

1 **Ethylene glycol**

2 Evaluation of the effects on reproduction, recommendation for classification

3 Subcommittee on the Classification of Substances Toxic to Reproduction

4 A committee of the Health Council of the Netherlands

5

6

The Health Council would like to give you the opportunity to comment on the draft advisory report. The draft has been presented to the Working Conditions Committee of the Social Economic Council of the Netherlands, and to experts of employer's organisations and trade unions. Other interested parties or persons are also invited to comment. The comments will be taken into account when drafting the final version of the advisory report.

Please follow the instructions for review, see www.healthcouncil.nl.

Please note that this is a draft report that will be finalised after comments, received during public consultation, have been considered. When citing from this report, please indicate that you are citing from a draft version.

Comments may be submitted until **16 June 2026** by e-mail to draftOSH@gr.nl.

Attn: Dr R.H. Mennen, scientific secretary
Subcommittee on the Classification of Substances Toxic to Reproduction
The Health Council of The Netherlands

1	Contents	
2	Samenvatting	4
3	Executive summary	8
4	1 Scope	11
5	1.1 Background	11
6	1.2 Committee and procedure	11
7	1.3 Labelling for lactation	13
8	1.4 Data	14
9	2 Identity of the substance	15
10	2.1 Name and other identifiers of the substance	15
11	2.2 Physicochemical properties	16
12	2.3 International classifications	16
13	3 Manufacture and uses	18
14	3.1 Manufacture	18
15	3.2 Identified uses	19
16	4 Toxicokinetics	21
17	4.1 Absorption	21
18	4.2 Distribution	21
19	4.3 Metabolism	22
20	4.4 Elimination	25
21	5 Mechanism of action and mechanism of reproductive toxicity	27
22	5.1 Glycolic acid as toxicant	27
23	5.2 Metabolic acidosis	27
24	5.3 Species differences	Fout! Bladwijzer niet gedefinieerd.
25	5.4 Translation to humans	28
26	6 Adverse effects on sexual function and fertility	29
27	6.1 Human data	29
28	6.2 Animal data	29
29	6.3 Evaluation of the data	33
30	6.4 Conclusion	35

1	7	Adverse effects on development	36
2	7.1	Human data	36
3	7.2	Animal data	36
4	7.2.1	Oral studies	36
5	7.2.2	Inhalation studies	44
6	7.2.3	Dermal studies	45
7	7.3	Evaluation of the data	46
8	7.4	Conclusion	51
9	8	Adverse effects on or via lactation	53
10	8.1	Animal data	53
11	8.2	Conclusion	53
12	9	Conclusions on classification and labelling	54
13		References	55
14		Committee	60
15	A	Supplementary tables	61
16		Fertility studies	61
17		Development studies	76

18
19

1 Samenvatting

2 Werknemers kunnen tijdens het werk worden blootgesteld aan stoffen die mogelijk
3 schadelijk zijn voor hun gezondheid. Op verzoek van het ministerie van Sociale Zaken
4 en Werkgelegenheid (SZW) heeft de Gezondheidsraad beoordeeld of ethyleenglycol
5 schadelijke eigenschappen heeft die invloed kunnen hebben op de voortplanting, en op
6 basis daarvan een classificatievoorstel opgesteld. Dit advies is tot stand gekomen in de
7 subcommissie Classificatie reproductietoxische stoffen, van de commissie Gezondheid
8 en beroepsmatige blootstelling aan stoffen (GBBS). Op www.gezondheidsraad.nl staat
9 informatie over de taken van deze vaste subcommissie van de Gezondheidsraad.

10 11 **Gebruik van ethyleenglycol**

12 Ethyleenglycol is een kleurloze vloeistof die vooral wordt gebruikt voor de productie
13 van polyestervezels, polymeren en harsen van polyethyleentereftalaat (PET). Het
14 wordt ook gebruikt als antivries- en koelvloeistof, bijvoorbeeld voor het vorstvrij maken
15 van vliegtuigen en voorruitens van auto's. Verder wordt het gebruikt als oplosmiddel in
16 verf en als weekmaker in plastics. Beroepsmatige blootstelling vindt plaats via
17 inademing, huid- of oogcontact. Dit komt bijvoorbeeld voor wanneer werknemers die
18 betrokken zijn bij ontdooiprocedures op luchthavens worden blootgesteld aan
19 ethyleenglycoldampen en nevel die vrijkomen bij het vernevelen van ontdooimiddelen
20 in de lucht.

21 22 **Classificeren naar bewijskracht**

23 Bij de beoordeling van effecten op de voortplanting kijkt de commissie zowel naar
24 effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de
25 ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie (productie
26 en afgifte van moedermelk) beoordeeld en effecten via de moedermelk op de zuigeling.
27 Als er aanwijzingen bestaan dat de stof schadelijke effecten heeft, stelt de commissie
28 voor om de stof te classificeren in gevarencategorieën die aangeven hoe groot de
29 bewijskracht is voor de schadelijke effecten, zie kader. Bij categorie 1 is de
30 bewijskracht het grootst en grotendeels gebaseerd op studies bij mensen (1A) of
31 dieren (1B). Bij categorie 2 is de bewijskracht beperkt en is er sprake van een
32 'verdenking'. De commissie kan ook adviseren om een stof niet te classificeren omdat
33 er onvoldoende gegevens beschikbaar zijn of omdat de stof waarschijnlijk niet
34 schadelijk is voor de voortplanting.

35
36 Een classificatievoorstel zegt iets over de bewijskracht voor de schadelijke
37 eigenschappen van een stof, maar niet over de mate waarin mensen op de werkplek

1 een gezondheidsrisico lopen. Dat hangt af van de mate waarin mensen op hun werk
2 worden blootgesteld aan de stof. Daar heeft de commissie geen zicht op.

3 4 **Geraadpleegde onderzoeken**

5 Er zijn geen onderzoeken bij mensen beschikbaar naar schadelijke effecten van
6 ethyleenglycol op de vruchtbaarheid, ontwikkeling van het nageslacht en lactatie. De
7 commissie baseert haar beoordeling op dierstudies.

8 9 *Vruchtbaarheid*

10 Uit de beschikbare onderzoeken komt geen eenduidig effect van ethyleenglycol op de
11 vruchtbaarheid naar voren. In onderzoek bij muizen werden na blootstelling aan
12 ethyleenglycol kleinere nestgroottes en minder levende pups waargenomen. Er konden
13 echter geen conclusies worden getrokken op basis van deze bevindingen, omdat het
14 onduidelijk was of deze effecten toegeschreven konden worden aan de vruchtbaarheid
15 of de ontwikkeling. Een studie in muizen en een studie in ratten toonden geen effect
16 van ethyleenglycol op de vruchtbaarheid. Andere onderzoeken waren van lage
17 kwaliteit, of de onderzoeksopzet was niet geschikt om de effecten op de
18 vruchtbaarheid te kunnen beoordelen. De opzet van de studies die de
19 voorplantingsorganen onderzochten was over het algemeen zeer beperkt. De
20 commissie beoordeelt de beschikbare gegevens als beperkt, en van onvoldoende
21 kwaliteit om een definitieve conclusie op te baseren.

22 23 *Ontwikkeling nageslacht*

24 Uit onderzoek bij ratten en muizen blijkt dat orale blootstelling aan ethyleenglycol een
25 negatieve invloed heeft op de ontwikkeling van het nageslacht. Hierbij gaat het om een
26 lager foetaal lichaamsgewicht, skeletafwijkingen, verlies van foetussen na innesteling,
27 en een lager aantal levende foetussen per nest. Bij konijnen zijn echter geen nadelige
28 effecten waargenomen bij een dosering die wel schadelijk was voor de moederdieren.
29 Dat komt mogelijk door verschillen tussen knaagdieren en konijnen in de mate van
30 accumulatie in de foetus van ethyleenglycol en zijn metaboliet glycolzuur die
31 verantwoordelijk is voor de nadelige effecten van het nageslacht. De commissie ziet op
32 basis van de literatuur echter geen bewijs dat een konijnenmodel een betere
33 voorspeller zou zijn voor het effect van ethyleenglycol op mensen. Daarom zijn de
34 knaagdierstudies niet uitgesloten in de evaluatie. De commissie concludeert dat er
35 duidelijk bewijs is voor een nadelig effect van blootstelling aan ethyleenglycol op de
36 ontwikkeling en adviseert classificatie in categorie 1B.

37
38

1 *Lactatie*

2 Er waren onvoldoende gegevens beschikbaar om de effecten van blootstelling aan
3 ethyleenglycol op of via lactatie te beoordelen.

4

5 **Advies aan het ministerie**

6 Op basis van de beschikbare onderzoeksgegevens adviseert de commissie om
7 ethyleenglycol:

- 8
- 9 • niet te classificeren voor effecten op de vruchtbaarheid, vanwege inconsistente
10 en onvoldoende gegevens;
 - 11 • te classificeren als stof die verondersteld wordt schadelijk te zijn voor de
12 ontwikkeling (categorie 1B) en te kenmerken met H360 (kan schade
13 toebrengen aan het ongeboren kind);
 - 14 • niet te classificeren voor de effecten op of via lactatie, vanwege een gebrek aan
gegevens.

PUBLIC DRAFT

Betekenis classificatievoorstellen reproductietoxische stoffen

In classificatievoorstellen gebruikt de Gezondheidsraad een indeling in gevarencategorieën. De categorieën zijn afgeleid van EU-verordening (EG) 1272/2008 en geven aan hoe sterk de bewijskracht is voor schadelijke effecten. Bij de categorie hoort ook een label met een EU-gevarenaanduiding die op verpakkingen kan worden gebruikt.

EU-gevarencategorieën voor voortplanting (*fertility* – F) en ontwikkeling (*development* – D)

- Categorie 1: Kan de vruchtbaarheid of het ongeboren kind schaden (EU-gevarenaanduiding H360 F/D)
- Categorie 1A: Stoffen waarvan bekend is dat zij toxisch zijn voor de menselijke voortplanting, hoofdzakelijk gebaseerd op onderzoek bij mensen (H360 F/D).
- Categorie 1B: Stoffen waarvan verondersteld wordt dat zij toxisch zijn voor de menselijke voortplanting, hoofdzakelijk gebaseerd op dierstudies (H360 F/D).
- Categorie 2: Kan mogelijk de vruchtbaarheid of het ongeboren kind schaden (H361f/d) - Stoffen die ervan verdacht worden dat zij toxisch zijn voor de menselijke voortplanting

EU-gevarencategorie voor effecten op of via lactatie

- Kan schadelijk zijn via de borstvoeding (H362). Stoffen waarvan is aangetoond dat zij de lactatie beïnvloeden of die in zodanige hoeveelheden in moedermelk aanwezig kunnen zijn dat er reden is tot bezorgdheid voor de gezondheid van het kind dat borstvoeding krijgt.

Betekenis voor de werkvloer

Werkgevers zijn op grond van de Arbowet wettelijk verplicht om gezondheids- en veiligheidsrisico's van het werken met stoffen zoveel mogelijk te voorkomen of te beperken. Op basis van de classificatievoorstellen van de Gezondheidsraad kan de minister van SZW besluiten stoffen op te nemen in de officiële lijst van kankerverwekkende, mutagene en voor de voortplanting giftige stoffen. Op die lijst staan kankerverwekkende en mutagene stoffen in categorie 1A en 1B en voor de voortplanting giftige stoffen in categorie 1A, 1B en 2. Afhankelijk van de classificatie vraagt de wetgever de werkgever aanvullende maatregelen te nemen om de werknemer te beschermen.

1

2

1 Executive summary

2 At the request of the Ministry of Social Affairs and Employment, the Health Council of
3 the Netherlands evaluated the effects of ethylene glycol on reproduction. Based on this
4 evaluation they made a recommendation for classification. The advisory report was
5 drafted by the subcommittee on the Classification of Substances Toxic to Reproduction
6 of the Dutch Expert Committee on Occupational Safety (DECOS) of the Health Council.
7 More information about the committee and its task can be found at
8 www.healthcouncil.nl.

9 Use of ethylene glycol

10 Ethylene glycol is a colourless liquid mostly used for the production of polyester fibres,
11 polymers, and polyethylene terephthalate (PET) resins. It is also used for its antifreeze
12 and coolant properties as a de-icing fluid for aircrafts and windshields for cars.
13 Additionally, it can be used as solvent in paints and as a softening agent in the
14 manufacture of plasticisers. Occupational exposure can occur through inhalation, and
15 skin and eye contact. For example, workers involved in airport de-icing operations can
16 be exposed when ethylene glycol vapour and mist is generated by spraying de-icing
17 formulation through the air.

19 Classification based on strength of evidence

20 To assess effects on reproduction, the committee evaluates the available literature on
21 the effects on male and female fertility and on the development of offspring. Moreover,
22 the committee considers effects of a substance on lactation and on the offspring via
23 lactation. If the data indicate hazardous properties, the committee recommends
24 classification into a hazard category. Classification is performed according to EU-
25 regulation (EC) 1272/2008 (see text box). When there are indications that a substance
26 has hazardous properties, the committee recommends classifying the substance into
27 hazard categories that indicate the strength of the evidence for hazardous effects (see
28 text box). For category 1, the strength of evidence is highest, and largely based on
29 studies in humans (1A) or animals (1B). For category 2, evidence is limited, and the
30 substance is categorised as a suspected toxicant. The committee can also recommend
31 not classifying a substance because of insufficient data or because the substance is
32 probably not hazardous for reproduction.

34
35 A recommendation for classification reflects the strength of evidence for the hazardous
36 properties of a substance, but it does not reflect the health risk for workers. The health

1 risk is based on the level of exposure to the substance in the workplace. The
2 committee does not have sufficient data on these exposure levels.

4 **Reviewed literature**

5 No human data were available for the evaluation of adverse effects of ethylene glycol
6 on fertility, development and lactation. The committee based their evaluation on animal
7 studies only.

9 *Fertility*

10 Overall, no consistent effects on fertility were observed across studies. Several studies
11 in mice showed potential adverse effects on fertility in terms of lower litter sizes and
12 lower number of live pups. However, definite conclusions could not be drawn as it was
13 unclear whether these effects could be attributed to fertility or development. One study
14 in rats and one in mice found no functional fertility effects. Other studies were of limited
15 quality, or were not optimally designed to assess fertility. Overall, the studies on
16 reproductive organs were very limited in design. The committee considers the available
17 data to be too limited and of too low quality to allow a definite conclusion on fertility.

19 *Development*

20 Studies in rats and mice clearly showed that exposure to ethylene glycol caused
21 adverse effects on development. The effects were mainly related to foetal body
22 weights, skeletal malformations, post-implantation loss and lower numbers of live
23 fetuses per litter. No adverse effects were found in rabbits up to dose levels causing
24 maternal toxicity. This could be because of differences between rodents and rabbits in
25 foetal accumulation of ethylene glycol and its metabolite glycolic acid, which is the
26 primary toxicant causing developmental toxicity. However, the committee did not find
27 conclusive evidence that shows that the rabbit model would be a more appropriate
28 model for evaluating the effects of ethylene glycol in humans. Therefore, the rodent
29 studies were not excluded from the evaluation. The committee concludes that based on
30 the clear evidence for an adverse effect on development, classification in Category 1B
31 is warranted.

33 *Lactation*

34 Due to a lack of relevant data, adverse effects of exposure to ethylene glycol on or via
35 lactation could not be evaluated.

37 **Recommendations to the ministry**

38 Based on the available scientific data, the committee recommends:

- 1 • not to classify ethylene glycol for reproductive toxicity, due to inconsistent and
- 2 insufficient data;
- 3 • to classify ethylene glycol in Category 1B, H360D, for developmental toxicity;
- 4 • not to classify ethylene glycol for effects on or via lactation, due to a lack of
- 5 data.
- 6

Classification for substances toxic to reproduction

The Health Council performs classification and labelling of substances according to the guidelines of the European Union (Regulation (EC) 1272/2008). The hazard categories described below indicate the strength of the evidence for hazardous properties of the substance. The substance is also labelled with an EU hazard statement code that can be used on packaging.

Classification for reproduction (fertility (F) and development (D)):

- Category 1: Known or presumed human reproductive toxicant - Causes adverse effects on fertility or the unborn child (Hazard statement code H360(F/D)).
- Category 1A: Known human reproductive toxicant – Substances that are known to be toxic for human reproduction, largely based on human studies (H360 (F/D)).
- Category 1B: Presumed human reproductive toxicant – Substances that are presumed to be toxic to human reproduction, largely based on animal studies (H360 (F/D)).
- Category 2: Suspected human reproductive toxicant – Can possibly affect fertility or the unborn child. Evidence from animal and/or human studies is limited (H361(f/d)).

Classification for lactation:

- Effects on or via lactation (H362) – Substances which have been proven to affect lactation or which are present in breast milk in such quantities that there is reason for concern for the health of the breastfed child.

Implications for the workplace

According to the Dutch Working Conditions Act, employers are legally required to prevent or minimize the health and safety risks of working with hazardous substances as much as possible. Based on the Health Council's recommendations for classification, the Minister of Social Affairs and Employment can decide to add substances to the official list of substances that are carcinogenic, mutagenic or toxic to reproduction. This list includes carcinogenic and mutagenic substances in categories 1A and 1B, and substances toxic to reproduction in categories 1A, 1B and 2. Depending on the classification, the government asks the employer to take additional measures to protect employees.

7

1 Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands regularly evaluates the toxic properties of substances used in the workplace. Based on these evaluations, the council makes recommendations for classification into hazard categories. The current report evaluates whether ethylene glycol (EG) is toxic to reproduction and whether classification is warranted. Based on the classification proposal, the minister can extend the existing list of compounds classified as reproductive toxicant or compounds with effects on or via lactation.

A recommendation for classification reflects the strength of evidence for the hazardous properties of a substance, but it does not reflect the health risk in the workplace. The assessment of health risk includes the level of exposure to the substance.

1.2 Committee and procedure

The classification was performed by the Health Council's Subcommittee on the Classification of reproduction toxic substances of the Dutch Expert Committee on Occupational Safety (DECOS). The members of the committee are listed on the last page of this report.

Classification of substances is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, see text box. The CLP regulation is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and offspring development as well as adverse effects on or via lactation.

Classification of substances toxic to reproduction

The Health Council performs classification and labelling of substances according to the guidelines of the European Union (Regulation (EC) 1272/2008). The hazard categories described below indicate the strength of the evidence for hazardous properties of the substance. The substance is also labelled with an EU hazard statement code that can be used on packaging.

Classification for reproduction (fertility (F) and development (D)):

- Category 1 Known or presumed human reproductive toxicant (H360F/D)
- Category 1A Known human reproductive toxicant
- Category 1B Presumed human reproductive toxicant
- Category 2 Suspected human reproductive toxicant (H361f/d)
- No classification for effects on fertility or development

Classification for lactation:

- Effects on or via lactation (H362)
- No labelling for lactation

Hazard statement codes:

H360F	May damage fertility.
H360D	May damage the unborn child.
H361f	Suspected of damaging fertility.
H361d	Suspected of damaging the unborn child.
H360FD	May damage fertility. May damage the unborn child.
H361fd	Suspected of damaging fertility. Suspected of damaging the unborn child.
H360Fd	May damage fertility. Suspected of damaging the unborn child.
H360Df	May damage the unborn child. Suspected of damaging fertility.
H362	May cause harm to breast-fed children.

Additional considerations to Regulation (EC) 1272/2008

If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the offspring, the compound is classified in category 1A, irrespective of the general toxic effects (see Regulation (EC) 1272/2008, 3.7.2.2.1.).

Adverse effects in a reproductive study, reported without information on the paternal or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in general toxicity studies.

Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se. The committee does not only use guideline studies (studies performed according to OECD (Organisation for Economic Cooperation and Development) standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1 The classification of compounds is the result of an integrated assessment of the nature
2 of all parental and developmental effects observed, their specificity and adversity, and
3 the dosages at which the various effects occur. The guideline necessarily leaves room
4 for interpretation, dependent on the specific data set under consideration. In the
5 process of using the regulation, the committee has agreed upon a number of additional
6 considerations, see text box.

