Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

1 December 2022

CLH report

Proposal for Harmonised Classification and Labelling

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

Chemical name:

Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol (glycerol formal)

EC Number: **N/A**

CAS Number: **N/A**

Index Number: **603-RST-VW-Y**

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION MASS OF 1,3-DIOXAN-5-OL AND 1,3-DIOXOLAN-4-YLMETHANOL

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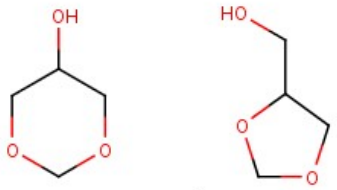
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON REACTION MASS OF 1,3-DIOXAN-5-OL AND 1,3-DIOXOLAN-4-YLMETHANOL

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol
Other names (usual name, trade name, abbreviation)	Glycerol formal
ISO common name (if available and appropriate)	Not applicable
EC number (if available and appropriate)	Not applicable
EC name (if available and appropriate)	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol
CAS number (if available)	-
Other identity code (if available)	Index Number: 603-RST-VW-Y
Molecular formula	$C_4H_8O_3$ (for both substances in the reaction mass)
Structural formula	
SMILES notation (if available)	1,3-dioxan-5-ol: OC1COCOC1 1,3-dioxolan-4-ylmethanol: OCC1COCO1
Molecular weight or molecular weight range	104.11 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The isomer 1,3-dioxolan-4-ylmethanol has a chirality centre and therefore occurs as a pair of enantiomers (no information on ratio of isomers available)
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not relevant
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

Glycerol formal is a glycerol acetal that is composed of about 60% 1,3-dioxan-5-ol and 40% 1,3-dioxolan-4-ylmethanol in an equilibrium. It can be prepared by reacting glycerol and formaldehyde in the presence of an acid catalyst[1]. The equilibrium between the isomers occurs via the open-chain form of the hemiacetal (see Figure 1).

[1] https://www.sigmaaldrich.com/catalog/product/ALDRICH/49920?lang=de&region=DE; See confidential annex I for typical concentration/concentration range for the constituents based on the information of the registrant

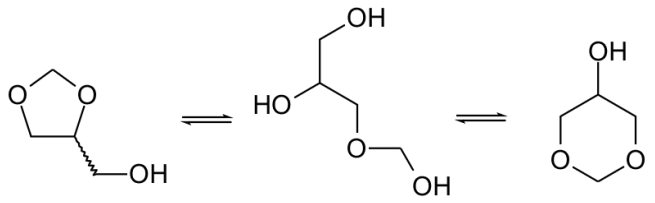


Figure 1: equilibrium between 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol via the open-chain form of the hemiacetal (https://commons.wikimedia.org/w/index.php?curid=19892044)

The isomer 1,3-dioxolan-4-ylmethanol has a chirality centre and therefore occurs as a pair of enantiomers.

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)*
1,3-dioxolan-4-ylmethanol **CAS number: 5464-28-8** **EC number: 226-758-4**	Confidential information	No harmonised classification available	Eye Irritation 2 (H319)
1,3-dioxan-5-ol **CAS number: 4740-78-7** **EC number: 225-248-9**	Confidential information	No harmonised classification available	Eye Irritation 2 (H319)

* ECHA C&L Inventory (2021) Information on Chemicals - Classification & Labelling Inventory, European Chemicals Agency. Online: http://echa.europa.eu/information-on-chemicals/cl-inventory

In several study reports available on ECHA Dissemination (2021) the test material is given as a mixture with constant ratio of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%) .

The following self-classification has been provided by the registrant for glycerol formal:

- Eye Irrit. 2 (H319)
- Repr. 2 (H361)

The test substance is glycerol formal in all studies where the test substance was explicitly stated. The purity is given in the study records below if available.

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Confidential information				

In several study reports available on ECHA Dissemination (2021) the following impurities of the test substance are mentioned: “stabilised with 0.02% ethylenediamine tetraacetic acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid”.

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

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
No information on additives available					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: For substance with no current entry in Annex VI of CLP

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATEs	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	603-RST-VW-Y	Reaction mass of 1,3-dioxan-5-ol and 1,3-dioxolan-4-ylmethanol	-	-	Repr. 1B	H360 Df	GHS08 Danger	H360 Df		-	

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

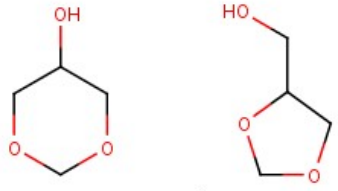
Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	hazard class not assessed in this dossier	No
Carcinogenicity	hazard class not assessed in this dossier	No
Reproductive toxicity	harmonised classification proposed; Repr. 1B (H360Df)	Yes
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	hazard class not assessed in this dossier	No
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There is no harmonised classification and labelling available for glycerol formal. The substance has not been included in former activities on harmonised classification.

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.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

The substance has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR properties is a community-wide action under article 36 of the CLP regulation.

5 IDENTIFIED USES

According to ECHA disseminated database (ECHA Dissemination, 2021) the substance is used at industrial sites in the following products: coatings, adhesives, sealants and elastomers. It is used as laboratory chemicals and as an intermediate. Professional uses also include the use in agrochemicals. According to ECHA disseminated database, the substance is also used in consumer products including home care products (air care products, anti-freeze and de-icing products, biocidal products, perfumes and fragrances, pharmaceuticals, polishes and wax blends and washing and cleaning products), fuels, agrochemicals and coatings, adhesives, sealants and elastomers. According to the registrant, the current consumer uses concern solely agrochemical products.

6 DATA SOURCES

Systematic searches for publications and other relevant data were performed based on the following databases:

- U.S. National Library of Medicine, Pubmed.gov
- TOXNET, ChemIDplus, IPCS, eChemPortal
- Medline, SciSearch, Biosis, PQscitech, Chemical Abstracts (HCA), Embase (at host STN International)

The REACH registration dossier for glycerol formal (last modified: 7 October 2020), publicly available from ECHA's disseminated database (ECHA Dissemination, 2021), has been analysed for study references, which then have been considered as data sources for this CLH report. Additionally, the confidential registration dossier was available for evaluation as well as several original study reports.

No relevant reviews and monographs with toxicological risk assessments on glycerol formal were identified.

Whenever secondary sources were encountered, it was attempted to retrieve the respective primary sources.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g., measured or estimated)
Physical state at 20°C and 101.3 kPa	Liquid	ECHA Dissemination (2021)	visual observation
Melting/freezing point	- 50 °C	ECHA Dissemination (2021)	measured, at 101.3 kPa
Boiling point	193.95 °C	ECHA Dissemination (2021)	calculated from measured data on isomers reported in scientific literature, at 101.3 kPa
Relative density	1.218	ECHA Dissemination (2021)	calculated from measured data on isomers reported in scientific literature, at 20 °C or 25 °
Vapour pressure	30 Pa	ECHA Dissemination (2021)	calculated from measured data, at 20 °C
Surface tension	44.49 mN/m	ECHA Dissemination (2021)	measured, at 25 °C
Water solubility	500 g/L	ECHA Dissemination (2021)	calculated from log Kow, at 20 °C
Partition coefficient n-octanol/water	- 0.99	ECHA Dissemination (2021)	measured
Flash point	99 °C	ECHA Dissemination (2021)	measured
Flammability	non flammable	ECHA Dissemination (2021)	study scientifically not necessary since flash point is over 60 °C
Explosive properties	nonexplosive	ECHA Dissemination (2021)	study scientifically not necessary based on structural assessment of the substance
Self-ignition temperature	> 400 °C	ECHA Dissemination (2021)	reported from secondary source (MSDS), measured at 101.3 kPa
Oxidising properties	no oxidising properties	ECHA Dissemination (2021)	study scientifically not necessary based on structural assessment of the substance
Granulometry	not applicable	ECHA Dissemination (2021)	
Stability in organic solvents and identity of	Not considered	ECHA Dissemination (2021)	study scientifically not necessary: In

Property	Value	Reference	Comment (e.g., measured or estimated)
relevant degradation products	to be critical		accordance with column 1 of REACH Annex IX, the study does not need to be conducted since stability of the substance is not considered to be critical.
Dissociation constant	No	ECHA Dissemination (2021)	study scientifically not necessary based on structural assessment of the substance and SPARC calculation of pKa is > 10
Viscosity	11.7 mm²/s	ECHA Dissemination (2021)	measured, at 25 °C

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetic assessment based on physical/chemical properties of the substance and available open literature. Weight of evidence	No additional information available than the one reported here. <u>Absorption:</u> Partition coefficient: -0.99 water solubility: ~500 mg/L → These properties of the substance are favourable for absorption. <u>Oral/GI absorption</u> Low molecular weight of the substance (104.11 g/mol) is favourable for absorption. The moderate log Kow value is favourable for passive diffusion. For risk assessment purposes, the oral absorption is set at 100% in the registration dossier. <u>Respiratory absorption</u> With a vapor pressure of 0.03 kPa, glycerol formal shows a low volatility. The moderate log Kow value is favourable for absorption directly across the respiratory tract epithelium by passive diffusion. For risk assessment purposes, the inhalation absorption is set at 100% in the registration dossier. <u>Dermal absorption</u> As water solubility is >10 g/L and the log Kow value is <1, glycerol formal may be too hydrophilic to cross the lipid rich environment of the stratum corneum. However, a dermal absorption of 100% is proposed for		Study report, 2013 reported from ECHA Dissemination (2021)

Method	Results	Remarks	Reference
	risk assessment purposes in the registration dossier. Distribution: Distribution of glycerol formal in the body is expected based on its low molecular weight. Accumulative potential: With a log Kow < 4, glycerol formal is not expected to have bioaccumulative potential. Metabolism and excretion: Based on the available data, no conclusions can be drawn on the metabolism and excretion of glycerol formal		

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

Information on toxicokinetic properties of glycerol formal is very limited. The only available information is the entry in the registration dossier in the section "basic toxicokinetics". In this entry oral, inhalation and dermal absorption of glycerol formal are estimated based on physico-chemical properties (low molecular weight, moderate log Kow, low volatility) of the substance. For risk assessment purposes 100% absorption was assumed by the registrants for all three exposure routes. Also based on the low molecular weight of the substance the registrants expected a distribution of glycerol formal in the body but no accumulative potential due to the log Kow < 4. Based on the available data no conclusions on metabolism and excretion could be drawn.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Evaluation not performed for this substance.

