Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether

EC Number: 203-906-6
CAS Number: 111-77-3

CLH-O-0000006857-59-01/F

Adopted

8 October 2020

8 October 2020

CLH-O-0000006857-59-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether**

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

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

The proposal was submitted by the **Netherlands** and received by RAC on **4 April 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **6 May 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **5 July 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Christine Bjørge**

Co-Rapporteur, appointed by RAC: **Stine Husa**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 October 2020** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	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	**Retain** GHS08 **Modify** Dgr	**Modify** H360D			
RAC opinion	603-107-00-6	2-(2-methoxyethoxy)ethanol; diethylene glycol monomethyl ether	203-906-6	111-77-3	Repr. 1B	H360D	GHS08 Dgr	H360D		Repr. 1B; H360D: C ≥ 3 %	
Resulting Annex VI entry if agreed by 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		Repr. 1B; H360D: C ≥ 3 %	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance 2-(2-methoxyethoxy)ethanol, commonly known as diethylene glycol monomethyl ether (DEGME), was previously assessed by the Technical Committee on Classification and Labelling (TC C&L) in 1996/1997 based on the information present in the Risk Assessment Report (RAR, 2000) prepared by the Netherlands, and was classified as Repr. 2; H361d. The dossier submitter (DS) was unable to retrieve the original classification proposal. All information dating after 1997 was considered new by the Dossier Submitter (DS; The Netherlands). This included more details on the formation and half-life of DEGME's metabolite which is a reproductive toxicant 2-methoxyacetic acid (MAA) in both rats and humans after oral exposure to DEGME (Aasmoe, Mathiesen, & Sager, 1999; Groeseneken *et al.*, 1988; Groeseneken *et al.*, 1989; Triskelion, 2017) as well as information on the reprotoxic potency of MAA (ECETOC, 2005). The DS was of the opinion that the metabolite MAA was probably not considered in the original classification proposal as it was not considered in the Risk Assessment Report (RAR, 2000), which was used as the basis for the original classification proposal.

Toxicokinetics

Absorption

In a study with Sprague Dawley rats, administered oral doses up to 2000 mg/kg bw, 95% was recovered in urine within 0-48 hours, mostly as metabolites, with the majority excreted within the 0-24 hours' time interval (Triskelion, 2017).

No information is available on absorption by inhalation.

In a dermal study, measurement of dermal penetration of DEGME through heat-separated human epidermal membranes showed a flux of 0.21 mg/cm^2/h from the pure chemical (Dugard *et al.*, 1984). McDougal *et al.* (2000) showed about 4-times lower flux in rat skin for DEGME in jet-8 fuel. It is noted that the concentration of DEGME in jet-8 fuel is low (0.08%) and the study with rat skin contained an additional skin layer in the form of some dermis which could reduce the flux (McDougal *et al*., 2000).

Metabolism and Excretion

ECETOC (2005) suggested two main pathways for the metabolism of the group of glycol ethers. The first pathway involving alcohol dehydrogenase resulted in alkoxy acetic acids and the second pathway involving microsomal P450 mixed function oxidation resulted in the formation of carbon dioxide via ethylene glycol or propylene glycol, see figure below.

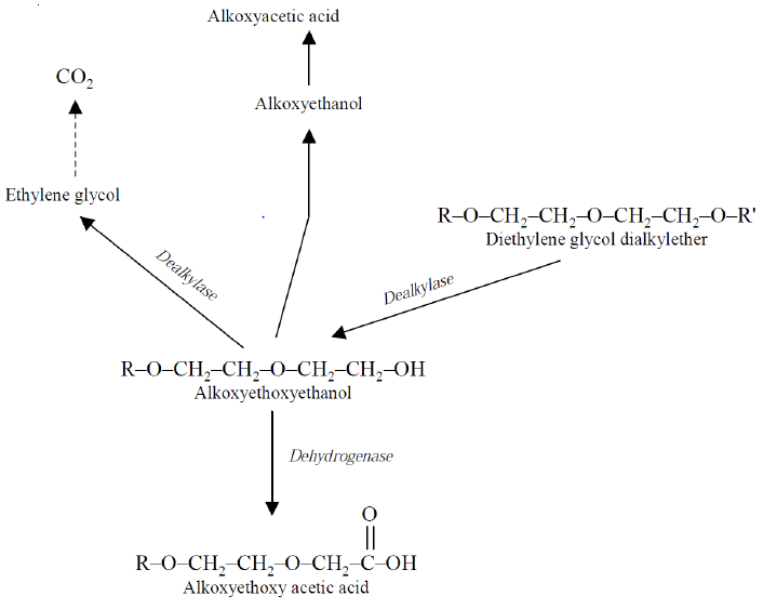

***Figure**: Proposed metabolic pathway for alkoxyethoxyethanols (ECETOC, 2005)*

Triskelion (2017) performed an excretion and metabolism study with male Sprague-Dawley rats exposed to a single dose of DEGME by gavage. Recovery of DEGME in urine (% of dosed) is described in the table below. In the study it was shown that approximately 1% of the reproductive toxicant MAA was formed after exposure to DEGME.

