Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol

EC Number: -
CAS Number: -

CLH-O-0000007209-71-01/F

Adopted

1 December 2022

1 December 2022

CLH-O-0000007209-71-01/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: **Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol**

EC Number: **-**

CAS Number: **-**

The proposal was submitted by the **Netherlands** and received by RAC on **2 November 2021.**

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 **7 February 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **8 April 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Tom Gebel**

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 **1 December 2022** by **consensus.**

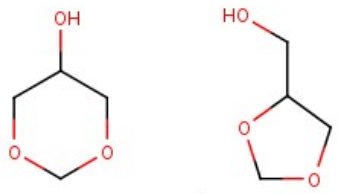
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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	No current Annex VI entry										
Dossier submitters proposal	TBD	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol	-	-	Repr. 1B	H360Df	GHS08 Dgr	H360Df			
RAC opinion	TBD	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol	-	-	Repr. 1B	H360Df	GHS08 Dgr	H360Df			
Resulting Annex VI entry if agreed by COM	TBD	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol	-	-	Repr. 1B	H360Df	GHS08 Dgr	H360Df			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol (glycerol formal) is a glycerol acetal, which is composed of ca. 60% 1,3-dioxan-5-ol and 40% 1,3-dioxolan-4-ylmethanol in an equilibrium.



The substance is used by consumers, by professional workers (widespread uses), in formulation or re-packing, at industrial sites and in manufacturing (ECHA Dissemination site: consulted 21/07/22). It does not have an entry in Annex VI to the CLP Regulation. The Dossier Submitter (DS) proposed a classification as Repr. 1B; H360Df. Reproductive toxicity is the only endpoint assessed in the CLH report, which is supplemented by an Annex with more detailed information. The reliability scores referred to throughout this document are from the CLH report and appear to be standard (Klimisch) scores.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

Overall, 8 animal studies were presented by the DS. No reliable studies explicitly addressing the endpoint "adverse effects on sexual function and fertility" were available in the registration dossier from 2021. However, the registrant provided study reports for two additional oral rat studies, which included exposure during mating and gestation and measurement of reproductive parameters (study report (1982b), study report (1982c)). No adverse effects on reproductive parameters or toxicity in dams were observed in these studies. The dose levels applied in these two studies were too low (max. 25 mg/kg bw/d) and therefore the DS gave both studies reliability scores of 3.

In consequence, the DS based the assessment on the available repeated dose toxicity studies. A 90-d repeated dose toxicity study (similar to OECD TG 408) in Sprague Dawley rats (study report, 1973) was considered as the key study. A reliability score of 2 is reported in the CLH report. Ten animals per sex and dose group received daily doses of 0, 12, 121, 1218 mg/kg bw/d via gavage. Two additional animals per sex and dose were used as recovery group and were held for six weeks after the end of exposure. Decreased relative reproductive organ weights (uterus, seminal vesicles, testes and epididymides) and histopathological effects in testes, seminal vesicles and epididymides were described. In addition to a reduced body weight gain lethality was observed in the highest dose group. Some deficiencies of this study were identified (e.g., large dose spacing, limit dose exceeded, no severity scores, no analysis of sperm parameters). All other studies received reliability scores of 3 or 4 by the DS. In two 90-d toxicity studies effects in male reproductive organs were also detected. However, the first study in rats was performed with

subcutaneous application and the second study in dogs was conducted with intramuscular application and used a low number of animals.

In summary, the DS concluded that the effects on the male reproductive organs provide some evidence with respect to classification, which justifies a classification in Category 2. No specific concentration limit was proposed.

Adverse effects on development

The DS described six studies in rats for the endpoint "adverse effects on development". Furthermore, a Frog Embryo teratogenesis Assay (FETAX) and several QSAR predictions were taken into account.

A prenatal developmental toxicity study from 1981 (similar to OECD TG 414), which received a reliability score of 2, was considered to be the key study by the DS (study report, 1981). Charles River CRCD rats were exposed from gestation day (GD) 6 – 17 to 0, 75, 150, 300 and 600 mg/kg bw/d via gavage. No clinical signs of toxicity and no adverse effects on body weight were observed in dams. In the absence of maternal toxicity, a pronounced and partly dose-dependent developmental toxicity was observed, including increased resorptions, a decreased number of live foetuses, an increased number of dead foetuses, a decreased number of live foetuses/pregnant female and a decrease in average foetal weight per litter. In addition, external malformations (e.g., anal atresia, tail malformations) and visceral malformations (e.g., ventricular septum defects) were observed, as well as several types of variations and a delay in ossification.

A further rat study (similar to OECD TG 414), which was given a reliability score of 2 by the DS, consisted of 5 experiments which included comparing the findings after intramuscular, subcutaneous and oral application (Aliverti et al. (1980)). The experiments also had different durations (days) of exposure and different rat strains were investigated. In experiment 4, oral exposure (0, 600, 1200 mg/kg bw/d) induced developmental toxicity (e.g., post-implantation loss, malformations) already at 600 mg/kg bw/d without maternal toxicity. Intramuscular and subcutaneous application also induced developmental toxicity (described in more detail under "Assessment and comparison with the classification criteria").

All other developmental toxicity studies (as well as a FETAX-assay and QSAR-predictions) received a reliability score of only 3 by the DS due to various limitations (e.g., only one dose tested, tested dose too low, subcutaneous administration, poor documentation, lack of information on purity, reduced study design). In two of these limited studies (Giavini and Prati (1980), Giavini et al. (1981)) cardiovascular malformations were observed.

In summary, the DS concluded that the clear and dose-dependent adverse effects on development (resorptions, decrease in foetal body weight, delay in foetal ossification, teratogenic effects), which were observed in rats in the reliable key study (similar to OECD TG 414) in the absence of maternal toxicity, supplemented by the results of the other studies, justifies a classification in Category 1B. No specific concentration limit was proposed.

Adverse effects on or via lactation

The DS proposed no classification due to lack of reliable data to assess adverse effects on or via lactation.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the proposed classification (Repr. 1B; H360Df) and reminded that two studies of limited quality are available which explicitly addressed the endpoint sexual function and fertility. Furthermore, it was proposed that an oral reproduction

study in female rats ("in utero study") (1982b) also be mentioned in the chapter on effects on or via lactation.