10.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

10.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

10.4 Skin corrosion/irritation

Evaluation not performed for this substance.

10.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

10.6 Respiratory sensitisation

Evaluation not performed for this substance.

10.7 Skin sensitisation

Evaluation not performed for this substance.

10.8 Germ cell mutagenicity

Evaluation not performed for this substance.

10.9 Carcinogenicity

Evaluation not performed for this substance.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

No studies explicitly addressing the endpoint "adverse effects on sexual function and fertility" are available in the registration dossier.

In the following table repeated dose studies relevant for the endpoint sexual function and fertility are reported.

Table 9: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
90-day toxicity study Similar to OECD TG 408 GLP: not specified Sprague Dawley rats 10 animals/sex /dose 2 additional animals/sex /dose were treated comparably but held for six weeks after end of exposure to look for regeneration. Reliability: 2 Deviations: ophalmological examinations, functional observation, water consumption were not recorded	Glycerol formal (ST 2060) Impurities: stabilised with 0.02% ethylenediamine tetraacetic acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid Mixture with constant ratio of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%)	Administration orally via gavage 0, 0.01; 0.10 and 1.00 mL/kg bw/d Using the density of 1.218 g/mL, the following doses were calculated by the dossier submitters: 0, 12, 121, 1218 mg/kg bw/d Vehicle: water Exposure duration: 90 days	4 animals died in the high dose group. No other clinical signs were observed. At the end of exposure, the gain in body weight in percent was 89.74, 94.87 and 66.67% (females) and 80.87, 68.31 and 57.36% (males) (low, middle, high dose group) compared to controls (100%) Intake and efficiency of feed were diminished dose-dependent in comparison to controls. Dose-dependent lower relative organ weights (in percent of body weight) are reported for uterus, seminal vesicles, testes and epididymis (uterus: 0.19% in control, 0.14% in highest dose group, seminal vesicles: 0.15% in control, 0.095% in highest exposure group, testes: 0.87% in control, 0.52% in highest dose group; epididymis: 0.30% in control, 0.18% in highest dose group) No indication is given to assume effects on testes (as described below) to be secondary to the reduced body or organ weights. **Control group (including regeneration animals):** No effects observed on reproductive organs. **12 mg/kg bw/d:** Uteri: inflammatory manifestations in 2/10 animals Testes: slight inhibition of	Study report, 1973 reported from ECHA Dissemination (2021) Study: 001 in section on repeated oral toxicity (key) Study reported in detail in Annex I (study 1)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			spermiogenesis in 1/10 animals **121 mg/kg bw/d:** Uteri: inflammatory endometrium in 3/10 animals Testes: partly inhibition of spermiogenesis in 2/10 animals with single atrophic seminiferous tubules in 1/10 **1218 mg/kg bw/d:** Uterus: inflammatory manifestations in 1/10 animals Testes: changes in all animals (10/10), from slight inhibition of spermiogenesis to total atrophy Seminal vesicles: considerable changes in 4 animals (plenty of excretion with swarms of desquamated cells) Epididymis: changes in 8/10 animals, consisting in abnormal content in ductus epididymidis and changes in the epididymal tubes. Data for recovery group: **12 mg/kg bw/d:** Uteri: inflammatory manifestations in 1/2 animals **121 mg/kg bw/d:** Testes: atrophic seminiferous tubules in low number and a slight interstitial oedema (no further details provided) in 1/2 animals **1218 mg/kg bw/d:** Testes: in both animals (2/2) tubules with disturbed spermiogenesis to complete atrophy Epididymis: degenerated cells from the seminal epithelium in the ductus epididymidis in 2/2 animals	
16 week toxicity study	Aqueous paste of diet containing glycerol	Administration orally via diet 316, 1000, 3162, 10000	No additional details or incidence data available than the ones reported below.	Study report (no date given) reported from ECHA Dissemination

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No guideline followed GLP: no Male Wistar rats 10 animals / dose group Reliability: 4	formal (no further information available)	ppm (corresponding to 15.8, 50, 158 and 500 mg/kg bw/d, converted cf. the CLP-Guidance) Exposure duration: 16 weeks	Examination of reproductive organs is not reported in this study. Reduced rate of weight gain in 3162 and 10000 ppm group	(2021) Study: 002 in section on repeated oral toxicity (supp)
90-day toxicity study Similar to OECD TG 408 GLP: yes CRCD rats 20 animals /sex/dose group Reliability: 3	Glycerol formal (no information on purity provided)	Administration orally via gavage 0, 1, 5, 25 mg/kg bw/d Vehicle: water Exposure duration: 90 days Rats used in this experiment have been previously "in-utero" exposed before oral gavage administration. The dosing of the females was 0, 1, 5, 25 mg/kg bw/d starting two weeks before mating until PND 20. Tested concentrations are quite low (maximum of 25 mg/kg bw/d) in comparison with limit concentration stated in OECD TG 408 (1000 mg/kg bw/d).	Ovaries, uterus, testes, epididymis, prostate were studied histopathologically No treatment related effects were observed. In one animal of the control and one animal of the highest dose group unilateral testicular degeneration was observed. The effect was more severe in the control animal. No effects were noted during the histopathological analysis of ovaries, uterus, epididymis, and prostate.	Study report 1982a reported from ECHA Dissemination (2021) Study: 003 in section on repeated oral toxicity (supp)
28-day study with i.v. application No guideline followed GLP: not specified New Zealand White rabbits 5 animals /dose group (3 females and 2 males in exposure groups and	Glycerol formal (ST 2060) impurities: stabilised with 0.02% ethylenediamine tetraacetic acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid Mixture with constant ratio of 5-hydroxy-1,3-dioxane (60%) and 4-	Administration intravenously (into the ear vein) daily in 30% dilution with 0.9 % NaCl solution 0, 0.024, 0.24 mL/kg bw/d Using the density of 1.218 g/mL, the following doses were calculated by the dossier submitters: 0, 29.2 and 292 mg/kg bw/d Vehicle: 0.9% NaCl solution Exposure duration: 28 days	Uterus, testes and epididymis were studied histopathologically No effects on body weight were observed. The study authors stated that differences in comparison to the organs of the control animals appeared only in the liver in dose group II (0.24 mL/kg). In the study report it is stated that in the control male “the tubuli contorti seminiferi show the picture of an only moderately active spermiogenesis”. In the low dose group, no effects on testes were observed (2 animals). In the high dose group 1/2 animals showed a reduced	Study report (no date given) reported from ECHA Dissemination (2021) Study: 001 in section on repeated dose toxicity, other routes (supp)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
4 females and 1 male in control) Reliability: 3 due to low number of animals	hydroxymethyl-1,3-dioxolane (40%)		spermiogenesis and abnormal elements such as polynuclear cells in the spermatic epithelium. The authors of the study concluded that differences in comparison to the organs of the control animals appeared only in the liver of the high dose group. Due to the low number of animals per dose group the results cannot be evaluated.	
90-day study with subcutaneous application No guideline followed GLP: not specified Sprague Dawley rats 10 animals/sex/dose 2 additional animals/sex/dose were treated comparably but held for six weeks after end of exposure to look for regeneration. Reliability: 3 due to subcutaneous application	Glycerol formal (ST 2060) impurities: stabilised with 0.02% ethylenediamine tetraacetic acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid Mixture with constant ratio of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%)	Administration subcutaneously (location not specified) daily in 30% (or 50%) dilution with 0.9 % NaCl solution 0, 0.24, 0.48 and 1.20 mL/kg bw/d Using the density of 1.218 g/mL, the following doses were calculated by the dossier submitters: 0, 292.3, 584.6, 1461.6 mg/kg bw/d dose I: 0.24 mL/kg (30% solution) dose II: 0.48 mL/kg (30% solution) dose III1: 1.20 mL/kg (30% solution) dose III2: 1.20 mL/kg (50% solution) Vehicle: 0.9% NaCl solution Exposure duration: 90 days	No clinical signs were observed. Relative increase in body weight in % of control at the end of exposure: Females: 155.5, 137.8, 155.5, 115.6%, males: 98.0, 79.8, 58.6, 60.6 (dose I, II, III1, III2). Values reported here as given by study authors. However, contradictory information on initial weight of control females is reported in figure 1-1 and table 3 of the original study report (initial body weight in control animals assumed to be quite high, as reported in table 3, compared to other exposure groups). Increase in body weight of females compared to control may be biased by this. Ovary, uterus, testes, epididymis and seminal vesicle studied histopathologically The relative organ weights (in percent of body weight) of the following organs were reduced in both dose groups III: testes (0.75/0.82/0.79/0.38/0.46), epididymis (0.25/0.25/0.25/0.16/0.19), ovaries (0.039/0.037/0.035/0.033/0.030) <u>Effects on testes and epididymis:</u> Control (including regeneration animals): Subtotal atrophy in testes in 1/12 animals. Also changes in epididymis and seminal vesicles of the same animal	Study report (no date given) reported from ECHA Dissemination (2021) Study: 002 in section on repeated dose toxicity, other routes (supp)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			dose I: testis changes (including reduced spermiogenesis) in 1/10 animals. dose II: disturbances of spermiogenesis with interstitial oedema in 5/10 animals. dose III1: atrophy of the seminiferous tubules connected with an interstitial oedema in 10/10 animals, also deviations from normal findings in epididymis and seminal vesicles. dose III2: changes in all animals, in 8/10 a subtotal to total atrophy of the seminal tubules, changes in the epididymis and seminal vesicles analogous to the picture of the testes. <u>Effects on ovaries reported in the highest dose group only:</u> dose III1: only small to medium-sized tertiary follicles dose III2: small to only middle-sized follicles in 8/10 animals. Data for recovery group: dose I: no changes observed (0/2) dose II: no changes observed (0/2) dose III1: Ovaries: only small to medium-sized tertiary follicles in 2/2 animals. Testis: a slight interstitial oedema in 1/2 animals and a partial atrophy in the other as well as changes of epididymes and seminal vesicle. dose III2: Ovaries: small to medium-sized tertiary follicles in 2/2 animals. Testes and epididymes: deviations from the normal findings in 1/2 animals	
90-day study with intramuscular application No guideline followed	Glycerol formal (ST 2060) impurities: stabilised with 0.02% ethylenediamine tetraacetic	Administration intramuscular (gluteal muscle) in 30% (or 50%) dilution with 0.9 % NaCl solution 0, 0.024, 0.24 mL/kg bw/d Using the density of 1.218 g/mL, the following doses	No clinical signs or changes in body or organ weights compared to control animals were observed. Ovary, uterus, testes and epididymis were studied histopathologically No effects on ovaries and uterus	Study report (no date given) reported from ECHA Dissemination (2021) Study: 003 in section on repeated dose toxicity, other routes (supp)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
GLP: not specified Beagle dogs 2 males and 2 females/exposure group, 3 males and 2 females for control group Reliability: 3 due to low number of animals	acid-sodium salt; 0.02% propylgallate and 0.01% thiodipropionic acid Mixture with constant ratio of 5-hydroxy-1,3-dioxane (60%) and 4-hydroxymethyl-1,3-dioxolane (40%)	were calculated by the dossier submitters: 0, 29.23, 292.3 mg/kg bw/d dose I: 0.024 mL/kg - (30% solution) dose II1: 0.24 mL/kg - (30% solution) dose II2: 0.24 mL/kg - (50% solution) Vehicle: 0.9% NaCl solution Exposure duration: 90 days	were reported. <u>Effects on testes and epididymis:</u> dose II1: negligible depression of spermiogenesis and also changes in the content of the tubules of the epididymis in 2/2 animals. dose II2: testis of 1/2 animals partly an atrophy of the seminiferous tubules and changes of the epididymis in the same animal	
Oral reproduction study in female rats "in utero study" Charles River CD rats 20 females/dose group Reliability: 3 due to low dosage	Glycerol formal (purity: >99%)	Administration oral via gavage in water 0, 1, 5, 25 mg/kg bw/d Exposure: 14 days prior to mating (with unexposed males), during mating and gestation until PND 20 of pups Pups were used for 90 day oral toxicity study reported above in this table	No clinical signs observed in females No effects on body weight of dams observed No effects on reproductive status, mating performance, length of gestation, and postimplantation survival rate observed	Study report, 1982b Study reported in detail in Annex I (study 2)
Fertility study CRCD rats 20 males/dose group Reliability: 3 due to low dosage	Glycerol formal (purity: presumably >99%)	Administration oral via gavage in water 0, 1, 5, 25 mg/kg bw/d Males used for this study were in utero exposed (see oral reproduction study in female rats reported one line above) Subsequently the males	No effect on male fertility was observed (time to mating, number of mated females/total number of females, number of pregnant females/total number of mated females) No effects observed on preimplantation loss, number of resorptions/number of implants, number of live foetuses/pregnant female.	Study report, 1982c Study reported in detail in Annex I (study 3)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
		were exposed for 90 days to 0, 1, 5, 25 mg/kg bw/d. In exposure week 11 they were cohabited with untreated females (two females/male). On GD 14 females were sacrificed and reproductive parameters were recorded.	A slight increase in preimplantation losses was observed in females mated to high dose males (not statistically significant). This was not considered treatment-related by the study authors but the result of a single female which had only one foetus. The second female mated with the same male had a normal litter size.	

