foetal toxicity: decrease in foetal body weight at 600 and 1800 mg/kg bw/day. At 1800 mg/kg bw/day: external malformations, visceral malformations of the cardiovascular system, increased skeletal variations. At 600 mg/kg bw/day: variations in ossification of sternebrae and vertebrae | 2, reliable with restriction. Method shares some similarity with OECD TG 414. Deviations include slightly animals than recommended (minimum 16), dosing started on GD7 and ended at GD17, not 5-20, endpoints unclear. | (Yamano et al., 1993) |
| Postnatal study.<br>Wistar Rats, >3 months old<br>8 females/dose group<br>Oral exposure to 0, 200, 600 and 1800 mg/kg bw/day DEGME (purity 99%) by gavage on GD7-17.<br>Rats sacrificed at 21 postpartum.<br>Non-guideline, non-GLP | Prolonged gestational period and reduced number of live pups at 1800 mg/kg bw/day. Reduced viability of pups at 600 and 1800 mg/kg bw/day. | 2, reliable with restriction. | (Yamano et al., 1993) |

#### 13.1.2.1 Introduction and overview study design (methods & endpoints)

Similar to the study by Hardin et al. (1986), a dose range finding study was performed first with fewer animals (4-6 females/group) but more (8) dose groups (0-4000 mg/kg bw/day). Additionally, groups of 5 non-pregnant rats were dosed as well. Based on the results, a second study was performed using 22 females/group (of which 14 were euthanized at GD 20 and the remaining 8 used for postnatal investigations)

at dose levels of 0, 200, 600 and 1800 mg/kg bw/day where all litters and pups were investigated for gross malformations. The study design is similar to OECD TG414 but with some important deficiencies that reduce the quality of the study. These deviations include fewer animals than recommended (14 instead of the recommended minimum of 16 pregnant females), shorter/different dosing time (GD 7-17 instead of the currently recommended GD5-GD20, or GD5-15 at the time of the study). Other improvements of the current guideline over the study by Hardin et al. (1986) insufficient acclimatization time, sufficient dose groups (3 plus control) in the main study, few more animals/group although still below the recommended number. Additional endpoints were investigated and include for non-pregnant rats some hematologic parameters, biochemical parameters, urinalysis on day 10 within 30 min after DEGME treatment and measurement of 11 organ weights on day 12 after overnight starvation. For pregnant females, thymus weights were measured after euthanasia at GD20. No histopathological investigation was included. General investigation with the remaining 8 dams from the main "teratology" study included length of gestation, no of stillborn/ live pups, litter sizes and external anomalies. On PND4 culling was performed to leave 8 pups per litter with approximately similar number males/females. During lactation, pups were examined for growth/external differentiation. Body weights were recorded on postnatal days 7, 14 and 21. At PND21 the pups were euthanized and skeletal observations performed using soft X-rays.

#### 13.1.2.2 Results

***Dose range finding study***

In non-pregnant treated rats, body weight gain and food consumption was statistically decreased at doses >3000 mg/kg bw/day (Fig. A2.1). The actual numbers represented in the figure were not listed. Urinary pH turned acidic at the lowest dose (125 mg/kg bw/day). Haematological parameters measured were decreased in a dose dependent manner. Statistically different decreases ($p < 0.01$) were seen at 4000 mg/kg bw/day in red blood cell count ($7.59 \times 10^6/mm^3$ vs $8.3 \times 10^6/mm^3$ in control), white blood cell count ($3.2 \times 10^3$/mm3 vs $5.1 \times 103/mm^3$), and haemoglobin concentrations (13.5 g/dl vs 15.5 g/dl in control). Haematocrit levels (41.3 or 39.6% vs 44.9% in control) were significantly decreased at dose levels of 3000 and 4000 mg/kg bw/day. Blood urea nitrogen (20.6 mg/dl vs 16.3 mg/dl in control) and relative kidney weights (table A2.3) were significantly increased at 4000 mg/kg bw/day suggesting nephrotoxicity. Total cholesterol, triglyceride, was significantly increased (64.1 mg/dl vs 33.9 in control) at 4000 mg/kg bw/day and alkaline phosphatase levels was significantly decreased at 2000 mg/kg bw/day only (6.4 KAU/L vs 8.7 KAU/L), although the levels were low as well (not significant) at 3000 and 4000 mg/kg bw/day (6.6 KAU/L). No signs of hepatotoxicity observed from haematological, biochemical or organ weight parameters. However, the relative organ weights of thymus and pituitary gland (table A2.3) were decreased in a dose-dependent manner with significant differences found at dose levels of 3000 and 4000 mg/kg bw/day.

In pregnant rats, maternal body weight and food consumption were decreased at >2000 mg/kg bw/day and >3000 mg/kg bw/day respectively (fig. A2.1 c/d). At 2000 mg/kg bw/day the male foetal weight and number of live foetuses was significantly decreased. At >3000 mg/kg bw/day the litters were completely resorbed.