7 Regarding fertility, the committee considers data on parameters related to male and
8 female fertility, such as seminal fluid volume and spermatozoa concentration, which are
9 related to male fertility. The committee excludes publications containing only data on
10 sex hormone levels from the assessment, because the relationship between these
11 hormone levels and functional fertility (ability to conceive children) is too uncertain.

12 In 2026, the president of the Health Council released a draft of the report for public
13 review. The committee has taken the comments received into account in deciding on
14 the final version of the report. These comments, and the replies by the committee, can
15 be found on www.healthcouncil.nl.

16 1.3 Labelling for lactation

17 The recommendation for classifying substances for effects on or via lactation is also
18 based on Regulation (EC) 1272/2008. The criteria define that substances which are
19 absorbed by women and have been shown to interfere with lactation, or which may be
20 present (including metabolites) in breast milk in amounts sufficient to cause concern for
21 the health of a breastfed child, should be classified and labelled. Unlike the
22 classification of substances for fertility and developmental effects, which is based on

1 hazard identification only (largely independent of dosage), the labelling for effects on or
2 via lactation is based on a risk characterization and therefore, it also includes
3 consideration of the level of exposure of the breastfed child.

4 Consequently, a substance should be labelled for effects on or via lactation when it is
5 likely that the substance is present in breast milk at potentially toxic levels. The
6 committee considers a concentration of a compound as potentially toxic to the
7 breastfed child when this concentration leads to exceeding the exposure limit for
8 children, or if that level is unknown, the exposure limit for the general population, e.g.
9 the acceptable daily intake (ADI).

10 **1.4 Data**

11 The committee evaluated the literature on reproductive toxicity of EG. A literature
12 search was performed using PubMed and Scopus. Only reproductive animal studies
13 were selected. No relevant cohort studies, case-control studies, cross-sectional
14 studies, or human experimental studies were available. The committee excluded
15 studies on ethylene glycol monomethyl ether, ethylene glycol monoethyl ether,
16 ethylene glycol monobutyl ether, ethylene glycol monophenyl ether, ethylene glycol
17 monohexyl ether and ethylene glycol diethyl ether, as these were beyond the scope of
18 the current literature summary.

19
20 The committee evaluated 20 animal studies: 6 fertility studies and 14 developmental
21 studies. Also, the REACH registration dossier of EG was consulted (publicly available
22 on ECHA website) and secondary sources, which included the EG Toxicological
23 overview by Public Health England, ATSDR Toxicological profile for EG (2010) and the
24 National Toxicology Program (NTP) monograph 2004 on EG. These were used to
25 retrieve information on substance identification, classification, manufacture, monitoring
26 and toxicokinetics.

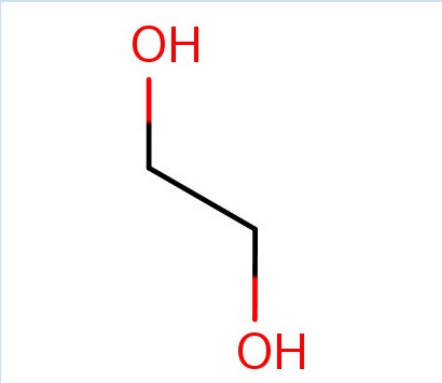
27
28

2 Identity of the substance

2.1 Name and other identifiers of the substance

The identity of EG is presented in Table 1 below.

Table 1 Substance identity and information related to the molecular and structural formula of EG.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Ethylene glycol
Other names (usual name, trade name, abbreviation)	Ethane-1,2-diol, 1,2-dihydroxyethane, 1,2-ethanediol, 1,2-ethylene glycol, 1-2 ethane-diol, 2-hydroxyethanol, ethylene alcohol, ethylene dihydrate, glycol, glycol alcohol, monoethylene glycol
ISO common name (if available and appropriate)	-
EC/EINECS number (if available and appropriate)	203-473-3
EC name (if available and appropriate)	Ethane-1,2-diol
CAS number	107-21-1
Molecular formula	C ₂ H ₆ O ₂
Structural formula	 <p>The structural formula shows a two-carbon chain (represented by a zigzag line) with a hydroxyl group (OH) attached to each carbon atom. The OH groups are written in red text.</p>
SMILES notation (if available)	OCCO
Molecular weight or molecular weight range	62.07

1

2 **2.2 Physicochemical properties**

3 The physicochemical properties of EG are presented in Table 2.

4 *Table 2 Summary of physicochemical properties of ethylene glycol¹*

Properties	Value
State of the substance at normal temperature and pressure	Colourless liquid (at 20°C and 1013 hPa)
Melting/freezing point	-13°C (at 1013 hPa)
Boiling point	197.4°C (at 1013 hPa)
Relative density	1.11 g/cm ³ (at 20°C)
Vapour pressure	12.3 Pa (at 25°C)
Surface tension	Not expected to be surface active
Water solubility	1000 g/L (at 20°C)
Partition coefficient n-octanol/water	-1.36 (at 25°C)
Flash point	111°C (at 1013 hPa)
Flammability	Non flammable
Explosive properties	Nonexplosive (100%)
Self-ignition temperature	398°C (at 1013 hPa)
Oxidising properties	Non oxidising (100%)
Granulometry	Not applicable
Stability in organic solvents and identity of relevant degradation products	Not applicable
Dissociation constant (pKa)	Not applicable
Viscosity	16.1 mPa·s (at 25°C)

5

6 **2.3 International classifications**7 EG currently has a harmonized classification in Annex VI of the CLP-Regulation (EC)
8 1272/2008 for:

9 Acute tox. 4 (H302: harmful if swallowed)

10

11

1 An NTP evaluation of EG did not demonstrate carcinogenicity.² Therefore, EG is not
2 listed as carcinogen by the NTP.¹

3
4 In Germany, EG is not included in the list of additional carcinogenic, mutagenic and
5 reprotoxic (CMR) substances in the context of worker protection.³

6
7 The state of California has included EG in their safe drinking water and toxic
8 enforcement act as a chemical known to cause developmental toxicity after ingestion
9 (since June 19, 2015).⁴

10
11 EG is currently not included in the list of substances NIOSH considers to be potential
12 occupational carcinogens.⁵

13
14 EG has the following classification in Australia:⁶

- 15 • Acute tox. 4 (H302: harmful if swallowed)
- 16 • STOT SE. 3 (H335, may cause respiratory irritation)

17
18 EG has the following classification in Japan:⁷

- 19 • Acute tox. 5 (H303: may be harmful if swallowed)
- 20 • Skin corrosion/irritation 3 (H316: causes mild skin irritation)
- 21 • Serious eye damage/eye irritation 2B (H320: Causes eye irritation)
- 22 • Repro. 1B (H360: may damage fertility or the unborn child)
- 23 • STOT SE 1 (H370: causes damage to organs; central nervous system, kidney,
24 heart, respiratory system)
- 25 • STOT RE 1 (H372: cause damage to organs through prolonged or repeated
26 exposure; central nervous system, respiratory system, heart)

27

3 Manufacture and uses

Ethylene glycol (EG) can be produced from carbon monoxide, ethylene oxide or conversion to ethylene carbonate. It is mostly used for the production of polyester fibres, polymers and resins. It also has antifreeze and coolant properties and can be used as solvent in paints and as a softening agent in the manufacture of plasticisers. Occupational exposure can occur through inhalation of EG vapour and mist, and skin and eye contact.

3.1 Manufacture

EG, also known as monoethylene glycol or glycol, can be produced from 3 different processes.

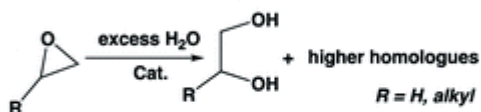
The first process is an industrial production route from carbon monoxide (Figure 1 (a)). It is used in countries producing large quantities of coal. The oxidative carbonylation of methanol to dimethyl oxalate provides an approach to the production of C1-based EG. Furthermore, dimethyl oxalate can be converted into EG in high yields (94.7%) by hydrogenation.

Because the methanol is recycled, only carbon monoxide, hydrogen, and oxygen are used.

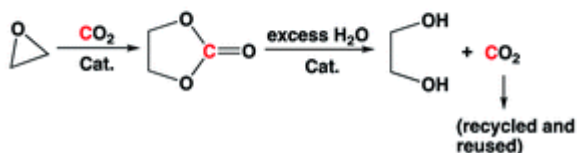
(a) Industrial methanol production route



(b) Hydration of epoxide to glycol

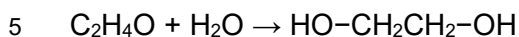


(c) Industrial shell OMEGA process to produce ethyleneglycol



1 Figure 1 The initial steps of each industrial route for the production of ethylene glycol.
2 The subsequent reaction steps are described in the accompanying text.⁸

3 The second production process is from ethylene oxide (Figure 1 (b)). The reaction with
4 water allows the production of EG in accordance with the following chemical equation:



6 This reaction, catalysed by acids or bases, generates EG with a yield of 90%. In
7 addition, several by-products are produced: oligomers diethylene glycol, triethylene
8 glycol, and tetraethylene glycol.

9 The third method of producing EG was developed by Shell. The equation is shown in
10 Figure 1 (c). The ethylene oxide is first converted with carbon dioxide (CO₂) to ethylene
11 carbonate.⁹ The latter is then hydrolysed in a second step to produce EG. The carbon
12 dioxide is released again in this step and can be fed back into the production process
13 circuit. The carbon dioxide partly results from ethylene oxide production, where a part
14 of the ethylene is completely oxidized. According to Shell, the conversion efficiency of
15 this process is over 99%.

16 **3.2 Identified uses**

17 EG is mostly used for the production of polyester fibres, polymers, and polyethylene
18 terephthalate (PET) resins. It is also used for its antifreeze and coolant properties, for
19 example as a de-icing fluid for windshields and aircrafts. Additionally, it can be used as
20 a desiccant for natural gas production as well as in hydraulic brake fluids, electrolytic
21 condensers, as solvent in paints and plastics, in inks and toners, washing and cleaning
22 products, dyes, as a softening agent in cellophane, and in the manufacture of
23 plasticisers, solvents, polishes and waxes, for non-metal-surface treatment products
24 and other coating products, leather treatment products, adhesives and sealants, and
25 biocides (e.g. disinfectants, pest control products).¹⁰⁻¹²

26 According to the REACH regulation, EG is manufactured in and / or imported to the
27 European Economic Area, at $\geq 1\,000\,000$ to $< 10\,000\,000$ tonnes per annum.¹³

28 It is estimated that workers are exposed mostly through inhalation, and skin and eye
29 contact. Inhalation is an occupational hazard, for example for workers involved in
30 airport de-icing operations by spraying the de-icing formulation through the air,
31 generating EG vapour and mist. Dermal exposure to EG in de-icing fluids may also be

- 1 hazardous, especially for workers who do not wear adequate skin protection during or
- 2 after application.¹⁰
- 3

PUBLIC DRAFT

4 Toxicokinetics

The major routes of exposure to EG are ingestion and dermal absorption. Inhalation exposure of EG is estimated to be low. EG is absorbed more quickly after ingestion than after dermal exposure. EG crosses the placenta and enters the developing foetus in rats and rabbits after oral administration. The most important metabolite of EG is glycolic acid (GA). Humans may metabolize GA at a higher rate than rats. EG is mainly metabolized by the liver and mostly excreted in the urine.

4.1 Absorption

Ingestion and dermal exposure are the major routes of exposure to EG. Following ingestion, EG is readily absorbed throughout the gastrointestinal (GI) tract. Distribution is rapid and occurs throughout body water as indicated by a volume of distribution of approximately 0.7–0.8 L/kg. Peak concentrations following ingestion occur within 1–4 hours.¹⁴ In contrast, dermal absorption of EG is expected to be very low and slow. Dermal absorption was studied in vitro using dermatomed human abdominal skin.¹⁵ Only $\leq 1\%$ of the applied EG was absorbed in 24 hours, of which $<0.7\%$ penetrated through the skin and $\sim 0.4\%$ remained in the skin.¹⁵

There is limited human data available on the toxicity of EG following inhalation. Theoretically, excessive inhalation exposure at room temperature is expected to be low because of the low vapour pressure of EG. Inhalation is theoretically possible at increased temperatures or where EG is aerosolised.¹⁴ In rats exposed nose-only to radiolabelled EG, the estimated absorption was 60–90% of the initial dose. Oral absorption is rapid and near 100%. In rats peak blood levels are reached within 1h and in mice, monkeys, dogs and pregnant rabbits between 1–3h. Pregnancy does not alter kinetics in rats. A single study indicates apparent dermal absorption of 26–32% and 60–84% of the applied dose in rat and mice respectively.¹⁰

4.2 Distribution

Estimated volume of distribution (Vd) after inhalation exposure was 0.78 and 0.91 L/kg in 2 volunteers. After oral exposure, EG distributes according to total body water with an apparent Vd of 0.54–0.56 L/kg based on data of 2 poisoned patients.¹⁴

In rats that inhaled radiolabelled EG, 75–80% of the initial body burden was distributed quickly throughout the body and it was estimated that 60% ended up in the respiratory

1 tract (mainly nasal cavity). In rats and mice, 96h after a single radiolabelled dose, 6-
2 22% and 3-11% of the radioactivity was retained in tissues for rat and mice,
3 respectively. Highest radioactivity was found in the liver of both species. In 2 rhesus
4 monkeys unmetabolized EG was evenly distributed throughout tissues (tissue-to-
5 plasma ratios of 0.85-1.91).¹⁰

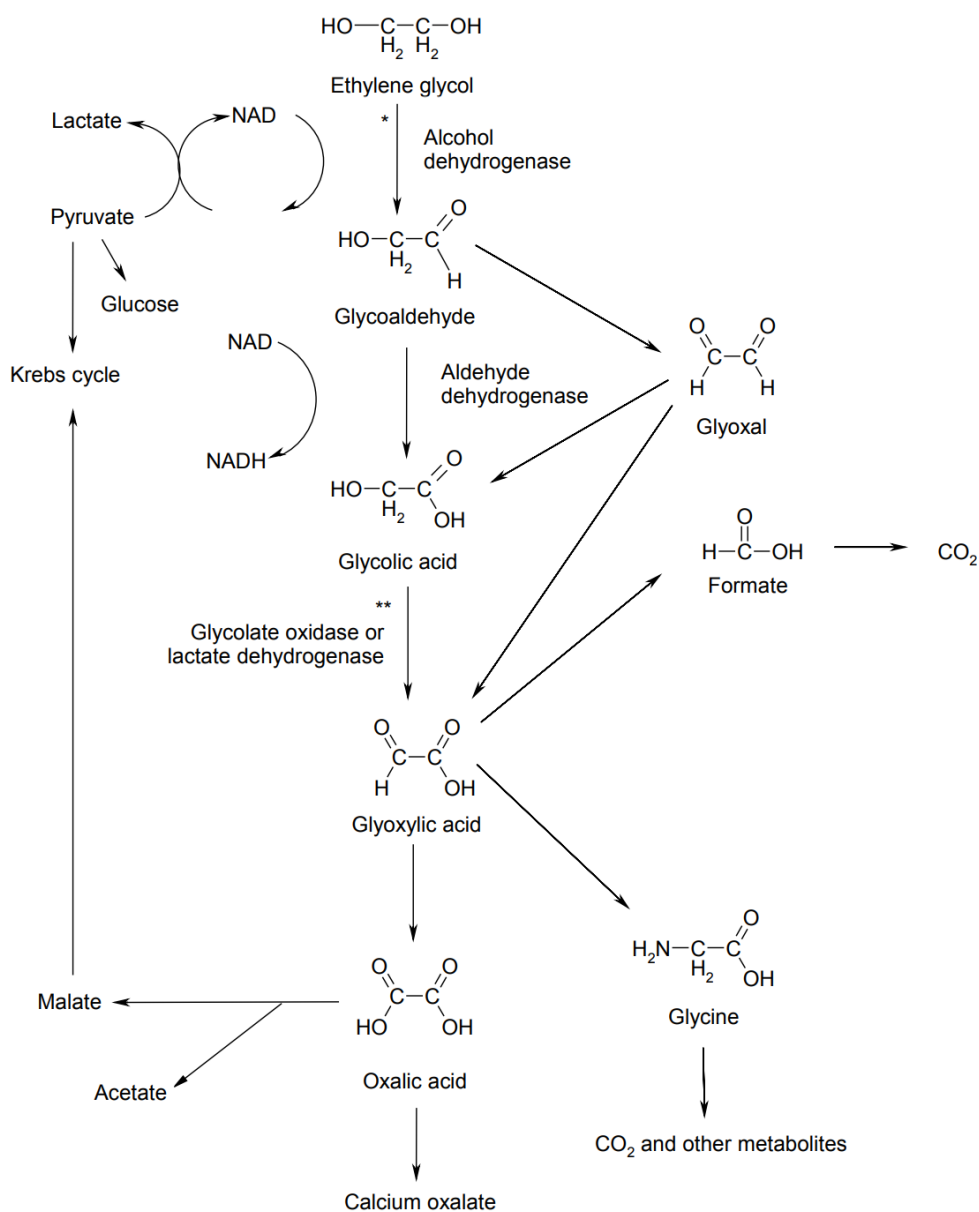
6 EG crosses the placenta and enters the developing foetus in rats and rabbits after oral
7 administration to pregnant dams. Unchanged EG concentrations in yolk sac and
8 embryos were 14-20% of maternal concentrations in a toxicokinetic study in rabbits.¹⁶
9 Extraembryonic fluid levels of EG were 2-fold higher in rats than in rabbits after a single
10 oral administration at GD10.¹⁰

11 After dermal exposure, levels of EG are the highest in pelt, namely 5-6% of the applied
12 dose or 8-15% of the applied dose when carcass and pelt are combined.¹⁰

13 **4.3 Metabolism**

14 The metabolism of EG was reviewed by the NTP-CERHR in 2004 and Slikker et al.^{10, 17,}
15 ¹⁸ Figure 2 provides an overview of the metabolism based on these 2 reviews. EG is
16 metabolised by alcohol dehydrogenase to glycoaldehyde, which is a process that can
17 be saturated. Glycoaldehyde is rapidly converted to glycolic acid (GA) by aldehyde
18 dehydrogenase. The following step is the oxidation to glyoxylic acid by GA oxidase or
19 lactic dehydrogenase. This is the major rate-limiting step in the metabolism. The
20 formed glyoxylic acid can be metabolized to create glycine, or malate, all of which may
21 be further broken down to generate respiratory CO₂, or to oxalic acid, which is
22 excreted in the urine. In excess, oxalic acid can form calcium oxalate crystals. Both GA
23 and oxalic acid are also products of normal protein and carbohydrate metabolism.

24 In volunteers who inhaled radiolabelled EG for 4h, GA concentrations peaked at 4-5h
25 after exposure. About 1% of the estimated dose was excreted as GA and 0.08-0.28%
26 was excreted as oxalic acid over 30h. In 8 intoxications, plasma glycolate levels ranged
27 from about 12 to 29 mM.