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

No reliable studies explicitly addressing the endpoint "sexual function and fertility" are available in the registration dossier. Six repeated dose toxicity studies (28-day and 90-day studies and one 16-week study) are reported in the registration dossier.

Although only three of these studies are oral toxicity studies (the other three studies used parenteral application forms - administration via the intravenous, intramuscular or subcutaneous route), all of these six repeated dose toxicity studies are considered and discussed for adverse effects on sexual function and fertility. The registrant also provided study reports for two additional studies not included in the registration dossier. Additional studies in the literature were not identified.

A 90-day repeated dose toxicity study from 1973 performed similar to OECD test guideline 408 and a reliability of 2 is reported (study report, 1973, ECHA Dissemination (2021)). This study is considered as the key study for effects on sexual function and fertility. The original study report was made available to the dossier submitters. Detailed information of this study can be found in Annex I.

The study was performed with ten Sprague Dawley rats per sex and dose group and two additional animals per sex and dose which were used as recovery group and were held for six weeks after the end of exposure. The animals were exposed via gavage to 0, 0.01, 0.10 and 1.00 mL/kg bw/d. Using the density of 1.218 g/mL for glycerol formal, the doses were transformed to the more common unit mg/kg bw/d (0, 12, 121, 1218 mg/kg bw/d). Water was used as vehicle and the rats were exposed daily for 90 days.

In the high dose group, 4 animals died (2/12 males and 2/12 females). No other clinical signs were observed. A delay in body weight gain was observed in all dose groups. At the end of exposure, weight gain in percent was 89.74, 94.87 and 66.67% in females and 80.87, 68.31 and 57.36% in males (low, middle, high dose group) compared to controls. This decrease is associated with a reduced intake of feed. No overt signs of toxicity were observed. Relative organ weights of testes, epididymis, seminal vesicle and uterus of animals of the highest dose group were reduced.

During gross pathology and histopathological analysis no effects were observed on the reproductive organs in the control group. In the low dose group examination of testes revealed slight inhibition of spermiogenesis in 1/10 animals, in the middle dose a partly inhibition of spermiogenesis in 2/10 animals with single atrophic seminiferous tubules in one of them was observed. The high dose group showed changes in testes in all animals (10/10), from slight inhibition of spermiogenesis to total atrophy; there were considerable changes in seminal vesicles of 4 animals (plenty of excretion with swarms of desquamated cells) and changes in the epididymis in 8/10 animals, consisting mainly in abnormal content in the ductus epididymidis and changes in the epididymal tubes. The study provides detailed descriptions of histopathological observations. However, no information on the severity of the effects is provided (e.g., severity scores) and no analysis of sperm parameters (number, quality etc.) was performed. Therefore, it is not specified whether the reduced

spermiogenesis results in oligospermia or even aspermia.

Effects in uteri of female animals were not dose-dependent. Inflammatory manifestations were observed in 2/10, 3/10 and 1/10 animals in the low, middle and high dose group.

Effects on testes were also observed in the animals held for six weeks after the end of exposure.

It is however noted that the adverse effects on reproductive organs were observed, at least partially, in the presence of general toxicity as mortality was observed in the high dose group (i.e. 2 out of 12 males and 2 out of 12 females died). It is not clear whether this may have contributed to the observed effects on reproductive organs, however this cannot be excluded. In relation to this, it is noted that the dose spacing may not be fully appropriate, i.e. the intervals between the chosen dose levels (12, 121, 1218 mg/kg bw/d) may be quite large. Thereby hampering the interpretation of the observed general toxicity and its potential association with the adverse effect on reproductive organs. Finally, the dose level of the high dose group (i.e. 1218 mg/kg bw/d) is slightly above the limit dose of 1000 mg/kg bw/d as mentioned in OECD TG 408. Nevertheless, it is considered likely that a slightly lower dose level of just below 1000 mg/kg bw/d may cause similar adverse effects on testis.

Overall, the dose-dependent increase of adverse effects on testes can be considered relevant and the basis for a classification, though some uncertainties are noted in relation to this oral 90-day rat study (study report, 1973).

In a second subchronic toxicity study (16 week study) with oral exposure, male Wistar rats (10 animals/dose group) were exposed to an aqueous paste of diet containing glycerol formal (no additional information on test material available) (study report, no date given according to ECHA Dissemination (2021). Due to the limited documentation of the study, it is considered as “not reliable”. Animals were exposed to 316, 1000, 3162 and 10000 ppm in the diet (corresponding to 15.8, 50, 158 and 500 mg/kg bw/d, converted cf. the CLP-Guidance). Examination of reproductive organs is not reported in this study.

The third subchronic oral toxicity study reported in the registration dossier is a 90-day gavage study with 20 animals per sex and dose group (study report 1982a, ECHA Dissemination (2021)). The study is similar to OECD test guideline 408 and performed under GLP conditions. However, the animals were exposed to very low doses of glycerol formal only (0, 1, 5, 25 mg/kg bw/d) compared to the limit doses stated in OECD test guideline 408 (1000 mg/kg bw/d). In addition, animals were previously "in-utero" exposed before oral gavage exposure (this “in utero exposure study” is available to the submitters of the CLH proposal and reported separately). Ovaries, uterus, testes, epididymis and prostate were studied histopathologically and no treatment related effects were observed. The only effect observed was unilateral testicular degeneration in one control and one high-dose group male. Again, only qualitative descriptions of histopathological findings are presented. The “in-utero exposure study” (study report, 1982b) is not included in the registration dossier. However, the study report was available to the dossier submitters and the study is presented in detail in Annex I. For the in-utero exposure female Charles River rats (20 animals/dose group) were exposed via gavage to 0, 1, 5 or 26 mg/kg bw/d starting 14 days prior to mating, during mating (with unexposed males) and gestation up to PND 20. No clinical signs were observed in females, and no effects were reported on reproductive status, mating performance, length of gestation, or postimplantation survival rate (for details see tables in Annex I, study 2). Due to the low administered dose the study was assigned a reliability of 3.