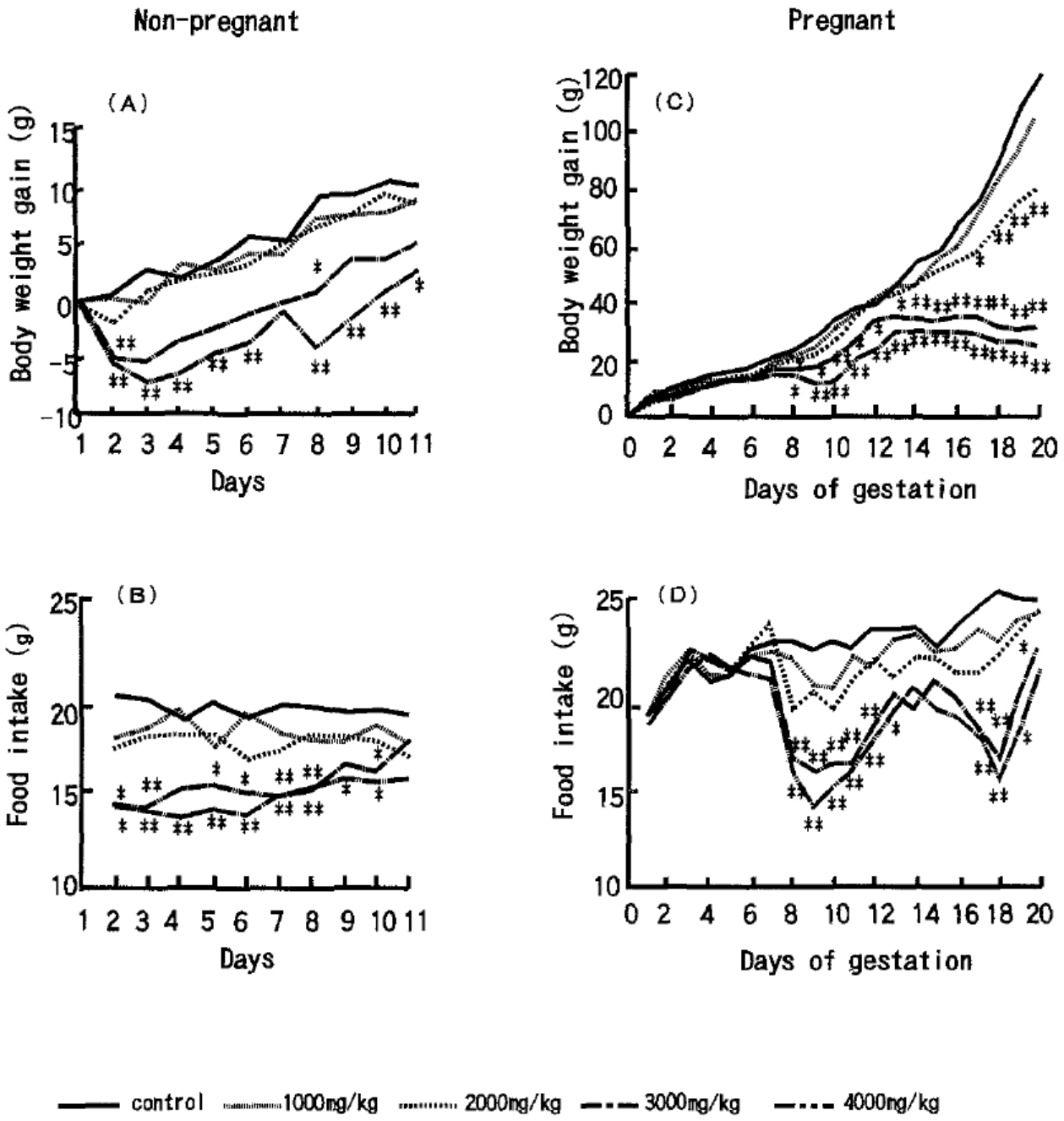



**Figure A2.1 Body weight gain (a/c) and food consumption (b/d) of non-pregnant and pregnant rats treated orally with DEGME (dose finding study). Non-pregnant rats were treated with DEGME for 11 consecutive days and pregnant rats from GD7 to GD17. Each value represents the mean of five rats (non-pregnant) or 4-6 rats (pregnant). Significantly different from control is depicted with * ($p < 0.05$) and ** ($p<0.01$).**

**Table A2.3 Effects of oral treatment with DEGME on relative organ weight in non-pregnant rats (dose finding study) orally treated for 11 consecutive days. Mean ± SE from five rats/group is represented.**

| Dose (mg/kg) | Liver | Kidney | Heart | Spleen | Stomach | Brain |
|---|---|---|---|---|---|---|
| Control | 2.90 ± 0.03 | 0.74 ± 0.01 | 0.36 ± 0.01 | 0.27 ± 0.01 | 0.64 ± 0.01 | 0.82 ± 0.03 |
| 125 | 2.90 ± 0.04 | 0.77 ± 0.01 | 0.37 ± 0.01 | 0.27 ± 0.01 | 0.63 ± 0.02 | 0.83 ± 0.02 |
| 250 | 2.84 ± 0.06 | 0.71 ± 0.01 | 0.33 ± 0.01 | 0.27 ± 0.01 | 0.59 ± 0.01 | 0.84 ± 0.03 |
| 500 | 2.81 ± 0.12 | 0.71 ± 0.01 | 0.35 ± 0.01 | 0.27 ± 0.01 | 0.60 ± 0.02 | 0.82 ± 0.03 |
| 1000 | 2.85 ± 0.10 | 0.71 ± 0.01 | 0.36 ± 0.01 | 0.27 ± 0.02 | 0.59 ± 0.01 | 0.83 ± 0.01 |
| 2000 | 2.75 ± 0.06 | 0.75 ± 0.02 | 0.35 ± 0.01 | 0.26 ± 0.01 | 0.61 ± 0.02 | 0.83 ± 0.01 |
| 3000 | 2.87 ± 0.07 | 0.77 ± 0.01 | 0.35 ± 0.01 | 0.25 ± 0.01 | 0.58 ± 0.02 | 0.83 ± 0.01 |
| 4000 | 2.93 ± 0.03 | 0.83 ± 0.01** | 0.37 ± 0.01 | 0.24 ± 0.01 | 0.63 ± 0.02 | 0.83 ± 0.02 |