1
 2 *Figure 2 Metabolic pathway for ethylene glycol (source: 10, 17, 18)*
 3 * Rate-limiting step; **Most rate-limiting step

4 GA was the major metabolite in the plasma of orally exposed male rats (single dose).
 5 During the first 12 hours after dosing, at 100 and 1000 mg/kg, glyoxylate and glyoxal as
 6 well as trace levels of glycoaldehyde were detected and at 10 mg/kg glyoxylate levels

1 exceeded glycolate levels. Plasma glycolate levels peaked 6h or 4 hours after a single
2 dose in rats or dogs respectively where EG peaked after 2 hours in both species.¹⁰

3 Enzymes metabolizing glycolate are more quickly saturated with bolus subcutaneous
4 dosing than with slow, continuous dosing, leading to 3-10 fold higher peak plasma
5 glycolate levels with bolus dosing.¹⁰

6 Multiple in vivo studies in rats and mice show increasing urinary excretion of GA and
7 other metabolites with increasing dose, probably corresponding to saturation of GA
8 metabolism. In male rats after a single radiolabelled oral dose (1000 mg/kg), GA
9 comprised 25% of the urinary radioactivity in the first 12 hours. In the following 12
10 hours, this amounted to 37% and oxalic acid was detected at 7.4%. At low dose (10
11 mg/kg), >90% of the urinary radioactivity was unmetabolized EG. The metabolism
12 pattern was similar in female rats. However, in mice only glycolate was detected with
13 increasing excretion at higher doses. Urinary excretion of EG and glycolate accounted
14 for 20.7 and 4.5% respectively, of a 2000 mg/kg dose in rats during 24 hours after
15 dosing.¹⁰

16 In percutaneously exposed rats and mice to radiolabelled EG (10 or 1000 mg/kg) most
17 of the radioactivity was excreted as EG. In rats this was 87-100% regardless of the
18 dose, but in mice glycolate represented a greater proportion of urinary radioactivity at
19 1000 mg/kg (up to 20%).¹⁰

20 Male rats exposed to ±1000 mg/kg EG in drinking water for 21 days had urinary oxalate
21 levels equivalent to 1.18% conversion of EG to oxalate. In another study, male Wistar
22 rats were treated with EG up to 400 mg/kg/day for 12 months. From >300 mg/kg/day,
23 GA levels in the kidney, blood and urine were increased compared to control. Oxalate
24 levels were increased at >300 mg/kg/day in the kidney but were similar across all
25 doses in blood and urine. It is suggested that the metabolism of GA occurs at doses
26 between 150 and 300 mg/kg/day in Wistar rats.¹⁰

27 In vitro data from a study in liver homogenates and a study using liver slices both
28 suggest that humans may metabolize GA at a higher rate than rats (approximately 2-4-
29 fold higher V_{max}/K_m values in human tissues). According to the NTP-CERHR, in vivo
30 human data to predict the saturation point in humans were lacking.^{10, 17}

31 One study suggested that pregnancy did not alter the pharmacokinetics in maternal
32 blood and urine when groups of pregnant and nonpregnant rats were treated with a
33 single dose at GD10 (for the pregnant rats). However, a single time point is considered

1 a too narrow exposure window to observe pregnancy-related changes in metabolism.
2 Foetal levels of EG or metabolites were not measured. In another study in rats, levels
3 of GA in embryos and extraembryonic fluid paralleled maternal levels but were 1.4-4-
4 fold higher than maternal levels. It is reported that there are species-specific
5 differences in the transfer of GA from maternal blood to the conceptus.¹⁰ These are
6 further discussed in Chapter 5.

7 **4.4 Elimination**

8 EG is primarily excreted in the urine, either as the parent molecule, GA, calcium
9 oxalate or glycine (and its conjugate hippurate). Oxalic acid is excreted in the urine and
10 may give rise to dihydrate and/or monohydrate oxalate crystals which may precipitate
11 in the kidney causing nephrotoxicity. Approximately 20% of a dose of EG may be
12 excreted unchanged by the kidneys. The elimination half-life in humans is estimated to
13 be in the range of 2.5–8.4 hours.¹⁷

14
15 In laboratory animals treated with radiolabelled EG the primary routes of excretion are
16 exhaled air and urine, regardless of the exposure route. After oral exposure, saturation
17 of metabolic pathways at higher doses leads to a greater urinary excretion and
18 decreased elimination via air.¹⁰

19
20 Rats exposed to EG vapour (32 mg/m³, 30 min) or aerosol (184 mg/m³, 17 min)
21 excreted 63% (over 4 days) or 75% (over 6 days) as CO₂. Urinary excretion was 20%
22 or 12% of the initial body burden after vapour and aerosol exposure respectively, while
23 faecal excretion was 3% and 1%, respectively.¹⁰

24
25 Elimination half-lives in plasma of laboratory animals after oral exposure at 1.4-2.5
26 hours in rats, 0.3-1.1 hours in mice, 3.5 hours in dog and 2.7-3.7h in monkeys at
27 multiple dose levels all include the 1000 mg/kg level. Data from intravenously exposed
28 animals show similar half-lives. Plasma elimination half-life was similar in pregnant rats
29 and nonpregnant rats.¹⁰

30
31 In male and female rats receiving a single oral dose between 10 and 1000 mg/kg, the
32 major excretory routes up to 96 hours of exposure were CO₂ exhalation (27-48% of
33 radioactivity), urine (21-43%) and faeces (2-4%). Female mice that were exposed
34 similarly as the rats excreted EG as exhaled CO₂ (22-55%) and exhaled volatile organic
35 compounds (3-11%), while excreting 24-56% via urine and 5-6% in faeces. The
36 majority was eliminated within 12 hours after dosing. An increase in urinary excretion
37 was evident between 10-100 mg/kg in female mice, between 10 and 400 mg/kg in
38 female rats and between 800 and 1000 mg/kg in male mice. This probably resulted

1 from saturation of enzymes that metabolize GA leading to increased excretion of this
2 metabolite via urine.¹⁰

3
4 Monkeys given a single oral dose (± 1000 mg/kg) excreted 24% of the dose as the
5 parent compound in urine within 48 hours. Dogs excreted about 50% of a single
6 administered oral dose (173 mmol/kg) via urine within 72 hours.¹⁰

7
8 Rats and mice were treated with dermal application of 10-1000 mg/kg undiluted or
9 1000 mg/kg 50% aqueous solution of radiolabelled EG under occlusion for 6 hours.
10 When measured over 96 hours after dosing, rats excreted 6-14% of the administered
11 dose, 4-8% was excreted in urine and 1% in faeces. Female mice exhaled volatile
12 organic compounds (21-34%) and CO₂ (10-16%). No dose-related shift in excretory
13 patterns was observed suggesting that metabolic pathways were not saturated with
14 dermal exposure.¹⁰

15 It should be noted that all animal studies reported in this chapter were performed at
16 doses/concentrations that are below the doses showing effects on development as
17 described in Chapter 7. Therefore it is unlikely that saturation of the EG metabolism
18 and accumulation of GA have influenced the outcome of the studies in this chapter on
19 kinetics.

20

21

5 Mechanism of action and mechanism of reproductive toxicity

The exact mechanism of toxicity of EG is unknown, but studies show that the primary toxicant is EG's metabolite glycolic acid. Metabolic acidosis may play a role in causing reproductive toxicity. A PBK model was developed to compare rat and human kinetics of EG.

5.1 Glycolic acid as toxicant

Toxicokinetic data and rat whole embryo culture (WEC) data concluded that not EG but GA is the active developmental toxicant.^{1,3-5, 8} Exposure to GA in rats produced effects matching the dysmorphogenesis seen after EG exposure in vivo in rodents at relevant concentrations.² Toxicokinetic studies in pregnant rats (GD 11) dosed with 1000 mg/kg EG via gavage showed that while EG levels were comparable in maternal blood, exocoelomic fluid and embryos, GA levels in exocoelomic fluid and embryos were higher compared to maternal blood GA levels, indicating that GA not only distributes to the rat embryo but also accumulates there. WEC studies in rabbits exposed to high concentrations of GA (≤ 12.5 mM) did not show effects, while in reported rat WEC studies, effects were seen at much lower concentrations of GA (≥ 3 mM).⁶ The absence of effects in rabbit WEC seem to reflect a difference in intrinsic sensitivity between rat and rabbits. However, there is a difference between rat and rabbit WEC studies regarding the role of the culture medium (in the rat it resembles the maternal blood, in the rabbit the yolk sac cavity fluid) and in the time of development of certain enzymes (particularly aldehyde dehydrogenase) in rats (GD 15), rabbits (GD 20), and humans (weeks 19/20 of pregnancy). Effects of EG on mice embryos occur even before these enzymes develop, making the concentrations of GA at that time dependent on maternal metabolism.

5.2 Metabolic acidosis

Pharmacokinetics play a central role in EG toxicity.¹ In humans, acute EG toxicity progresses from initial central nervous system depression attributed to high circulating EG levels, to metabolic acidosis associated with acid metabolites of EG, such as GA, and ultimately renal (and neurological) damage as a result of oxalic acid build up, which can precipitate with calcium to form crystals¹.

Metabolic acidosis is a disturbance of the acid-base balance in which the blood becomes too acidic because of too much acid, too little bicarbonate, or both. Metabolic

1 acidosis can contribute to GA developmental toxicity. Developmentally toxic effects of
2 GA can occur in the absence of metabolic acidosis, but the effects are enhanced in the
3 presence of metabolic acidosis.^{19, 20} The metabolic acidosis and hyperosmolarity
4 resulting from EG exposure in rats were suspected to contribute to an increased
5 occurrence of foetal abnormalities and reduced foetal body weight.¹⁹

6 **5.3 PBK model for humans**

7 A Physiologically based kinetic (PBK) model was developed to compare rat and human
8 kinetics of EG.³⁰ The model was validated by comparing internal dose surrogates in
9 rats and humans and by the use of human clinical case reports. The authors concluded
10 that humans are unlikely to achieve blood levels of GA associated with developmental
11 toxicity in rats.^{30, 31} The committee agrees that the PBK model may be useful in risk
12 assessment to translate from rat to human and make a prediction at the maternal level
13 by translating the external exposure scenarios into internal exposure scenarios in the
14 form of maternal blood levels of EG and GA. It should be noted that based on these
15 models alone, no conclusion can be drawn regarding the percentage of EG or GA
16 accumulating in the rat or human embryo, as the models do not include an embryonic
17 compartment. Importantly, no PBK model was developed that would allow a
18 comparison between rabbits and humans. It is not clear to what extent the rabbit model
19 would be relevant for extrapolation to human systemic and compartmental exposure
20 scenarios as a rabbit PBK model is lacking.

21

22

6 Adverse effects on sexual function and fertility

No human data are available on the effects of EG on sexual function and fertility. The available animal studies show inconsistent results, and the data are limited and of low quality. The committee concludes that the data do not provide sufficient basis for classification of EG for effects on fertility.

6.1 Human data

No human data were available related to EG and reproductive effects.

6.2 Animal data

An overview of the in vivo studies on adverse effects on sexual function and fertility is provided in Annex A Table 1.

Studies on fertility

A non-guideline fertility assessment by Lamb et al. (1985) performed continuous breeding of CD-1 mice. A total of 20 animals per sex per group were dosed at 0.25, 0.5, and 1% EG in drinking water. Doses were equal to 0, 410, 840 and 1640 mg/kg bw/day. A control group consisted of 40 animals per sex. Exposure occurred during a pre-mating period (7 days), a cohabitation and breeding period (98 days), a segregation period (21 days) and continued throughout the life of the offspring. High dose and control pups that were born during the segregation period were mated for 7 days to assess fertility of the offspring. No treatment-related effects were observed in the parental animals on body weight, water consumption or clinical signs of general toxicity. There were 2 deaths in the 0.5% group (but not in the 1% group), as well as 3 in the control group. According to the authors, at least 1 of the 2 deaths in the 0.5% group could be treatment-related as oxalate crystals were found in the renal tubules. For the F₀-generation, all pairs produced litters during continuous breeding, resulting in a fertility index of 100% in all treatment groups. At the highest dose, however, there were decreased numbers of litters per fertile pair (4.5 vs. 4.9 in controls), fewer pups per litter (10.2 vs. 10.8 in controls) and a lower pup weight (although <10% difference with control values). The proportion of pups born alive was unaffected by treatment. The fertility index (no. of pregnant compared to no. of cohabitated) of the F₁-parents was 80% for the control group and 61% for animals receiving 1% EG, although this difference was not statistically significant. The number of pregnant females compared

1 to the number of mated females was unaffected. It should be noted that only 7 days
2 were allowed for mating of F₁ parents, whereas 14 days is the current standard in
3 guideline studies. Although not statistically significant, at the high dose, the number of
4 live pups per litter (8.4 vs. 10.4) and proportion of pups born alive (0.88 vs. 0.97 was
5 lower).³² The committee notes that although the study was not performed according to
6 the current guidelines, the effects on the number of litters per fertile pair and the lower
7 number of pups per litter in the F₀ and F₁ generation of the highest dose group were at
8 dose levels where no general toxicity was observed. It should be noted that possible
9 treatment related mortality was noted at 0.5 % EG exposure, but not at the highest
10 dose level of 1.0% EG. Although there is a statistically significant decrease in the
11 number of litters in the F₀ generation, the effect is relatively small.

12 As follow-up of Lamb et al. (1985), a non-guideline continuous breeding study was
13 performed by Gulati et al. (1986) in CD-1 mice.³³ Similarly, 20 animals per sex per
14 group were dosed at 0.5, 1.0, and 1.5% EG in drinking water, equal to 0, 897, 1798
15 and 2826 mg/kg bw/day. A control group consisted of 40 animals per sex. Mice were
16 exposed during the premating period (7 days), during the breeding period (98 days),
17 the segregation period (21 days), offspring was killed except for the last litters (F₁),
18 where exposure continued throughout the life of the offspring. Weaned animals of the
19 last litters from the high dose and controls were mated in a cross-over design.
20 Additionally, weanlings (F₁) of all treatment groups were mated with partners of the
21 same treatment group. In both the cross-over and the second-generation studies
22 females were allowed to litter. Exposure occurred during premating, cohabitation,
23 segregation and throughout the life of offspring. In the continuous breeding study, 2
24 control animals (1 male and 1 female) and 4 animals at 1.5% EG (3 males and 1
25 female) died during either the pre-cohabitation or cohabitation phase. Moreover,
26 statistically significant lower body and liver weights were observed in P₀ parental male
27 mice in the 1.5% exposed group. No effects on body weight or treatment-related
28 lesions in kidney, liver, ovary, uterus, or vagina were observed in female mice. The
29 fertility index (number of fertile / number of cohabited pairs) was 88% in the 1.5%
30 exposure group (16 cohabited) and 100% in the other exposure groups (20 cohabited)
31 and controls (38 cohabited). The number of live pups in the consecutive litters (9.99 vs.
32 11.81 live pups per litter in controls) and their weight (1.46 vs. 1.57 g in controls for
33 combined weight) were significantly decreased. The proportion of pups born alive was
34 unaffected by treatment with EG. In the cross-over study, the mating index (no. with
35 copulatory plugs/no. cohabited) was higher in EG treated groups and the fertility
36 index (No. fertile/No. cohabited) was 50% in all groups including controls that were
37 mated with controls. In the cross-over mating of control females with males exposed to
38 1.5% EG, live pups per litter and the proportion of pups born alive were unaffected. In

1 males at 1.5% EG, sperm motility and density (not statistically significant) were lower
2 compared to control and the percentage of abnormal sperm was higher compared to
3 control. Histologic changes were observed in the testes from many of the male
4 animals, consisting of degeneration of seminiferous tubules, loss of spermatozoa,
5 spermatids, spermatogonia and spermatocytes and vacuolization of epithelial cells.
6 Histologic changes were also noted in the kidneys of treated males. EG treatment did
7 not interfere with oestrous cycle. No differences in organ weights were noted in both
8 sexes after correction for body weight at necropsy. In addition, the reproductive
9 performance of the F₁-animals was evaluated by mating 20 F₁ generation animals/sex
10 of all treatment groups around day 74 of age. Mating was continued until a copulatory
11 plug was found or for 7 days. No effects on body weight were noted, but 18% (i.e. 9 out
12 of 49) weaned males at 1.5% EG died prior to cohabitation (vs. 0, 0 and 3% in the
13 control, 0.5, 1.0% EG groups). No female pups died in any of the treatment groups. No
14 statistically significant changes were noted in mating index (85, 90, 95 and 75% at 0,
15 0.5, 1.0 and 1.5% EG) or fertility index (75, 85, 90 and 65%, at 0, 0.5, 1.0 and 1.5%
16 EG). Moreover, EG treatment did not affect the number of live pups per litter, the
17 proportion of pups born alive and the sex ratio, although pup body weight was reduced
18 at all 3 dose levels (without a dose relationship). No differences in organ weights were
19 noted in both sexes after correction for body weight at necropsy, with the exception of
20 testis and epididymis weights for males exposed to 1.0 and 1.5% EG. The groups
21 exposed to 0.5% and 1% EG showed a significant reduction in sperm density and the
22 1% and 1.5% dose groups showed a significant reduction in sperm motility in F₁ mice.
23 Although not statistically significant, the percentage of abnormal sperm was higher in
24 males treated at 1.0 and 1.5% EG. In females, EG treatment did not interfere with
25 oestrous cycle.³³ The committee notes excessive toxicity (18% mortality) in the F₁
26 generation at 1.5% EG. Although for the F₀ generation the fertility index in controls was
27 100%, ideally the exposed groups would have been compared to historical control data
28 to see whether the effects in the high dose were due to exposure or whether these
29 occurred within the range of normal biological variation. In the F₂ generation, no
30 statistically significant changes were noted in mating and fertility indices and these
31 indices were generally more variable (as compared to 100% in most groups of the F₀-
32 generation).

33 DePass et al. 1986 performed a non-guideline three generation reproduction study.³⁴
34 Fisher 344 Rats (10 males and 20 females per group) were treated with 40, 200, and
35 1000 mg/kg bw/day EG in diet (calculated exposures based on dietary intake were 40-
36 50, 200-300 and 1000-1300 mg/kg/day for males and 40-60, 200-300 and 900-1200
37 mg/kg/day for females). Rats were exposed starting 7 weeks of age, during a 7-week
38 pre-mating period and throughout breeding. The F₁ and F₂ offspring (10 males and 20

1 females per group randomly chosen from the litters) was exposed throughout life and
2 subsequently mated at approximately 100 days of age. No treatment related effects on
3 mortality, diet intake or body weight changes were observed. No treatment related
4 effects on fertility index, gestation index, gestation survival index or days from first
5 mating to litter were observed for all 3 generations. Moreover, pup survival indices
6 (PND 0-4, 4-14 and 4-21) were unaffected, as well as pup body weights.³⁴ Effects on
7 developmental toxicity described within this study are discussed in Chapter 7.2. The
8 committee concludes that this study did not show effects of EG on fertility up to the limit
9 dose of 1000 mg/kg/day.

10 Harris et al. (1992) performed a non-guideline 21-day developmental toxicity study in
11 which also a few fertility parameters were examined.³⁵ CD-1 mice (10/sex/group) were
12 exposed to 0, 250, 700, and 2500 mg/kg/day EG in water by oral gavage. Proven fertile
13 males were exposed from study day 3 to 7 and during cohabitation (day 8 to 13) and
14 killed on day 20. Females were exposed from study day 0 to day 21. No treatment
15 related effects on clinical signs, body weight, liver and kidney histopathology, testes
16 weight or sperm number or mobility were observed in male mice. Continuous exposure
17 of females did not result in clinical signs or mortality and the number of pregnant
18 females was unaffected. At the highest dose (2500 mg/kg/day), EG exposure resulted
19 in fewer live (7.4 vs. 10.0 in control) and more dead implants (1.4 vs. 0.4 in control).
20 Although not statistically significant, the total number of implantation sites in females at
21 2500 mg/kg/day was lower, i.e. 8.9 vs. 10.4 in control.³⁵ The committee evaluates the
22 evidence for an effect on fertility as not convincing. Mice were exposed during a
23 relatively short period, not covering the entire spermatogenic cycle (males).
24 Nevertheless, exposure at the high dose level resulted in a lower number of live
25 implantations and a higher number of dead implantations and although not statistically
26 significant, a lower number of implantation sites was noted in exposed females.
27 Despite the short exposure scenario, the data could be indicative of an effect on
28 fertility.

29 *Studies on reproductive organs*

30 A non-guideline study was performed by Hong et al. (1988) with male and female
31 B6C3F1 mice.³⁶ EG was administered via oral gavage for 4 consecutive days at doses
32 of 0, 200, 400, and 1000 mg/kg bw/day (7/sex/group). No mortality occurred.
33 Statistically significant reduction in body weight was observed for only female mice in
34 the 1000 mg/kg bw/day group. No histological evidence of organ or tissue damage and
35 no significant effects on testicular weight were observed.³⁶ The committee considers an
36 exposure duration of 4 days as too short to be able to draw firm conclusions. The
37

1 committee does not take this study into consideration in its evaluation for effects on
2 fertility.