***Table:** Recovery of DEGME in urine (% of dosed)*

	500 mg/kg bw	**1000 mg/kg bw**	**2000 mg/kg bw**
	%/(sd)	**%/(sd)**	**%/(sd)**
MEAA	94.5 (7.7)	91.1 (8.5)	87.2 (4.4)
MAA	1.4 (0.1)	1.1 (0.1)	0.8 (0.1)
DEG	2.4 (0.3)	1.7 (0.6)	1.5 (0.3)
DEGME-glucuronide	1.0 (0.1)	0.8 (0.1)	0.7 (0.1)
DEGME	3.4 (0.4)	3.6 (0.7)	4.9 (0.7)

sd = standard deviation

On this basis the following metabolism scheme was suggested.

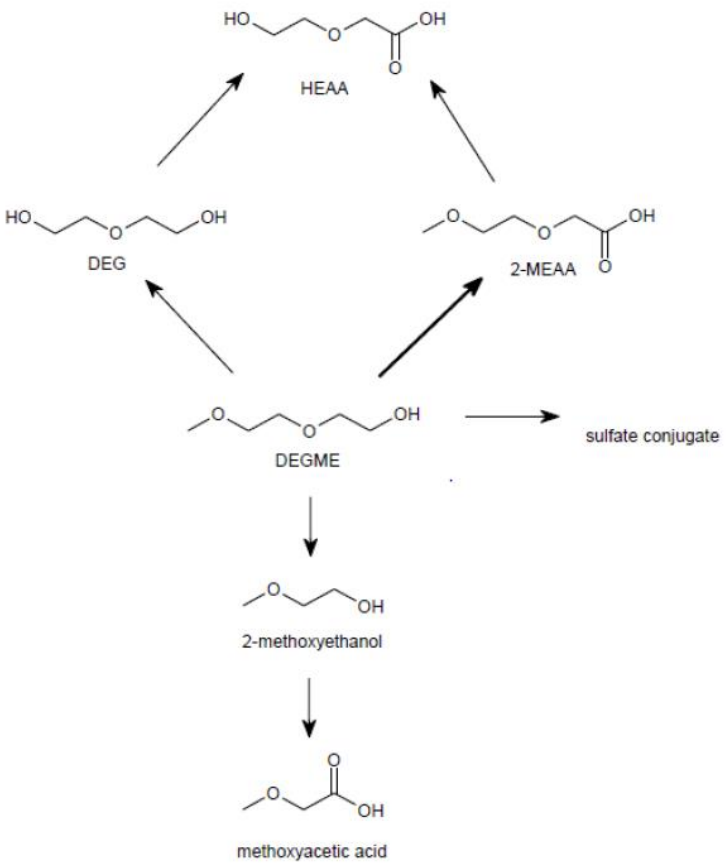

***Figure**: Proposed metabolic pathway of DEGME (Triskelion, 2017).*

Alkoxyacetic acids, including MAA, are reported to be eliminated slowly (ECETOC, 2005). The elimination seems to be slower in humans as compared to rodents. Triskelion (2017) found the ratio of MAA between 24h/48h clearance to be smaller in comparison to the other metabolites after exposure to DEGME, indicating slower clearance.

Groeseneken *et al.* (1989) found the half-life of MAA in seven healthy male volunteers to be 77 hours based on urinary excretion after exposure to DEGME. The recovery of MAA relative to the inhaled DEGME indicated that the greatest amount of DEGME was metabolised by alcohol dehydrogenase. In comparison, Moss *et al.*, (1985) described the clearance half-life of MAA to be 19.7h in SD rats. Aasmoe *et al.*, (1999) found the clearance half-life from plasma in male/female Wistar rats to be 13.2h/18.6h, respectively, based on plasma and urinary excretion after exposure to MAA. RAC notes that a longer half-life in humans compared to rats can be assumed in general because of allometric scaling.