(organ weight/body weight × $10^2$)

| Dose(mg/kg) | Adrenal G. | Thymus | Ovary | Pituitary G. |
|---|---|---|---|---|
| Control | 39.02 ± 2.89 | 114.49 ± 3.46 | 47.17 ± 1.18 | 6.64 ± 0.47 |
| 125 | 40.59 ± 0.92 | 119.44 ± 7.89 | 48.40 ± 0.86 | 6.23 ± 0.24 |
| 250 | 40.54 ± 1.10 | 115.37 ± 7.85 | 46.79 ± 1.17 | 6.14 ± 0.60 |
| 500 | 41.78 ± 1.90 | 124.11 ± 6.15 | 50.85 ± 2.10 | 5.81 ± 0.52 |
| 1000 | 39.00 ± 1.65 | 101.26 ± 8.75 | 48.54 ± 0.66 | 5.73 ± 0.27 |
| 2000 | 39.17 ± 0.97 | 85.46 ± 2.24 | 43.36 ± 1.78 | 5.34 ± 0.16 |
| 3000 | 37.27 ± 2.56 | 49.56 ± 2.15 | 44.92 ± 1.51 | 5.05 ± 0.24* |
| 4000 | 41.88 ± 2.53 | 35.62 ± 1.77** | 50.78 ± 2.38 | 4.97 ± 0.30* |

(organ weight/body weight ×$10^5$)

*Significantly different from control at $p < 0.05$
** Significantly different from control at $p < 0.01$

**Table A2.4 Effects of oral treatment with DEGME on pregnant rats and their foetuses (dose finding study).**

| | diEGME (mg/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | 125 | 250 | 500 | 1000 | 2000 | 3000 | 4000 |
| No. of dams | 6 | 5 | 4 | 4 | 5 | 6 | 6 | 5 |
| No. of corpora lutea[a] | 15.8 ± 0.6 | 16.6 ± 0.9 | 15.3 ± 0.3 | 15.5 ± 0.6 | 15.6 ± 0.8 | 15.8 ± 0.9 | 17.7 ± 0.9 | 18.8 ± 2.2 |
| No. of implants | 15.0 ± 0.4 | 15.0 ± 0.5 | 15.0 ± 0.4 | 14.5 ± 0.6 | 15.2 ± 0.5 | 14.3 ± 0.7 | 14.2 ± 1.3 | 13.1 ± 2.1 |
| No. of live fetuses | 14.7 ± 0.5 | 14.0 ± 0.6 | 14.3 ± 0.9 | 13.8 ± 0.8 | 12.8 ± 1.1 | 5.2 ± 1.3 | 0* | 0* |
| Incidence of dead or resorbed fetuses (%) | | | | | | | | |
| Early stage | 2.2 | 6.7 | 3.3 | 3.5 | 7.9 | 44.0 | 96.7 | 100.0* |
| Late stage | 0 | 0 | 1.7 | 1.8 | 8.0 | 21.1* | 3.3 | 0 |
| Sex ratio Male/Female | 49/39 | 37/33 | 28/29 | 31/24 | 41/23 | 18/13 | — | — |
| Fetal weight[a] (g) | | | | | | | | |
| Male | 3.2 ± 0.08 | 3.1 ± 0.06 | 3.1 ± 0.06 | 3.4 ± 0.38 | 2.7 ± 0.07 | 2.2 ± 0.04* | — | — |
| Female | 3.0 ± 0.09 | 2.8 ± 0.10 | 3.0 ± 0.04 | 3.2 ± 0.39 | 2.5 ± 0.06 | 2.2 ± 0.11 | — | — |
| Incidence of fetuses with malformations (%) | 0 | 0 | 0 | 0 | 2 [1] (1)[b] | 13 [3] (2)[c] | | |
| Incidence of fetuses with other anomalies (%) | 0 | 0 | 0 | 0 | 0 | 18 [5] (3)[d] | | |

[a] Mean ± SE
[b] Omphalocele
[c] Anasarca (2 fetuses), anury (1 fetus)
[d] Dorsum subcutaneous hematoma
[ ]: No. of fetuses with case
( ): No. of conceived mothers with case

*Significantly different from control at $p < 0.05$

**Table A2.5 Effects of oral treatment with DEGME on pregnant rats and their foetuses (main teratology study).**

| | diEGME (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 200 | 600 | 1800 |
| No. of dams | 14 | 14 | 14 | 14 |
| Body wt (g) | 336 ± 4.51 | 331 ± 6.11 | 328 ± 6.23 | 317 ± 4.69* |
| Thymus wt (mg) | 228 ± 7.32 | 208 ± 7.39 | 218 ± 11.45 | 181 ± 8.02** |
| No. of corpora lutea[a] | 16.4 ± 0.5 | 16.6 ± 0.5 | 16.7 ± 0.6 | 16.4 ± 0.5 |
| No. of implants[a] | 14.6 ± 0.9 | 13.5 ± 1.0 | 13.7 ± 1.0 | 14.6 ± 0.6 |
| No. of live fetuses[a] | 13.5 ± 0.9 | 12.6 ± 1.0 | 12.9 ± 1.1 | 7.9 ± 0.9** |
| Incidence of dead or resorbed fetuses (%) | | | | |
| Early stage | 6.9 | 7.0 | 5.1 | 21.3** |
| Late stage | 0 | 0 | 1.7 | 24.8** |
| Sex ratio Male/Female | 93/96 | 86/90 | 101/80 | 54/57 |
| Fetal weight [a] (g) | | | | |
| Male | 3.3 ± 0.17 | 2.9 ± 0.14 | 2.6 ± 0.12* | 2.1 ± 0.06** |
| Female | 3.1 ± 0.15 | 2.8 ± 0.13 | 2.5 ± 0.13* | 2.0 ± 0.05** |