3 Bolon et al. (1997) studied ovary samples of mice from 18 NTP continuous breeding
4 studies in an additional evaluation of the data generated by Gulati et al. (1986).³⁷ CD1
5 mice (20/sex/group) were exposed to 1% EG in drinking water (equal to 1798 mg/kg
6 bw/day). The study also included 0% controls (40/sex). Mice were exposed during the
7 pre-mating period (7 days), during the breeding period (98 days), the segregation period
8 (21 days) and throughout the life of the offspring. Offspring (20/sex/group) was dosed
9 with 0.5%, 1% or 1.5% EG in drinking water (equal to 897, 1798 and 2826 mg/kg
10 bw/day). General toxicity parameters were not reported in the Bolon paper. No
11 statistically significant changes in ovarian follicle counts were noted in both the F0 and
12 F1 generation.³⁷ The committee considers the study design to be of limited value to
13 deliver conclusive information.

14 **6.3 Evaluation of the data**

15
16 There are no reported cases of EG-induced fertility effects in humans.
17 In general, EG dose levels in the animal studies described above were high. According
18 to the OECD guidelines, a limit dose of 1000 mg/kg bw/day should be chosen for
19 practical purposes in the set-up of the experimental design in case a compound shows
20 no maternal toxicity at lower dose levels. However, the committee considers fertility
21 effects at high doses as being relevant for the hazard evaluation, provided the effects
22 were found in the absence of maternal toxicity.

23 24 **Overall fertility**

25 Two well-performed mice studies with EG in drinking water by Lamb et al. (1985) and
26 the follow-up study by Gulati et al. (1986) showed potential fertility effects, although the
27 studies were not performed according to the current guidelines.^{32, 33} Despite the high
28 dose levels up to 1% EG in drinking water (equal to 1640 mg/kg bw/day), no general
29 toxicity occurred in the parental animals in the fertility assessment study by Lamb et al.
30 (1985).³² In the F0 generation, effects were observed in the 1% exposed group in terms
31 of a lower number of litters per fertile pair and a lower average number of pups per litter
32 compared to controls.³² The reported lower litter size in the F1 generation (not
33 statistically significant) was not convincing to the committee. There is no information on
34 the numbers per individual litter; only average numbers were reported. It is unclear
35 whether the effects on litter size can be attributed to fertility or development, since the
36 study was not designed to assess this (i.e. the number of implantation sites is not
37 counted).

1
2 In the follow-up continuous breeding study by Gulati et al. (1986) it was also unclear
3 whether the lower number of live pups could be attributed to an effect on fertility or
4 development, since also in this study, the study design does not allow for assessment
5 of the number of implantation sites.³³ In this study the dosages were higher than in the
6 study by Lamb et al. and general toxicity was observed at the highest dose for both
7 generations (up to excessive toxicity in the F1-generation at 1.5% EG in drinking water;
8 equal to 2826 mg/kg bw/day), especially in male mice.³³ Effects were reported on
9 testes histopathology, testis and epididymis weights, sperm motility and density and
10 abnormal sperm, but the fertility and mating indices were not affected.³³

11
12 No fertility effects were observed in a well performed three-generation study in rats
13 (although not performed according to the current guidelines) administering EG via diet
14 with dosages up to 1000 mg/kg bw/day.³⁴ No treatment related effects on general
15 toxicity endpoints were observed in terms of mortality, diet intake or body weight
16 changes. No treatment related effects on fertility index, gestation index, gestation
17 survival index or days from first mating to litter were observed for all 3 generations.³⁴

18 A non-guideline 21-day developmental toxicity study in mice with EG via oral gavage
19 with exposures up to 2500 mg/kg bw/day also studied a few fertility parameters such as
20 effects on implantation sites, testes and sperm parameters.³⁵ Limitations of the study
21 are the limited number of animals used and the exposure duration, which is relatively
22 short to examine fertility effects. No significant effect was observed on testes weight,
23 sperm mobility or number.³⁵ Despite the above-mentioned limitations, the committee
24 deems the evidence as relevant and indicative of a fertility effect, because of the lower
25 uterine implants in the top-dose group (7.4 ± 1.2 vs 10.0 ± 0.9 in control groups).³⁵

26 ***Reproductive organ parameters***

27 Regarding effects on the testes, Gulati et al. (1986) showed no effect on mice testes
28 weight after correction for terminal body weight in mice.³³ Histopathological
29 examinations showed degenerations of seminiferous tubules, loss of spermatozoa,
30 spermatids, spermatogonia and spermatocytes and vacuolization of epithelial cells.
31 Also, effects on sperm motility, density and abnormal sperm were observed. However,
32 these effects on sperm did not result in functional fertility effects in this study.³³

33
34 Two other studies in mice did not report effects on testes and sperm parameters, but
35 the exposure durations were too short to be able to draw firm conclusions with regards
36 to the testes and sperm parameters.^{35, 36} The studies that specifically evaluated mice

1 female reproductive parameters also had limitations in design and exposure duration,
2 making it difficult to draw firm conclusions.^{36, 37}

3 **6.4 Conclusion**

4 All studies described have limitations. Overall, 2 mice studies reported lower litter sizes
5 and lower number of live pups at high dose levels for which it is unclear whether they
6 can be attributed to fertility or development because the number of implantation sites
7 could not be specified in these studies.^{32, 33} One three-generation study showed no
8 fertility effects in rats.³⁴ A lower number of live uterine implants was observed in a
9 developmental study in mice. However, this study was not optimally designed to
10 assess fertility endpoints.³⁵ The studies on reproductive organs were overall very
11 limited in design. One well-designed study in mice reported effects on the
12 histopathology of the testes and sperm parameters, although no functional fertility
13 effects were observed.³³

14
15 A possible explanation for the inconsistent results between studies may be the applied
16 dose levels. The study with negative results used lower doses than the studies in which
17 lower litter sizes and lower number of live pups were observed or the study with an
18 effect on the live uterine implants.³²⁻³⁵ The dose levels at which findings were noted
19 were high (i.e. >limit dose of 1000 mg/kg/day),^{32, 33, 35} in one study even resulting in
20 general toxicity, but as findings were also noted at dose levels without excessive
21 general toxicity, these studies are considered appropriate for hazard evaluation related
22 to effects on fertility.³³

23
24 In conclusion, no human data were available to draw a conclusion on adverse effects
25 of EG on sexual function and fertility. Based on the animal studies, no consistent
26 effects were observed across the studies and the committee evaluates the available
27 data to be too limited and of too low quality to allow a definite conclusion on fertility.
28 The committee therefore deems the data to be inconclusive and do not provide
29 sufficient basis for classification of EG for effects on fertility.
30

7 Adverse effects on development

No human data are available on developmental effects of EG. Animal studies showed developmental effects of exposure to EG in rodents, but not in rabbits. However, the committee did not find conclusive evidence to exclude the rodent studies from the evaluation. The committee concludes that classification in category 1B is warranted.

7.1 Human data

No human data were available related to EG and developmental effects.

7.2 Animal data

7.2.1 Oral studies

An overview of the in vivo studies on adverse effects on development is provided in annex A table 2-4.

Rat studies

Maronpot et al. 1983 performed a non-guideline prenatal development study with Fischer 344 rats.³⁸ EG was administered to pregnant dams via the diet at target doses of 0, 40, 200, and 1000 mg/kg bw/day. Females were mated with males in order to achieve 20 pregnant females per treatment level. Dams were treated from GD 6 to 15 and a c-section was performed on GD21. General toxicity in the dams was not observed, i.e. no clinical signs of toxicity were noted and corrected maternal body weight gain was unaffected. Foetal weight, foetal length, total number of implantation sites and litter size was unaffected by treatment. There was no increased incidence in major malformations in litters of EG treated females. At the highest dose level, preimplantation loss was higher compared to the control, although not statistically significant. Also, higher number of ossified and unossified vertebral centra were observed at this dose level (14.2% vs 1.8% in control and 26.0% vs 11.4%, respectively).³⁸ The committee notes that only the targeted dose was reported and not the actual test article intake. It was also considered that the observed (statistically non-significant) preimplantation loss was probably unrelated to EG exposure, as the treatment was initiated after completion of implantation (from GD 6 onwards). The observed effects on ossification may indicate (minimal) embryotoxicity.

1 A non-guideline developmental toxicity study was performed by Price et al. (1985).³⁹
2 The study was GLP compliant and performed by the NTP. The authors performed a
3 study in rats and mice (described later). Female CD rats were treated with EG at 0,
4 1250, 2500 or 5000 mg/kg/day in water via oral gavage from GD6 through 15. C-
5 section was performed on GD20. Two replicates of the study were performed making
6 the total number of animals per group 20. All treated rats showed piloerection and
7 treated rats at the mid and the high dose showed an increased water consumption
8 compared to control. There was a dose-related decrease in all maternal body weight
9 parameters and gravid uterine weight. Maternal weight gain during treatment (GD6-
10 GD15) was statistically significantly lower in all treatment groups. Maternal liver weight
11 (high dose) and relative kidney weight (mid and high dose) were significantly
12 decreased compared to controls. There was a decrease in the number of live foetuses
13 per litter at mid dose (11.90 ± 0.60) and high dose (11.04 ± 0.79) vs 13.54 ± 0.28 in the
14 control), and in the average foetal body weight at mid dose (2.916 ± 0.056 g) and high
15 dose (2.388 ± 0.089 g) compared to the control (3.404 ± 0.052 g). The following
16 developmental toxicity parameters were affected in a dose-related manner: an increase
17 in post-implantation loss per litter (high dose only, $21.34 \pm 5.24\%$ vs $4.70 \pm 1.23\%$ in
18 control), an increase in the percentage of malformed live foetuses per litter (mid dose
19 ($25.11 \pm 4.84\%$) and high dose ($75.53 \pm 6.42\%$) compared to the control ($1.37 \pm 0.97\%$),
20 and the percentage of litters with one or more malformed live foetuses at the low
21 (39.29%), mid (68.97%) and high dose (96.15%). For all morphological defects, a dose
22 response relationship was observed. External malformations were significantly
23 increased at the high dose (15 vs 0 in control), visceral malformations were increased
24 at the low (6 vs 0) and high dose (8 vs 0), and skeletal malformations and the mid (19
25 vs 2) and high dose (24 vs 2). The authors reported malformations which included,
26 amongst others, cleft palate, cleft lip, anophthalmia, meningoencephalocele,
27 gastroschisis, exencephaly, anomalies of great vessels and malformations of ribs,
28 arches and centra.³⁹ The committee points out that a dose-response relationship was
29 only found for skeletal malformations. Anomalies of the great vessels, hydroureter and
30 hydronephrosis that are generally considered variations were reported incorrectly as
31 malformations.

32 A non-guideline three generation reproduction study was performed by DePass et al. in
33 1986.³⁴ Fisher 344 Rats (n=10 males and 20 females/group) were treated with 0.04,
34 0.2, and 1.0 g/kg/day EG (purity >99.9%) in diet. Rats were exposed during a 7-week
35 pre-mating period and throughout breeding. Offspring (n=10 males and 20
36 females/group randomly chosen from the litters) were exposed throughout life and
37 breeding was started at 100 days of age. No treatment related effects on mortality, diet
38 or body weight changes were observed. One second generation female and one

1 second generation male in the high dose group showed mild focal interstitial nephritis
2 but this condition was also observed in 2 control group pups. One third generation
3 female in the high dose group showed mild focal tubular hyperplasia but this condition
4 was also observed in 2 control male pups. Neonatal body weights at day 4, 14, or 21
5 postpartum were not affected after treatment with EG.³⁴ The committee considers the
6 available data too limited to allow a conclusion for developmental related effects.

7 A non-guideline developmental study was performed by Price et al. (1988) (GLP
8 compliant; performed by the NTP).⁴⁰ Female CD rats (N=4-5/group/replicate, multiple
9 replicates to generate at least 20 F1 litters per dose group) were treated with EG at 0,
10 250, 1250 and 2250 mg/kg bw/day via oral gavage during GD6-20. Dams were
11 euthanized on PND 1. Pups were fostered by untreated dams as of post-natal day 1.
12 Litters were culled to 8 pups per litter. A reduced maternal body weight, reduced
13 gestational weight gain, increased kidney weight and reduced uterine weight were
14 observed in the high dose group. Statistically significantly longer gestational periods
15 were observed in the 1250 (21.58±0.07 vs 21.26±0.07 in control) and 2250 mg/kg
16 bw/day (21.84±0.09 vs 21.26±0.07) exposure groups. A reduced live litter size and
17 increased mortality per litter was observed in the high dose group (11.87±0.48 vs
18 13.67±0.33 in control). Offspring showed reduced body weight on PND 1 and 22 in the
19 high dose groups. A reduced kidney and brain weight was observed on PND 22 and 63
20 in the high dose groups. Exposure to 2250 mg/kg bw/day also resulted in a reduced
21 time to achieve wire grasping (9.71±0.18 days vs 11.98±0.78 days in control).
22 Hydrocephalus (n=6 of 88 pups examined at PND 22) was observed in the high dose.
23 EG exposure did not result in external or visceral defects but did show an increase in
24 skeletal malformations in the high dose group, such as malformed ribs, centra and
25 sternebrae. In this group, 30% of the male and 60% of the female offspring showed
26 malformations, compared to 0% in control groups.⁴⁰ The committee notes that
27 selectively downsizing the litters could have caused bias. It is unclear how the findings
28 on hydrocephalus relate to the historical control data. The reduced kidney and brain
29 weight in the offspring were reported as absolute values, whereas these are likely
30 secondary to the effects seen on the lower body weight of the pups. Overall, clear
31 developmental effects were present, even at late postnatal age.

32 A non-guideline developmental study was performed by Marr et al. (1992) and was a
33 follow-up to the studies by Price et al. (1985, 1988).⁴¹ Pregnant Sprague-Dawley rats
34 (n=7 per group) were treated with 2500 mg/kg/day EG via oral gavage from GD 6-15. A
35 vehicle control group was included. Dams were euthanized on GD 18, 20 or PND 1, 4,
36 14, 21 or 63. The focus of the study was on skeletal malformations and ossification and
37 whether these effects could be outgrown over time. Maternal gestational weight gain

1 was statistically significantly 3-6% lower compared to control at GD 11, 15, 18 and 20.
2 EG did not affect the duration of gestation. Foetal body weight was approximately 25%
3 lower than controls at GD 18 and 20 and pup weight was 10% lower than controls on
4 PND 1. Skeletal malformations occurred in 100% of the exposed litters at GD 18
5 ($76.3 \pm 11.3\%$ vs 0% in control), 20 ($88.4 \pm 3.4\%$ vs $1.1 \pm 1.1\%$) and PND1 ($95.2 \pm 4.8\%$ vs
6 0%), 4 ($83.4 \pm 6.1\%$ vs $1.0 \pm 0.5\%$), and 21 ($87.5 \pm 7.2\%$ vs 0%). Also, the percentage of
7 malformed pups or fetuses per litter was 75% or higher in exposed groups versus less
8 than 1% per litter in control groups, except for PND 63 where only 28% of exposed
9 pups was malformed. Agenesis of the ribs and short ribs were common malformations
10 (24-41%, and 6-34%, respectively). According to the authors, skeletal remodelling in
11 the postnatal period may explain the lower percentage in malformations on PND 63.
12 The percentage of total ossification was statistically significantly lowered at all time
13 points (GD20: $24.85 \pm 1.46\%$ vs $37.42 \pm 1.19\%$, PND1: $66.49 \pm 1.33\%$ vs $78.64 \pm 0.45\%$,
14 PND 4: $84.32 \pm 0.95\%$ vs $92.58 \pm 0.98\%$, PND 14: $94.08 \pm 0.54\%$ vs $99.75 \pm 0.13\%$,
15 PND21: 95.22 ± 0.50 vs $100 \pm 0.0\%$) except PND 63. This could partially be attributed to
16 lower ossification of sternbrae and centra.⁴¹ The committee notes that only 1 dose
17 was tested in this study. The calculation of statistically significant ossification data
18 changes (lower percentage) when the pup body weight is taken into account as a
19 covariate in the analysis. Nonetheless, partial recovery of skeletal malformations and
20 delayed ossification later in life was observed.

21 A non-guideline developmental study in rats and mice was performed by Neep-
22 Bradley et al. (1995).⁴² The study in mice will be described later in this document. Per
23 group, 25 rats were treated once daily with EG from GD5 through GD15 at dose levels
24 of 0, 150, 500, 1000 and 2500 mg/kg bw/day via oral gavage. Dams were euthanized
25 on GD21. Maternal toxicity was observed at 2500 mg/kg bw/day. Body weight gain was
26 significantly lower at GD6-9 and GD15-18 and the final body weight was also lower
27 compared to controls. During treatment, water consumption was significantly
28 increased. Gravid uterine weight was decreased compared to controls and absolute
29 and relative kidney weights as well as relative liver weights were increased at 2500
30 mg/kg bw/day. In addition, the relative liver weight was increased at 1000 mg/kg
31 bw/day, but whether this effect is adverse remains uncertain in absence of any
32 histopathology. In foetuses, most malformations occurred at a dose of 2500 mg/kg
33 bw/day. A number of external and soft tissue malformations were observed such as
34 gastroschisis (7/21 vs 0/24 in control litters), hydrocephaly (10/21 vs 0/24), severe
35 lateral ventricle dilation (6/21 vs 0/24), umbilical hernia (5/21 vs 0/24) and foetal
36 atelectasis (19/21 vs 12/24). Moreover, increased incidences of 31 skeletal
37 malformations were observed primarily in the thoracic region, for example missing ribs
38 (20/21 vs 0/24) or thoracic arches (17/21 vs 0/24). At 500 mg/kg bw/day an increased

1 incidence in poorly ossified supraoccipital was observed (8/22 vs 2/24). At 1000 mg/kg
2 bw/day, there was one foetus with gastroschisis (non-significant effect), and increased
3 incidences of skeletal malformations were observed, including extra 14 thoracic
4 centrum and arches (8/23 vs 1/24), missing rib (8/23 vs 0/24), and missing thoracic
5 arches (6/23 vs 0/24).⁴² The committee concludes that maternal toxicity is evident in
6 the high dose but is considered unrelated to the observed developmental effects. The
7 committee considers the maternal NOAEL to be 1000 mg/kg bw/day. The foetal
8 NOAEL would be 500 mg/kg bw/day, a dose where some developmental toxicity was
9 observed. Overall, the committee considers this study as robust.

10 Carney et al., 1999 performed a non-guideline, GLP compliant developmental toxicity
11 study to investigate the toxicokinetics of EG.²⁰ Female Sprague-Dawley rats (25/group)
12 were exposed to a single dose of 2,500 mg/kg/day EG or distilled water (control) via
13 gavage on gestation day 10 and necropsy took place on GD 21. In treated dams, an
14 increase in kidney weight and liver weight was observed, while a decrease in maternal
15 body weight was observed on GD 12, 16, and 21. No effects were observed on
16 pregnancy rate, litters with viable foetuses, corpora lutea/dam, implantations/dam, %
17 preimplantation loss/dam, foetuses/litter, and foetal sex ratio (M:F). Only a significant
18 difference was observed in the percentage resorbed implantations (15.9 at 2,500
19 mg/kg vs 2.5 at 0 mg/kg). Significant increased external malformations were found at
20 2,500 mg/kg/day, including meningoencephalocele (3.5% foetuses affected (25.0%
21 litters affected)), exencephaly (5.2 (25.0)), cleft lip (3.5 (29.2)), cleft palate (2.8 (29.2)),
22 and omphalocele (9.8 (54.2)), as well as visceral malformations, including dilated
23 cerebral ventricles (15.9 (33.3)). Also skeletal malformations, including hemivertebrae
24 (60.0 (95.8)), extra vertebrae (5.9 (29.2)), missing vertebrae (24.4 (62.5)), fused
25 vertebrae (36.3 (75.0)), fused centra (8.9 (33.3)), fused ribs (62.4 (95.8)), extra ribs (4.5
26 (20.8)), missing ribs (60.2 (91.7)), skeletal variations, and a decrease in foetal
27 bodyweight (3.75±0.58 g vs 0 5.53±0.30 g in control) were found at 2500 mg/kg/day.²⁰
28 The committee notes that only one dose was tested and was administered at a single
29 timepoint, so no dose-response relationship could be assessed.