In summary, the alkoxyacetic acid metabolites showed a relatively slow clearance in comparison to other metabolites from DEGME. Further, the DS indicated that the clearance in humans is slower than in rats, and consequently that the internal human concentration after repeated exposure will be higher compared to rodents. Further, it was shown that approximately 1% of the reproductive toxicant MAA was formed after exposure to DEGME in male rats.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS included five studies for the evaluation of adverse effects on sexual function and fertility. Four studies with rats (three with males only), and one study with male Guinea pigs. Male rats showed reduced testis weights (from 1800 mg/kg bw/d and upwards), testicular atrophy and degenerated spermatozoa (at 3600 mg/kg bw/d). These effects were accompanied by a reduction in bodyweight and organ weights.

A metabolism study with DEGME showed that limited amounts of MAA is formed in rats. MAA has been reported to cause testicular damage in rats and mice (at single oral or i.p. doses of 118 mg/kg bw and higher), and to reduce fertility in mice (at doses of 140 mg/kg bw/d and higher) and is therefore classified for effects on fertility (Repr. 1B). As only a limited amount of MAA is formed (around 1%) after exposure to DEGME, it is uncertain whether sufficient amounts of MAA are formed at dose levels of DEGME relevant for classification.

The DS considered that, based on absence of human data and relevant data on functional fertility in animals, classification for effects on sexual function and fertility is not warranted.

Development

The DS included three oral studies with rats, one oral study with mice and one dermal study with rabbits for the assessment of adverse effects on development.

Severe developmental effects were reported in several species (i.e. reduced foetal viability in rats, mice and rabbits; increased visceral malformations in rats). In mice and rabbits, the effects were observed at dose levels also inducing maternal lethality (approximately 10%).

Two studies with rats reported specific and severe developmental effects showing a dose-response relationship. However, these effects were observed at doses ≥1800 mg/kg bw/d, at which also effects on maternal body weight occurred. Cardiac malformations (malformations of the aortic arch; ventricular septal defects) observed in rats were not considered causally related with the maternal toxicity reported. One malformation of the aortic arch and one ventricular septal defect were observed at 720 and 600 mg/kg bw/d, respectively (not statistically significant). Further, a reduction in postnatal viability was reported in rats at 600 mg/kg bw/d (not statistically significant) in the absence of maternal toxicity, however, observed to be significant at the high dose of 1800 mg/kg bw/d.

2-methoxyacetic acid is a metabolite of DEGME and is classified in Category 1B for developmental toxicity. MAA causes malformations of the heart (dilated ductus arteriosus and dilated aortic arch) at a single dose of 186 mg/kg bw/d and higher on GD12. It is likely that MAA is responsible for the effects observed after exposure to DEGME. The DS also noted that the half-life of MAA in humans is approximately 3.1-6.8 times longer than in rats, which indicates 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 (plasma/urinary) half-life of MAA in rats is estimated to be in the range of 12.8–21.8h, while in humans the half-life of MAA is higher (77.1 ± 9.5h). On this basis the DS indicated that reprotoxic effects of DEGME through the metabolite MAA cannot be excluded.

The DS proposed to classify the DEGME as developmental toxicant in Category 1B; H360D, based on increased visceral malformations and postnatal mortality starting at concentrations below the limit dose of 1000 mg/kg bw/d for reproductive toxicity and reaching statistical significance at concentrations above the limit-dose in rats in the absence of maternal toxicity. This is further supported by the formation of MAA in potentially teratogenic amounts and by MAA longer half-life in humans compared to rats (3.1-6.8 folds), suggesting that developmental effects may occur in humans at lower external dose levels compared to rats.

Setting of Specific Concertation Limits (SCL)

Derivation of an SCL was considered in the CLH report based on the limited effects of DEGME at dose levels close to the limit dose of 1000 mg/kg bw/d. The ED_{10} was estimated to be above 400 mg/kg bw/d (no calculation needed) resulting in an SCL of 3% (Guidance on the application of CLP criteria, v5.0, 2017, hereafter CLP guidance). However, the available data was 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 TG 414. Further, no oral developmental study in rabbits and no (multi)generation toxicity study in rats were available. 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 therefore no SCL was proposed by the DS.

Adverse effects on or via lactation

No studies were found regarding the effects of DEGME following lactational exposure in animals or in humans, therefore, no data were available for comparison with the CLP criteria.