[a]Mean ± SE

*Significantly different from control at $p < 0.05$

** Significantly different from control at $p < 0.01$

**Table A2.6 External observations of foetuses from dams treated orally with DEGME (main teratology study).**

| | diEGM (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 200 | 600 | 1800 |
| No. of fetuses examined | 189 | 176 | 181 | 111 |
| Incidence of fetuses with malformations (%) | 0.0 | 0.0 | 0.0 | 14.1**[12](9) |
| Anasarca | 0.0 | 0.0 | 0.0 | 8.7**[7](7) |
| Anury | 0.0 | 0.0 | 0.0 | 9.4**[8](7) |
| Peromelia | 0.0 | 0.0 | 0.0 | 1.8 [1](1) |
| Incidence of fetuses with other anomalies(%) | 0.0 | 0.0 | 0.0 | 13.5**[15](7) |
| Dorsum subcutaneous hematoma | 0.0 | 0.0 | 0.0 | 13.5**[15](7) |

[ ]: No. of fetuses with case

( ): No. of conceived mothers with case

** Significantly different from control at $p < 0.01$

**Table A2.7 Visceral observations of foetuses from dams treated orally with DEGME (main teratology study).**

| | diEGME (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 200 | 600 | 1800 |
| No. of fetuses examined | 98 | 91 | 93 | 59 |
| Incidence of fetuses with malformations (%) | 0.0 | 0.0 | 2.4 [1] (1) | 28.0** [18] (9) |
| Double aortic arch | 0.0 | 0.0 | 0.0 | 1.0 [1] (1) |
| Right aortic arch | 0.0 | 0.0 | 0.0 | 9.6* [5] (4) |
| Ventricular septal defect | 0.0 | 0.0 | 2.4 [1] (1) | 18.4** [13] (6) |
| Agenesis of ductus arteriosus | 0.0 | 0.0 | 0.0 | 1.4 [1] (1) |
| Incidence of fetuses with variations (%) | 3.5 [4] (3) | 5.0 [5] (5) | 35.3** [32] (13) | 100.0** [59] (14) |
| Thymic remnant in the neck | | | | |
| Unilateral | 0.7 [1] (1) | 2.0 [2] (2) | 20.6** [20] (11) | 11.1 [8] (5) |
| Bilateral | 0.0 | 0.0 | 4.8 [2] (1) | 88.9** [51] (14) |
| Total | 0.7 [1] (1) | 2.0 [2] (2) | 25.4** [22] (12) | 100.0** [59] (14) |
| Dilated renal pelvis | | | | |
| Unilateral | 2.8 [3] (2) | 2.1 [2] (2) | 11.4 [10] (6) | 36.4** [19] (11) |
| Bilateral | 0.0 | 0.0 | 0.9 [1] (1) | 16.4** [11] (6) |
| Total | 2.8 [3] (2) | 2.1 [2] (2) | 12.3 [11] (6) | 52.8** [30] (13) |
| Kinked ureter | 0.0 | 0.9 [1] (1) | 0.9 [1] (1) | 0.0 |

[ ]: No. of fetuses with case
( ): No. of conceived mothers with case
*Significantly different from control at $p < 0.05$
** Significantly different from control at $p < 0.01$

**Table A2.8 Skeletal observations of foetuses from dams treated orally with DEGME (main teratology study).**

| | diEGME (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 200 | 600 | 1800 |
| No. of fetuses examined | 91 | 85 | 88 | 52 |
| Incidence of fetuses with malformations (%) | 0.0 | 0.0 | 0.0 | 13.9** [5] (5) |
| Agenesis of digitorum pedis and manus | 0.0 | 0.0 | 0.0 | 3.6 [1] (1) |
| Agenesis of sacro-coccygeal vertebrae | 0.0 | 0.0 | 0.0 | 10.4* [4] (4) |
| Incidence of fetuses with variations (%) | 3.2 [3] (3) | 1.2 [1] (1) | 5.8 [6] (5) | 96.2** [49] (14) |
| Cervical ribs | 2.3 [2] (2) | 0.0 | 2.2 [2] (2) | 1.4 [1] (1) |
| Rudimentary lumbar ribs | 0.0 | 0.0 | 0.8 [1] (1) | 0.0 |
| Splitting of vertebral bodies | | | | |
| Thoracic | 0.0 | 0.0 | 0.0 | 69.9** [33] (14) |
| Lumbar | 0.0 | 1.2 [1] (1) | 1.0 [1] (1) | 78.4** [38] (13) |
| Incomplete ossification of occipitale | 0.9 [1](1) | 0.0 | 2.8 [3] (2) | 85.5** [44] (14) |
| Degree of ossification[a] | | | | |
| No. of sternebrae | 4.2 ± 0.22 | 3.6 ± 0.19 | 2.8 ± 0.24** | 0.3 ± 0.11** |
| No of proximal and middle phalanges | | | | |
| Fore limb | 3.6 ± 0.34 | 3.2 ± 0.22 | 2.9 ± 0.17 | 1.6 ± 0.12** |
| Hind limb | 3.9 ± 0.04 | 3.8 ± 0.08 | 3.5 ± 0.11 | 2.0 ± 0.19** |
| No. of ossification centers of vertebrae | | | | |
| Thoracic | 12.6 ± 0.09 | 12.2 ± 0.08 | 11.7 ± 0.15** | 9.0 ± 0.24** |
| Lumbar | 6.0 | 6.0 | 6.0 | 5.3 ± 0.18** |
| Sacral and caudal | 6.7 ± 0.22 | 5.9 ± 0.19 | 4.2 ± 0.29** | 1.1 ± 0.21** |