As an “add-on” to the 90-day toxicity study with the in utero exposed rats, a fertility study was performed (study report, 1982c; study also not included in the registration dossier but available to the dossier submitters and presented in detail in Annex I). For this study male in utero exposed rats were treated with 0, 1, 5, 25 mg/kg bw/d. After 11 weeks of exposure, they were cohabited with untreated females (two females/male). On GD 14 females were sacrificed and reproductive parameters were recorded. No effects on male or female fertility or reproductive parameters were observed (for details see table in Annex I, study 3). As noted for the “in utero study” and the 90-day toxicity study with in utero treated rats, the reliability of the study was considered as 3 due to the low administered doses.

The following section summarises the repeated dose toxicity studies that have been performed with intravenous, subcutaneous or intramuscular application. The original study reports of these studies are available to the dossier submitters.

In a 28-day toxicity study with new Zealand White rabbits 5 animals per dose group (male and female in total) were intravenously exposed to 0, 0.024, 0.24 mL/kg bw glycerol formal (study report, no date given, reported in ECHA Dissemination (2021). Using the density of 1.218 g/mL, doses of 0, 29.2 and 292 mg/kg bw/d were calculated by the dossier submitters. Due to the application route and the limited number of exposed animals a reliability of 3 was assigned by the dossier submitters. Uterus, testes and epididymis were studied histopathologically. The study authors stated that differences in comparison to the organs of the control animals appeared only in the liver in dose group II (0.24 mL/kg). In the study report it is stated that the one control male showed "only moderately active spermiogenesis". In the low dose group, no effects on testes were observed in both animals. In the high dose group 1/2 animals showed a reduced spermiogenesis and abnormal elements such as polynuclear cells in the spermatic epithelium. Due to the low number of animals per dose group, however, the results cannot be interpreted. No effects on body weight were observed. Despite the intravenous application which results per definition in a complete bioavailability of the substance, the highest dose was only 300 mg/kg bw/d. Moreover, the exposure period was only 28 days.

Results from a 90-day repeated dose study with subcutaneous application (location not further specified) in Sprague Dawley rats confirmed the results obtained in the 90-day oral toxicity key study reported above. The study did not follow any OECD test guideline due to its route of application. Therefore, only a reliability of 3 was assigned by the dossier submitters. Nevertheless, the study is of generally good quality.

Ten animals per sex and dose group were exposed daily to 0.24, 0.48 and 1.20 mL/kg bw glycerol formal (study report, no date given, ECHA Dissemination (2021)). Using the density of 1.218 g/mL, doses of 0, 292.3, 584.6, 1461.6 mg/kg bw/d were calculated by the dossier submitters. The substance was administered in 30% 0.9%-NaCl solution except for the high dose which was administered in a 30% or 50% 0.9%-NaCl solution (dose I: 0.24 mL/kg (30% solution), dose II: 0.48 mL/kg (30% solution), dose III1: 1.20 mL/kg (30% solution), dose III2: 1.20 mL/kg (50% solution)). In female animals, weight gain in all test groups was faster compared to the control group. At the end of exposure, the relative increase in body weight in % of control was 155.5, 137.8, 155.5 and 115.6%, compared to control (dose I, II, III1, III2, see explanatory note in Table 9 on the high weight gain in female animals in the exposure groups). Male animals in the low exposure group showed similar weights compared to control animals at the end of exposure (98.0%). At 0.48 mL/kg and 1.20 mL/kg a reduction was observed (dose II: 79.8%, dose III1: 58.6% and dose III2: 60.6%). In the highest dose groups the weights of testes, epididymis and ovaries, were reduced. The reproductive organs were studied histopathologically. In dose group I testis changes in 1/10 animals were observed. In the middle dose group (dose II) disturbances of spermiogenesis with interstitial oedema were found in 5/10 animals. In the two highest exposure groups (dose group III1 and III2) atrophy of the seminiferous tubules combined with an interstitial oedema was found in all 10 animals accompanied by deviations from normal findings in epididymis and seminal vesicles (dose group III1). In dose group III2 changes in testes were observed in all 10 animals. In 8/10 animals a subtotal to total atrophy of the seminal tubules with changes in the epididymis and seminal vesicles analogous to the picture of the testes was found. In female animals, effects on ovaries were reported for the highest dose groups: In dose group III1 only small to medium-sized tertiary follicles were detected, in dose group III2 only small to middle-sized follicles were found in 8/10 animals. Effects on testes and ovaries were also observed in the animals held for six weeks after the end of exposure.

In a second 90-day toxicity study (no guideline mentioned) with non-oral application, Beagle dogs (2 males and 2 females/exposure group, 3 males and 2 females in control group) were administered glycerol formal intramuscularly in the gluteal muscle. Due to the application route and the limited number of exposed animals a reliability of 3 was assigned by the dossier submitters. The animals were exposed to 0, 0.024, 0.24 mL/kg bw. Using the density of 1.218 g/mL, doses of 0, 29.23 and 292.3 mg/kg bw/d were calculated by the dossier submitters. Glycerol formal was administered in a 30% or 50% dilution with 0.9 % NaCl (only the highest dose was tested in two dilutions (30%, dose II1 and 50%, dose II2) corresponding to the same final glycerol formal content). Ovary, uterus, testes and epididymis were studied histopathologically. No clinical signs or changes in body or organ weights compared to control animals were observed. No effects on uterus and ovaries were reported. In the two high dose groups minimal depression of spermiogenesis and also changes in the content of the tubules of the epididymis were observed in both male animals (dose II1). In dose II2 testis of one of two male animals showed partly an atrophy of the seminiferous tubules and changes of the epididymis.

The studies reported in section 10.10.4 on adverse effects on development are mostly performed similar to OECD TG 414. This means that pregnant female animals were exposed for a relevant time-period to test for effects on the developing organism. Effects on male and also female fertility or sexual function are not monitored with these studies. Therefore, these studies cannot be evaluated in this context.

Overall, results from the 90-day gavage study in Sprague Dawley rats reported as key study show dose-dependent adverse effects on male reproductive organs. These effects are expected to impair reproductive function as they interfere with spermiogenesis. However, several uncertainties are noted. The adverse effects on testis were, at least partially, observed in the presence of general toxicity, as mortality was observed at the high dose. In addition, it is noted that the dose selection was not fully appropriate (dose intervals being quite large), hampering the interpretation of the observed general toxicity and its potential association with the adverse effect on reproductive organs. Further, the study is a repeated dose study and did not include mating of exposed animals and measures of fertility (e.g., number of pregnant females). In addition, no sperm parameters were obtained. Effects on female animals are observed but to a lesser degree and are not dose-dependent. Due to severe limitations, the two other oral toxicity studies are not reliable and cannot be used to support the findings observed in the key study.

However, the findings in the key study (effects on male reproductive organs) are supported by findings in two 90-day toxicity studies with either subcutaneous application in rats or intramuscular application in Beagle dogs. Both studies have their limitations (reliability 3) mainly because of the application route and in case of the dog study because of the limited number of animals, but the results are considered as solid. The study with intravenous application in rabbits cannot be used in a supportive way, since number of animals per sex and dose group was too low.

The only studies that included exposure during mating and measurements of resultant reproductive parameters were study report (1982b) and study report (1982c). It is however noted that the applied dose levels in these two studies are quite low. Therefore, the absence of effects in these studies cannot be used to justify the absence of reproductive effects in general.

Overall, only evidence from a non-mating study is available (study report 1973). In this study detailed histological examinations of the reproductive organs are provided but no severity scores for the observed effects are given (e.g., for atrophy of testes). The effects are dose-dependent and considered relevant for humans. Using this study as a basis for classification is associated with uncertainties, since the adverse effects on testis were observed, at least at the high dose level, in the presence of general toxicity (mortality). In addition, there are some uncertainties related to the dose selection (dose intervals being quite large), thereby hampering the interpretation of the observed general toxicity and its potential association with the adverse effect on reproductive organs. Further, classical reproductive effects (e.g., sperm parameters, number of pregnant females) are not available and no information is provided on the outcome of mating.

It should however be acknowledged that humans are much more sensitive to impairments of sperm production or sperm quality compared to rats. This means that while rats still can produce the same number of offspring with a substantially reduced number of fertile sperm, impaired fertility in men can be observed at only slightly reduced sperm numbers or sperm quality. According to Working (1988) the number of sperm per human ejaculate is typically only two-to four-fold higher than the number at which fertility is significantly reduced, while this number is up to 1400-fold in rats or rabbits. Slight impairment of sperm number or quality in rats will therefore have less dramatic effects compared to humans.

Based on these considerations the effects on testes provide some evidence for classification of glycerol formal for effects on sexual function and fertility (see following section).

10.10.3 Comparison with the CLP criteria

For potential classification with regard to adverse effects on sexual function and fertility, criteria from CLP Regulation (EC, 2008)[2] in combination with explanations from the Guidance on the Application of the CLP

[2] REGULATION (EC) No 1272/2008 considering all ATPs published until January 2021

criteria (ECHA, 2017) were applied. Any adverse effect of glycerol formal on the female and male reproductive system, on the onset of puberty, gamete production and transport, reproductive cycle, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems were considered. For potential classification of glycerol formal, classification criteria were analysed accordingly:

Comparison with Category 1 criteria

- Known human reproductive toxicant (1A)

  Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility […] 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) (EC, 2008).

- Presumed human reproductive toxicant (1B)

  The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility […] in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. (EC, 2008).

- Suspected human reproductive toxicant (Cat 2)

  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. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects (EC, 2008).

Further, the Regulation states that

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 (EC, 2008).

There are no epidemiological data to support classification of glycerol formal in Category 1A.