Another MSCA also supported the proposed classification but considered the classification with respect to adverse effects on sexual function and fertility as borderline to no classification.

One company confirmed that it has no knowledge of further toxicological and ecotoxicological studies of relevance.

Assessment and comparison with the classification criteria

Adverse effects on sexual function and fertility

No human data could be identified for the assessment of adverse effects on sexual function and fertility. In consequence, the assessment was based on the available eight animal studies. In the descriptions of the studies (below), the oral studies are presented first, starting with the only study for which a reliability score of 2 is reported in the CLH report. All other studies received scores of 3 or 4 from the DS. Two oral studies included mating of animals and determined reproduction parameters (study report (1982b), study report (1982c)). Unfortunately, the applied doses in these studies were very low, which may explain why no effects were seen. Thus, the assessment was based on the repeated dose toxicity studies, which investigated the reproductive organs. Three studies used unusual routes of exposure (intravenous, subcutaneous and intramuscular application).

Oral studies

Oral 90-d study in rats (1973)

ECHA dissemination site (2022): 001 in section on repeated oral toxicity (key study)

An oral 90-d study (similar to OECD test guideline 408) was performed in Sprague Dawley rats. Mentioned as deviations from the guideline were missing ophthalmological examinations, functional observations and data on water consumption. The test substance consisted of a mixture of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%), the two components of glycerol formal. The mixture was stabilised by 0.02% ethylenediamine tetraacetic acid-sodium salt, 0.02% propylgallate and 0.01% thiodipropionic acid. Ten animals per sex and dose group received doses of 0, 12, 121, 1218 mg/kg bw/d via gavage, with water as the vehicle. Two additional animals per sex and dose were included in a recovery group and were retained untreated for six weeks after the end of exposure. A reliability score of 2 is reported in the CLH report. Two males and two females of the high dose group died (1 female after 3 d, 1 female after 77 d, 1 male after 5 d and 1 male after 74 d). Including the animals of the recovery group, the mortality rate was 16.7% in both sexes (20% excluding the animals of the recovery group). Furthermore, one control animal of the recovery group died on the 124th day (ECHA Dissemination site: consulted 28/07/22). No other clinical signs were observed. The mean body weights of females after 90 d were 252 g, 257 g and 254 g in the low, middle and high dose, respectively, compared to 268 g in the control group. In males the mean body weights after 90 d were 422 g, 419 g and 400 g in the low, mid and high dose, respectively (control group: 435 g). It is noted that the initial mean body weight of the groups already showed some variability (see table below). In males for example, the initial mean weight of the control group was about 15% lower compared to the high dose group. The weight gain in the low, mid and high dose groups was 89.74%, 94.87% and 66.67%, respectively, in females and 80.87%, 68.31% and 57.36%, respectively, in males, compared to the control animals. This decrease was accompanied by a reduced feed consumption and feed efficiency, particularly in males. However, there is no information available about the standard variation or statistical significance of body weight data and feed consumption. Thus, the relevance of the apparent differences is not clear. Furthermore,

a higher hemosiderin content of the spleen was mentioned for the animals of the highest dose. With respect to reproductive toxicity a decrease of the relative weight of reproductive organs (testes, epididymides, seminal vesicle and uterus) was observed, especially in the high dose group (see table below). A slight inhibition of spermiogenesis in 1/10 animals was determined in the low dose group, a partial inhibition of spermiogenesis in 2/10 animals with single atrophic seminiferous tubules in one of them in the mid dose group and changes in spermiogenesis in all surviving animals (8/8) in the high dose group ranging from slight inhibition to total atrophy. In the high dose group, the seminal vesicles were affected in 4/8 animals (plenty of excretion with swarms of desquamated cells) and the epididymides in all animals, mainly abnormal content in the ductus epididymidis and changes in the epididymal tubes. Effects on testes and epididymides were also observed in the recovery group: In the mid dose group, atrophic seminiferous tubules in low number and a slight interstitial oedema were detected in 1/2 animals. In the high dose group, the two animals showed inhibited spermiogenesis, single completely atrophic tubules and traces of an interstitial oedema. Furthermore, degenerated cells from the seminal epithelium in the ductus epididymidis were observed in both animals. The relative uterus weight was also reduced, and some inflammation was observed in the uteri, but the histopathological effects were not dose-related and a concern with respect to female fertility is not sufficiently substantiated.

***Table**: Oral 90-d study in rats from 1973: mortality, body weight (gain) and feed consumption data compared to reproductive parameters*

Dose (mg/kg bw/d)	0	12	121	1218
Number of animals/sex	10	10	10	10
Females				
Mortality, body weight (gain) and feed consumption				
Mortality	0	0	0	2
Mean initial bw (g)	190	182	183	202
Mean bw (g) after 90 d	268	252	257	254
Relative weight gain in % of control	-	89.74	94.87	66.67
Mean feed consumption (g/animal)	1310	1250	1272	1181
Relative feed efficiency in % of control	100	94.11	97.82	73.95
Relative weights of reproductive organs (% of bw)				
Uterus	0.19	0.16	0.16	0.14
Number of animals with histopathological effects in reproductive organs				
Uterus	0	2/10	3/10	1/8
Males				
Mortality, body weight (gain) and feed consumption				
Mortality	0	0	0	2
Mean initial bw (g)	252	274	294	295
Mean bw (g) after 90 d	435	422	419	400
Relative weight gain in % of control	-	80.87	68.31	57.38
Mean feed consumption (g/animal)	1934	1927	1846	1684
Relative feed efficiency in % of control	100	81.18	71.56	65.96
Relative weights of reproductive organs (% of bw)				
Testes	0.87	0.89	0.89	0.52
Epididymides	0.30	0.29	0.27	0.18
Seminal vesicles	0.15	0.12	0.12	0.095
Number of animals with histopathological effects in reproductive organs				

Dose (mg/kg bw/d)	0	12	121	1218
Number of animals/sex	10	10	10	10
Testes	0	1/10	2/10	8/8[a]
Epididymides	0	0	0	8/8
Seminal vesicles	0	0	0	4/8

a: 8 animals were investigated histopathologically (Annex I of CLH-report, p. 8). The two excluded animals were probably those that died prematurely.