Comments received during consultation

The proposed classification as Repr. 1B; H360D was supported by 4 commenting Member State Competent Authorities (MSCAs), while one MSCA was of the opinion that the current classification as Repr. 2; H361d should be retained. One MSCA commented on the presence of the impurity 2-methoxyethanol (2-MEA) in DEGME at levels of 0-0.4%. 2-MEA is classified as Repr. 1B for fertility and development. The MSCA was of the opinion that the presence of 2-MEA in DEGME should be taken into account in the classification of DEGME. However, according to DEGME consortium the impurity is no longer present in DEGME at concentrations warranting classification.

One commenting Industry/Trade association was of the opinion that the current classification as Repr. 2; H361d should be retained, because developmental toxicity was only seen to any significant extent at very high doses and above the limit dose for classification, and that the effects seen were due to the small amounts of MAA formed during metabolism. Further, the developmental toxicity was observed alongside maternal toxicity. In addition, they highlighted the low potency of DEGME. The DS responded in the RCOM that they remained of the opinion that DEGME should be classified as a reproductive toxicant in Category 1B for effects on development. However, they also acknowledged that based on the low potency of DEGME a corresponding SCL of 3% could be set. They performed a Bench Mark Dose (BMD) analysis to assess the potential effects of DEGME at the limit dose level (1000 mg/kg bw/d) in the two developmental toxicity studies by Hardin *et al.* (1986) and Yamano *et al.* (1993), and based on the BMD analysis the DS concluded the following:

- Increased cardiac 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. BMD analyses indicates DEGME is expected to cause at or below the limit dose:

    - an increase of 10% skeletal variations/malformations (with an unclear fraction of high concern malformations),
    - a reduction in foetal body weight of at least 10%,
    - an increase of >1% cardiac malformations
    - An increase of >10% postnatal mortality at PND4 (already present at PND2).
- Formation of MAA in potentially teratogenic amounts (1% corresponds to 10 mg/kg bw/d at the limit dose, MAA is known to cause malformations from 39 mg/kg bw/d in rats, but lower concentrations have not been tested).
- The half-life of MAA is longer in humans compared to rats and it may accumulate after repeated exposure. It is acknowledged the formation rate of MAA is expected to be slower as well, but the relevant source of this information could not be evaluated. It therefore remains unclear if MAA is indeed formed more slowly in humans compared to rats resulting in equipotency in both species.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

The DS included five repeated dose toxicity studies for the evaluation of adverse effects on sexual function and fertility. Four studies with rats (three with males only), and one study with male Guinea pigs. No mating studies were available. The main results of the studies are included in the table below.

Method	**Results**	**Klimisch score, remarks**	**Reference**
Fischer rats, age 6-8 weeks 10/sex/dose group 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. 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 *et al.*, 1985
Sprague-Dawley rats, age unknown. 50 males/dose group Oral exposure (gavage) to 613 mg/kg bw/d DEGME (purity 99.5%) for 3-21 days. At 2-day intervals starting at day 3, 5 rats were sacrificed and testis histopathologically examined. 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 4-8 males/dose group Oral exposure to 0 and 2000 mg/kg bw/d DEGME (purity >98%) for 1, 2, 5 and 20 days. Non-guideline, non-GLP	At 2000 mg/kg bw/d the animals had lower body weight compared to control animals (ca -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 4-8 males/dose group	Statistically significant reduction of body weight and relative testis weight after >5 days at 2000 mg/kg bw/d.	2, reliable with restriction	Kawamoto *et al.*, 1990

Method	Results	Klimisch score, remarks	Reference
Oral exposure to 0, 500, 1000 and 2000 mg/kg bw/d DEGME (purity >98%) by gavage for 20 days. Non-guideline, non-GLP			
Hartley Guinea pigs, age 6-8 weeks 6 males/dose group Dermal exposure to 0, 40, 200 and 1000 mg/kg bw/d DEGME for 6 hours/d, 5 days/week for 90 days. Non-guideline, non-GLP	No effects seen on examined reproductive organs including the testis. Spleen weight decreased at >200 mg/kg bw/d and mild fatty change in livers in all treated animals.	2, reliable with restriction	Hobson *et al.*, 1986
Albino COBS, CD, BR rats, age unknown 10 males/dose group Oral exposure to 0, 900, 1800, 3600 mg/kg bw/d DEGME by gavage for 6 weeks (5 days/week). Similar to OECD TG 407, but not all endpoints examined longer time period and males only. Non-GLP, but well reported	All mentioned changes were statistically significant: At 1800 and 3600 mg/kg bw/d, there was an increased heart weight and reduced absolute liver weight. At 3600 mg/kg bw/d, body, absolute spleen and brain weight were reduced while relative kidney weight was increased, and the relative testis weight was reduced and was accompanied with degenerated spermatozoa and hypospermia in 50% of rats. At 1800 mg/kg bw/d, the relative testis weight was increased.	2, reliable with restriction	Krasavage & Vlaovic, 1982

In the study by Miller *et al.* (1985), Fisher rats (males/females) were exposed to DEGME at doses of 0, 150, 500 or 1080 mg/m^3 (0, 30, 100 or 216 ppm) by inhalation (6 h/d, 5 d/week, 13 weeks). This study did not show any exposure related effects on reproductive organs and no general toxicity or treatment related deaths.