No studies explicitly addressing reproduction (e.g. studies according to OECD TG 421, 422, one- or two-generation studies, EOGRTS) are available in the registration dossier. Though the studies described in study report (1982b) and study report (1982c) included exposure during mating and measurement of reproductive parameters, it is noted that the applied dose levels in these two studies are quite low. No adverse effects on reproductive parameters were observed in these two studies. The repeated dose animal studies listed in Table 9 and described in detail in chapter 10.10.2 show dose-dependent histopathological changes in testes and epididymis of exposed animals. The observations from a 90-day oral rat study are supported by data from a less reliable subchronic study with dogs with intramuscular administration and a further subchronic rat study with subcutaneous administration. The changes include inhibition of spermiogenesis and atrophy. Such effects can in general be considered relevant for humans since they are expected to impair reproductive function by leading to a reduction of fertile sperm. However, these effects occurred in the 90-day oral rat study, at least at the high dose level, in the presence of general toxicity (i.e. mortality). In addition, there are some uncertainties related to the dose selection (dose intervals being quite large), thereby hampering the

interpretation of the observed general toxicity and its potential association with the adverse effect on reproductive organs. As discussed in the previous section, no reliable mating studies are available and the histopathological effects in testes are described by the study authors without assigning severity scores.

There are also indications of adverse effects in female reproductive organs, however these effects are ambiguous and cannot be seen as a firm basis for classification.

Based on these considerations the effects on the male reproductive organs provide some evidence for classification of glycerol formal for effects on sexual function and fertility and this would justify a classification in Category 2.

10.10.4 Adverse effects on development

Table 10: Summary table of animal studies on adverse effects on development

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Prenatal Developmental Toxicity Study
Similar to OECD TG 414
GLP: yes
Charles River CRCD rats
25 pregnant females/dose group, 24 in control group
Reliability: 2
due to the reduced exposure window. Generally, exposure in OECD TG 414 recommended from preimplantation to the day of caesarean section.</td>
<td>Glycerol formal (purity: >99%)
Administration oral via gavage in water
0, 75, 150, 300, 600 mg/kg bw/d
From GD 6 – 17
Sacrifice on gestation day 20</td>
<td>P0 generation:
- No clinical signs of toxicity in any of the treatment groups. No adverse effects on body weight are reported
- NOAEL > 600 mg/kg bw/d (nominal) (highest dose tested, absence of adverse effects)

F1 generation:
Reproductive status
<table>
<tr><td>Dose in mg/kg bw/d</td><td>0</td><td>75</td><td>150</td><td>300</td><td>600</td></tr>
<tr><td>Total number of females</td><td>24</td><td>25</td><td>25</td><td>25</td><td>25</td></tr>
<tr><td>Total number of resorptions</td><td>11</td><td>3</td><td>6</td><td>5</td><td>28</td></tr>
<tr><td>No. resorptions/no. implants) per female</td><td>0.03</td><td>0.01</td><td>0.02</td><td>0.02</td><td>0.07*</td></tr>
<tr><td>Total number of foetuses</td><td>308</td><td>313</td><td>300</td><td>262</td><td>288</td></tr>
<tr><td>Number of alive foetuses</td><td>308</td><td>313</td><td>300</td><td>260</td><td>272</td></tr>
<tr><td>No. alive foetuses /pregnant female</td><td>12.8</td><td>13.0</td><td>12.5</td><td>12.4</td><td>11.3*</td></tr>
<tr><td>Number of dead foetuses</td><td>0</td><td>0</td><td>0</td><td>2</td><td>16</td></tr>
<tr><td>Average weight (g)/litter</td><td>3.68</td><td>3.69</td><td>3.38*</td><td>3.16*</td><td>2.81*</td></tr>
</table>
* statistically different from control P≤0.05
At 600 mg/kg bw/d an increase in the total number of resorptions was observed (11/3/6/5/28). The number of resorptions/number of implants per female was significantly (P ≤ 0.05) increased (0.03/0.01/0.02/0.02/0.07). The number of alive foetuses/pregnant female was significantly (P ≤ 0.05) decreased (12.8/13.0/12.5/12.4/11.3) and the number of dead foetuses was increased (0/0/0/2/16).</td>
<td>Study report, 1981 reported from ECHA Dissemination (2021)
Study: 001 developmental toxicity (key)

Study reported in the Annex I with extended tables (study 4)</td>
</tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
A dose-dependent statistically significant decreases (P ≤ 0.05) in average foetal weight per litter was observed (3.68/3.69/3.38/3.16/2.81).

<u>External foetal examination:</u>
<table>
<tr><td>Dose in mg/kg bw/d</td><td>0</td><td>75</td><td>150</td><td>300</td><td>600</td></tr>
<tr><td>No. examined foetuses</td><td>308</td><td>313</td><td>300</td><td>260 (2)</td><td>272 (15)</td></tr>
<tr><td>- No. with malformations</td><td>0</td><td>3</td><td>1</td><td>7 (0)</td><td>13 (2)</td></tr>
<tr><td>- No. of malformations</td><td>0</td><td>3</td><td>3</td><td>9 (0)</td><td>21 (2)</td></tr>
<tr><td>No. of examined litters</td><td>24</td><td>24</td><td>24</td><td>21</td><td>24</td></tr>
<tr><td>- No. with malformations</td><td>0</td><td>2</td><td>1</td><td>6</td><td>10</td></tr>
</table>
Dead foetuses reported in parenthesis

External malformations in foetuses were increased in the two highest dose groups. Anal atresia (0/0/0/2/7) and tail malformations (0/1/0/4/10) were observed in foetuses from dams administrated 300 and 600 mg/kg bw/d, and anasarca in foetuses from the 600 mg/kg bw/d group (0/0/0/0/3+2 in dead foetuses).

<u>Visceral foetal examination:</u>
<table>
<tr><td>Dose in mg/kg bw/d</td><td>0</td><td>75</td><td>150</td><td>300</td><td>600</td></tr>
<tr><td>No. examined foetuses</td><td>91</td><td>100</td><td>93</td><td>86 (2)</td><td>88 (15)</td></tr>
<tr><td>- No. with malformations</td><td>3</td><td>0</td><td>2</td><td>4 (0)</td><td>31 (7)</td></tr>
<tr><td>- No. of malformations</td><td>4</td><td>0</td><td>2</td><td>4 (0)</td><td>44 (10)</td></tr>
<tr><td>- No. with variations</td><td>0</td><td>1</td><td>6</td><td>6 (0)</td><td>13 (0)</td></tr>
<tr><td>- No. of variations</td><td>0</td><td>1</td><td>6</td><td>6 (0)</td><td>13 (0)</td></tr>
<tr><td>No. of examined litters</td><td>24</td><td>24</td><td>24</td><td>21</td><td>24</td></tr>
<tr><td>- No. with malformations</td><td>1</td><td>0</td><td>1</td><td>3</td><td>16</td></tr>
<tr><td>- No. with variations</td><td>0</td><td>1</td><td>2</td><td>5</td><td>8</td></tr>
</table>
Dead foetuses reported in parenthesis

Visceral malformations in foetuses were observed in the highest dose group: 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 variation was observed (0/0/6/6/12).

<u>Skeletal foetal examination:</u>
<table>
<tr><td>Dose in mg/kg bw/d</td><td>0</td><td>75</td><td>150</td><td>300</td><td>600</td></tr>
<tr><td>No. examined foetuses</td><td>308</td><td>313</td><td>300</td><td>260 (1)</td><td>272 (11)</td></tr>
<tr><td>- No. with malformations*</td><td>0</td><td>3</td><td>15</td><td>46 (0)</td><td>66 (2)</td></tr>
<tr><td>- No. of malformations*</td><td>0</td><td>3</td><td>16</td><td>46 (0)</td><td>67 (3)</td></tr>
<tr><td>- No. with variations</td><td>105</td><td>177</td><td>264</td><td>252 (0)</td><td>269 (2)</td></tr>
</table>
</td><td></td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>- No. of variations</td><td>116</td><td>236</td><td>451</td><td>702 (0)</td><td>1239 (2)</td></tr>
<tr><td>No. of examined litters</td><td>24</td><td>24</td><td>24</td><td>21</td><td>24</td></tr>
<tr><td>- No. with malformations</td><td>0</td><td>2</td><td>8</td><td>13</td><td>17</td></tr>
<tr><td>- No. with variations</td><td>23</td><td>24</td><td>24</td><td>21</td><td>24</td></tr>
</table>
Dead foetuses reported in parenthesis

*Wavy ribs were considered as malformation by the study authors and therefore included in this table. According to the DEVTOX database[3] wavy ribs are considered as variation

Skeletal variations in foetuses were observed dose dependently: wavy ribs (0/3/14/46/62+1 in dead foetus) cervical ribs (3/3/0/7/ 25+2 in dead foetuses), lumbar rib (35/56/54/56/96), extra lumbar vertebra (0/0/0/16/54).

A dose-dependent delay in foetal ossification (variation), primarily of the skull bones, vertebra, and sternebra, in foetuses of all treatment groups was observed: incomplete ossification of skull bone (2/21/41/95/112), incomplete ossified cervical vertebra (0/3/16/86/188), incomplete 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), incompletely ossified pelvic bone (1/5/31/82/175).</td><td></td></tr>
<tr><td>Prenatal Developmental Toxicity Study

Similar to OECD TG 414

GLP: yes

Charles River CRCD rats

25 pregnant females/dose group

Reliability: 3, since only one very low dose was tested</td><td>Glycerol formal (purity: >99%)

Administration oral via gavage in water

0, 10 mg/kg bw/d

From GD 6 – 17

Sacrifice on gestation day 20</td><td><u>P0 generation:</u>

- There were no clinical signs of toxicity among females. No adverse effects on body weight are reported

- NOAEL > 10 mg/kg bw/d (nominal) (highest dose tested, absence of adverse effects)

<u>F1 generation:</u>

- There were no adverse effects on the reproductive status of dams as indicated by the numbers of resorptions and live and dead foetuses per litter.