Some deficiencies of the study could be identified, which hampered the evaluation. The top dose was too high, because 2 males and 2 females died before the end of the study. The direct cause of death was apparently pulmonary oedema. Thus, the relevance of the observed effects at this dose is questionable. The top dose (1218 mg/kg bw/d) was above the limit dose of 1000 mg/kg bw/d, but only slightly. The dose spacing was larger than usual (factor of 10) and unfortunately, a dose of about 300 mg/kg bw/d was not tested. Below the high dose, the relevant effects at the mid dose are limited to low incidences of histopathological effects in the testes and small reductions of the relative weights of the epididymides and seminal vesicles. A statistical analysis of the data is missing. As stated by the DS, no information on the severity of the effects was provided (e.g., severity scores) and no analysis of sperm parameters (number, quality etc.) was performed.

Oral 16-week-study in rats (no date given)

ECHA dissemination site (2022): 002 in section on repeated oral toxicity (supporting)

A further oral study was performed in male Wistar rats (10/dose). Glycerol formal was applied via the diet with doses of 316, 1000, 3162, 10000 ppm for 16 weeks. The doses correspond to 15.8, 50, 158 and 500 mg/kg bw/d (conversion according to the Guidance on the Application of the CLP criteria (CLP guidance; ECHA, 2017). There was no information available on the purity of the substance. No specific guideline was mentioned. A reduced body weight gain was observed at 158 and 500 mg/kg bw/d. The examination of reproductive organs was not reported. Due to the poor documentation the study received a score of 4 and was considered not reliable by the DS.

Oral reproduction study in female rats ("in utero study") (1982b)

ECHA dissemination site (2022): 001 in the section on toxicity to reproduction (supporting)

Female Charles River CRCD rats (20/dose group) were administered via gavage doses of 0, 1, 5 and 25 mg/kg bw/d 14 d prior to mating (with unexposed males), during mating and gestation until postnatal day (PND) 20. The purity of glycerol formal was higher than 99%. No signs of clinical effects were observed and no effects on reproductive status, mating performance, length of gestation or post-implantation survival rate were detected. The reliability of this study is mainly reduced because of the too low doses, which induced no treatment related effects. Thus, a reliability score of 3 was given by the DS. Pups were used for the 90-d oral toxicity study reported (study report 1982a, see below) and the oral fertility study in male rats (study report 1982c, see below).

Oral fertility study in male rats (1982c)

ECHA dissemination site (2022): 002 in section on toxicity to reproduction (supporting)

Male Charles River CRCD rats (20/dose group), which were exposed *in utero* in the oral reproduction study in female rats (1982b), received via gavage doses of 0, 1, 5 and 25 mg/kg bw/d for 90 d. The purity of glycerol formal was presumably higher than 99%. They were cohabited with untreated females (two females/male) in week 11. Females were sacrificed on GD 14 and reproductive parameters were recorded. With respect to male fertility no effects were

detected as to time to mating, number of mated females/total number of females, or number of pregnant females/total number of mated females. Furthermore, no effects were determined in relation to preimplantation loss, number of resorptions/number of implants, number of live foetuses/pregnant female. The reliability is mainly reduced because of the too low doses, which induced no treatment related effects. Thus, a reliability score of 3 was assigned by the DS.

Oral 90-d study in rats (1982a)

ECHA dissemination site (2022): 003 in section on repeated oral toxicity (supporting)

Another oral 90-d study (similar to OECD TG 408) was performed in Charles River CRCD rats (20 animals/sex/dose group) with doses of 0, 1, 5 and 25 mg/kg bw/d via gavage under conditions of GLP. There is no information about the purity available. Rats used in this experiment had been previously "in utero" exposed in the oral reproduction study in female rats (1982b). No histopathological effects were detected in ovaries, uterus, testes, epididymides and prostate, and overall, no treatment related effects were observed. One animal of the control and one animal of the highest dose group showed unilateral testicular degeneration. However, the effect was more severe in the control animal. The reliability of this study is mainly questioned because of the too low doses, which induced no treatment related effects. This was the reason for a reliability score of 3 given by the DS.

<u>Studies with intravenous, subcutaneous and intramuscular application</u>

28-d-study in rabbits with intravenous application (no date given)

ECHA Dissemination site (2022): 001 in section on repeated dose toxicity, other routes (supporting)

A 28-d study with New Zealand White rabbits was conducted with intravenous application. Five animals/dose group (3 females/2 males in exposure groups and 4 female/1 male in the control group) received doses of 0, 29.2 and 292 mg/kg bw/d glycerol formal in a 0.9% NaCl solution as the vehicle. The test substance consisted of a mixture of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%), the two components of glycerol formal. The mixture was stabilised by 0.02% ethylenediamine tetraacetic acid-sodium salt, 0.02% propylgallate and 0.01% thiodipropionic acid. No specific guideline was mentioned. No effects on body weight were observed. The histological examinations of the liver revealed enlarged cells charged with fat in the high dose group. Uterus, testes and epididymides were also investigated histopathologically. 1/2 males of the high dose group showed a reduced spermiogenesis and abnormal elements such as polynuclear cells in the spermatic epithelium. However, testes effects were also reported for the one control male. The tubuli contorti seminiferi showed the picture of an only moderately active spermiogenesis. Due to the unusual route of application and the limited number of animals a reliability score of 3 was assigned to this study by the DS.