31 *Mice studies*

32 Schuler et al. (1984) performed a non-guideline developmental study with female CD-1
33 mice.⁴³ First, a dose range-finding study was performed to determine the lethal dose.
34 For the developmental study, pregnant dams (n=50) were treated with 11090 mg/kg
35 (LD10) EG via oral gavage from GD7 to GD14. A vehicle control group was included
36 (n=50). Pups were sacrificed 3 days after birth. Mortality in treated pregnant dams
37 (5/50) was higher than in controls (0/50), but not statistically significant. The number of
38 viable litters was decreased in treated dams compared to the controls (15/37 vs 29/29

1 in control) and the number of dead pups per litter was increased (1.5 vs 0.1). The
2 number of live pups per litter (2 vs 9), the pup postnatal survival (40% vs 100%), pup
3 weight gain (0.2 g vs 7 g) and pup birth weight (1.4 vs 1.7) were all statistically
4 significant decreased compared to controls.⁴³ It is unclear to the committee when or
5 why maternal animals died. Although no statistics were performed on mortality of the
6 dams, the committee assessed this as high, since the observed mortality is reaching
7 the limit of common practice (10%). The number of viable litters was low, also in the
8 control group. Nonetheless, the dose group showed lower number of litters, and a
9 lower survival compared to controls. Since only one dose was tested, no dose-
10 response relationship could be assessed.

11 A non-guideline GLP-compliant developmental toxicity study was performed by Price et
12 al. (1985, NTP study) in rats (described earlier) and mice.³⁹ Female CD-1 mice were
13 treated with EG at 0, 750, 1500 or 3,000 mg/kg/day via oral gavage from GD6 through
14 GD15. C-section was performed on GD17. Two replicates of the study were performed
15 making the total number of animals per group 20. Dams of the mid and high dose
16 group showed a significantly decreased weight gain during treatment, a decreased liver
17 weight and decreased gravid uterine weights. There was an increase in post-
18 implantation loss per litter reaching statistical significance at the high dose level. In
19 addition, a dose-related decrease in live litter size was observed in the high dose group
20 (9.83 ± 0.56 vs 11.88 ± 0.49 in control), and a dose-related decrease in average foetal
21 body weight per litter was observed in all dose groups (low (0.882 ± 0.017 g), mid
22 (0.787 ± 0.024 g) and high dose (0.712 ± 0.022 g) compared to control (0.974 ± 0.013 g)).
23 There was a dose-related increase in the percentage of malformed foetuses per litter
24 (low dose ($10.0 \pm 1.96\%$), mid dose ($37.77 \pm 6.30\%$) and high dose ($56.54 \pm 6.80\%$)
25 compared to control ($0.25 \pm 0.25\%$)). Furthermore, the percentage of litters with at least
26 one malformed foetus was increased in all dose groups (low (66.67%), mid (81.82%)
27 and high dose (96.65%) compared to control (4.0%)). External malformations, including
28 exencephaly, meningoencephalocele, cleft palate, cleft lip, and facial cleft were
29 significantly increased in the high dose group compared to the control (8 litters vs 0 in
30 control), as well as visceral malformations, including aortic stenosis, hydronephrosis,
31 hydroureter, retrotracheal or retrooesophageal pulmonary artery (7 vs 0). Skeletal
32 malformations, including malformed ribs, malformed arches, and malformed centra
33 were significantly increased at all dose levels (low dose (15 litters), mid dose (17), and
34 high dose (22) vs control (1)).³⁹ According to the committee, this study in mice is more
35 convincing than the rat study by the same authors. The reported malformations were
36 not always malformations but in some cases variations (see also summary on rats).
37 Nonetheless, clear developmental effects of EG were observed in terms of skeletal
38 malformations at dose levels where no maternal toxicity occurred. Since the reduction

1 in the number of implantation sites per litter was not dose-related, it was considered
2 unlikely to be related to EG treatment.

3 Neeper-Bradley et al. (1995) performed a non-guideline developmental study on
4 female CD-1 mice (n=30/group).⁴² They were treated once daily with EG from GD5
5 through GD15 at dose levels of 0, 50, 150, 500, and 1500 mg/kg bw/day via oral
6 gavage. Dams were euthanized on GD18. Although not statistically significant, some
7 deaths occurred in treatment groups 50, 500 and 1500 mg/kg bw day. There was a
8 slightly lower maternal weight gain and body weights at 1500 mg/kg bw/day (not
9 statistically significant). Foetal body weights per litter were reduced at 1500 mg/kg
10 bw/day (1.156±0.11 g vs 1.325±0.09 g in controls). Exencephaly was noted in 2 litters
11 at 500 mg/kg bw/day and 3 litters at 1500 mg/kg bw/day, however this was not
12 statistically significant. Some skeletal variations were observed at 500 mg/kg bw/day
13 (Extra rib 14, first lumbar arch, bilateral (17/24 vs 4/19 control litters)). At 1500 mg/kg
14 bw/day, skeletal malformations occurred, including fused thoracic arches (8/21 vs
15 0/19), fused ribs (15/21 vs 1/19), extra thoracic centrum and arches (10/21 vs 0/19),
16 and extra bilateral rib and thoracic arches (10/21 vs 0/19).⁴² The committee concluded
17 that maternal toxicity at the high dose was considered minimal. Developmental effects
18 were found at the high dose and included decreased foetal weight and skeletal
19 malformations.

20 A non-guideline fertility assessment by continuous breeding was performed by Lamb et
21 al. in 1985, who also reported some developmental effects (see also Chapter 6.2).³²
22 This study was a dose-range finding study for the study of Gulati et al. 1986. CD-1
23 mice (20/sex/group) were dosed at 0.25, 0.5 and 1% EG in drinking water (equals to
24 410, 840 and 1640 mg/kg bw/day). A control group of 40 mice per sex was present.
25 Exposure occurred during a premating period, cohabitation and breeding, segregation
26 and throughout the life of the offspring. No general toxicity occurred in the parental
27 animals, but some deaths occurred in all groups. Regarding developmental effects, a
28 lower live pup weight and lower number of live pups per litter were observed in the high
29 dose compared to controls. Also, the number of live pups per litter and live pup weights
30 were lower (not significant). A pattern of skeletal defects was observed in the offspring
31 in treated mice only, although this was not quantified. Skull, sternbrae, ribs and
32 vertebrae were affected in both males and females.³² The committee points out that
33 since this is a fertility study, the data on development are limited. However, the effects
34 on number of live pups and pup weight together with the reported skeletal defects
35 provide an indication that developmental toxicity becomes apparent at the high dose
36 where minimal parental toxicity was observed.

1 As a follow-up to the study of Lamb et al. (1985), a non-guideline continuous breeding
2 study was performed by Gulati et al. in 1986 (also described in Chapter 6.2).³³ CD-1
3 mice were dosed at 0.5, 1.0 or 1.5% EG in drinking water, vehicle controls were
4 included (equals to 0, 897, 1798 and 2826 mg/kg bw/day). The animals were exposed
5 during pre-mating, breeding, segregation, and throughout the life of the offspring. The
6 pup numbers per litter were lower at 1.5% as compared to controls ($p < 0.05$). Both
7 absolute and adjusted pup weights (male, female, and combined) were also adversely
8 affected in a dose-related pattern at all 3 dose levels ($p < 0.05$). A significant number of
9 pups delivered by breeding pairs in the 1.0 and 1.5% EG dose groups showed distinct
10 facial deformities, including in some cases cleft palate and ablepharon. Weanlings of
11 the last litter of the continuous breeding phase were mated on PND 74 ± 10 . A
12 significant number of these weanlings showed facial abnormalities during the early
13 post-partum phase. There were no effects on body weight, kidney and liver weights in
14 the males and females of the parental generation. In males no effects were noted on
15 seminal vesicle, right cauda and prostate gland, but the relative weight of the right
16 testis and right epididymis were significantly lower in the 1.0 and 1.5% groups. EG
17 treatment had no significant effect with respect to the number of live pups per litter
18 (male, female, and/or combined), proportion of pups born alive, and the sex ratio. The
19 adjusted live pup weight (males and females combined) was minimally reduced
20 ($p < 0.05$) at all 3 dose levels.³³ The committee notes that the study is primarily intended
21 to assess fertility rather than development. In the continuous breeding phase, there
22 were some indications for developmental effects (facial abnormalities), but the effects
23 on development (i.e. pup weight) in the second generation were considered minimal.

24 A non-guideline 21-day developmental toxicity study was performed by Harris et al. in
25 1992.³⁵ CD-1 mice (10 per group) were exposed to 0, 250, 700, and 2500 mg/kg/day
26 EG in drinking water. Females were treated between GD 8 and 14. No adverse clinical
27 signs were observed. The 2500 mg/kg/day EG exposure group showed a significant
28 decrease in mean litter weight.³⁵ The committee considers this screening study as
29 limited. A decrease in mean litter weight was observed only in the high-dose group.
30 However, it is not clear how many litters were included. Compared to the control, this
31 may or may not be considered an adverse effect.

32

33 *Rabbit studies*

34 Tyl et al. (1993) studied New Zealand White rabbits (N=23-24/dose) administered with
35 EG by gavage on GD 6-19 at doses of 0, 100, 500, 1000, and 2000 mg/kg bw/day.⁴⁴
36 After exposure to 2000 mg/kg bw/day, 42.1% mortality, three early deliveries and one
37 spontaneous abortion were observed. Maternal absolute kidney weight (but not relative

1 weight) was slightly increased at 2000 mg/kg bw/day (to 106.3% of the control value of
2 the right kidney and 107.6% of the control of the left kidney). This was accompanied by
3 renal lesions, which were limited to the cortical renal tubules and included intraluminal
4 crystals, epithelial necrosis, and tubular dilation and degeneration. The death of the
5 dams at 2000 mg/kg bw/day was directly related to the acute renal failure from crystal
6 deposition, consistent with third-stage toxicity, including the presence of oxalate
7 crystals. No maternal toxicity occurred at ≤ 1000 mg/kg bw/day. There was no indication
8 of developmental toxicity at any dose tested, including no effects on pre- or post-
9 implantation loss, number of foetuses, foetal body weight, or sex ratio (% male
10 foetuses) per litter, and no evidence of teratogenicity.⁴⁴ The committee assesses this
11 study as negative for developmental toxicity. Exposure to a dose of 2000 mg/kg bw/day
12 showed significant maternal toxicity in the kidney, without effects on the developing
13 offspring. At the lower dose of 1000 mg/kg bw/day no abnormalities were observed.

14 **7.2.2 Inhalation studies**

15 A non-guideline developmental inhalation study was performed by Tyl et al. (1995a).⁴⁵
16 Female CD rats and CD-1 mice were exposed to EG for 6 hours per day from GD6
17 through GD15. EG was nebulized, and animals were exposed to 0, 150, 1000, 2500
18 mg/m³ EG aerosols (2.4 μ m) in inhalation chambers. Depending on the lung retention
19 and the level of grooming of the contaminated fur, the authors estimated a theoretical
20 dose of 947 mg/kg/day corresponding to an exposure of 2500 mg/m³. No mortality was
21 observed within this study. No effects on food or water consumption, body weight or
22 weight gain were observed in rats. The highest exposure group in rats showed an
23 increase in liver weight. No effects on gestational parameters or malformations were
24 observed in rats. CD-1 mice showed a significant reduction in maternal body weight
25 and uterus weight in the 1000 and 2500 mg/m³ exposure groups. Moreover, mice
26 showed a reduction in viable implants in the 2500 mg/m³ exposure group. Also in mice,
27 a statistically significant increase was observed in the incidences of a number of
28 external, visceral and skeletal malformations in the 1000 and 2500 mg/m³ exposure
29 groups, such as cleft palate, exencephaly, misshapen nasopharynx, and protruding
30 tongue. Wet fur due to deposition of EG was observed in all exposure groups. Based
31 on the amount of EG in extract from fur, oral exposure was calculated to be the major
32 route of exposure to the extent of 64-90%.⁴⁵ The committee notes that both rats and
33 mice received the same exposure levels that may not have been in proportion to the
34 species (see also Chapter 7.3). No effects were observed in rats although potentially
35 maternal toxicity effects in the liver were noted in the high exposure group. In mice,
36 clear maternal toxicity effects and developmental effects were observed in the mid and

1 high exposure groups. This difference may be explained by the wet fur and subsequent
2 oral ingestion of EG in mice.

3 A non-guideline developmental inhalation study was performed by Tyl et al. in 1995b.⁴⁶
4 Female CD-1 mice were exposed to EG for 6 hours per day from GD6 through GD15.
5 EG was nebulized, and animals were exposed to 0, 150, 1000, 2500 mg/m³ EG
6 aerosols (1.7 µm) through nose-only or to 0 or 2100 mg/m³ EG aerosols through whole
7 body exposure. Mortality was observed in controls and the 2500 mg/m³ EG nose-only
8 exposure group, found to be due to asphyxiation related to the exposure method and
9 not EG related. Nose-only EG exposure did not result in alterations in maternal body
10 weight, weight gain or liver weight. Increased kidney weight was observed in the 1000
11 and 2500 mg/m³ exposure groups. A non-significant reduction in live foetuses per litter
12 was observed in the 1000 and 2500 mg/m³ exposure groups and a significant
13 reduction in foetal body weight was observed in the 2500 mg/m³ exposure group. No
14 treatment-related increases in external or visceral malformations were observed. A
15 significant increase in the incidence of fused ribs was observed in the 2500 mg/m³
16 exposure group.⁴⁶ The committee concludes that skeletal malformations developed in
17 both whole body and nose-only exposure routes.

18 **7.2.3 Dermal studies**

19 A non-guideline developmental dermal study was performed by Tyl et al. in 1995c.⁴⁷
20 Female CD-1 mice were exposed daily to EG from GD 6 through 15. EG was dissolved
21 in water and applied in concentrations of 0%, 12.5%, 50% or 100% on the skin for 6
22 hours per day during which the skin was occluded and the mice were restrained. The
23 concentrations correspond to 0, 404, 1677 and 3549 mg/kg bw/day. Oral EG (3000
24 mg/kg bw/day) was used as a positive control. Mice were euthanised on GD 18. There
25 were no deaths in the dermal exposure groups and all clinical signs during treatment
26 were associated with the restrain conditions. Gestational weight change increased
27 significantly in high dose mice. Minimal-grade renal tubular lesions were observed in
28 3/30 mice in the high dose group. The number of dams with fully resorbed litters was
29 higher in all treatment groups compared to controls. There was a significantly reduced
30 number of late resorptions per litter at 12.5% as well as a significantly reduced number
31 of dead foetuses per litter. There were no increased incidences in malformations, but 2
32 variations occurred in the high dose group, namely a poorly ossified parietal skull bone
33 and the majority of the intermediate phalanges of the hindlimb were unossified.
34 Incidences of both variations were statistically significantly increased. Multiple signs of
35 general and reproductive toxicity occurred in the positive control group, such as
36 maternal death, lower foetal body weights per litter and various soft tissue and skeletal

1 malformations.⁴⁷ The committee concludes that dermal exposure did not cause
2 detrimental effects that could lead to classification, since only variations were observed
3 and no malformations.

4 **7.3 Evaluation of the data**

5 In general, EG dose levels in the animal studies described above were high. According
6 to the OECD guidelines, a limit dose of 1000 mg/kg bw/day should be chosen for
7 practical purposes in the set-up of the experimental design in case a compound shows
8 no maternal toxicity at lower dose levels. However, the committee considers
9 developmental effects at high doses relevant for the evaluation, provided the effects
10 were found in the absence of maternal toxicity.
11

12 *Rodent studies*

13 Developmental studies in rats and mice via oral gavage were published by Price et al.
14 (1985).³⁹ Foetuses of rats exposed to EG doses exposed to 1250, 2500 and 5000
15 mg/kg bw/day were noted with dose-related increased skeletal malformations which
16 were accompanied by effects on maternal and foetal body weights. Post-implantation
17 loss was observed at 5000 mg/kg/day and there was a lower number of live foetuses
18 per litter at doses where maternal toxicity also occurred. The studies in mice exposed
19 to doses up to 3000 mg/kg bw/day showed even more convincing results than the data
20 observed in rats. Skeletal malformations caused by EG were observed at dose levels
21 where no maternal toxicity occurred. EG exposure also resulted in increased post-
22 implantation loss, a lower live litter size and lower average foetal body weights.³⁹

23 Price et al. also conducted a developmental study (1988) in rats with doses up to 2250
24 mg/kg bw/day via oral gavage.⁴⁰ A slightly longer exposure duration was applied, as
25 well as a long follow-up period for the offspring (up to PND 63) as compared to the rat
26 study described above. EG caused an increase in skeletal malformations when rats
27 were exposed to 2250 mg/kg bw/day. Malformations were found in 30% of the male
28 and 60% of the female offspring at 2250 mg/kg bw/day, compared to 0% in controls.
29 Clear developmental effects were present, even at late postnatal age.⁴⁰

30 Developmental toxicity studies in rats and mice via oral gavage were conducted
31 according to EPA guidelines published by Neeper-Bradley et al. (1995).⁴² Rats were
32 dosed up to 2500 mg/kg bw/day and developmental effects were observed that were
33 considered unrelated to maternal toxicity. External, soft tissue and skeletal
34 malformations were observed. Overall, the committee considers this study as robust. In

1 the mice study, doses up to 1500 mg/kg bw/day were administered. Maternal toxicity at
2 1500 mg/kg bw/day was minimal. At this dose, developmental effects were found, such
3 as decreased foetal weights and skeletal malformations.⁴²

4 Two studies in rats and two studies in mice also showed developmental effects, but
5 these effects were less severe.^{32, 33, 38, 41} The studies had a different set-up in terms of
6 follow-up period or administration route. In general, the reported skeletal effects
7 concerned variations that may be an indication of foetal/embryonal toxicity. These
8 studies do not contradict the findings observed by Price et al. (1985, 1988) and
9 Neeper-Bradley et al. (1995).^{39, 40, 42}

10 In a study with EG administered through diet at doses up to 1000 mg/kg bw/day in rats,
11 minimal embryotoxicity was observed.³⁸ The increased incidences in ossification state
12 of the vertebral centra at not maternally toxic doses are variations and not
13 malformation, and could be a sign of delayed maturation.

14 As a follow-up of the studies by Price et al., Marr et al. 1992 studied whether or not
15 skeletal variations can recover over time by administrating rats one dose of 2500 mg
16 EG/kg bw/day on GD10.⁴¹ The observed delayed ossifications were partly recovered
17 later in life. Even though delayed ossification is reversible, this does not mean that a
18 compound should not be classified as a hazard.

19 In 2 fertility studies in mice with EG in drinking water, some developmental endpoints
20 were assessed as well, although the data were limited. In the first study, EG was dosed
21 up to 1640 mg/kg bw/day and effects were observed on number of live pups and pup
22 weights together with skeletal malformations (craniofacial defects).³² In the follow-up
23 study EG was dosed up to 2826 mg/kg bw/day and minimal embryotoxicity was
24 observed as well.³³ In the continuous breeding phase of this study, there were some
25 indications of developmental effects (facial abnormalities) in the first generation, but the
26 effects on development in the second generation were minimal. The question remains
27 whether there were very few malformations or whether only a limited number of pups
28 was included in the examination. The committee concludes there may be an effect on
29 development in this study, but it is minimal.

30 Other mice studies had methodological limitations and were of less importance to the
31 committee's conclusion. EG was administered by the oral route (drinking water or
32 gavage). A study with only one dose group of 1400 mg/kg bw/day, described mortality
33 of the dams.⁴³ The number of litters was low, also in the control group. Nonetheless, at
34 1400 mg/kg bw/day, a lower number of litters was noted and a lower survival compared
35 to controls. A limited screening study with doses up to 2500 mg/kg bw/day showed

1 minimal effects on litter weight and number of live pups, but no malformations were
2 noted.³⁵ However, it was not clear how many litters were included in the evaluations.