[a]Mean ± SE
[ ]: No. of fetuses with case
( ): No. of conceived mothers with case

***Main teratology study***

Maternal body weight gain, food consumption (figure 3) and thymus weight (table A2.5) were decreased significantly at 1800 mg/kg bw/day. According to Yamano et al. (1993), the reduction in body weight at later stages of gestation might be explained by smaller foetus weight. Considering an average of 8 foetuses per dam and an average (significant) weight loss of (1.2 g x 54 males+1.1 g x 57 females)/111 males+females = 1,15 g, then 8 x 1.15 g = 9.2 g weight loss on average per dam that can be attributed to foetal weight loss. The measured weight in the high dose group is on average 317 g. 317 + 9.2 is 328.2 g which is likely not significantly different anymore from the control group since the mid dose group is not significantly different either (with an average of 328 g although a bit more variation, SD = 6.23 vs 4.69 in the high dose group). Therefore indeed the maternal body weight loss is likely, at least to a great extent, attributable to the foetal weight loss and cannot be considered maternal/general toxicity.

Foetal body weight is decreased in a dose-dependent relationship and significant also at 600 mg/kg bw/day (table A2.5). Some significant increases in external observations including anury (9.4%), anasarca (14.1%) were observed in foetuses as well as subcutaneous hematomas (13.5%) (table A2.6). A dose-dependent increase in visceral malformations and variations were observed (table A2.7). Limited visceral malformations were seen at 600 mg/kg bw/day (2.4 % vs 0% in control and low dose) and a significant increase in combined visceral malformations at 1800 mg/kg bw/day (28%) with the majority being ventrical septal defects (18.4%), which was significantly increased by itself. Significant visceral variations were seen already at 600 mg/kg bw/day including unilateral (20.6%, significant) or bilateral (4.8%) thymic remnants in the neck. At 1800 mg/kg bw/day, these thymic remnants were also seen in all (100%) foetuses. Additionally, dilated renal pelvis was found in 52.8% (significantly increased) of the foetuses at 1800 mg/kg bw/day. Skeletal malformations (table A2.8) were only seen in foetuses also with external anomalies in the high dose group and include significantly increased incidence of agenesis of sacro-coccygeal vertebrae (10.4% of total 13.9% skeletal malformations). Significant reduction in skeletal ossification was observed at 600 and 1800 mg/kg bw/day. Significant incidences of skeletal variations were observed in 96.2% of foetuses in the high dose group (including splitting of vertebrae thoracic and lumbar bodies and incomplete occipitae ossification).

***Postnatal study***

All dams in each group delivered live pups although the number was mentioned to be significantly smaller at 1800 mg/kg bw/day (37 compared to around 100 in the other dose groups). No details about pup weight or length just after birth have been given. Duration of gestation was significantly increased ($p<0.01$) at the high dose by 1.7 days (table A2.9). On PND4, the percentage of live pups was non-significantly reduced at 600 mg/kg bw/day to around 62% and significantly reduced at 1800 mg/kg bw/day to around 5% (fig. 3). At this stage (PND 4) the number of dams with live pups reduced from 8 to 6 at 600 mg/kg bw/day and to 1 at 1800 mg/kg bw/day. Body weight gain of pups was slightly reduced at 600 mg/kg bw/day at PND 21 and strongly decreased for the single litter surviving at PND21 from the high dose group (fig. 4). Postnatal developmental parameters measured such as skeletal malformations were not significantly different between the groups.