90-d study in rats with subcutaneous application (no date given)

ECHA Dissemination site (2022): 002 in section on repeated dose toxicity, other routes (supporting)

A 90-d study with subcutaneous application was performed in Sprague Dawley rats (10 animals/sex/dose) with calculated doses of 0, 292.3, 584.6 and 1461.6 mg/kg bw/d. The test substance consisted of a mixture of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%), the two components of glycerol formal. The mixture was stabilised by 0.02% ethylenediamine tetraacetic acid-sodium salt, 0.02% propylgallate and 0.01% thiodipropionic acid. Glycerol formal was applied as a 30% dilution in a 0.9% NaCl solution in all three dose groups. A second high dose group received the dose of 1461.6 mg/kg bw/d as a 50% dilution in 0.9% NaCl solution (high dose II). No specific guideline was mentioned. A recovery group consisted of 2 additional animals/sex/dose held for six weeks after end of exposure. No signs of

clinical effects were observed. The dosed females showed a higher body weight increase (low dose: 155.5% of control, mid dose: 137.8%, high dose I: 155.5%, high dose II: 115.6%). This apparent increase might be caused by the higher initial body weight of the control animals. The males showed a reduced body weight gain compared to control in the mid and high dose (low dose: 98.0%, mid dose: 79.8%, high dose I: 58.6%, high dose II: 60.6%). Furthermore, in the high dose groups reduced weights of the thyroid and hypophysis, enlarged cells in the hypophysis and haematological alterations (e.g., changes in erythrocyte count, decrease of leucocyte count) were determined.

With respect to reproductive toxicity, the relative weights of reproductive organs (testes, epididymides and ovaries) were decreased in the two high dose groups (see table below). Histopathology of the male reproductive organs revealed in 1/10 control animals a subtotal atrophy in the testes and changes in the epididymides and seminal vesicles. In the low dose group 1/10 animals showed testes alterations (including reduced spermiogenesis). In the mid dose group disturbances of spermiogenesis with interstitial oedema were found in 5/10 animals. In the high dose group I, atrophy of the seminiferous tubules combined with interstitial oedema was found in all animals, which was accompanied by changes in the epididymides and seminal vesicles. Also in the high dose group II changes in the testes were observed in all animals. In 8/10 animals a subtotal to total atrophy of the seminal tubules with alterations in the epididymides and seminal vesicles were found. The high dose recovery groups also showed effects in male reproductive organs. A slight interstitial oedema in 1/2 animals and a partial atrophy in the other as well as alterations of epididymides and seminal vesicle were detected. In the second high dose recovery group changes in testes and epididymides were observed in 1/2 animals.

In females of the high dose group I, only small to medium-sized tertiary follicles of ovaries were observed. In the second high dose group (II) small to middle-sized follicles were found in 8/10 animals. These effects did also not reverse after 6 weeks of recovery.

The study received a reliability score of 3 from the DS, mainly because of the unusual route of exposure (subcutaneous application). Furthermore, the top dose of 1461.6 mg/kg bw/d, which induced pronounced effects, is higher than the (oral) limit dose of 1000 mg/kg bw/d.

***Table**: 90-d study in rats with subcutaneous application: body weight gain and reproductive parameters*

Dose (mg/kg bw/d)	0	292.3	584.6	1461.6 (I)	1461.6 (II)
Number of animals/sex	10	10	10	10	10
Females					
Body weight gain					
Relative weight gain in % of control	-	155.5	137.8	155.5	115.6
Relative weights of reproductive organs (% of bw)					
Ovaries	0.039	0.037	0.035	0.033	0.030
Number of animals with histopathological effects in reproductive organs					
Ovaries	0	0	0	10/10	8/10
Males					
Body weight gain					
Relative weight gain in % of control	-	98.0	79.8	58.6	60.6
Relative weights of reproductive organs (% of bw)					
Testes	0.75	0.82	0.79	0.38	0.46
Epididymides	0.25	0.25	0.25	0.16	0.19
Number of animals with histopathological effects in reproductive organs					
Testes	1/10[a]	1/10	5/10	10/10[a]	10/10[a]

a: also effects in epididymides and seminal vesicles

90-d study in dogs with intramuscular application (no date given)

ECHA Dissemination site: 003 in section on repeated dose toxicity, other routes (supporting)

A 90-d study with intramuscular application was performed in Beagle dogs. Two males and 2 females/exposure group were administered calculated doses of 0, 29.23 and 292.3 mg/kg bw/d. The test substance consisted of a mixture of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%), the two components of glycerol formal. The mixture was stabilised by 0.02% ethylenediamine tetraacetic acid-sodium salt, 0.02% propylgallate and 0.01% thiodipropionic acid. Three males and two females were used as control animals. The low dose was applied as 30% solution in 0.9% NaCl. The high dose was administered as a 30% solution in 0.9 % NaCl (dose II1) and in a second high dose group as a 50% solution in 0.9 % NaCl (dose II2). No guideline was mentioned. No clinical signs or changes in body or organ weights were detected. The histopathological examination of the thyroid detected a desquamation of the epithelium in single follicles in one animal of the high dose group II1 and partly colloid-poorer follicles with desquamation of the epithelium in 2/2 animals in the high dose group II2. The histopathological examination of ovary, uterus, testes and epididymides did not detect effects at the low dose. In the first high dose group (II1) minimal/negligible depression of spermiogenesis and changes in the content of the tubules of the epididymides were observed in 2/2 males. In the second high dose group (II2) 1/2 males showed a partial atrophy of the seminiferous tubules in testes and changes of the epididymides. No effects on the uterus and ovaries were reported. The reliability score given by the DS was 3 because of the low number of animals and the unusual route of exposure.

Summary of adverse effects on sexual function and fertility and conclusion on classification

Two studies included mating of animals and determined reproduction parameters (study report (1982b), study report (1982c)). No adverse effects were identified with respect to sexual function and fertility and general toxicity up to a dose of 25 mg/kg bw/d. Thus, the maximum dose was too low to derive a robust conclusion on adverse effects on sexual function and fertility because no general toxicity was induced.