3 Studies on inhalation or dermal exposure showed less convincing developmental
4 effects than studies on oral exposure, and had methodological limitations. Inhalation
5 studies used analytical concentrations of 0, 119, 888, and 2090 mg/m³ (2090 mg/m³
6 equals to 606 mg/kg bw/day in rats, and 1074 mg/kg bw/day in mice. In rats, a
7 respiratory volume of 0.8 l/min/kg and an exposure duration of 6 hours a day resulted
8 in a conversion factor of 0.29 m³/kg/day. In mice, a respiratory volume of 1.43 l/min/kg
9 and an exposure duration of 6 hours per day resulted in a conversion factor of 0.514
10 m³/kg/day).⁴⁵ The studies showed no developmental effects in rats at dose levels
11 where no maternally toxic effects were observed, while in mice clear maternally toxic
12 effects and developmental effects were observed in the 888 and 2090 mg/m³ exposure
13 groups.⁴⁵ This difference between rats and mice may be attributed to the much lower
14 exposure expressed as mg/kg bw/day in rats as compared to mice. Additionally, the
15 inhalation studies (whole body) also involved oral exposure because of ingestion of EG
16 deposition during grooming of wet fur.

17 A publication studying developmental effects in mice with nose only exposure (3 doses
18 up to 2505 mg/m³) resulted in lower foetal body weights and increased skeletal
19 malformations.⁴⁶ A dermal study in mice with doses up to 3549 mg/kg bw/day resulted
20 in increased variations (poorly ossified parietal skull bone; unossified hindlimb
21 phalanges) but no malformations were observed.⁴⁷

22 *Rabbit studies*

23 In contrast to the various studies in rats and mice that showed developmental toxicity, a
24 study in rabbits did not show any sign of developmental toxicity related to EG
25 administration up to maternally toxic dose levels.⁴⁴

26

27 *Species differences*

28 The underlying causes for the interspecies discordance are important to be able to
29 extrapolate in vivo developmental toxicity data to humans and conclude on potential
30 human relevance of developmental effects observed in rodents. The differences can
31 partly be explained by differences in distribution and accumulation of GA in maternal
32 versus embryonic compartments. An abstract summarizing animal studies that
33 compared pharmacokinetics and metabolism of EG in pregnant rats and rabbits
34 showed that levels of GA in maternal blood and extraembryonic fluids (EEF) were 3 to
35 36-fold lower in rabbits than in rats.²¹ In rats, concentrations of GA were higher in EEF

1 than in maternal blood.²¹ A study by Carney et al. that compared the administration of a
 2 teratogenic dose of EG to pregnant rats on GD 10 or rabbits on GD 9 showed similar
 3 maximum EG levels in maternal blood in rats and rabbits after a dose of 1000 mg/kg
 4 body weight (bw)/day (see Table 3 for specific concentrations). However, the maximum
 5 GA levels in embryos differed by a factor 10 between both species.^{16, 22} The maximum
 6 concentration of GA in the rat embryo was 150% of that in the maternal blood, while in
 7 the rabbit embryo it was only 30% of the maximum maternal blood concentration.^{16, 22}
 8 Maximal levels of GA in rabbit maternal blood were only 46% of the respective levels in
 9 rats.^{16, 22}

10
 11 Table 3: Overview of maximal concentrations of EG or GA in rats or rabbits as was
 12 studied by Carney et al. 2008 and summarised in the REACH registration dossier.^{16, 23}

Species	EG dose	EG maternal blood	GA maternal blood	GA embryo	GA embryo/GA maternal blood
rats	1000 mg/kg/bw/day	886 µg/g	314-363 µg/g	478.6 µg/g	~150%
rabbits	1000 mg/kg/bw/day	890.5 µg/g	145.6 µg/g	45.7 µg/g	~30%
GA rabbits/GA rats		~100%	~46%	~10%	

13
 14 The toxicokinetic profile suggests that the lower GA levels in rabbits were due to a
 15 slower rate of maternal metabolism of EG to GA and a slow uptake of GA via the rabbit
 16 yolk sac.^{16, 22} In conclusion, these data show that embryonic exposure to GA in the
 17 rabbit is lower after systemic maternal exposure to EG due to a difference in maternal
 18 biotransformation from EG to GA and a difference in embryonic penetration of GA. This
 19 could explain the absence of developmental toxicity in rabbit in vivo studies as
 20 compared to rats.

21
 22 However, other research has shown contradictory information regarding penetration of
 23 the rabbit embryo. In Carney's study, GA levels in rabbit embryos remained below 50
 24 µg/g and were approximately 30% of maximum maternal blood levels (see Table 3),¹²
 25 while in a publication by Moore et al. that studied EG (metabolite) maternal-foetal
 26 distribution in rats and rabbits, a peak in rabbit embryonic GA values was seen that
 27 was comparable to the maximum maternal GA blood levels, and embryonic levels were

1 higher than maternal blood levels during the elimination phase.^{16, 24} Differences in
2 embryonic penetration between rat and rabbit embryos exist. However, the data by
3 Moore et al. 2024 do not exclude the possibility of GA accumulation above maternal
4 blood levels occurring in the rabbit embryo as well.

5
6 Other possible explanations for the differences in distribution between species reported
7 in the literature are differences in visceral yolk sac biology and in the position of the
8 carrier mediated transporters, located in the placenta, that are responsible for GA
9 crossing the placental barrier.^{16, 22, 25} The rat yolk sac completely surrounds the embryo,
10 is incorporated into the developing placenta and is the major source of maternal-foetal
11 exchange from GD7-7.5 to ~GD12.²⁶ The rabbit yolk sac is not closely apposed to the
12 uterus during early organogenesis and does not completely enclose the embryo until
13 relatively later in development (~GD13).²⁶ During early development, the human yolk
14 sac is connected to the midgut and later, in the first trimester, remains connected via
15 the vitelline duct.²⁶ In short, the inverted yolk sac placenta exists in rodents but not in
16 rabbits or humans which may have implications for GA distribution to the embryo in
17 different species. This difference in yolk sac orientation between rabbits and rats may
18 explain the differences in GA distribution between species. The early rabbit yolk sac
19 might be a relatively inefficient transporter because of its orientation and this was,
20 according to the authors, supported by (unpublished) limited data for EG and GA given
21 to GD9 rabbits.²⁶

22
23 In rat and rabbit WECs, the disposition of GA in the embryos and visceral yolk sacs
24 was studied.²⁵ The disposition of GA was inhibited by the presence of a high
25 concentration of a d-lactic acid competitor in the rat embryos, and incubation of rat
26 embryos under acidic conditions increased the disposition of GA into the embryo
27 compared to alkaline conditions.²⁵ Because of the abilities of a competitor to inhibit GA
28 disposition, the authors suggest this disposition to be carrier mediated via
29 monocarboxylic acid transporters (MCT) which differ in anatomical position across
30 species.²⁵ The transporters in rat and rabbit placentae (MCT1/4) are located in
31 opposite polarity across the trophoblast: MCT1 were positioned at the maternal side of
32 the rat and mouse trophoblast, whereas MCT4 were located at the foetal side.^{27, 28} In
33 rabbits, MCT1 was predominantly localised to the foetal side and MCT4 to the maternal
34 side of the trophoblast.²⁷ In humans, MCT1 is predominantly located at the basal (foetal
35 facing) plasma membrane and MCT4 at the microvillous (maternal facing) membrane.²⁹

36
37 A study by Moore investigated the distribution of GA between maternal blood and the
38 conceptus at the beginning and end of placentation in both rats and rabbits (rat at
39 GD11 and GD16, rabbit at GD10 and GD19). This study showed for the rat GA

1 accumulation (GD11) in EEF and embryo after 6 hours (C_{max} ca 1.5-fold maternal
2 C_{max} at t=3). In the rabbit (GD10), however, after 9 hours the GA concentration in the
3 embryo was higher than C_{max} in the maternal blood, which was reached at t=6 hours
4 (C_{max} embryo ca 1.3 times higher). This may indicate that accumulation of GA also
5 occurs in the rabbit, but passage into the embryo seems to take more time. No
6 accumulation was seen in either species after dosing on GD 16 or 19. This suggests
7 that GA partitions from maternal blood into the rat conceptus only during early
8 pregnancy, before placentation is complete, which correlates with the MCT1
9 expression in the maternally facing epithelium of the syncytiotrophoblast during early
10 development.²⁴

11 **7.4 Conclusion**

12 There are no reported cases of EG-induced developmental effects in humans.

13 Developmental toxicity is observed in rats and mice but not in rabbits up to dose levels
14 causing maternal toxicity. The developmental effects reported in rodents were mainly
15 related to foetal body weights and skeletal malformations, in addition to post-
16 implantation loss and lower number of live foetuses per litter.^{32, 33, 38-42} Additional
17 studies in mice in which EG was administered by the oral route (gavage or drinking
18 water) had methodological limitations and showed less clear developmental effects.^{35,}
19 ⁴³ Studies with inhalation or dermal exposure routes in rats and mice showed no or less
20 convincing developmental effects.⁴⁵⁻⁴⁷ This could be related to relatively low doses or
21 limited uptake. Overall, the observed malformations are serious and irreversible.
22 Moreover, the effects are considered not to be a secondary non-specific consequence
23 of other toxic effects, and thus directly related to EG treatment.

24 Based on the information on the mechanism of toxicity, the committee did not find
25 conclusive evidence to exclude the rodent studies from the evaluation. In rat embryos,
26 GA accumulation occurs whereas limited accumulation is seen in rabbit embryos.
27 There is suggestive information on similarities between humans and rabbits related to
28 yolk sac physiology and MCT orientation. However, this is not sufficiently convincing for
29 the committee to consider the rodent model irrelevant for potential human
30 developmental effects. Moreover, there is a lack of information on the mechanism of
31 action, which further hampers the selection of the rabbit model as the more appropriate
32 model for human effects of EG/GA.

33 Except for one study, all studies were dosed above the limit dose of 1000 mg/kg/day.
34 Despite these high dose levels, limited maternal toxicity was seen at the highest dose

1 levels. The committee is of the opinion that specific developmental effects, also at
2 doses above the limit dose as set by the OECD guidelines, should not be ignored
3 based on limit dose considerations especially in absence of maternal toxicity.

4 Based on the above considerations, there is clear evidence for an adverse effect on
5 development. The committee concludes that classification in category 1B is warranted.

6

PUBLIC DRAFT

8 Adverse effects on or via lactation

Because of limited data, the committee proposes not to classify EG for effects on or via lactation.

8.1 Animal data

The number of studies available evaluating the influence of EG on or via lactation is limited.

A three-generation study in rats exposed to 40, 200, and 1000 mg/kg bw/day EG in diet reported mean pup weights at lactation days 4, 14 and 21 for the F1, F2 and F3 generations.³⁴ This study is also described in sections 6.2 (fertility) and 7.2 (development). No adverse effects on the pups during or related to lactation were observed. EG treatment did not affect neonatal body weight at days 4, 14, or 21 postpartum. There were no treatment-related histopathologic findings in weanlings.

Pup weaning was also included in a continuous breeding study in mice with doses equal to 0, 410, 840 and 1640 mg EG/kg bw/day.³² This study is also described in sections 6.2 (fertility) and 7.2 (development). The publication shared no information about the pups during lactation. However, the follow-up study that exposed mice with doses equal to 0, 897, 1798 and 2826 mg EG/kg bw/day (also described in sections 6.2 and 7.2) reported no effect on the average pup weight at weaning (i.e. post-lactation) as compared to controls.³³

8.2 Conclusion

Because of the limited data for effects of EG on or via lactation, the committee proposes not to classify due to a lack of data.

1 **9 Conclusions on classification and labelling**

2 The committee recommends classification according to Regulation (EC) 1272/2008 of
3 the European Union.

4 **Proposed classification for fertility**

5 No classification for reproductive toxicity due to inconsistent and insufficient data.

6 **Proposed classification for developmental toxicity**

7 Category 1B, H360D

8 **Proposed labelling for effects on or via lactation**

9 No classification for effects on or via lactation due to a lack of data.

PUBLIC DRAFT

References

Literatuur

- 1 National Toxicology Program N, National Institute of Environmental Health Sciences.,
2 *Report on Carcinogens: Listed Substances*. Research Triangle Park, NC: U.S.
3 Department of Health and Human Services, 2021.
4 https://ntp.niehs.nih.gov/ntp/roc/content/listed_substances_508.pdf.
- 5
6
7
8 2 National Toxicology P. *Toxicology and Carcinogenesis Studies of Ethylene Glycol*
9 *(CASRN 107-21-1) in B6C3F1 Mice (Feed Studies)*. Research Triangle Park, NC: U.S.
10 Department of Health and Human Services, Public Health Service, 1993; NIH
11 Publication No. 93-3154. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr413.pdf.
- 12 3 Ausschuss für Gefahrstoffe. *TRGS 905: Verzeichnis krebserzeugender,*
13 *keimzellmutagener oder reproduktionstoxischer Stoffe*. Dortmund: Bundesanstalt für
14 Arbeitsschutz und Arbeitsmedizin (BAuA), 2021.
15 [https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-](https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf)
16 [Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf](https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf).
- 17 4 Office of Environmental Health Hazard Assessment. *Proposition 65: Chemicals Known*
18 *to the State to Cause Cancer or Reproductive Toxicity*. Sacramento, CA: California
19 Environmental Protection Agency, 2019.
20 <https://oehha.ca.gov/media/downloads/proposition-65/p65list091319.pdf>.
- 21 5 Control CfD and Prevention. *Occupational Cancer*. National Institute for Occupational
22 Safety and Health (NIOSH) 2023.
- 23 6 Safe Work Australia. *Hazardous Chemical Information System (HCIS): Chemical*
24 *Details*. Safe Work Australia 2023.
- 25 7 Nite Chemical Management Center. *GHS Classification Results: IMCG-0094*. National
26 Institute of Technology and Evaluation (NITE) 2023.
- 27 8 Kothandaraman J and Heldebrant DJ. There are no conflicts to declare. *Catalytic*
28 *coproduction of methanol and glycol in one pot from epoxide, CO(2), and H(2)*. RSC
29 Adv 2020; 10(69): 42557–63.
- 30 9 Engineering C. *Ethylene Glycol Production*. 2015.
31 <https://www.chemengonline.com/ethylene-glycol-production/?printmode=1>
- 32 10 ATSDR. *TOXICOLOGICAL PROFILE FOR ETHYLENE GLYCOL*. U.S. DEPARTMENT
33 OF HEALTH AND HUMAN SERVICES; Public Health Service; Agency for Toxic
34 Substances and Disease Registry, 2010.

- 1 11 Australian government; Department of Climate Change E, the Environment and Water.
2 *Fact sheet Ethylene glycol (1,2-ethanediol)*. 2022.
3 [https://www.dcceew.gov.au/environment/protection/npi/substances/fact-](https://www.dcceew.gov.au/environment/protection/npi/substances/fact-sheets/ethylene-glycol-12-ethanediol)
4 [sheets/ethylene-glycol-12-ethanediol](https://www.dcceew.gov.au/environment/protection/npi/substances/fact-sheets/ethylene-glycol-12-ethanediol)
- 5 12 Yue H, Zhao Y, Ma X and Gong J. *Ethylene glycol: properties, synthesis, and*
6 *applications*. Chemical Society Reviews 2012; 41(11): 4218–44.
- 7 13 ECHA. *Substance information ethylene glycol*. 2022.
8 <https://echa.europa.eu/fr/substance-information/-/substanceinfo/100.003.159>
- 9 14 Public Health England. *Ethylene glycol toxicological overview*. 2015.
10 [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachmen-](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/455702/Ethylene_Glycol_PHE_TO_210815.pdf)
11 [t_data/file/455702/Ethylene_Glycol_PHE_TO_210815.pdf](https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/455702/Ethylene_Glycol_PHE_TO_210815.pdf).
- 12 15 Saghir SA, Bartels MJ and Snellings WM. *Dermal penetration of ethylene glycol through*
13 *human skin in vitro*. International Journal of Toxicology 2010; 29(3): 268–76.
- 14 16 Carney EW, Tornesi B, Markham DA, Rasoulpour RJ and Moore N. *Species-specificity*
15 *of ethylene glycol-induced developmental toxicity: toxicokinetic and whole embryo*
16 *culture studies in the rabbit*. Birth Defects Res B Dev Reprod Toxicol 2008; 83(6): 573–
17 81.
- 18 17 NTP-CERHR. *Monograph on the Potential Human Reproductive and Developmental*
19 *Effects of Ethylene Glycol*. 2004; 1556-2271.
- 20 18 Slikker Jr W, Andersen ME, Bogdanffy MS, Bus JS, Cohen SD, Conolly RB, et al. *Dose-*
21 *dependent transitions in mechanisms of toxicity*. Toxicology and Applied Pharmacology
22 2004; 201(3): 203–25.
- 23 19 Khera KS. *Chemically induced alterations in maternal homeostasis and histology of*
24 *conceptus: their etiologic significance in rat fetal anomalies*. Teratology 1991; 44(3):
25 259–97.
- 26 20 Carney EW, Freshour NL, Dittenber DA and Dryzga MD. *Ethylene glycol developmental*
27 *toxicity: unraveling the roles of glycolic acid and metabolic acidosis*. Toxicol Sci 1999;
28 50(1): 117–26.
- 29 21 Carney E, Pottenger L, Bartels M, Jackh R and Quast J. *Comparative pharmacokinetics*
30 *and metabolism of ethylene glycol in pregnant rats and rabbits*. Toxicology Letters
31 1998; 95(1001): 208–.
- 32 22 Carney EW, Tornesi B, Liberacki AB, Markham DA, Weitz KK, Luders TM, et al. *The*
33 *impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in*
34 *pregnant CD rats*. Toxicol Sci 2011; 119(1): 178–88.
- 35 23 ECHA. *REACH registration dossier of ethylene glycol*
36 <https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/15973>

- 1 24 Moore NP, Bogaards JJP, Buscher BAP, Wolterbeek APM and Cnubben NHP.
2 *Disposition of glycolic acid into the embryo following oral administration of ethylene*
3 *glycol during placentation in the rat and rabbit*. Toxicol Sci 2024; 198(1): 31–9.
- 4 25 Ellis-Hutchings RG, Moore NP, Marshall VA, Rasoulpour RJ and Carney EW.
5 *Disposition of glycolic acid into rat and rabbit embryos in vitro*. Reprod Toxicol 2014; 46:
6 46–55.
- 7 26 Carney EW, Scialli AR, Watson RE and DeSesso JM. *Mechanisms regulating toxicant*
8 *disposition to the embryo during early pregnancy: an interspecies comparison*. Birth
9 Defects Research Part C: Embryo Today: Reviews 2004; 72(4): 345–60.
- 10 27 Moore NP, Picut CA and Charlap JH. *Localisation of lactate transporters in rat and*
11 *rabbit placentae*. International journal of cell biology 2016; 2016(1): 2084252.
- 12 28 Nagai A, Takebe K, Nio-Kobayashi J, Takahashi-Iwanaga H and Iwanaga T. *Cellular*
13 *expression of the monocarboxylate transporter (MCT) family in the placenta of mice*.
14 Placenta 2010; 31(2): 126–33.
- 15 29 Settle P, Mynett K, Speake P, Champion E, Doughty I, Sibley C, et al. *Polarized lactate*
16 *transporter activity and expression in the syncytiotrophoblast of the term human*
17 *placenta*. Placenta 2004; 25(6): 496–504.
- 18 30 Corley RA, Bartels MJ, Carney EW, Weitz KK, Soelberg JJ, Gies RA, et al.
19 *Development of a physiologically based pharmacokinetic model for ethylene glycol and*
20 *its metabolite, glycolic Acid, in rats and humans*. Toxicol Sci 2005; 85(1): 476–90.
- 21 31 Corley RA and McMartin KE. *Incorporation of Therapeutic Interventions in*
22 *Physiologically Based Pharmacokinetic Modeling of Human Clinical Case Reports of*
23 *Accidental or Intentional Overdosing with Ethylene Glycol*. Toxicological Sciences 2005;
24 85(1): 491–501.
- 25 32 Lamb JC, Maronpot R, Gulati DK, Russell VS, Hommel-Barnes L and Sabharwal P.
26 *Reproductive and developmental toxicity of ethylene glycol in the mouse*. Toxicology
27 and Applied Pharmacology 1985; 81(1): 100–12.
- 28 33 Gulati D and Lamb IV J. *Ethylene glycol: reproduction and fertility assessment in CD-1*
29 *mice when administered in drinking water*. National Toxicology Program Report NTP-
30 84-FACB-034 1986.
- 31 34 DePass LR, Woodside MD, Maronpot RR and Weil CS. *Three-generation reproduction*
32 *and dominant lethal mutagenesis studies of ethylene glycol in the rat*. Fundamental and
33 Applied Toxicology 1986; 7(4): 566–72.
- 34 35 Harris MW, Chapin RE, Lockhart AC and Jokinen MP. *Assessment of a short-term*
35 *reproductive and developmental toxicity screen*. Fundam Appl Toxicol 1992; 19(2):
36 186–96.