In the study by Cheever *et al.* (1988), Sprague-Dawley rats (males) were exposed to DEGME at doses of 5.1 mmol/kg bw (613 mg/kg bw) by gavage for up to 20 days. Five rats were killed at 2-day intervals from days 3 through 21. No effects on testis were observed in the histopathological examination. No early deaths or overt signs of toxicity were observed.

In the study by Kawamoto *et al*. (1990), Wistar rats (males, 4-8/group) were exposed to 2000 mg/kg bw/d DEGME by oral gavage for 1, 2, 5 and 20 days in a time course study. 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.

Further, Kawamoto *et al.* (1990) performed an accompanying dose-response study with oral exposure to DEGME at doses of 0, 500, 1000 or 2000 mg/kg bw/d (4-8/group) for 20 days. Body weight gain was reduced at 2000 mg/kg bw/d compared to the control group from day 10 onwards. At 2000 mg/kg bw/d the testis weight was statistically significantly reduced relative to the body weight. The relative thymus weight was decreased at 1000 and 2000 mg/kg bw/d.

In a repeated dose toxicity study by Krasavage and Vlaovic (1982), male rats (10/group) were exposed to DEGME by oral gavage at doses of 0, 900, 1800 or 3600 mg/kg bw/d for 6 weeks. The high dose group showed decreased relative testis weight and atrophy, accompanied by

evidence of degenerated spermatozoa in the epididymis and hypospermia, in 50% of the rats. At this dose, clear signs of systemic toxicity were reported.

In a 90-day study by Hobson *et al*. (1986), Hartley Guinea pigs (males, 6/dose) were exposed to DEGME dermally (occlusive) at doses of 0, 40, 200 and 1000 mg/kg bw/d for 5 days/week and 6 hour/d. The average body weight decreased in a dose-related manner, however, not statistically significantly different from controls. The relative and absolute spleen weight decreased in the mid and high dose group. An increase in serum lactate dehydrogenase was observed in the high dose group. A mild change in liver fat was observed in all treated groups compared to controls. No testicular lesions were observed, nor were body weight and the relative and absolute weights of the testes, seminal vesicles and prostate affected.

In the postnatal developmental toxicity study by Yamano *et al.* (1993), rats were exposed to DEGME at doses of 0, 200, 600 and 1800 mg/kg bw/d during GD7-17 and the rats were sacrificed postnatal day 21. In the study, the duration of gestation was significantly increased by approximately 1.7 days in the high dose group. A metabolism study with DEGME showed that limited amounts of MAA is formed in rats (Triskelion, 2017). MAA has been reported to cause testicular damage in rats and mice (at single oral or i.p. doses of 118 mg/kg bw and higher), and to reduce fertility in mice (at doses of 140 mg/kg bw/d and higher) and is therefore classified for effects on fertility (Repr. 1B). As only a limited amount of MAA is formed (around 1%) after exposure to DEGME, it is uncertain whether sufficient MAA is formed at dose levels of DEGME around the limit dose recommended in the OECD TG 414 (1000 mg/kg bw/d).

Human data

No data on the effects of DEGME on human fertility is available.

In summary, the studies available show reduced testis weights (from 1800 mg/kg bw/d and upwards), testicular atrophy and degenerated spermatozoa (at 3600 mg/kg bw/d) in male rats exposed to DEGME. However, these effects were accompanied by a reduction in bodyweight and organ weights. The effects on testis weight at these levels can be considered secondary to general toxicity and are not considered relevant for classification. No data are available on functional reproduction parameters.

Overall, RAC agrees with the DS proposal and is of the opinion that based on the absence of human data and relevant data on functional fertility in animals, **no classification for adverse effects on sexual function and fertility is warranted based on inconclusive data.**

Adverse effects on development

The DS included three studies with rats, one study with mice and one study with rabbits for the assessment of adverse effects on development. None of the studies were performed according to OECD Test Guidelines or GLP. The main results of the studies are included in the table below.