Thus, the assessment was based on the repeated dose toxicity studies, which investigated the reproductive organs and the general toxicity in sufficient detail. The only study, which received a reliability score of 2 was the oral 90-d study in rats from 1973. Clear effects on male reproductive organs (testes, epididymides, seminal vesicle) were observed in the highest dose (1218 mg/kg bw/d), which included reduced organ weights and histopathological effects. This dose, which exceeded the limit dose of 1000 mg/kg bw/d, also induced mortality in two males, thus the dose was too high to be considered for classification purposes. If effects on male reproductive organs would only have been observed in the highest dose group, the high mortality could justify "no classification" for this endpoint. However, histopathological testes effects were also observed in 2/10 animals of the mid dose group and in 1/2 animals in the mid dose recovery group. In the low dose group only slight inhibition of spermiogenesis was observed in 1/10 animals. A reduced body weight gain of males was observed, but a statistical analysis was not performed, and the relevance is not clear. Some further deficiencies of this old study were noted, which include the large dose spacing (factor of 10), missing investigations of sperm parameters and uncertainties about the severity of effects. The other repeated dose toxicity studies suffer from a limited reliability (score of 3 or 4), but it is noted, that the 90-d study with subcutaneous application in rats induced effects on male reproductive organs at 1461.6 mg/kg bw/d, exceeding the (oral) limit dose, but also at 584.6 mg/kg bw/d with respect to the testes, all in the presence of a reduced body weight gain. Furthermore, the 90-d study with intramuscular application in dogs detected some effects at a dose of 292.3 mg/kg bw/d in testes and changes of the epididymides, accompanied by a desquamation of the epithelium in the thyroid as general toxicity.

According to the CLP Regulation (Annex I, Table 3.7.1 (a)), "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)*."

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, 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*" ("Suspected human reproductive toxicant").

RAC acknowledges, that human data or conclusive mating studies of sufficient reliability are not available. However, it is stated in the CLP regulation, that a classification can also be based on repeated dose toxicity studies (Annex I, 3.7.2.5.3.): *"Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads."*

Summary of effects in male reproductive organs below the limit dose of 1000 mg/kg bw/d

With respect to the designated key study (90-d, oral, rats, 1973) effects in the male reproductive organs below the limit dose were slight reductions of the relative weight of epididymides and seminal vesicles and histopathological testes alterations in single animals (2/10) at the mid dose of 121 mg/kg bw/d, accompanied by a reduced body weight gain. Effects in the mid dose group did not fully reverse in the recovery group. A slight inhibition of spermiogenesis in 1/10 animals was observed at the low dose (12 mg/kg bw/d) as a borderline effect. The 90-d rat study with subcutaneous application, implying a 100% systemic availability, detected histopathological testes effects in 5/10 animals in the mid dose group (584.6 mg/kg bw/d) in the presence of a reduced body weight gain. These effects reversed after 6 weeks of recovery. The 90-d study in dogs with intramuscular administration, which also means a 100% systemic availability, showed histopathological effects in testes and epididymides at 292.3 mg/kg bw/d, accompanied by a desquamation of the epithelium in the thyroid as general toxicity.

RAC concludes that there is some evidence of a potential to damage male reproductive organs and in light of the presumed low sperm reserve of humans **RAC concurs with the DS, that classification in Category 2 (Repr. 2; H361f) is justified**.

The generic concentration limit of 3% ("group 2", medium potency) is justified, if the ED10 is higher than 4 mg/kg bw/d, but below 400 mg/kg bw/d. No effects were observed below a dose of 4 mg/kg bw/d. With respect to the oral key study (90-d, rat, 1973) very slight effects were determined at 12 mg/kg bw/d (slight inhibition of spermiogenesis in 1/10 rats) and a partial inhibition of spermiogenesis in 2/10 animals with single atrophic seminiferous tubules in one of them at 121 mg/kg bw/d. At the highest dose of 1218 mg/kg bw/d various changes in testes and epididymides were observed in all animals. Thus, the ED10 is between 4 and 400 mg/kg bw/d and the generic concentration limit is justified, as proposed by the DS.

With respect to female fertility, there are slight indications of adverse effects in female reproductive organs; however, these effects are not considered sufficient for classification.

Adverse effects on development

No human data could be identified for the assessment of adverse effects on development. In consequence, the assessment was based on the available six rat studies and supplemental data. The oral studies are presented first, starting with the designated key study with a reliability score of 2.

Oral studies

Oral prenatal developmental toxicity study (1981)

ECHA dissemination site (2022): 001 developmental toxicity (key)

An oral prenatal developmental toxicity study (similar to OECD TG 414) was performed in 25 pregnant Charles River CRCD rats per dose group (24 animals in the control). Rats were exposed from GD 6 – 17 to 0, 75, 150, 300 or 600 mg/kg bw/d glycerol formal via gavage. The purity of glycerol formal was higher than 99%. On GD 20 the animals were sacrificed. The study received a reliability score of 2, a minor limitation referred to the reduced exposure window compared to the current version of OECD TG 414.

Clinical signs or effects on the body weight of dams were not observed. Thus, there was no maternal toxicity up to the highest dose. With respect to developmental effects, the total number of resorptions was increased in the highest dose group (see table below). Furthermore, the number of resorptions/number of implants per female was significantly increased in this dose group. The total number of live foetuses was decreased, and the number of dead foetuses increased. The number of live foetuses/pregnant female was significantly decreased in the highest dose group. The average foetal weight per litter was significantly and dose-dependently decreased at ≥ 150 mg/kg bw/d.

The DS reported that external malformations were increased in the two highest dose groups, including anal atresia (0/0/0/2/7) and tail malformations (0/1/0/4/10). Anasarca was increased at 600 mg/kg bw/d (0/0/0/0/3+2 in dead foetuses) (see table below).

Furthermore, the DS showed that visceral malformations were detected in the highest dose group as ventricular septal defects (3/0/2/4/26+6 in dead foetuses) and retroesophageal aortic arch malformations (0/0/0/0/3+1 in dead foetus). In addition, azygous branching variations were increased (0/0/6/6/12), starting at 150 mg/kg bw/d.

With respect to skeletal findings, skull bone malformations were mentioned for the highest dose group (0/0/0/0/3). Wavy ribs were observed (0/3/14/46/62+1 in dead foetus) and considered as malformation by the study author and included as such in the corresponding table. Referring to the DEVTOX database the DS reminded that wavy ribs could also be considered to be a variation. Furthermore, skeletal variations like cervical ribs (3/3/0/7/25+2 in dead foetuses), lumbar ribs (35/56/54/56/96) and extra lumbar vertebra (0/0/0/16/54) were observed. In addition, a dose-dependent delay in foetal ossification (variation), primarily of the skull bones, vertebra, and sternebra was observed in all treatment groups: incomplete ossification of skull bone (2/21/41/95/112), incompletely ossified cervical vertebra (0/3/16/86/188), incompletely ossified thoracic vertebra (1/6/26/58/149), incompletely ossified sternebra (73/137/251/244/264), incompletely ossified lumbar vertebra (0/2/6/16/78), incompletely ossified sacral vertebra (0/2/13/18/66) and incompletely ossified pelvic bone (1/5/31/82/175).