- No effects were recorded on foetal weight.

- There was no evidence of a teratogenic or foetotoxic effect.</td><td>Study report, 1981 reported from ECHA Dissemination (2021)

Study: 002 developmental toxicity (key)

Study reported in detail in Annex I (study 5)</td></tr>
<tr><td>Prenatal Developmental Toxicity Study</td><td>Glycerol formal (purity: 99%)

Administratio</td><td>For detailed result tables see Annex I

Test is divided in 5 experiments</td><td>Aliverti et al. (1980)

Reported in ECHA</td></tr>
</table>

[3] https://www.devtox.org/nomenclature/ml_organ.php?lan=en (Accessed on 1.2.2021)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Similar to OECD TG 414 GLP: no Sprague-Dawley (for experiments 1, 2, 3 and 4); Wistar (for experiment 5) rats 10 pregnant females/dose group Reliability: 2	n intramuscular (im); subcutaneous (sc); oral (po) Controls received physiological saline Doses / Concentrations: 0 mL/kg, 0.25 mL/kg (300 mg/kg) in experiment 1 0.50 mL/kg (600 mg/kg) in experiments 1, 2, 3, 4 and 5 1.0 mL/kg (1200 mg/kg) in experiments 1 and 4 From GD 6 – 15 in experiments 1, 3, 4 and 5 or from GD 6-15, 7-8, 9-10, 11-12, 13-14, 15-16 in experiment 2. Sacrifice on gestation day 21	<u>P0 generation:</u> No maternal toxicity was observed in any of the tests. <u>F1 generation</u> **Experiment 1** Glycerol formal was administered at 3 dose levels via intramuscular application to Sprague-Dawley from GD 6-15 → No maternal toxicity was observed → Postimplantation loss rate increased dose-dependently from 4.4% (control) to 63.7% (highest dose). The mean weight of live foetuses decreased from 4.1 g (control) to 3.2 g (highest dose). Significant differences from control values were demonstrated in all treated groups. The number of females with malformed foetuses increased dosed dependently (0/2/6/7). The incidence of visceral malformations increased dose-dependently (malformation rate (MR): 0/0/28/75). Malformations were particularly found in the cardiovascular system. Typical of these malformations was a ventricular septal defect involving a more or less extensive communication between the two ventricules sometimes accompanied by cardiomegaly, atrial hypertrophy, and right and retroesophageal aortic arch. Foetuses found dead in the uterus had widespread subcutaneous oedema and ventricular septal defects involving extensive intraventricular communication. The malformation rate of skeletal costal defects was 0/15/10/22. Skeletal anomalies were mostly limited to wavy ribs. **Experiment 2** Glycerol formal was administrated at 600 mg/kg bw/d at different time periods during gestation to determine the day(s) of gestation when the embryo was most sensitive to the teratogenic effects. → In the positive control group (exposed to glycerol formal from GD 6-15), the embryotoxic and teratogenic effects observed in experiment 1 were reproduced. The same treatment for only 2 consecutive days (any period tested) between GD 7 and 16 induced no cardiovascular malformations. Postimplantation loss rates were always significantly lower than the positive control group rate. **Experiment 3 and 4** Embryotoxic and teratogenic effects of glycerol formal administration was investigated by comparing subcutaneous to intramuscular route (exp. 3) and oral to intramuscular route (exp. 4). → Administration by subcutaneous and oral routes (GD 6-15) led to similar effects compared to those observed after intramuscular administration. Postimplantation loss rates and visceral malformation rates for were even higher after oral exposure	Dissemination (2021) Study: 003 developmental toxicity (Supp.) Study reported in the Annex I with extended tables (study 6)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
Experiment 5

Embryotoxic and teratogenic effects of glycerol formal administration by subcutaneous and intramuscular routes were investigated in Wistar (W) rats instead of Sprague-Dawley (SD) rats.
<table>
<tr><th>Experiment No.</th><th>Strain</th><th>Dose (ml/kg)</th><th>Route</th><th>Postimplantation loss rate (%)</th></tr>
<tr><td rowspan="2">1</td><td>SD</td><td>0</td><td>im</td><td>4.4</td></tr>
<tr><td>SD</td><td>0.50</td><td>im</td><td>19.8*</td></tr>
<tr><td rowspan="2">5</td><td>W</td><td>0</td><td>im</td><td>5.0</td></tr>
<tr><td>W</td><td>0.50</td><td>im</td><td>15.7 NS</td></tr>
</table>
* Statistically different (p< 0.05) from control
NS Non significantly different (p> 0.05) from the control value

<table>
<tr><th rowspan="3">Experiment No.</th><th rowspan="3">Strain</th><th rowspan="3">Dose (ml/kg)</th><th rowspan="3">Route</th><th colspan="6">Abnormalities</th></tr>
<tr><th colspan="3">Visceral cardiovascular defects</th><th colspan="3">Skeletal costal defects</th></tr>
<tr><th>EF</th><th>AF</th><th>MR</th><th>EF</th><th>AF</th><th>MR</th></tr>
<tr><td rowspan="2">1</td><td>SD</td><td>0</td><td>im</td><td>59</td><td>0</td><td>0</td><td>50</td><td>0</td><td>0</td></tr>
<tr><td>SD</td><td>0.50</td><td>im</td><td>53</td><td>15</td><td>28*</td><td>40</td><td>4</td><td>10*</td></tr>
<tr><td rowspan="2">5</td><td>W</td><td>0</td><td>im</td><td>20</td><td>0</td><td>0</td><td>18</td><td>2</td><td>11</td></tr>
<tr><td>W</td><td>0.50</td><td>im</td><td>56</td><td>5</td><td>8.9 NS</td><td>35</td><td>16</td><td>46*</td></tr>
</table>
* Statistically different (p< 0.05) from control
EF number of examined foetuses (EF)
AF number of abnormal foetuses (AF)
MR malformation rate in percent
NS Non significantly different (p> 0.05) from control

→ Glycerol formal administrated at 0.50 mL/kg either im. or sc. on GD 6 - 15 to Wistar rats induced a lower postimplantation loss rate and a lower frequency of cardiovascular malformations compared to Sprague Dawley rats. However, skeletal costal defect were higher in Wistar compared to Sprague-Dawley rats.
</td><td></td></tr>
<tr><td>Prenatal Developmental Toxicity Study

Similar to OECD TG 414

GLP: no

Sprague-Dawley rats

40 pregnant females per exposure group; 20 in control group</td><td>Glycerol formal (no information on purity available)

Administration subcutaneously

No vehicle

0.5 mL/kg bw/d (presumably 600 mg/kg bw/d as given in the study by</td><td><u>P0 generation:</u>

Maternal toxicity was not investigated.

<u>F1 generation</u>

- 193 foetuses from 40 treated females and 119 foetuses from 20 control females were examined.

- About 40% of the foetuses of the treated rats showed anomalies of the interventricular septum; this malformation was associated in nearly 50% of the cases with serious anatomical alterations of the main blood vessels departing from the heart.

- The anomalies of the interventricular septum were of different types and gravity. In most cases, these anomalies were located at the interventricular foramen, i.e., between the muscular septum and the endocardial cushions.

- The importance of the ventricular septum defect proved to be very variable: from little orifices to large communications concerning also</td><td>Giavini and Prati (1980)

Reported in ECHA Dissemination (2021)

Study: 004 developmental toxicity (Supp.)</td></tr>
</table>

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td>Reliability: 3, since no information on maternal toxicity is provided and no information on purity</td><td>Aliverti et al. reported above)
From GD 6 – 15
Sacrifice on gestation day 21</td><td>a large part of the muscular septum, the atrioventricular valves and the interatrial septum. In the latter, in extreme forms, the heart appeared as a unique cavity with a sketch of division between the ventricules; the atria, in particular the right atrium, were very enlarged.
At least 5 different types of malformations of the main vessels originating from the heart were observed (see table below):
<table>
<tr><td></td><td>Number of foetuses</td></tr>
<tr><td>Examined</td><td>193</td></tr>
<tr><td>With cardiovascular malformations</td><td>76</td></tr>
<tr><td>With ventricular septum defect</td><td>39</td></tr>
<tr><td>With ventricular septum defect and</td><td></td></tr>
<tr><td>Double aortic arch</td><td>20</td></tr>
<tr><td>Right aortic arch</td><td>10</td></tr>
<tr><td>Aortic-pulmonary window</td><td>1</td></tr>
<tr><td>Absence of innominate artery</td><td>1</td></tr>
<tr><td>Coarctation of the aorta</td><td>4</td></tr>
<tr><td>Dextrocardia</td><td>1</td></tr>
</table></td><td></td></tr>
<tr><td>Prenatal Developmental Toxicity Study
No guideline followed
Sprague-Dawley rats
Number of rats not given
Reliability: 3</td><td>Glycerol formal (no purity given)
Administration subcutaneously
1 mL/kg bw/d (1200 mg/kg bw/d)
From GD 6 – day to sacrifice of dams
Sacrifice on gestation day 13, 14, 15, 16 or 17</td><td>Developmental retardations in the heart were studied
Marked dilation of blood vessels were already observed on GD 13. In addition, swelling of the embryo was observed.
Theory of the authors: substance causes malformations by interfering with embryonic osmoregulatory system that leads to changes in circulating fluids and consequent changes of the blood flow in the heart and aortic arch system.</td><td>Giavini et al. (1981)</td></tr>
<tr><td>Oral reproduction study in female rats
Charles River CD rats
20 females/dose</td><td>Glycerol formal (purity: >99%)
Administration oral via gavage in water
0, 1, 5, 25</td><td>No clinical sign observed in females.
- No dose-dependent effects observed on body weight of pups.
- No externally visible effects observed in the offspring
The number of dead pups from PND 8 - 14 was significantly (P≤0.05) higher in rats from the 1 mg/kg/day group. There was no increase in mortality in this group on PND 1 or PND 21 and no increase in dead pups was seen in the 5 or 25 mg/kg/day groups.</td><td>Study report, 1982b</td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
group Reliability: 3, due to low dosage	mg/kg bw/d Exposure: 14 days prior to mating (with unexposed males), during mating and gestation until PND 20 of pups Pups were used for 90 day toxicity study reported in Table 9		