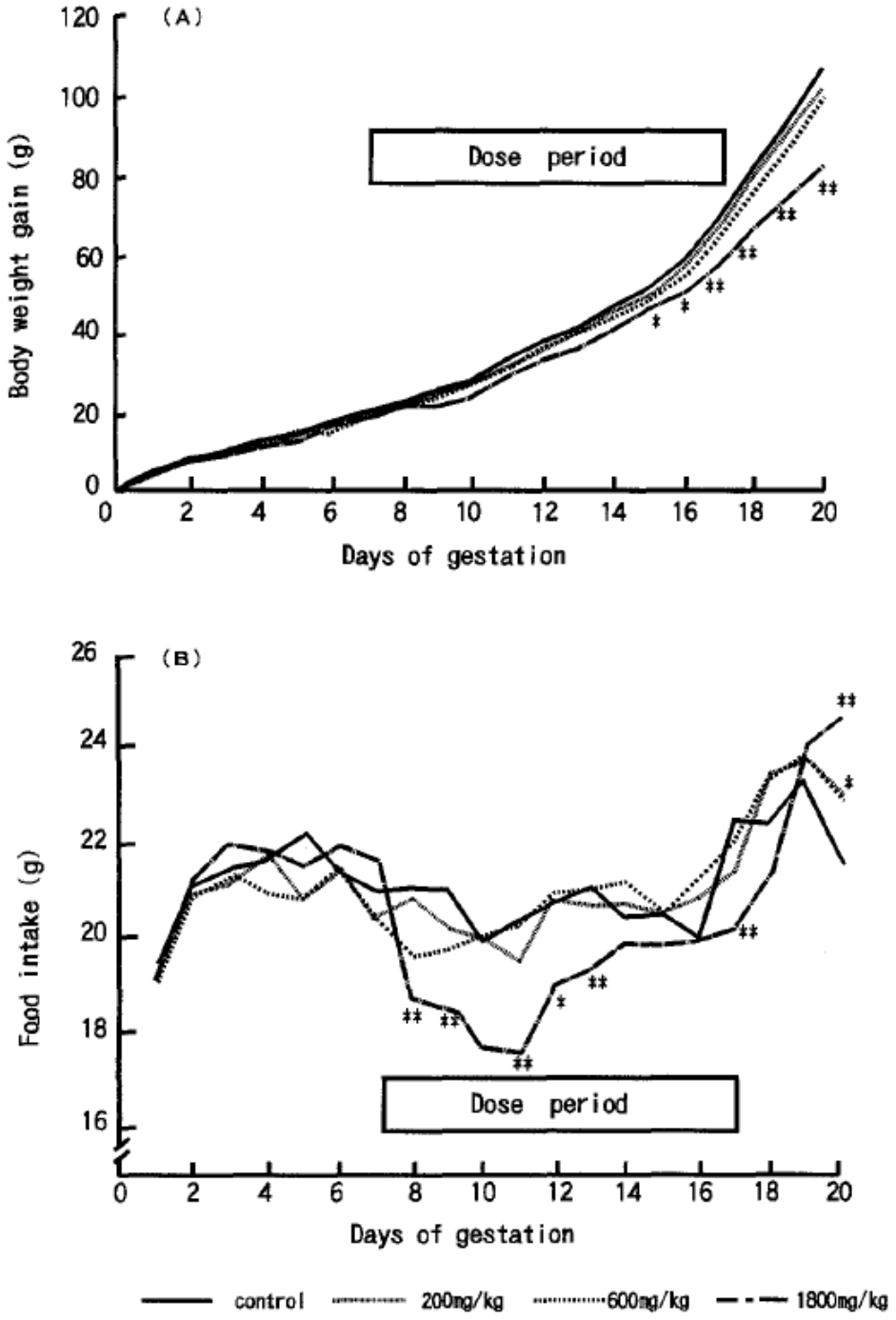



**Figure 2 Body weight gain (a) and food consumption (g) of pregnant rats treated orally with DEGME from 7-17 days of gestation. Each value represents the mean of 22 rats.**

*Significantly different from control at $p < 0.05$
** Significantly different from control at $p < 0.01$

**Table A2.9 Postnatal observations of foetuses from dams treated orally with DEGME (main teratology study).**

| | diEGME (mg/kg) | | | |
|---|---|---|---|---|
| | 0 | 200 | 600 | 1800 |
| No. of dams | 8 | 8 | 8 | 8 |
| Duration of gestation[a] (days) | 22.1 ± 0.13 | 22.4 ± 0.18 | 22.5 ± 0.19 | 23.8 ± 0.25** |
| No. of implants[a] | 13.6 ± 0.98 | 14.4 ± 0.65 | 13.6 ± 0.75 | 13.8 ± 1.31 |
| No. of new borns[a] | 12.5 ± 0.96 | 12.6 ± 0.60 | 11.6 ± 1.18 | 4.6 ± 0.82** |
| Sex ration Male/Female | 42/58 | 54/47 | 46/47 | 23/14 |
| Viability at weaning[b] | 100.0 | 98.4 | 100.0 | 100.0 |
| External differentiation[a] (days) | | | | |
| No. of litters examined | 8 | 8 | 6 | 1 |
| Detachment of ears | 2.0 ± 0.23 | 2.1 ± 0.20 | 2.1 ± 0.27 | 2.0 |
| Hair growth | 7.2 ± 0.05 | 7.1 ± 0.17 | 7.4 ± 0.18 | 9.5 |
| Teeth appearance | 10.9 ± 0.18 | 10.4 ± 0.19 | 11.3 ± 0.31 | 12.0 |
| Opening of eyelids | 14.5 ± 0.13 | 14.7 ± 0.21 | 14.9 ± 0.32 | 14.5 |
| Incidence of pups with external anomalies(%) | | 0.9 | 3.0 | 3.1 |
| Anury | 0.0 | 0.9 [1] (1) | 3.0 [2] (2) | 3.1 [1] (1) |

[a] Mean ± SE

[b] $\frac{\text{No. of offpring on day 21}}{\text{No. of offspring on day 4}} \times 100$