Method	Results	Klimisch score, Remarks	Reference
Dose-finding study Sprague-Dawley rats, age unknown 9 females/group Oral exposure (gavage) 0, 1000, 1495, 2235, 3345, 5175 mg/kg bw/d DEGME (purity unknown) on GD7-16 Sacrifice on GD21	5175 mg/kg bw/d: Gestational bw reduced, 2/9 dams died. 3345 and 5175 mg/kg bw/d, litter size reduced to 10 and 0% respectively vs. 91.2% in control. 2235 and 3345 mg/kg bw/d: foetal weight decreased. Total number of skeletal variations, visceral and cardiovascular malformations increased at 2235 mg/kg bw/d.	2, reliable with restriction	Hardin *et al.* 1986

Method	Results	Klimisch score, Remarks	Reference
Main study: Sprague-Dawley rats, age unknown 25 females/group Oral exposure (gavage) 0, 720, 2165 mg/kg bw/d DEGME (purity unknown) GD7-16 Sacrifice on GD20	2165 mg/kg bw/d decreased maternal weight (approx. 7%), 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. 720 mg/kg bw/d: skeletal variations (ribs) and reduced ossification of the appendicular skeleton and dilated renal pelvis.	2, reliable with restriction Method shares some similarity with OECD TG 414. Deviations: dosing started on GD7, not GD5. Rats shipped from CRL on day 4 after gestation, received on day GD5 and dosed on GD7.	Hardin *et al.*, 1986
Dose finding study Wistar rats, age >3 months old 4-6 females/dose group Oral exposure (gavage) 0, 125, 250, 500, 1000, 2000, 3000 and 4000 mg/kg bw/d DEGME (purity 99%) GD7-17 Rats sacrificed on GD20	Maternal body weight gain was reduced at ≥2000 mg/kg bw/d. At ≥3000 mg/kg bw/d reduced maternal food consumption. There was a dose-dependent decrease in number of live foetuses. No live foetuses at 3000 and 4000 mg/kg bw/d.	2, reliable with restriction Method shares some similarity with OECD TG 414. Deviations include fewer animals than recommended, dosing started on GD7, not GD5.	Yamano *et al.*, 1993
Teratology study Wistar rats, age >3 months old 14 females/group Oral exposure (gavage) 0, 200, 600 and 1800 mg/kg bw/d DEGME (purity 99%) GD7-17 Rats sacrificed on GD20	Parental toxicity: at 1800 mg/kg bw/d decreased maternal bw gain (approx. 6%), food consumption and thymus weight. Foetal toxicity: decrease in foetal bw at 600 and 1800 mg/kg bw/d. At 1800 mg/kg bw/d: external malformations, visceral malformations of the cardiovascular system, increased skeletal variations. At 600 mg/kg bw/d: 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 GD5.	Yamano *et al.,* 1993
Postnatal study Wistar rats, age >3 months old 8 females/group Oral exposure (gavage) 0, 200, 600 and 1800 mg/kg bw/d DEGME (purity 99%) GD7-17 Rats sacrificed on day 21 postpartum	Prolonged gestational period and reduced number of live pups at 1800 mg/kg bw/d. Reduced viability of pups at 600 and 1800 mg/kg bw/d.	2, reliable with restriction	Yamano *et al.*, 1993
CD-1 mice, 6-8 weeks 50 females/group Oral exposure (gavage) 0, 4000 mg/kg bw/d DEGME (purity 99%) for 8 days starting on GD7	At 4000 mg/kg bw/d: 5/50 dams died. Reduced percentage of viable litters (16% vs. 97% in controls). Reduced number of live pups per litter (3 vs 10 in the control), and pup survival over days 1-3 postpartum (31% vs 100% in the controls).	2, reliable with restriction	Schuler *et al.*, 1984
New Zealand White rabbits, age not specified 25 females/group Dermal exposure to 0, 50, 250, 750 mg/kg bw/d DEGME (purity 99.2%) GD6-18 Foetuses examined on GD29	Parental toxicity: at 750 mg/kg bw/d 2/25 died and 1/25 at 50 mg/kg bw/d. At 750 mg/kg bw/d decreased weight gain at GD9-11 and haematological changes in parental animals (i.e. decrease in red blood cells and packed cell volume values). At 250 and 50 mg/kg bw/d no clinical signs of treatment were reported. Foetal toxicity: at >250 mg/kg bw/d, delayed ossification of the hyoid bone and cervical spur of the vertebrae. At 750 mg/kg bw/d, 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 *et al.*, 1986