- 1 36 Hong HL, Canipe J, Jameson CW and Boorman GA. *Comparative effects of ethylene glycol and ethylene glycol monomethyl ether exposure on hematopoiesis and histopathology in B6C3F1 mice*. J Environ Pathol Toxicol Oncol 1988; 8(7 Spec No): 27–38.
2
3
4
- 5 37 Bolon B, Bucci TJ, Warbritton AR, Chen JJ, Mattison DR and Heindel JJ. *Differential follicle counts as a screen for chemically induced ovarian toxicity in mice: results from continuous breeding bioassays*. Fundam Appl Toxicol 1997; 39(1): 1–10.
6
7
- 8 38 Maronpot RR, Zelenak JP, Weaver EV and Smith NJ. *Teratogenicity study of ethylene glycol in rats*. Drug Chem Toxicol 1983; 6(6): 579–94.
9
- 10 39 Price CJ, Kimmel CA, Tyl RW and Marr MC. *The developmental toxicity of ethylene glycol in rats and mice*. Toxicol Appl Pharmacol 1985; 81(1): 113–27.
11
- 12 40 Price CJ, George, J.D., Marr, M.C., Kimmel, C.A., Schwetz, B.A., Morrissey, R.E.,
13 *Developmental toxicity evaluation of ethylene glycol (CAS No. 107-21-1) in CD Rats*.
14 National Toxicology Program (NTP), National Institute of Environmental Health
15 Sciences, 1988; NTP-88-079.
- 16 41 Marr MC, Price CJ, Myers CB and Morrissey RE. *Developmental stages of the CD (Sprague-Dawley) rat skeleton after maternal exposure to ethylene glycol*. Teratology 1992; 46(2): 169–81.
17
18
- 19 42 Neeper-Bradley TL, Tyl RW, Fisher LC, Kubena MF, Vrbanic MA and Losco PE.
20 *Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice*. Fundam Appl Toxicol 1995; 27(1): 121–30.
21
22
- 23 43 Schuler RL, Hardin BD, Niemeier RW, Booth G, Hazelden K, Piccirillo V, et al. *Results of testing fifteen glycol ethers in a short-term in vivo reproductive toxicity assay*. Environ Health Perspect 1984; 57: 141–6.
24
25
- 26 44 Tyl RW, Price CJ, Marr MC, Myers CB, Seely JC, Heindel JJ, et al. *Developmental toxicity evaluation of ethylene glycol by gavage in New Zealand white rabbits*. Fundam Appl Toxicol 1993; 20(4): 402–12.
27
28
- 29 45 Tyl RW, Ballantyne B, Fisher LC, Fait DL, Savine TA, Dodd DE, et al. *Evaluation of the developmental toxicity of ethylene glycol aerosol in the CD rat and CD-1 mouse by whole-body exposure*. Fundam Appl Toxicol 1995; 24(1): 57–75.
30
31
- 32 46 Tyl RW, Ballantyne B, Fisher LC, Fait DL, Dodd DE, Klonne DR, et al. *Evaluation of the developmental toxicity of ethylene glycol aerosol in CD-1 mice by nose-only exposure*. Fundam Appl Toxicol 1995; 27(1): 49–62.
33
34
- 35 47 Tyl RW, Fisher LC, Kubena MF, Vrbanic MA and Losco PE. *Assessment of the developmental toxicity of ethylene glycol applied cutaneously to CD-1 mice*. Fundam Appl Toxicol 1995; 27(2): 155–66.
36
37

- 1 48 Tyl RW, Price CJ, Marr MC, Myers CB, Seely JC, Heindel JJ, et al. *Developmental*
2 *toxicity evaluation of ethylene glycol by gavage in New Zealand white rabbits.*
3 Toxicological Sciences 1993; 20(4): 402–12.
- 4 49 Tyl RW, Ballantyne B, Fisher LC, Fait DL, Dodd DE, Klonne DR, et al. *Evaluation of the*
5 *developmental toxicity of ethylene glycol aerosol in CD-1 mice by nose-only exposure.*
6 Toxicological Sciences 1995; 27(1): 49–62.
7

PUBLIC DRAFT

1 **Committee**

2 **The members of the Subcommittee on the Classification of Substances Toxic to** 3 **Reproduction**

4 Drs. W.M.L.G. Gubbels-Van Hal, former director IGCON BV, former consultant
5 registration and toxicology, Oss, *chair*

6 Dr. M.M.H.J. van Gelder, assistant professor in Pharmaco Epidemiology,
7 Radboudumc, Nijmegen

8 Prof. dr. L.J.M. Smits, Professor of Clinical Epidemiology and Risk-Based Care,
9 Maastricht University

10 Dr. V.C. Lenters, Lecturer, Amsterdam Institute for Life and Environment (A-LIFE), Vrije
11 Universiteit Amsterdam (*from January 1st, 2024*)

12 Prof. J. Legler, Professor of Toxicology, Institute for Risk Assessment Sciences (IRAS),
13 Utrecht University (*from January 1st, 2024*)

14 Dr. M.W.G.D.M. van de Loo, Study Director Developmental and Reproductive
15 Toxicology, Charles River Laboratories, Den Bosch

16 Dr. E.C.M. Tonk, regulatory toxicologist, Charles River Laboratories, Den Bosch
17

18 **Consulted expert**

19 Dr. R. Greupink, assistant professor in FarmTox, Radboudumc, Nijmegen
20

21 **Observer**

22 Dr. ir. L. Geraets, RIVM, Bilthoven
23

24 **Scientific secretaries**

25 Dr. R.H. Mennen, Health Council of the Netherlands, Den Haag

26 L. Souhoka, Health Council of the Netherlands, Den Haag

1
2
3
4
5
6

A Supplementary tables

Fertility studies

7
8
9

Table 1 Oral studies on adverse effects of ethylene glycol on sexual function and fertility

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
Lamb et al., 1985	Mice, COBS CrI:CD1, (ICR)BR outbred albino N=20/sex/group Controls: 40/sex	Reproductive study (non-guideline, non-GLP). Fertility assessment by continuous breeding (NTP protocol) with a preparatory dose range finding study.	Purity: 99.6% <u>Concentrations:</u> 0, 0.25, 0.5 and 1.0% (w/v), oral via drinking water. According to the authors, this is equal to 410, 840	<u>P0 Parents</u> No general toxicity in parental animals, i.e. clinical signs, effects on body weight or water consumption. Some deaths	<u>P0 parents:</u> - Decreased number of litters per fertile pair at high dose (4.5±0.2) versus control (4.9±0.08), P<0.01. - Decreased mean number of live pups per litter high dose	High dose levels: above 1000 mg/kg bw/day. The authors measured drinking water consumption per pair of mice for 2 weeks. This

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p><u>Design:</u></p> <ul style="list-style-type: none"> - Premating period of 7 days - 98 days cohabitation and breeding (male+female), all pups born during this period were killed - 21 days segregation period, pups born during this period were kept alive - Exposure occurred during premating, cohabitation, segregation and throughout the life of offspring. - High dose and control pups (N=20/sex/group) born during segregation were mated. <p><u>Examinations:</u></p>	<p>and 1640 mg/kg bw/day, based on daily water consumption and mean body weight data.</p> <p><u>F1 parents:</u> 0, 1.0% (w/v), oral via drinking water. This is equal to 1640 mg/kg bw/day, based on daily water consumption and mean body weight data.</p> <p>Dosage solutions were within 98 – 107% of intended concentrations.</p>	<p>occurred in all groups. One death in the 0.5% group could be treatment related according to the authors (oxalate crystals in renal tubules).</p> <p><u>F1 parents</u> No general toxicity observed.</p>	<p>(10.2±0.3) versus control (10.8±0.5), P<0.05.</p> <p><u>F1 parents:</u></p> <ul style="list-style-type: none"> - Fertility was lower in high dose animals (61%) compared to controls (81%), not significant. - Number of live pups per litter and live pup weights were lower in high dose animals (not significant). 	<p>could be used to calculate actual dose levels. Furthermore, they reported a decreased number of litters per fertile pair of parental animals (F0) in the highest dose group.</p> <p>However, no general toxicity was found at these dose levels.</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>- Fertility and reproductive performance of the adults and F1 generation. Viability, sex and weight of the pups.</p> <p>- Organ weights of F1 offspring and skeletal examination of a proportion of F1 mice.</p> <p>- Histology of the head of F1 mice.</p> <p><u>Statistics:</u></p> <p>- Results presented as Mean \pmSE.</p> <p>- Tests used differ per parameter. Mostly Chi-Square approximation to Kruskal Wallis is used for group comparisons and Mann-Whitney U or Fisher exact test for pairwise</p>				

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		comparisons. An ANOVA was carried out for assessment of body weight.				
Gulati et al., 1986	Mice, Crl:CD-1 (ICR)BR outbred albino N=20/sex/group Controls: 40/sex	Reproductive study (non-guideline, GLP-compliant). Fertility assessment by continuous breeding Design: <u>Task 2</u> - Premating period of 7 days - 98 days cohabitation and breeding (1 male+ 1 female), all pups born during this period were killed <u>Task 3</u> - Weaned animals of the last litter of task 2 from the high dose group (N=20/sex/group) and	Purity: 99.6% Concentrations: 0, 0.5, 1, 1.5% (weight/volume) 0, 897, 1798 and 2826 mg/kg bw/day, oral in drinking water Dosage concentrations were within 94 and 104% of intended concentration at all time points (task 2 four times, task 3 once and task 4 three times)	<u>Task 2</u> Parental animals: - No treatment-related effects on body weight, water consumption significantly increased in males a 1.5% <u>Task 3</u> Parental animals: - No treatment-related effects on body, liver or kidney weight observed in female mice. - Significant reduction in body (42.29±0.10g in 1.5% group vs 46.45±1.04g in	<u>Task 2</u> - Non-significant reduction in fertility index (14/16 (88%) in 1.5% ethylene glycol group vs 38/38 (100%) in control group). - Reduced live pup count (11.8/litter in controls, 9.99/litter at 1.5%) (p<0.05) - Reduced pup weight (absolute and adjusted for litter size (<10%, p<0.05) <u>Task 3</u> -No significant effect on mating and fertility index	Follow-up from the study by Lamb et al. (1985), but higher dose tested. Parental NOAEL 1.0% Fertility NOAEL 0.5% based on effects on sperm in task 4.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>untreated males or females were mated.</p> <p>-unexposed cohabitation for max 1 week</p> <p>- Females were allowed to litter, all pups born were killed.</p> <p><u>Task 4</u></p> <p>- Weaned pups from the last litters of task 2 (F1)</p> <p>-Exposure for 74 days, thereafter during mating and until weaning of the offspring (F2 generation)</p> <p><u>Examinations:</u></p> <p>- Fertility (mating index + fertility index), viability and weight of pups/litters.</p> <p>- body weight and organ weight</p>		<p>control group) and liver weight (1.93±0.05g in 1.5% group vs 2.11±0.07g in control group) observed in male mice.</p> <p>- kidney degeneration, dilation, and regeneration of tubules and deposition of crystals (oxalate) in the tubules</p> <p><u>Task 4</u> Parental parents (F1):</p> <p>- No treatment-related effects on body weight and kidney weight (abs + rel), significant lower liver weight (abs, not rel) in females</p>	<p>-Significant effect (<10%) on adjusted live pup weight in 1.5% group males/control females</p> <p>-Increased % abnormal sperm at 1.5%- (8.28±2.02 vs 5.06±0.53 in control groups, P<0.05).</p> <p>-Reduced sperm mobility (80.6±8.05 vs 94.3±0.51 in control group, P<0.05)</p> <p>-Degeneration of seminiferous tubules (85% 1.5% males and 57% of control), loss of spermatozoa, spermatids, spermatogonia, and spermatocytes, and vacuolization of epithelial cells.</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>-sperm parameters -external abnormalities in pups</p> <p><u>Statistics:</u> Data is displayed as mean \pmSE. Cochran-Armitage or Jonckheere test for trends. Statistics differ per parameter, most used are Chi-Square, Kruskal-Wallis, Wilcoxon rank-sum and Fisher's exact test.</p>		<p>- No treatment-related effects on body weight, sign decreased liver weight (1.93\pm0.05g in 1.5% group vs 2.11\pm0.07g in control group) in males</p>	<p><u>Task 4</u> -No effects on mating and fertility index - Significant effect (<<10%) on adjusted live pup weight in all dose groups 0.5% - Reduced sperm density (801\pm61 compared to 1036\pm63 in control group, P<0.05) 1.0% - Reduced sperm density (855\pm63) compared to 1036\pm63 in control group, P<0.05) - Reduced sperm motility (92.1\pm1.47 vs 94.6\pm0.89 in control groups, P<0.05). 1.5%</p>	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
DePass et al., 1986	Rats, Fisher 344 N= 10 males/ group and 20 females/ group	Three-generation reproduction study (non-guideline, non-GLP). <u>Design:</u> - Premating period of 7 days - During breeding, period not mentioned. - Offspring was exposed throughout life and started	Purity: 99.93% <u>Concentrations:</u> Approximately 0.04, 0.2 and 1.0 g/kg bw/day Oral, diet 2 untreated control groups.	No effects on mortality, diet consumption or body weight observed.	- Reduced testes weight (0.120±0.006 g vs 0.140±0.005 g in control group - Reduced sperm motility (84.1±5.02 vs 94.6±0.89 in control groups, P<0.05). - Degeneration of seminiferous tubules in 60% of males at 1.5% vs 40% in controls No treatment related effects on fertility index, gestation index, gestation survival index or days from first mating to litter observed.	Concentrations in diet vary, but dose-levels stay constant.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		breeding on 100 days of age. <u>Examinations:</u> Fertility, behaviour and histopathology, kidney lesions. <u>Statistics:</u> continuous data using Barlett's test. T-test for equal and unequal variance. Frequency data using chi-square and Fisher's exact tests.				
Carney et al., 1999	Rat, CD, Sprague-Dawley, females. N=25/group time-mated rats	Developmental study (non-guideline, GLP compliant) in which also fertility parameters were examined. Design: Treatment from GD 6 to 15.	Purity EG: 99.98% Concentration: 2500 mg/kg, by oral gavage Solution within 99-107% of target concentration	Decreased maternal body weights on GD 12, 16, 21. Decreased body weight gain intervals (except the 0-6 pre-dosing period).	No effect observed on pregnancy rate, litters with viable foetuses, corpora lutea/dam, implantations/dam, % preimplantation loss/dam, foetuses/litter, and foetal sex ratio (M:F).	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>Necropsy on GD 21.</p> <p>Examinations: maternal body weight, organ weights (liver, kidney, gravid uterus), corpora lutea, implantations, pre-implantation loss, resorptions, litter size, sex ratio</p> <p>Statistics: Bartlett's test, ANOVA, Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction, Fisher exact test</p>	Control: deionized water via gavage	<p>Increased absolute and relative kidney weights.</p> <p>Increased liver weight, with absolute liver weight being significantly different than control.</p> <p>One dam exhibited vaginal bleeding on GD 19 and 20 and was found to have a completely resorbed litter on GD 21.</p> <p>Mean feed-consumption values were decreased by 6-13%.</p>	<p>% Resorbed implantations: 0 mg/kg: 2.5 2500mg/kg: 15.9* P=0.05</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
Harris et al., 1992	Mice, Crl:CD-1 N=10/sex/group	Short-Term Reproductive and Developmental Toxicity Screening study (non-guideline, non-GLP). Design 1: - Treatment of pregnant female mice during GD8-14 Design 2: - Treatment of males (SD3-20) and females (SD0-20) - Cohabitation on study day 8 - Animals are sacrificed on study day 21. Examinations: - Liver, kidney and testes histology in male mice.	Purity not mentioned. Oral gavage Concentrations: 0, 250, 700, and 2500 mg/kg/day, formulation were within 93 to 100% of target. This is equivalent to 0, 50, 140 and 500 mg/kg bw/day conform the CLP guidance. Positive control: Ethylene glycol monomethyl ether, 0, 70, 250, and 700 mg/kg/day in drinking water. This is equivalent to 0,14, 50, and 140 mg/kg bw/day	No adverse clinical signs observed. No mortality observed.	Males: no effects on testes weight, sperm mobility or number observed. Females (study design 1): - No effects on numbers of live or dead implants observed. Females (study design 2), EG in mg/kg/day: no. pregnant: 0: 9/10 250: 9/10 700: 7/10 2500: 9/10 no. live implants/female: 0: 10.0 ± 0.9 250: 10.7 ± 0.4 700: 11.1 ± 0.5 2500: 7.4 ± 1.2*	Although no significant effect was observed on testes weight, sperm mobility or number, potential fertility effects cannot be excluded based on the data.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>- Fertility (impregnation, no. live implants per female, no. dead implants/female, total implants/female) and litter weight.</p> <p>Statistics: Cochran-Armitage test for linear trend followed by Fisher's exact test. Kruskal-Wallis analysis of variance for dose group comparison and Jonckheere's test for dose-responses. Mann-Whitney U test for pairwise comparisons.</p>	<p>conform the CLP guidance [19].</p> <p>Dosage solutions were between 93-100% of target concentrations.</p>		<p>P<0.05</p> <p>no. dead implants/female: 0: 0.4 ± 0.2 250: 0.8 ± 0.1 700: 0.3 ± 0.3 2500: 1.4 ± 0.4*</p> <p>P<0.05</p> <p>total implants/female: 0: 10.4 ± 1.0 250: 11.4 ± 0.3 700: 11.4 ± 0.4 2500: 8.9 ± 1.1</p> <p>- No effects on fertility observed.</p> <p>Positive control: - Reduction in testes weight, sperm mobility and number (P<0.05) in 250 and 700 mg/kg/day exposure groups.</p>	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
					- Study design 1: reduced number of live implants (P<0.01) in 250 and 700 mg/kg/day groups. - Study design 2: reduced impregnation (P<0.05) in 700 mg/kg/day group.	
Hong et al., 1988	Mice, B6C3F1, males and females N=7/sex/group	4-day mouse study (non-guideline, non-GLP). Histological examinations on day 1 post exposure: lung, heart, liver, kidneys, adrenal glands, spleen, thymus, stomach, bone marrow, urinary bladder, small and large intestine, uterus (female) or testes (male). Statistics:	Purity: 99.6% Concentrations: 0, 50, 100 or 250 mg/kg bw/day by oral gavage. Positive control: Ethylene glycol monomethyl ether, 0, 50, 100 or 250 mg/kg bw/day by oral gavage.	No mortality observed. Significant reduction in body weight for female mice (P<0.01) observed in 250 mg/kg bw/day group (21.4±0.3 g vs 23.4±0.2 g in control groups). No histological evidence of organ or tissue damage observed.	No effect on testicular weight observed in male mice. Positive control: Significant (P<0.01) decreases in testicular weight in 250 mg/kg bw/day group (3.3±0.1 g vs 4.1±0.2 g in control group)	Short study duration of 4 days. Positive control has a specific working mechanism and is therefore not directly comparable to ethylene glycol.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		Wilk-Shapiro test for normality, one-way analysis of variance and Dunnett's test for multiple comparison with control group.				
Bolon et al., 1997	Mice, Crl:CD-1 (ICR)BR outbred albino mice (CD1) N=20/sex/group Controls: 40/sex	Retrospective study (non-guideline, GLP compliant), ovaries obtained from 18 Reproductive Assessment by Continuous Breeding (RACB) bioassays in NTP archive. Design: - Premating period of 7 days - 98 days cohabitation and breeding (male+female), all pups born during this period were killed - 21 days segregation period, pups born	Concentrations: Parental: 0%, 1.5% (w/v), oral via drinking water. This is equivalent to 0 and 3000 mg/kg bw/day conform the CLP guidance [19]. Offspring: 0%, 0.5%, 1%, 1.5% (weight/volume), oral via drinking water. This is equivalent to 0, 1000, 2000 and 3000 mg/kg bw/day conform the CLP guidance.	Not mentioned.	Parental animals: Non-significant increase in ovarian follicle count in 1.5% group. Offspring: 0.5%, 1% and 1.5% ethylene glycol exposures resulted in non-significant reductions in number of small, growing and antral ovarian follicles observed versus control. Positive control: - Parental: Non-significant reduction in ovarian follicle counts.	Ovary samples used from Gulati et al., 1986. Poor study design.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on sexual function and fertility	Remarks
		<p>during this period were kept alive</p> <ul style="list-style-type: none"> - Exposure occurred during pre mating, cohabitation, segregation and throughout the life of offspring. - High dose and control pups (N=20/sex/group) born during segregation were mated. <p>Examinations: Ovarian follicle counts (N=10)</p> <p>Statistics: Group comparisons using Kruskal-Wallis non-parametric ANOVA and dose response trends using Mann-Witney U test.</p>	<p>Purity not mentioned.</p> <p>Positive control: ethylene glycol monomethyl ether, 0, 0.03, 0.1, 0.2 and 0.3% (w/v) oral via drinking water.</p>		<ul style="list-style-type: none"> - Offspring: significant reduction ($P < 0.05$) in small, growing and antral ovarian follicle counts compared to controls. 	