[ ]: No. of fetuses with case

( ): No. of conceived mothers with case

** Significantly different from control at $p < 0.01$

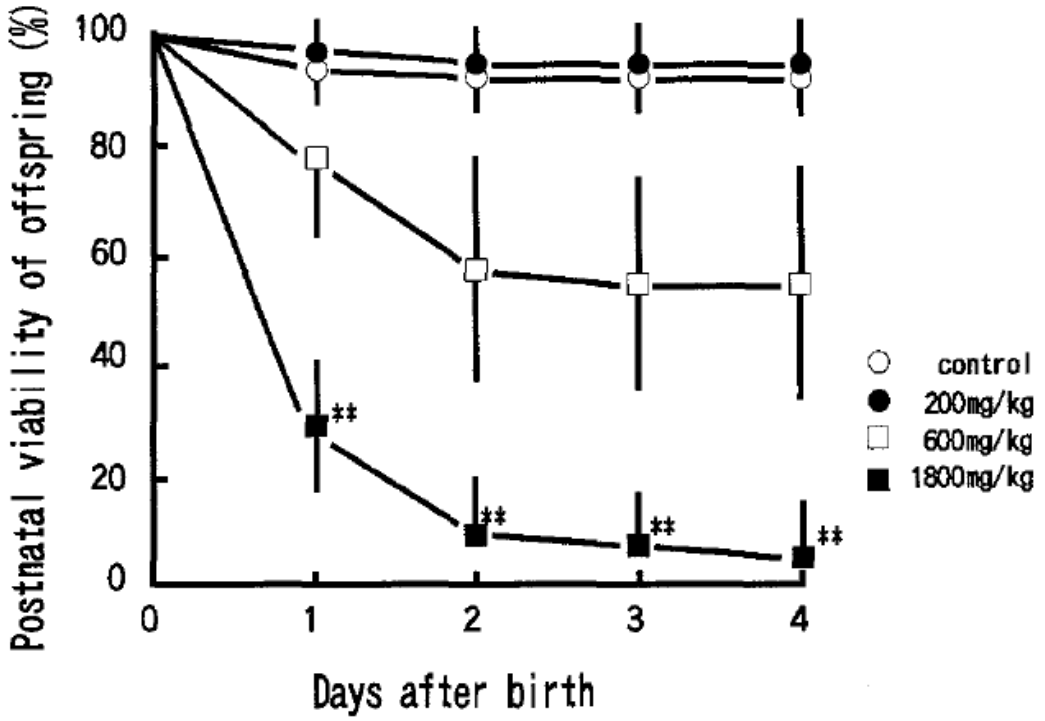



**Fig. 3.** Postnatal viability of offspring from the dams treated orally with diEGME. Means of litter means from 8 dams in each group and SE are represented.** Significantly different from control at $p < 0.01$

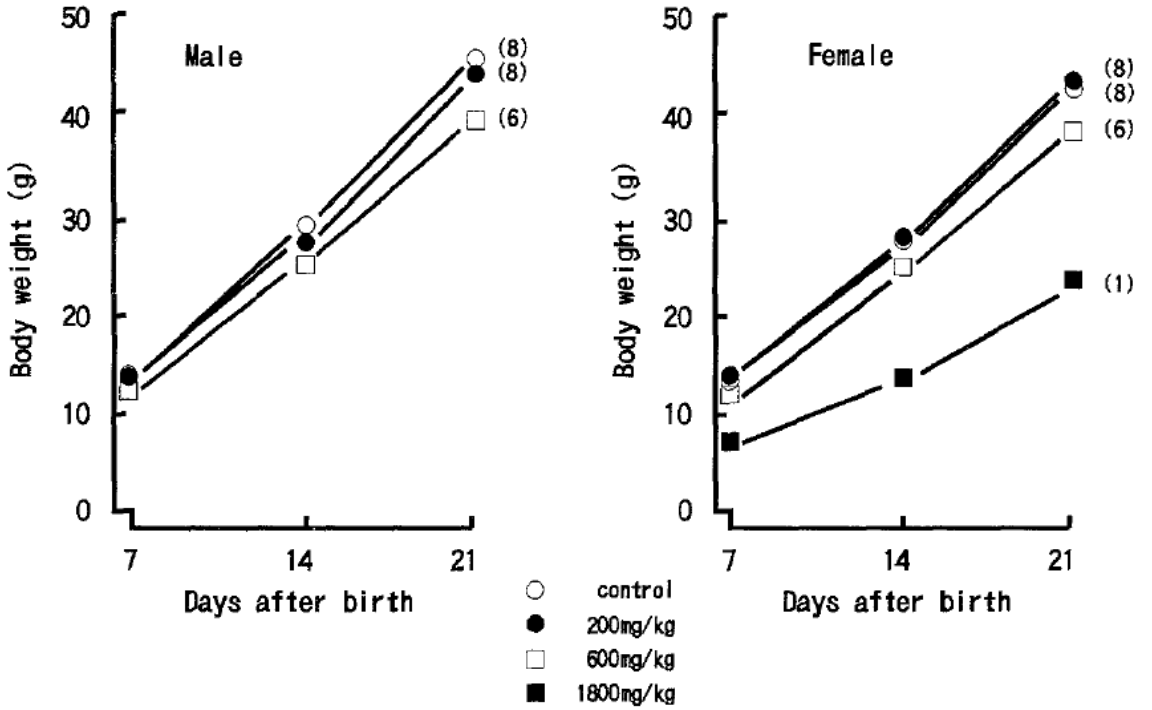



**Figure 4. Body weight changes of offspring from dams treated orally with DEGME. Means of litters of each group are represented. Male offspring from dams treated with 1800 mg/kg bw/day all died before PND5 (Postnatal study).**

#### 13.1.2.1 Discussion & Summary

The hematologic changes observed in this study were according to Yamano et al. (1993) very similar as seen with EGME which can support the idea of a similar mechanism possibly via metabolic activation of MAA. The most sensitive organ was the thymus, of which the weight was reduced in a dose dependent manner in both dams (significant at 1800 mg/kg bw/day) and foetuses (significant at 600 mg/kg bw/day). The spectrum of DEGME developmental toxicity is found to be similar to the study by Hardin et al. (1986) regarding visceral malformations, but not skeletal malformations.

In summary, in non-pregnant rats, significant general toxicity and haematological or biochemical changes occur at high concentrations (>3000 mg/kg bw/day). Thymus and pituitary weights were reduced at these high dose levels as well.

In pregnant rats, maternal body weight and food consumption were decreased already at smaller doses at >2000 mg/kg bw/day. At 2000 mg/kg bw/day the male foetal weight and number of live foetuses was significantly decreased. At >3000 mg/kg bw/day the litters were completely resorbed.

*Teratology study.* Maternal body weight gain, food consumption and thymus weight were decreased significantly at 1800 mg/kg bw/day. According to Yamano et al. (1993), the reduction in body weight at later stages of gestation is likely explained by to smaller foetus weight, which is supported by calculations of foetal weight reduction and adding them to the maternal weight removing the significant difference in weight between maternal animals and control animals. Additionally, the body weight of pregnant rats was affected at lower doses than non-pregnant rats in the dose-range finding study.