In the two oral rat studies, developmental effects were reported after exposure to DEGME (Hardin *et al.*, 1986; Yamano *et al.*, 1993). Hardin *et al*. (1986) reported a reduction in live litters, increased incidence of malformations in the cardiovascular system (aortic arch and ventricular septum), in the ribs (rudimentary and wavy/ fused) and reduced foetal weight following *in utero* exposure to high doses of DEGME (>2000 mg/kg bw/d). These dose-levels also induced maternal weight loss, possibly due to loss of foetuses. In the main study, at 720 mg/kg bw/d (and not significantly in the dose-finding study at 1000 mg/kg bw/d which may be related to the smaller number of animals used per dose group), an increase in skeletal malformations (total number of rudimentary and wavy/fused ribs) was reported in the absence of maternal toxicity. For further details regarding the malformations in the main study, see table below:

Table: *Incidences of skeletal and visceral malformations from the main study by Hardin et al., 1986.*

Malformations	**0 mg/kg bw/d**	**720 mg/kg bw/d**	**2165 mg/kg bw/d**
Skeletal malformations			
Number examined **Litter (foetuses)/% foetuses**	22 (123)	21 (111)	20 (89)
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
Visceral malformations			
Number examined **Litter (foetuses)/% foetuses**	22 (129)	21 (115)	21 (82)
Cardiovascular:			
Double aortic arch	0	0	7** (9)/12
Right aortic arch	0	1 (1)/1	6** (6)/10
Right ductus arteriosus	0	0	1 (1)/5
Ventricular septal defect	0	0	14*** (27)/39
Total:	0	1 (1)/1	15*** (33)/46

Differ significantly from corresponding control at *p<0.05; **p<0.01; ***p<0.001

In the range finding rat study, by Yamano *et al.*, 1993, no live litters were observed at 3000 and 4000 mg/kg bw/d resulting in a reduced maternal body weight gain at these doses. In the main study (Yamano *et al.*, 1993) at 1800 mg/kg bw/d in the absence of maternal toxicity, the incidence of resorptions was increased, malformations of the cardiovascular system (aortic arch, ventricular septum) and skeletal variations were reported, and the foetal weight was reduced. At 600 mg/kg bw/d, a decrease in foetal weight and an increase in variations, both skeletal (not statistically significant) and visceral (statistically significant), were reported. Further details of the malformations and variations is included in the table below.

Table: *Incidences of visceral malformations and variations from the main study by Yamano et al. (1993)*

Doses mg/kg bw/d	**0**	**200**	**600**	**1800**
Number of foetuses	98	91	93	59
Visceral malformations (%)				
Cardiovascular:				
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)
Total	0.0	0.0	2.4 [1] (1)	28.0** [18] (9)
Visceral variations (%)				
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)

[]: No. of foetuses with case

(): No. of conceived mothers with case

Differ significantly from corresponding control at *p< 0.05; **p<0.01

In the postnatal study in rats by Yamano *et al*. (1993), the number of pups delivered was significantly decreased at 1800 mg/kg bw/d (13.5, 12.6, 12.9 and 7.9* in the control, 200, 600 and 1800 mg/kg bw/d dose group, respectively) and duration of gestation was significantly increased (by approximately 1.7 days in the high dose group). On PND4, the percentage of live pups was non-significantly reduced at 600 mg/kg bw/d (to around 62%) and significantly reduced at 1800 mg/kg bw/d (to around 5%). The absence of a statistically significant effect at 600 mg/kg bw/d could be related to the low number of animals used per dose group (8 animals/group) and could therefore be considered as biological relevant. Body weight gain of pups was slightly reduced in the 600 mg/kg bw/d dose group at PND 21 (litter weigh 38g vs. 44g in controls litters) and strongly decreased for the single litter surviving at PND21 from the high dose group (litter weight 24g vs. 44g in control litters).

In the mouse study, the animals were orally exposed to only one single dose of DEGME, 4000 mg/kg bw/d (Schuler *et al.*, 1984). Effects were reported including a statistically significant decrease 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.

When rabbits were dermally exposed to DEGME up to 750 mg/kg bw/d (Scortichini *et al.*, 1986), maternal toxicity was observed in the high dose group, and a non-significant increase in embryonic resorptions and developmental variations was also reported. At 250 mg/kg bw/d, cranial variations were noted in the offspring in the absence of maternal toxicity.

In addition, one study was included assessing the postnatal development in AlpkAP (Wistar-derived) rats following subcutaneously administration of DEGME to 0, 255, 510 and 1020 mg/kg bw/d on GD6-20 (15 dams/group) (Doe, 1984). The pups were examined on PND2 and 5. No gross pathology or histopathological assessment was performed. No maternal toxicity was reported. A non-significant reduction in pup survival was reported on PND5 in the high dose group.