Table 11: Summary table of other studies relevant for developmental toxicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Frog Embryo teratogenesis Assay (FETAX) Reliability: 3	Glycerol formal (purity 98%)	Embryos of Xenopus laevis 200 embryos/concentration Vehicle: FETAX solution Two trials with 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75% Exposure for 96 h (media changed at 24 h intervals)	**Malformations:** - Pooled 96 h LC_{50}: 1.61% - Pooled 96 h EC_{50}: 1.12% **Teratogenic Index (TI):** - Trial 1: 1.51, trial 2: 1.37 **NOEL(mortality):** - Trial 1: 1.25, trial 2: 1.00 **NOEL(malformation):** - Trial 1: 0.5, trial 2: 0.5 **NOEL(length):** - Trial 1: 0.75, trial 2: <0.25 A concentration dependency of appearance of malformations was observed. Malformations included cephalic, skeletal and ocular malformations, abnormalities in pigmentation, gut coiling and swimming behaviour at	Dresser et al. (1992)

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			exposure concentrations >0.5% were observed.	
QSAR prediction Reliability: 3	Glycerol formal	An original algorithm, namely activity distribution diagrams, was applied to predict teratogenic potential of a large sets of structurally heterogeneous compounds including glycerol formal No guideline followed	Glycerol formal teratogenicity is considered as uncertain (not classified) according to activity distribution diagrams algorithm. No further information available	García-Domenech et al. (2001) Reported in ECHA Dissemination (2021) Study: 005 developmental toxicity (Supp.)
QSAR prediction Reliability: 3	1,3-Dioxan-5-ol (one of the two constituents of glycerol formal)	According to REACH guidance on QSAR R.6, May/July 2008	Prenatal Development Toxicity Index (PDTI): +1 The predicted value for the current substance is PDTI = +1 and thus the theoretical model suggests that the chemical could promote prenatal toxicity. No further information available	Study report, 2012 reported from ECHA Dissemination (2021) Study: 006 developmental toxicity (supp)
QSAR prediction Reliability: 3	Presumably 1,3-dioxolan-4-ylmethanol (one of the constituents of glycerol formal) The CAS and EC number do not match (EC number from 1,3-Dioxan-5-ol is given in registration dossier)	According to REACH guidance on QSAR R.6, May/July 2008	Prenatal Development Toxicity Index (PDTI): +1 The predicted value for the current substance is PDTI = +1 and thus the theoretical model suggests that the chemical could promote prenatal toxicity. No further information available	Study report, 2012 reported from ECHA Dissemination (2021) Study: 007 developmental toxicity (supp)

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

Overall, five studies in rats are available, delivering results for the endpoint “adverse effects on development”. In addition, a Frog Embryo teratogenesis Assay (FETAX) is published and several QSAR predictions are reported in the registration dossier for glycerol formal.

In a prenatal developmental toxicity study from 1981 similar to OECD TG 414 (study report 1981, ECHA Dissemination (2021) reliability 2) 25 pregnant Charles River CRCD rats per dose group (24 animals in the control group) were exposed from GD 6 – 17 to 0, 75, 150, 300 or 600 mg/kg bw/d glycerol formal via gavage. On GD 20 animals were sacrificed. Generally, exposure in a study following OECD TG 414 is

recommended from preimplantation to the day of caesarean section. In the current study exposure of dams was from GD 6 - 17 with sacrifice of animals on GD 20. Presumably this exposure schedule was selected based on the publication by Aliverti et al. (1980) (results of this publication are mentioned in the study report) who applied an exposure window of 10 days from GD 6 – 15, also in Sprague-Dawley rats.

This study is considered as the key study for effects on development. The original study report was available to the dossier submitters. Detailed information of this study can be found in Annex I.

No clinical signs of toxicity in the dams are reported in any of the treatment groups. In addition, no adverse effects on body weight were observed. In the highest dose group, an increase in the total number of resorptions (11/3/6/5/28) was detected. Thus, the number of resorptions/number of implants per female was significantly ($P \leq 0.05$) increased (0.03/0.01/0.02/0.02/0.07). The total number of live foetuses was decreased (308/313/300/262/288) and the number of dead foetuses increased (0/0/0/2/16). The number of live foetuses/pregnant female was significantly ($P \leq 0.05$) decreased in the highest dose group (12.8/13.0/12.5/12.4/11.3). Besides, a dose-dependent statistically significant decrease ($P \leq 0.05$) in average foetal weight per litter was found in foetuses from 150, 300 and 600 mg/kg bw/d groups (3.68/3.69/3.38/3.16/2.18).

External malformations in foetuses were increased in the two highest dose groups. They consisted mainly in anal atresia (0/0/0/2/7), tail malformations (0/1/0/4/10), and anasarca (0/0/0/0/3+2 in dead foetuses).

Visceral malformations were observed in the highest dose group and consisted mainly in cardiovascular defects, primarily defects of the ventricular septum (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, high numbers of azygous branching variation (0/0/6/6/12) were found.

Skeletal examination revealed several types of variations. Wavy ribs (classified as malformation by the study authors) were observed dose-dependently (0/3/14/46/62+1 in dead foetus). Cervical ribs (3/3/0/7/ 25+2 in dead foetuses), lumbar rib (35/56/54/56/96) and extra lumbar vertebra (0/0/0/16/54) were observed with increased incidences in higher doses.

A dose-dependent delay in foetal ossification (considered as variations), primarily of the skull bones (2/21/41/95/112), vertebra (cervical vertebra (0/3/16/86/188), thoracic vertebra (1/6/26/58/149), sacral vertebra (0/2/13/18/66)), sternebra (73/137/251/244/264), lumbar vertebra (0/2/6/16/78), and pelvic bone (1/5/31/82/175) were observed in foetuses of all treatment groups.

Overall, the increase in resorptions which results in a significantly increased number of resorptions/number of implants per female, in the absence of maternal toxicity alone would be considered as a relevant adverse effect justifying classification. In addition, statistically significant decrease in foetal body weight accompanied by a delay in foetal ossification were observed. Finally, the substance showed teratogenic effects in this reliable key study: external and visceral malformations were observed dose-dependently.

Since the prenatal toxicity study described above did not allow the derivation of a NOAEL for developmental toxicity, the study was repeated with a lower dose of glycerol formal (0, 10 mg/kg bw/d; study report, 1981, ECHA Dissemination (2021)). Due to the administration of only one very low dose the dossier submitters assigned a reliability of 3 to this study. The study protocol and the evaluated endpoints were the same as in the study with higher doses. The original study report was available to the dossier submitters. At 10 mg/kg bw/d no adverse effects on the reproductive status of dams (indicated by the numbers of resorptions and live and dead foetuses per litter) and no effects on foetal weight were reported. Furthermore, no substance-related malformations or variations were observed (for details see tables in Annex I, study 5). Due to the low dose, the relevance of this study in the context of classification is limited.

In an oral reproduction study in rats (Study Report 1982b; study not included in the registration dossier, but available to the dossier submitters) female Charles River rats (20 animals/dose group) were exposed via gavage to 0, 1, 5 or 26 mg/kg bw/d starting 14 days prior to mating, during mating (with unexposed males) and gestation up to PND 20. No clinical signs or dose-dependent effects were observed in females. No externally visible effects were observed in offspring. Due to the low administered dose the study was assigned a reliability of 3 (for details see tables in Annex I, study 2).

Three publications from 1980 and 1981 investigating the cardiovascular malformations of rat foetuses after

glycerol formal application are available. Two of these studies are reported in the registration dossier. Aliverti et al. (1980) performed several experiments and exposed pregnant Sprague Dawley or Wistar rats via intramuscular, subcutaneous or oral administration to 0, 0.25, 0.50 or 1.0 mL/kg bw/day (this corresponds to 0, 300, 600 or 1200 mg/kg bw/d). The study is considered as reliable (RL2). Complete tables with the results are presented in the Annex I.

In the first experiment glycerol formal was administrated at 3 doses level (0, 300, 600, 1200 mg/kg bw/d) via intramuscular application to Sprague-Dawley from GD 6-15. In the absence of maternal toxicity, the postimplantation loss rate increased dose-dependently from 4.4% (control) to 63.7% (highest dose). The mean weight of live foetuses decreased from 4.1 g (control) to 3.2 g (highest doe). Significant differences from control values were demonstrated in all treated groups. Moreover, the number of females with malformed foetuses increased dosed dependently (0/2/6/7) as well as the incidence of visceral malformations (malformation rate (MR): 0/0/28/75). Malformations were particularly found in the cardiovascular system. Typical of these malformations was a ventricular septal defect involving a more or less extensive communication between the two ventricles, sometimes accompanied by cardiomegaly, atrial hypertrophy, and right and retroesophageal aortic arch. The malformation rate of skeletal costal defects was 0/15/10/22. Skeletal anomalies were mostly limited to wavy ribs. Foetuses found dead in the uterus had widespread subcutaneous oedema and ventricular septal defects involving extensive intraventricular communication.

In the second experiment glycerol formal was administrated to Sprague-Dawley rats at 600 mg/kg bw/d for different time periods during gestation to determine the day(s) of gestation when the embryo was most sensitive to the teratogenic effects. It was shown that the window of exposure during gestation selected in experiment 1 (GD 6-15) covered best the critical period for cardiovascular malformations and an only two day long exposure period within this window did not lead to the same severity of effects.