Table: *Selected parameters of the oral prenatal developmental toxicity key study in rats (1981) from the annex of the CLH-report (some types of effect are specified in italics)*

Dose (mg/kg bw/d)	0	75	150	300	600
No. of females	24	25	25	25	25
Reproductive status					
Total no. of resorptions	11	3	6	5	28
No. resorptions/no. implants per female	0.03	0.01	0.02	0.02	0.07*
Total no. of foetuses	308	313	300	262	288
No. of alive foetuses	308	313	300	260	272
No. alive foetuses/pregnant female	12.8	13.0	12.5	12.4	11.3*
No. of dead foetuses	0	0	0	2	16
Average weight (g)/litter	3.68	3.69	3.38*	3.16*	2.81*
External foetal examination (dead foetuses in parenthesis)					
No. examined foetuses	308	313	300	260 (2)	272 (15)
- No. with malformations	0	3	1	7	13 (2)
- No. of malformations	0	3	3	9	21 (2)
-- No. of anal atresia	*0*	*0*	*0*	*2*	*7*
-- No. of tail malformations	*0*	*1*	*0*	*4*	*10*
-- No. of anasarca	*0*	*0*	*0*	*0*	*3 (2)*
No. of examined litters	24	24	24	21	24
- No. with malformations	0	2	1	6	10
Visceral foetal examination (dead foetuses in parenthesis)					
No. examined foetuses	91	100	93	86 (2)	88 (15)
- No. with malformations	3	0	2	4	31 (7)
- No. of malformations	4	0	2	4	44 (10)
-- No. ventricular septal defects	*3*	*0*	*2*	*4*	*26 (6)*
-- No. retroesophageal aortic arch	*0*	*0*	*0*	*0*	*3 (1)*
- No. with variations	0	1	6	6	13
- No. of variations	0	1	6	6	13
No. of examined litters	24	24	24	21	24
- No. with malformations	1	0	1	3	16
- No. with variations	0	1	2	5	8
Skeletal foetal examination (dead foetuses in parenthesis)					
No. examined foetuses	308	313	300	260 (1)	272 (11)
- No. with malformations**	0	3	15	46	66 (2)
- No. of malformations**	0	3	16	46	67 (3)
*-- No. of wavy ribs***	*0*	*3*	*14*	*46*	*62 (1)*
- No. of malformations without wavy ribs	0	0	2	0	5 (2)
-- No. of skull bone malformations	*0*	*0*	*0*	*0*	*3*
- No. with variations	105	177	264	252	269 (2)
- No. of variations	116	236	451	702	1239 (2)
No. of examined litters	24	24	24	21	24
- No. with malformations**	0	2	8	13	17
- No. with variations	23	24	24	21	24

* statistically different from control P≤0.05

** Wavy ribs were considered as malformation by the study author and were included in the overall number of malformations. However, according to the DEVTOX database (https://www.devtox.org/nomenclature/ml_organ.php?lan=en) wavy ribs are considered to be a variation (consulted 01/08/2022).

RAC notes in support of the DS proposal, that various parameters of the reproductive status (e.g., resorptions, live foetuses, foetal weight) were clearly affected, starting at 150 mg/kg bw/d, with

a significantly reduced foetal weight/litter. Statistically significant increases in the incidences of resorptions/number of implants per female and a reduced number of live foetuses/pregnant female, all in the absence of maternal toxicity, were also found. With respect to external findings, RAC considers the increased incidence of anal atresia (0/0/0/2/7) to be the main malformation. Some uncertainties surround the tail malformations, because it is not clear what specific type of findings were summarised. With regard to the visceral findings, the ventricular septal defects (3/0/2/4/26 (6)) clearly stand out and are considered as the key malformation induced by glycerol formal. With respect to skeletal findings, RAC is of the opinion that it is more appropriate to consider the wavy ribs as a variation, which should be subtracted from the overall incidence of skeletal malformations. The incidence of skull malformations was slightly increased in the high dose (0/0/0/0/3), but it is not clear what specific findings were summarised and considered as malformations.

Oral prenatal developmental toxicity study (1981)

ECHA dissemination site (2022): 002 developmental toxicity (key)

This study did not detect a NOAEL for developmental toxicity. Thus, a further oral prenatal developmental toxicity study with only one low dose of 10 mg/kg bw/d and a control group was performed. The study design and the investigated endpoints were the same as in the study described above. No adverse effects were determined. The study is of limited relevance for classification purposes because the tested dose was below any effect level (reliability score by the DS: 3).

Oral reproduction study in female rats ("in utero study") (1982b)

ECHA dissemination site (2022): 001 in section on toxicity to reproduction (supporting)

The oral reproduction study in female rats, which was already summarised in the chapter on adverse effects on sexual function and fertility, is also taken into account with respect to developmental toxicity as supplemental information. The low doses of 0, 1, 5 and 25 mg/kg bw/d did not induce effects in dams or offspring (reliability score by the DS: 3).

<u>Studies comparing subcutaneous, intramuscular and oral application</u>

Prenatal developmental toxicity study divided into 5 experiments comparing different routes of exposure, dosing periods and rat strains: Aliverti et al. (1980)

ECHA dissemination site (2022): 003 developmental toxicity (supplemental)

The overall design of the 5 experiments was similar to OECD TG 414. The experiments differed by the route of application, the days of exposure, the doses and the rat strain used. The number of pregnant females per dose group was not consistently the same. The number of females with positive vaginal smears ranged from 5 to 13 and the number of females with signs of implantation varied from 5 to 11 (table 1 of the publication; table 13 of the annex of the CLH report). The animals were sacrificed on GD 21. The purity of glycerol formal was described as higher than 99%. Maternal toxicity was not observed in the experiments though in the high dose group of experiment 4 fatalities occurred (see below). The observed abnormalities were divided into external, visceral cardiovascular defects and skeletal costal effects. Furthermore, a malformation rate (No. abnormal foetuses/No. examined foetuses in %) was calculated. It is not fully clear what types of findings were included for the calculation of the malformation rate. This should be kept in mind in the following description. However, it is stated in the publication that malformations were mostly limited to cardiovascular defects (particularly ventricular septal defects) and wavy ribs. Ventricular septal effects are malformations, but wavy ribs should be considered as a variation. A reliability score of 2 was assorted to the study by the DS.