It should be noted that DEGME is metabolised in rats to small amount of MAA (approximately 1%). MAA induces comparable cardiovascular malformations as DEGME but, at much lower concentrations compared to DEGME.

Human data

Only one case report is available. In this report, retrocaval ureter, with anomalies in both the cardiovascular and skeletal system was described (Karaman *et al*., 2002). The mother worked in the textile industry; therefore, maternal exposure to DEGME was expected. As the report did not include blood concentration measurements or workplace monitoring, no conclusions can be drawn about a possible correlation between the observed anomaly and exposure to DEGME.

Summary

In several species, severe developmental effects were reported (i.e. reduced foetal viability in rats, mice and rabbits; increased visceral and skeletal 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 may be considered more appropriate for classification in Category 2 than 1B (CLP Regulation, Annex I, 3.7.2.4.3).

In the two rat studies, developmental effects were reported, that showed a dose-response relationship (Hardin *et al.*, 1986; Yamano *et al.*, 1993). These effects were reported following exposure to relative high doses of DEGME (≥1800 mg/kg bw/d), at which also effects on maternal body weight occurred (6-7%), but this reduction could be related to the reduced foetal viability at this dose level. The specific developmental effects reported at relatively high doses, should not be totally ignored based on the limit dose at 1000 mg/kg bw/d normally used in the assessment of reproductive toxicity, especially because the studies were not based on test guidelines and have a more limited power (e.g. fewer animals per dose group in the Yamano *et al.* (1993) study). 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).

The occurrence of the cardiac malformations (malformations of the aortic arch; ventricular septal defects) in rats were not considered causally related with the maternal toxicity reported. It should be noted that the cardiac malformations were also observed in rats at doses below 1000 mg/kg bw/d. One malformation of the aortic arch at 720 mg/kg bw/d (Hardin *et al*., 1986) and one ventricular septal defect at 600 mg/kg bw/d (Yamano *et al.*, 1993) were also reported, however, at low incidences. Although not statistically significant at these dose levels, these effects may be suggestive of a dose-response relationship.

Furthermore, a reduction in postnatal viability (not statistically significant) was reported in rats at 600 mg/kg bw/d in the absence of maternal toxicity while this effect was observed to be significant at 1800 mg/kg bw/d suggesting a dose dependency (Yamano *et al.*, 1993).

In addition, in the BMD analyses based on foetal data, performed by the DS and included in the RCOM, it was shown that it was highly likely that toxicologically significant effects (>1% cardiac malformations, >10% lower foetal pup weights, >10% pup mortality) could occur at or below the limit dose level of 1000 mg/kg bw/d.

Comparison with the CLP criteria

RAC agrees with the DS and considers that DEGME should be classified in Repr. 1B based on clear evidence of developmental toxicity based on the following:

The two main concerns reported in the developmental toxicity studies were an increased incidence of visceral malformations and postnatal mortality starting at doses below the limit dose recommended in the OECD TG 414 (1000 mg/kg bw/d) and reaching statistical significance at doses above the limit dose in the rat in the absence of maternal toxicity.

The formation of the teratogenic MAA following exposure to DEGME was 1%. MAA has been shown to causes malformations from 39 mg/kg bw/d in rats, but lower concentrations have not been tested.

MAA is shown to have a 3.1 to 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.

Setting of specific concentration limit (SCL)

The DS considered the setting of a SCL due to the limited effects of DEGME at dose levels close to the limit dose of 1000 mg/kg bw/d. Based on the available data, the upper confidence limit for the ED_{10} exceeded the threshold of 400 mg/kg bw/d resulting in an SCL of 3% for Repr. 1B (CLP guidance).

A BMD analysis based on foetal data performed by the DS and included in the RCOM clarified that it is highly likely that toxicologically significant effects (>1% cardiac malformations, >10% lower foetal pup weights, >10% pup mortality) will occur at or below the limit dose level at 1000 mg/kg bw/d. Therefore, RAC agrees with the DS and considers that DEGME should be classified as a reproductive toxicant in category 1B, but with a low potency and the corresponding SCL of 3%.

Adverse effects on or via lactation

No studies were found regarding the effects of DEGME following lactational exposure in animals or in humans. The DS did therefore not include any comparison with the CLP criteria.

Overall, RAC considers that classification as **Repr. 1B; H360D** with an **SCL of 3%** is warranted for DEGME

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).