Foetal body weight is decreased in a dose-dependent relationship and significant also at 600 mg/kg bw/day. Some significant increases in external observations were observed in foetuses as well as subcutaneous hematomas. A dose-dependent increase in visceral malformations and variations were observed. Limited visceral malformations were seen at 600 mg/kg bw/day and a significant increase in combined visceral

malformations at 1800 mg/kg bw/day. Significant visceral variations were seen already at 600 mg/kg bw/day including thymic remnants in the neck. Additionally, dilated renal pelvis was found in 52.8% (significantly increased) of the foetuses at 1800 mg/kg bw/day. Only limited skeletal malformations were seen in foetuses also with external anomalies in the high dose group. Significant reduction in skeletal ossification was observed at 600 and 1800 mg/kg bw/day. Significant incidences of skeletal variations were observed in 96.2% of foetuses in the high dose group.
*Postnatal investigation.* All dams in each group delivered live pups but the number was significantly smaller at 1800 mg/kg bw/day. Duration of gestation was significantly increased at the high dose. On PND4, the percentage of live pups was non-significantly reduced at 600 mg/kg bw/day and significantly reduced at 1800 mg/kg bw/day. At this stage (PND 4) the number of dams with live pups reduced from 8 to 6 at 600 mg/kg bw/day and to 1 at 1800 mg/kg bw/day. Body weight gain of pups was slightly reduced at 600 mg/kg bw/day at PND 21 and strongly decreased for the single litter surviving at PND21 from the high dose group (fig. 4). Postnatal developmental parameters measured such as skeletal malformations were not significantly different between the groups.

## 13.2 ANNEX 2, Summary of reproductive toxicity profile of 2-methoxyacetic acid

### 13.2.1 Introduction

The metabolite 2-methoxyacetic acid (MAA), which is formed during the metabolism of DEGME is considered responsible for the observed developmental toxicity of DEGME. MAA has a harmonised classification as Repr. 1B H360FD and an entry as SVHC on the ANNEX XV candidate list based on endocrine properties and adverse reproductive effects in *in vitro* and *in vivo* studies. In support for the proposal to classify DEGME as reproductive toxicant category 1B, the toxicological profile regarding *in vivo* reproductive effects of MAA is presented here shortly. Most of the data has been derived from the proposal to put MAA on the candidate list (see ECHA website) and ECETOC (2005).

### 13.2.2 Effects on fertility and development

ECETOC (2005) reported the lowest dose tested in short-term repeated dose toxicity tests (8 mg/kg bw/day) in the longest treatment study with hamsters (5 weeks) still affected the fertility. In this study, Male Syrian golden hamsters received 0, 80, 160 or 650 MAA as a single dose or 0, 8, 32, and 64 mg/kg bw/day for 5 weeks. The spermatozoa were recovered at weekly intervals and their fertilising capability was assessed *in vitro*. Decreased fertilisation capability was observed at all dose levels in all subacture treated animals and from week 3 and 4 onwards in the single dosed animals.
In a 2 week oral rat study with 8 days of repeated dosing up to 300 mg/kg bw/day, effects on the thymus, testes and haematological effects were observed with a NOAEL of 30 mg/kg bw/day. Many studies report effects on the male reproductive system including decrease in testicular weight, histological damage and depletion of spermatocytes (ECETOC, 2005).
In pregnant rats (Wistar), oral administered MAA causes reduction in litter size and resorptions (4-53.8% at 190-380 mg/kg bw/day) at day GD20 and malformed foetuses (0-98.9% at 190-380 mg/kg bw/day) (Ritter et al., 1985). The malformations included malformations to the heart, dilated ductus arteriosus and dilated aortic arch, as well as ventral polydactyly (Ritter et al., 1985). In SD rats given MAA via drinking water at doses of 39 and 79 mg/kg bw/day between GD7-18, clear teratogenic effects were observed including cardiovascular malformations in 15% of the low dose group and complete resorptions at the high dose group (ECETOC, 2005).
In CD-1 mice, single oral administration of MAA causes a dose related increase in paw malformations, similar to dose response induced by EGME. In a 2-generation continuous breeding study with CD-1 mice

(2/sex/group) given MAA via the drinking water at approximately 0, 140, 240 and 390 mg/kg bw/day, the high dose group females did not became pregnant. In the mid-dose group, the fertility index was 95% but pup lethality was 75.4% and all pups died by PND4. The mid and lower dose groups had fewer litters per pair and reduced viable animas per litter. In the surviving F1 generation continuously exposed to the low dose, there was no mating and fertility index was 0. In males in the high dose group, weights of testis, epididymis and seminal vesicle were reduction as was sperm motility.
Overall, MAA exerts pronounced foetotoxic, embryotoxic and teratogenic effects in all species investigated (rats, mice, rabbits, monkey and *Drosophila*) and also via all routes of exposures.
MAA is classified for Repr 1B H360FD.

### 13.2.3 Conclusions and relation of effects caused by MAA and DEGME

Regarding fertility, most effects reported from repeated dose toxicity studies indicate the male reproductive system is affected, which has been observed at high doses with DEGME as well, but generally in the presence of general toxicity. Additionally, the thymus is reported to be affected as well, and similar cardiovascular malformations were reported. Furthermore, reductions in litter size and pup viability have been reported as well as increased resorptions for MAA, EGME and DEGME at doses without general toxic effects.

The similar toxicological profiles suggest MAA is likely the main contributor to the reproductive effects caused by DEGME as is also suspected to be the case for EGME. The potency of DEGME is much smaller but this coincides well with the small fraction of MAA produced by metabolism of DEGME in comparison to EGME.