Experiment 1: intramuscular application (Sprague-Dawley rats)

In the first experiment pregnant Sprague-Dawley rats received intramuscular doses of 0, 300, 600 and 1200 mg/kg bw/d glycerol formal between GD 6 and 15. No effects in dams were observed. The post-implantation loss rate raised significantly and dose-dependently (4.4%/8.5%/19.8%/63.7%). The foetal body weight was significantly decreased in all dose groups (4.1 g/3.6 g/3.1 g/3.2 g). The number of females with malformed foetuses (0/2/6/7) increased (no. of females with signs of implantation: 9/8/8/9)). In particular, the malformation rate of visceral cardiovascular defects (0%/0%/28%/75%) was statistically significantly increased in the mid and high dose groups, which particularly affected the cardiovascular system (e.g., ventricular septal defects). The inspection of dead foetuses in the uterus detected widespread subcutaneous oedema and ventricular septal defects. Furthermore, the malformation rate of skeletal costal defects was significantly increased (0%/15%/10%/22%). Skeletal anomalies were mainly limited to wavy ribs.

Experiment 2: intramuscular application (Sprague-Dawley rats)

To identify the most sensitive time period during gestation pregnant Sprague-Dawley rats received two intramuscular doses of 0 and 600 mg/kg bw/d glycerol formal on two days in 5 different groups ranging from GD 7-8, 9-10, 11-12, 13-14 to 15-16. A positive control group was exposed daily from GD 6-15, which confirmed the results from experiment 1. The exposure for only two days in any of these test periods did not induce cardiovascular malformations. The post-implantation loss rate was always significantly lower than in the positive control group, ranging from 0.7% (GD 9-10) to 26.9% (GD 13-14) compared to 47.3% of the positive control group (GD 6-15). The malformation rate of skeletal costal defects was significantly reduced in three groups (GD 7-8: 7%, GD 9-10: 8.9%, GD 11-12: 0%) compared to the positive control group (GD 6-15: 44%)

Experiment 3: subcutaneous compared to intramuscular application (Sprague-Dawley rats)

In experiment 3, Sprague-Dawley rats received doses of 0 and 600 mg/kg bw/d via subcutaneous and intramuscular injection (GD 6-15). The post-implantation loss rate was significantly increased (subcutaneous: 35.7%, intramuscular: 28.7%) compared to the control (3.5%), which received the vehicle via subcutaneous injection. The malformation rate for visceral cardiovascular defects was also significantly increased (subcutaneous: 47%, intramuscular: 35.3%) compared to the control group (0%). Comparing both routes of application widely similar effects were observed as already described in experiment 1. The malformation rate of skeletal costal defects was significantly increased (subcutaneous: 24%, intramuscular: 65%) compared to the control group (0%).

Experiment 4: oral application (Sprague-Dawley rats)

In this experiment pregnant rats were administered oral doses of 0, 600 and 1200 mg/kg bw/d (GD 6-15). The number of females with signs of implantation was 6, 11 and 5 for the control, low and the high dose group. The post-implantation loss rate increased (1.6%/55.9%/95.9%) significantly as well as the rate of visceral malformations (0%/74%/100%), but in the high dose group there were only two live foetuses, thus only 1 foetus was available for the determination of skeletal and visceral effects each. The malformation rate of skeletal costal defects was also higher in the low dose group (0%/20%/0%). The mean foetal weight was decreased (3.7 g/3.0 g/2.4 g). In the high dose group, 2 animals died in course of the experiment, which the study authors attributed to technical errors. In general, similar developmental effects occurred at 600 mg/kg bw/d compared to subcutaneous and intramuscular application of experiment 3. Following oral exposure, the post-implantation loss rate (55.9%) and the malformation rate of visceral cardiovascular defects (74%) was somewhat higher.

Experiment 5: subcutaneous compared to intramuscular application (Wistar rats)

In experiment 5, Wistar rats (instead of Sprague-Dawley rats) were exposed via subcutaneous and intramuscular injection with doses of 0 and 600 mg/kg bw/d (GD 6-15). Compared to the data of Sprague-Dawley rats (experiment 1 and 3) some differences were observed. With respect to intramuscular application the post-implantation loss rate was somewhat lower in Wistar rats (Wistar: 15.7%, Sprague-Dawley: 19.8%) as well as the rate of cardiovascular malformations (Wistar: 8.9%, Sprague-Dawley: 28%) while the malformation rate of skeletal costal effects was higher (Wistar: 46%, Sprague-Dawley: 10%). With respect to the subcutaneous application the post-implantation rate was also lower in Wistar rats and not statistically significant (Wistar: 10.2%, Sprague-Dawley: 35.7%) as well as the rate of cardiovascular malformations (Wistar: 13%, Sprague-Dawley: 47%) and again the rate of skeletal costal effects was higher (Wistar: 47%, Sprague-Dawley: 24%).

Studies with subcutaneous application

Prenatal developmental toxicity study with subcutaneous administration: Giavini and Prati (1980)

ECHA dissemination site (2022): 004 developmental toxicity (supporting)

Pregnant Sprague-Dawley rats (40 per exposure group; 20 in control) were exposed to doses of 0 and 600 mg/kg bw/d via daily subcutaneous injections on GD 6-15 (sacrifice on GD 21). There was no information about the purity of the applied substance. The study design was similar to OECD TG 414. 193 foetuses from the 40 animals, which received glycerol formal, and 119 foetuses of the control group were investigated. 76 foetuses of the dosed group (about 40%) showed cardiovascular malformations, in 39 foetuses ventricular septum defects were detected, which differed by type and gravity. In 20 foetuses the ventricular septum defect was associated with a double aortic arch. Ten foetuses showed a right aortic arch and 4 foetuses a coarctation of the aorta. An aortic-pulmonary window, the absence of innominate artery and dextrocardia was detected in one foetus. Maternal toxicity was not investigated and considering the unusual route of exposure and the missing information about the purity a reliability score of 3 is considered appropriate by the DS.