In experiments 3 and 4 the intramuscular application of the substance was compared to subcutaneous (experiment 3) and oral (experiment 4) application. For oral application animals were treated with 0, 600 or 1200 mg/kg bw/d from GD 6-15. Similar effects compared to those observed after intramuscular administration were observed. Postimplantation loss rates and visceral malformation rates were even higher after oral exposure.

In experiment 5 the embryotoxic and teratogenic effects of glycerol formal were investigated in Wistar rats instead of Sprague-Dawley rats. Glycerol formal administrated (600 mg/kg bw/d) either intramuscularly or subcutanously on GD 6-15 induced a lower postimplantation loss rate (15.7% in Wistar rats compared to 19.8% in Sprague-Dawley rats) and a lower frequency of cardiovascular malformations in Wistar rats compared to Sprague Dawley rats (malformation rate in Wistar rats: 8.9% versus 28% in Sprague-Dawley rats). However, skeletal costal defect were higher in Wistar compared to Sprague-Dawley rats (Wistar rats: 46%, Sprague-Dawley rats: 10%).

In the second publication reported in the registration dossier (Giavini and Prati, 1980) pregnant Sprague Dawley rats were exposed subcutaneously to 600 mg/kg bw/d from GD 6–15. About 40% of the foetuses showed anomalies of the interventricular septum. Giavini and Prati studied these cardiovascular malformations histopathologically. In about 50% of the cases this malformation was associated with serious anatomical alterations of the main blood vessels departing from the heart. The authors of this CLH dossier assigned a reliability of 3 to this study (due to only one applied dose, the lack of information on purity and an overall reduced study design).

In a further publication from the same group (Giavini et al., 1981) Sprague-Dawley rats were subcutaneously exposed to 1200 mg/kg bw/d from GD 6 until the day of sacrifice of dams (i.e. GD 13, 14, 15, 16 or 17). The developmental retardations in the heart were studied. Marked dilation of blood vessels were already observed on GD 13. In addition, swelling of the embryo was observed. A reliability of 3 was assigned by the authors of this CLH dossier due to the lack of information on purity and only one applied dose.

A Frog Embryo teratogenesis Assay (FETAX), which can be considered as a screening assay for developmental toxicity, showed a concentration-dependent appearance of malformations (Dresser et al., 1992). These malformations included cephalic, skeletal and ocular malformations, abnormalities in pigmentation, gut coiling and swimming behaviour at exposure concentrations >0.5%. Due to the screening character of the test a reliability of 3 was assigned by the dossier submitters.

For the QSAR predictions reported in the registration dossier a reliability of 3 was assigned by the dossier submitters. This is mainly due to very limited reporting. In one case (García-Domenech et al. (2001) glycerol formal teratogenicity is considered as uncertain (not classified) by the study authors. For the individual constituents of the reaction mass of glycerol formal (1,3-Dioxan-5-ol and 1,3-dioxolan-4-ylmethanol) a Prenatal Development Toxicity Index (PDTI) of +1 was assigned. This means that the theoretical model suggests that the chemicals could promote prenatal toxicity.

Taking together the available information, adverse effects on development of glycerol formal (resorptions, decrease in foetal body weight, delay in foetal ossification, teratogenic effects) have clearly been shown in a reliable study performed similar to OECD TG 414. In this study, the substance was applied to rats via gavage. These results are supported by a reliable publication from Aliverti et al. (1980) and further studies with lower reliability.

10.10.6 Comparison with the CLP criteria

For potential classification of adverse effects on development, criteria from the CLP Regulation (EC, 2008) supported by explanations from the Guidance on the Application of the CLP criteria (ECHA, 2017) were applied. The manifestations of developmental toxicity (resorptions, decrease in foetal body weight, delay in foetal ossification, teratogenic effects) were considered. For potential classification of glycerol formal, classification criteria were analysed accordingly.

Comparison with Category 1 criteria

- Known human reproductive toxicant (Cat 1A)

  Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on […] development in humans, or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans.

  The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B (EC, 2008).

  There are no epidemiological data to support classification of glycerol formal in Category 1A.

- Presumed human reproductive toxicant (Cat 1B)

  The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect […] on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. (EC, 2008)

  …The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. (EC, 2008)

  Clear adverse effects on development (resorptions, decrease in foetal body weight, delay in foetal ossification, teratogenic effects) were observed in rats in a reliable study similar to OECD test guideline 414. The effects were dose-dependent and occurred in the absence of maternal toxicity.

  A classification in Category 1B is justified based on the observed effects.

- Suspected human reproductive toxicant (Cat 2)

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

Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification.

Adverse effects on development were observed in rats in a reliable study similar to OECD test guideline 414 (reliability 1). The severity of these effects (resorptions, decrease in foetal body weight, delay in foetal ossification, teratogenic effects) is considered clear evidence of an adverse effect on development and therefore relevant for classification. The evidence is sufficiently convincing to place the substance in Category 1B.

10.10.7 Adverse effects on or via lactation

No human or animal studies are available.

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

No human or animal studies are available.

10.10.9 Comparison with the CLP criteria

For potential classification on adverse effects via lactation, criteria from CLP Regulation (EC, 2008) were applied. For potential classification of glycerol formal, classification criteria were analysed accordingly:

- *…"However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the:*

  (a) human evidence indicating a hazard to babies during the lactation period; and/or

  No human data available are available.

  (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

  (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk."

  No one or two generation studies are available which could show adverse effects in the offspring due to transfer in the milk. In addition, no toxicokinetic studies are available which could indicate that the substance in present in potentially toxic levels in breast milk.

  Therefore, no additional labelling of the substance for "adverse effects on or via lactation" is warranted.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

a) Sexual function and fertility

Results from repeated dose studies indicate adverse effects on reproductive organs. However, some uncertainties are noted.

Therefore, a classification for effects on sexual function and fertility (Cat. 2, H361f) is warranted for glycerol formal.

"*Specific concentration limits and generic concentration limits are limits assigned to a substance indicating a threshold at or above which the presence of that substance in another substance or in a mixture as an identified impurity, additive or individual constituent leads to the classification of the substance or mixture as hazardous*" (EC, 2008).

Only studies with oral exposure were considered for the potency determination.

For the endpoint "sexual function and fertility" the generic concentration limit of 3% ("group 2", medium potency) can be applied for the following reasons:

- No effects were observed at doses below 4 mg/kg bw/d (i.e., effective dose with a 10% effect level above the background (ED_{10}) is above 4 mg/kg bw/d).
- Effects were observed between 12 and 1218 mg/kg bw/d. Slight effects were already seen in the low dose (10%, 1/10 animals with slight inhibition of spermiogenesis), and middle dose (20%, 2/10 animals with partly inhibition of spermiogenesis) groups (12 – 121 mg/kg bw/d), severe effects in the high dose group (1218 mg/kg bw/d; changes in testes in 100%, 10/10 animals from slight inhibition of spermiogenesis to total atrophy). Therefore, the ED_{10} is between 4 and 400 mg/kg bw/d, the substance is assigned to "group 2", medium potency and the generic concentration limit should be applied.

b) Developmental toxicity

Results from reliable studies on developmental toxicity indicate adverse effects on the development of the offspring independent of maternal toxicity.

Therefore, a classification for effects on development of the offspring (Cat. 1B, H360D) is warranted for glycerol formal.

For the endpoint "developmental toxicity" the generic concentration limit of 0.3% ("group 2", medium potency) can be applied for the following reasons:

- No effects were observed at doses below 4 mg/kg bw/d (i.e., the effective dose with a 10% effect level above the background (ED_{10}) is above 4 mg/kg bw/d).
- Effects were observed between 75 and 600 mg/kg bw/d. The average weight per litter was significantly reduced at 150 mg/kg bw/d. Increasing numbers of external malformations were observed starting at 300 mg/kg bw/d, visceral malformations were observed in the highest dose group, skeletal anomalies were observed starting at a dose of 150 mg/kg bw/d. Since the effects of "reduced average weight per litter" showed the lowest LOAEL for the endpoint developmental toxicity, this effect was selected for the calculation of the ED_{10} as given in the Guidance on the Application of CLH criteria for continuous data (ECHA, 2017):

Dose in mg/kg bw/d	0	75	150	300	600
Average weight (g)/litter	3.68	3.69 NOAEL	3.38* LOAEL	3.16*	2.81*

* statistically different from control P<0.05

- For effects that are measured as changes in magnitude, the ED_{10} is defined as the dose at which a change of 10%, compared to the concurrent control group, is observed
- The ED_{10} is 166.45 mg/kg bw/d because at this dose level the average weight/litter is calculated to be 90% of the control value. A 10% reduction of the control value of 3.68 g gives 3.312 g. Interpolation between 75 and

150 mg/kg bw/day to a dose level which would be expected to result in an average weight/litter of 3.312 g gives a value of 166.45 mg/kg bw/day.

- (150 - 75) / (3.69 - 3.38) = 241.94
- 3.69 - 3.312 = 0.378
- 0.378 x 241.94 = 91.45
- 91.45 + 75 = 166.45 mg/kg bw/d
- Therefore, the ED_{10} is between 4 and 400 mg/kg bw/d, the substance is assigned to "group 2", medium potency and the generic concentration limit should be applied.

c) Effects via lactation

In the absence of any studies indicating effects via lactation **no classification for effects via lactation is warranted for glycerol formal.**

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				
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.

<u>Experiment 1: intramuscular application (Sprague-Dawley rats)</u>

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.

10.11 Specific target organ toxicity-single exposure

Evaluation not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Evaluation not performed for this substance. However, an evaluation of the repeated dose toxicity studies is included in the overall assessment of the endpoint reproductive toxicity in section 10.10.

10.13 Aspiration hazard

Evaluation not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Evaluation not performed for this substance.

12 EVALUATION OF ADDITIONAL HAZARDS

Evaluation not performed for this substance.

13 ADDITIONAL LABELLING

Not relevant

14 ANNEXES

Please see separate documents for Annex I and confidential Annex I.

15 REFERENCES

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