Prenatal developmental toxicity study with subcutaneous administration: Giavini et al. (1981)

Pregnant Sprague-Dawley rats were administered a daily dose of 1200 mg/kg bw/d subcutaneously from GD 6 to varying days of sacrifice (GD 13, 14, 15, 16 or 17). There is no information about the purity of the applied substance. No guideline is mentioned, and the number of animals is not given (reliability score by the DS: 3). The study focussed on the developmental retardations in the heart. Already at GD 13 a marked dilation of blood vessels was observed. Furthermore, swelling of the embryo was reported. The authors put forward the theory that the malformations were induced by interference with the embryonic osmoregulatory system.

Other studies and information

Information on a frog embryo teratogenesis assay (FETAX) was submitted (Dresser et al. (1992)), which investigated embryos of Xenopus laevis (200/concentration) in concentrations of 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5 and 1.75% in FETAX solution in two trials. The exposure duration was 96 h with media change at 24 h intervals. Among others various malformations (e.g., cephalic, skeletal and ocular malformations) were observed at concentrations of 0.75% and higher. Since the frog is not a common species for the investigation of developmental effects, the relevance of this study is very limited (reliability score by the DS: 3).

Three QSAR predictions were also provided on glycerol formal or its components. The models partly suggest that a prenatal toxicity could be promoted. In light of the various rat studies, the

QSAR predictions are considered as supplemental information of limited relevance (reliability score by the DS: 3).

Summary of adverse effects on development and conclusion on classification

Clear evidence of developmental toxicity in the absence of maternal toxicity was detected in the oral prenatal developmental toxicity study (1981) in rats, which is considered to be the key study for the assessment. External and visceral malformations (e.g., ventricular septal defects), resorptions, a decreased foetal body weight and a delay in foetal ossification was observed at 600 mg/kg bw/d and partly at lower doses in a dose-dependent manner. With respect to oral exposure these effects are complemented by experiment 4 of Aliverti et al. (1980), which detected at 600 mg/kg bw/d in the absence of maternal toxicity a pronounced post-implantation loss and especially cardiovascular malformations such as ventricular septal defects. The other studies demonstrated that developmental toxicity (e.g., ventricular septal defects) also occurred after subcutaneous and intramuscular injection. Ventricular septal defects can be considered as key malformations, and these were seen in various experiments.

According to the CLP Regulation (Annex I, Table 3.7.1 (a)), "*Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B)*."

"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, 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*" ("Suspected human reproductive toxicant").

Human data is not available but based on animal data and considering the criteria of the CLP Regulation and the corresponding CLP guidance (ECHA, 2017), **RAC concurs with the DS that classification in Category 1B (Repr. 1B; H360D) is justified**.

The generic concentration limit of 0.3% ("group 2", medium potency) is justified, if the ED10 is higher than 4 mg/kg bw/d, but below 400 mg/kg bw/d. No developmental effects were observed below a dose of 4 mg/kg bw/d. Based on the oral key study the DS selected the average foetal weight per litter as starting point for the calculation of an ED10, because this parameter showed the lowest LOAEL for the endpoint developmental toxicity. The average foetal weight per litter was significantly reduced at 150 mg/kg bw/d (3.38 g) compared to the control (3.68 g), which represents an 8.2% reduction. Thus, an ED10 is very close to 150 mg/kg bw/d and the generic concentration limit of 0.3% ("group 2", medium potency) would be appropriate, as calculated in detail by the DS in the CLH report.

The average foetal weight per litter was only slightly reduced at 150 mg/kg bw/d (8.2%) and RAC is of the opinion that a calculation based on the key malformation (ventricular septal defects) should be added (table below). The calculation includes the dead foetuses and is in line with example 2 (p. 426) of the CLP guidance (ECHA, 2017).

***Table**: Data for the calculation of an ED10 based on ventricular septal defects (including dead foetuses)*

Dose in mg/kg bw/d	0	75	150	300	600
No. examined foetuses	91	100	93	88	103
No. ventricular septal defects	3	0	2	4	32
Ventricular septal defects in % of examined foetuses	3.3%	0%	2.2%	4.5% (NOAEL for classification)	31% (LOAEL for classification)

Determination of the ED10 value:

Control ventricular septal defects is 3.3%. The ED10 rate would be 13.3%. Interpolation between NOAEL (classification) (4.5% at 300 mg/kg bw/d) and LOAEL (classification) (31% at 600 mg/kg bw/d) leads to an ED10 of 399 mg/kg bw/d.

Calculation:

(600-300) / (31-4.5) = 11.3 mg/kg bw/d per % steepness. Going from 4.5% to 13.3% requires addition of 8.8%. This equals 8.8% * 11.3 mg/kg bw/d per % = 99 mg/kg bw/d

99 mg/kg bw/d plus 300 mg/kg bw/d as starting point = 399 mg/kg bw/d.

The ED10 lies close to 400 mg/kg bw/d. Considering the high severity of effect (ventricular septal defects) as modifying factor (CLP guidance (ECHA, 2017): 3.7.2.6.5, p. 412) the medium potency group is justified, which confirms the evaluation of the DS.

Adverse effects on or via lactation

In the oral reproduction study in female rats (1982b), which is summarised in the chapter on adverse effects on sexual function and fertility, offspring animals were exposed during lactation. No effects were observed in dams or offspring, but this may have been due to the too low doses (0, 1, 5 or 25 mg/kg bw/d) used in the study. Thus, **no robust conclusions can be drawn with respect to adverse effects on or via lactation and no classification can be derived due to inconclusive data**.

Overall, RAC concludes that classification as Repr. 1B; H360Df is justified. No specific concentration limits are proposed.

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).