Version date: Monday 16 March 2026

1
2

PUBLIC DRAFT

Version date: Monday 16 March 2026

1 **Development studies**

2

3 *Table 2 Oral studies on effects of ethylene glycol on development*

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Maronpot et al., 1983	Rat, Fischer 344, females. N=20/group	Prenatal development study (non-guideline, non-GLP). Design: Treatment of pregnant dams from GD 6 to 15. C-section on GD 21. Examinations: visceral (nasal cavity, eyes, brain) and skeletal alterations and malformations. Statistics:	Purity: >99.9% Concentrations: 0, 40, 200 and 1000 mg/kg bw/day (targeted dose) in diet. Negative controls: regular diet. Positive control: hydroxyurea 500 mg/kg (ip) on GD11.	No effect on body weight gain (corrected for litter weight) and no clinical signs.	No effect on foetal length, foetal weight, total implantations or litter size. 1000 mg/kg bw/day: Preimplantation loss was higher (median: 23.0% IQR: 9.0-42.0%) compared to control (median: 10.0% IQR: 2.0-29.2%) but not statistically significant. There was no increased incidence of major malformation in litters of ethylene glycol treated females. 1000 mg/kg bw/day:	Minimal embryotoxicity due to delayed foetal maturation (increased incidences of poorly ossified and unossified vertebral centra) Bodyweight only measured on day 6, 11, and 21, which was not synchronized with the treatment. Only targeted dose was reported.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Continuous data: F-test (with paired group F-max test or t-test) - Non-parametric data: multiple sum of ranks test. - Binominal data: Fischer exact test 			<ul style="list-style-type: none"> - Increased incidence of poorly ossified (14.2% versus 1.8%, P<0.001) and unossified vertebral centra (26.0% versus 11.4%, P<0.001) compared to control. Major malformations did occur in the positive controls. 	<p>Treatment only started after implantations and no further resorptions occurred.</p> <p>Implantation loss is possibly non-related to the treatment.</p>
Price et al., 1985	Rat, CD, females N=>10/group/replicate (so total of 20/group)	<p>Developmental study (NTP study, GLP compliant, blinded).</p> <p>Design: Treatment of pregnant rats from GD6 through 15. 2 replicates of the teratology evaluation were conducted.</p>	<p>Purity: >99% Vehicle: water</p> <p>Concentrations: 0, 1250 (low), 2500 (mid) or 5000 (high) mg/kg bw/day, via oral gavage</p> <p>Negative control: vehicle</p>	<p>Maternal toxicity: No maternal deaths Piloerection in all treated groups but not in controls.</p> <p>- Dose-related lower maternal body weight parameters and</p>	<p>Developmental toxicity:</p> <ul style="list-style-type: none"> - Increased post-implantation loss (resorptions + dead fetuses) per litter at the high dose (21.34± 5.24%) compared to control (4.70± 1.23%), P<0.05. - Higher percentage litter with post implantation loss at one or more sites 	<p>The doses selected were based on a preliminary toxicity study performed using timed-pregnant rats and mice (Price et al., 1984a, b). the reduction in live litter size at the mid dose may</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>Caesarean section was performed on GD 20.</p> <p>Examinations: maternal body weight on GD 0, 3, 6-15, 18 and 20. maternal liver and kidney and gravid uterine weights, uterine contents (number of implantation sites, resorptions, dead foetuses, and live foetuses) and visceral, skeletal and morphological abnormalities.</p> <p>Statistics: - Data are presented as mean ± SEM</p>	<p>Actual dose levels were within 10% of the calculated levels.</p>	<p>gravid uterine weight compared to control.</p> <p>- Decreased weight gain during treatment (all P<0.01):</p> <p>Control: 42.03±1.96g</p> <p>1250 mg/kg: 34.81±1.73g</p> <p>2500 mg/kg: 29.45±1.38g</p> <p>5000 mg/kg: 20.68±1.93g</p> <p>- Decreased weight on GD20 (mid and high dose, P<0.01):</p> <p>Control: 366.06±3.94g</p> <p>2500 mg/kg: 345.69±5.86g</p>	<p>at the high dose (74.1 vs 39.3 in control)</p> <p>- Decreased number of live foetuses per litter at mid dose (11.90±0.60) and high dose (11.04±0.79), vs 13.54±0.28 in control, P<0.05.</p> <p>- Decreased average foetal body weight per litter at mid dose (2.916±0.056 g) and high dose (2.388±0.089 g) compared to control (3.404±0.052 g).</p> <p>- Increase in percentage live foetuses malformed per litter at mid dose (25.11±4.84%) and high dose (75.53±6.42%) compared to control (1.37±0.97%).</p> <p>- Increase in percentage litters with one or more malformed live foetuses</p>	<p>have been related to the lower number of implantation sites per litter at that dose relative to controls.</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Dose response: test for linear trend - ANOVA with post hoc tests - test for linear trend on proportions and chi-square test (nominal data). One tailed- fisher exact test for pairwise comparison. 		<p>5000 mg/kg: 324.99±6.64g</p> <p>- Decreased weight gain during gestation (mid and high dose, P<0.01):</p> <p>Control: 129.50±3.06g</p> <p>2500 mg/kg: 108.26±4.06g</p> <p>5000 mg/kg: 90.17±6.35g</p> <p>- Decreased gravid uterine weight (mid and high dose, P<0.01):</p> <p>Control: 73.04±1.63g</p> <p>2500 mg/kg: 58.00±2.97g</p>	<p>at low dose (39.29%, P<0.01), mid dose (68.97%, P<0.001) and high dose (96.15%, P<0.001).</p> <p>Morphologic defects: Dose response relationship observed for all types of malformations.</p> <p>-External malformations in 15 litters at high dose compared to 0 control litters (P<0.001), amongst others cleft palate, cleft lip, anophthalmia, meningoencephalocele, gastroschisis, exencephaly.</p> <p>-Visceral malformations in 6 litters at low dose (P<0.05), 3 litters at the mid dose (n.s.) 8 at high dose (P<0.01) compared</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
				5000 mg/kg: 46.61±3.75g - Decreased absolute (but not relative) liver weight (high dose, P<0.05): Control: 15.47±0.26g 5000 mg/kg: 13.70±0.35g - Relative (but not absolute) kidney weight increased (mid and high dose, P<0.05): Control: 0.517±0.012g 2500 mg/kg: 0.573±0.008g 5000 mg/kg: 0.615±0.021g	to 0 control litters. Increase mainly due to anomalies of great vessels. - Skeletal malformations in 19 litters at mid dose and 24 litters at high dose versus 2 control litters (P<0.001), including malformed ribs (short, missing, branched and/or fused), malformed arches (enlarged, small, fused and/or missing) and malformed centra (misaligned, unilateral ossification, off centre, fused and/or missing) of malformed sternbrae (fused or scrambled).	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
				- Increased water consumption during and after treatment (mid and high dose, P<0.05).		
DePass et al., 1986	Fisher 344 Rats N= 10 males/ group and 20 females/ group	Three-generation reproduction study (non-guideline, non-GLP). Design: - During 7-week pre-mating period - During breeding, time period not mentioned. - Offspring was exposed throughout life and started breeding on 100 days of age. Examinations:	Purity: 99.93% Concentrations: Approximately 0.04, 0.2 and 1.0 g/kg bw/day in diet as calculated by the authors. 2 untreated control groups.	No effects on mortality, diet consumption or body weight observed.	No effects on observed neonatal body weight at day 4, 14, or 21 postpartum.	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>neonatal body weight, histopathology, kidney lesions.</p> <p>Statistics: continuous data using Barlett's test. T-test for equal and unequal variance. Frequency data using chi-square and Fisher's exact tests.</p>				
Price et al., 1988	<p>COBS CD (SD)BR outbred albino rats</p> <p>N= 4-5 females per dose group/replicate, as well as 3-6 sperm positive females to each associated group of untreated foster dams.</p>	<p>Developmental study (NTP study, GLP compliant).</p> <p>Design: Treatment of pregnant rats from GD6-20</p> <p>Dams were euthanised on</p>	<p>Purity: 99.6% Vehicle: water</p> <p>Concentrations: 0, 250, 1250 and 2250 mg/kg bw/day in water via oral gavage.</p> <p>Dose formulations</p>	<p>Maternal toxicity:</p> <p>- No effect on body weight GD0-15, reduced body weight in high dose group on GD20 (P<0.01, 352.0±4.8 g compared to</p>	<p><u>P0 parents</u></p> <p>-Longer gestational periods (P<0.01) in high dose groups. Control: 21.26±0.07 days 1250 mg/kg: 21.58±0.07 days 2250 mg/kg: 21.84±0.09 days</p> <p>-Reduced live litter size on PND 1 (P<0.01) in high dose group</p>	<p>All litters with fewer than eight surviving pups on postnatal day 4, as well as those which could not be culled in accordance with the stated sex ratio</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
	Multiple replicates to come to: N= at least 20 F1 litters per/dose group	PND1. Pups were cross-fostered to untreated mothers on PND 1 and were euthanised PND 4, 22 or 63. Examinations: Maternal organ weights (kidney, liver and post-pregnancy uterus), uterine contents, developmental landmarks and sexual maturation, offspring organ weights, and visceral, skeletal and morphological abnormalities. Statistics: - Data are presented as mean ± SEM	were analysed by gas chromatography and compared to an internal standard to verify concentration prior to administration to the test animals.	367.8±4.3 g in control group). – Reduced gestational weight gain GD6-20 (P<0.01) in high dose group (84.3±3.5 g compared to 106.2±3.0 g in control group). No effect on BW on PND 1 after littering. - Increased kidney weight (P<0.01) in high dose group (2.42±0.07 g compared to 2.20±0.05 g in control group).	(11.87±0.48 compared to 13.67±0.33 in control group). -Increased mortality per litter PND1-4 (P<0.01) in high dose group (5.15±1.06% compared to 2.07±1.12% in control group) <u>F1 offspring:</u> -Reduced body weight in high dose group on PND1 (P<0.01) and PND22 (P<0.05). -Reduced absolute kidney weight in high dose group (P<0.05) on PND22 and PND63. -Reduced absolute brain weight in high dose group (P<0.05) on PND22 and in females on PND63. Relative kidney weight females at mid and high affected on PND63	requirements were also sacrificed. This could have led to potential bias.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - General Linear Model analysis - one-way ANOVA and pairwise comparisons - Chi-square test (nominal data). One tailed- fisher exact test for pairwise comparison. 		<ul style="list-style-type: none"> - No significant effect on liver weight observed - Reduced uterine weight on PND 1 ($P < 0.05$) in high dose group (2.08 ± 0.14 g compared to 4.62 ± 0.14 g in control group). No effect on number of uterine implantation sites per dam. 	<ul style="list-style-type: none"> - Reduced time to achieve wire grasping in high dose group ($P < 0.05$) (9.71 ± 0.18 days compared to 11.98 ± 0.78 days in control group). No effects on other developmental landmarks - No significant effect on external and visceral defects observed. - Increase in skeletal defects ($P < 0.001$), litters with malformed pups in high dose group (female 60.0% and male 30% compared to 0% in control group), including malformed ribs (missing, short, fused), malformed centra (fused, ossification, off centre) and fused sternbrae. 	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Marr et al., 1992	Rat, Sprague-Dawley, N= 7/dose group/time point of euthanasia	<p>Developmental study (non-guideline, non-GLP).</p> <p>Design: Treatment of pregnant rats from GD 6-15.</p> <p>Dams were euthanized on GD 18, 20 or PND 1, 4, 14, 21 or 63.</p> <p>Examinations: - Foetus (GD 18 or 20) /pup weight (PND 1, 4, 7, 14, 21 and 63) - Examination of skeleton: The regions of the skeleton that were examined included the sternum,</p>	<p>Purity>99%</p> <p>Concentrations: 0, 2500 mg/kg bw/day in water via oral gavage.</p>	<p>Maternal weights were lower by 3-6% compared to control at GD 11, 15, 18 and 20 (all P<0.05). Maternal body weight gain during treatment (GD 6-15) was 27% lower compared to control and maternal weight gain was 13% lower compared to control during gestation.</p> <p>There was no effect of ethylene glycol treatment on</p>	<p>- Foetal body weight was 26 and 24% lower than control at GD 18 and 20, respectively (P<0.05 for both time points). - Pup body weight was 10% lower than control at PND 1 (P<0.05). - Pup body weight was 8-5% lower between PND 4 to 63 (not statistically significant)</p> <p>Skeletal malformations: - Increased percentage malformed per litter, exposed versus control: GD18: 76.3±11.3% versus 0%, P<0.05 GD 20: 88.4±3.4% versus 1.1±1.1%, P<0.05 PND 1: 95.2±4.8% versus 0%, P<0.05 PND 4: 83.4±6.1% versus 1.0±0.5%, P<0.05</p>	<p>Only one dose tested.</p> <p>The number of statistically significant ossification data should be lower since the pup body weight should have been a covariate in the analysis.</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>carpals, tarsals, phalanges, and vertebral centra.</p> <p>Statistics: - Results are presented as mean \pm SEM - Continuous data: t-test or Mann-Whitney U test. Categorical data: Fisher's Exact test - Proportions of ossification: general linear model (ANOVA) (with and without body weight as covariate)</p>		<p>gestational day of delivery, i.e., gestational length,</p>	<p>PND 14: 77.5\pm16.5% versus 0%, P<0.05 PND 21: 87.5\pm7.2% versus 0%, P<0.05 PND 63: 28.2\pm8.3% versus 7.5\pm7.5%, not significant - Percentage of litters with malformations was 100% at GD18, GD 20, PND1, PND 21 (all P<0.05 compared to control) and 100% at PND 4 and 80% at PND 63 (not significant). - Agenesis of the ribs (incidence 24-41%) and short ribs (incidence 6-34%) were common. - Bipartite cartilage and bipartite ossification centres, incidence peaks at PND 1 and 21. - Increase in variations in exposed group. Rudimentary rib on</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					lumbar arch I was most common. Ossifications (ANOVA without foetal body weight as covariate): -GD20: - Percent total ossification lowered (24.85±1.46% versus 37.42±1.19%, P<0.05) - Percent sternbrae, centra, forelimb distal phalanges and metatarsals ossified were lowered (P<0.05). PND1: - Percent total ossification lowered (66.49±1.33% versus 78.64±0.45%, P<0.05). - Percent sternbrae and centra ossified lowered (P<0.05). PND 4:	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					<ul style="list-style-type: none">- Percent total ossification lowered (84.32±0.95% versus 92.58±0.98%, P<0.05).- Percent sternebrae, centra, forelimb middle phalanges ossified were lowered (P<0.05). PND 14: <ul style="list-style-type: none">- Percent total ossification lowered (94.08±0.54% versus 99.75±0.13%, P<0.05)- Percent sternebrae and centra ossified lowered (P<0.05). PND21: <ul style="list-style-type: none">- Percent total ossification lowered (95.22±0.50 versus 100±0.0%, P<0.05).- Percent sternebrae and centra ossified lowered (P<0.05). PND 63:	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Neeper-Bradley et al., 1995	Rat, CD, females N= 25/group	Developmental study (EPA TSCA testing guideline, GLP compliant). Design: Females were treated daily from GD 5 through 15. Dams were euthanized on GD 21. Examinations: - Maternal liver, kidney and gravid uterine weight. Kidney histology. - Corpora lutea, number of live and dead fetuses, resorptions.	Purity: >99.9% Vehicle: water Concentrations: 0 (vehicle), 150, 500, 1000 and 2500 mg/kg bw/day, via oral gavage. Dosing solutions were 103-108% of nominal concentrations	Maternal toxicity: 1000 mg/kg bw/day: - Increased relative liver weight (P<0.05). 2500 mg/kg bw/day: - Body weight gain was reduced at GD6-9 (P<0.05) and GD15-18 (P<0.01). - During treatment, water consumption	- Percent sternebrae ossified lowered (P<0.05) Developmental effects: 500 mg/kg bw/day: Skeletal variations: - Increased incidence in poorly ossified supraoccipital, P<0.05 1000 mg/kg bw/day: - Reduced foetal body weight per litter (4.981±0.31 g versus 5.245±0.26 g in controls), P<0.05. Malformations external/soft tissue: - Gastroschisis in one foetus, not significant. Malformations skeletal: - Extra 14 thoracic centrum and arches	Maternal toxicity is evident in the high dose and since the liver effects are unlikely to explain any developmental toxicity (although no histopathology was performed), the committee would deduce a NOAEL at 1000 mg/kg bw/day.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Sex of foetuses, variations and malformations. Statistics: - Data presented as mean \pmSD - Continuous data: ANOVA and t-tests with Bonferroni post-hoc test. - Non-parametric data: Kruskal-Wallis followed by Mann Withney U when appropriate. - Incidence data: Fisher exact test 		<ul style="list-style-type: none"> was increased (P<0.01). - Reduced final body weight (365.08\pm33.0g versus 382.17\pm30.6g in controls), P<0.01. -Reduced gravid uterine weight (74.042\pm24.0g versus 98.013\pm30.6g in controls), P<0.01. - Increased absolute and relative kidney weight and relative liver weight (all P<0.01). 	<ul style="list-style-type: none"> (8/23 versus 1/24 control litter), P<0.05. - Missing rib (8/23 versus 0/24 control litters), P<0.01. - Missing thoracic arches (6/23 versus 0/24 control litters), P<0.05. Variations skeletal: Increased incidence of multiple skeletal variations involving ossification sites at the cervical and thoracic region. 2500 mg/kg bw/day: - Reduced foetal body weight per litter (4.033\pm0.40g versus 5.245\pm0.26g in controls), P<0.01. Malformations external/soft tissue: 	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					<ul style="list-style-type: none">- Gastroschisis (7/21 versus 0/24 litters in controls), P<0.05.- Hydrocephaly (10/21 versus 0/24 control litters), P<0.05.- Lateral ventricle dilated (6/21 versus 0/24 control litters), P<0.05.- Umbilical hernia (5/21 versus 0/24 control litters), P<0.05.- Atelectasis (19/21 versus 12/24 control litters), P<0.01. <p>Skeletal malformations:</p> <ul style="list-style-type: none">- Cervical arch 7 missing (6/21 versus 0/24 control litters), P<0.05.- Extra 14 thoracic centrum and arches (18/21 versus 1/24 control litter), P<0.01.	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					<ul style="list-style-type: none">- Lumbar centra skewed (11/21 versus 0/24 control litters), $P < 0.05$.- Rib missing (20/21 versus 0/24 control litters), $P < 0.01$.- Ribs fused (17/21 versus 0/24 control litters), $P < 0.01$.- Thoracic arch missing (17/21 versus 0/24 control litters), $P < 0.01$.- Thoracic arches fused (13/21 versus 0/24 control litters), $P < 0.01$.- Thoracic centrum and arch missing (5/21 versus 0/24 control litters), $P < 0.05$. <p>Variations skeletal: Increased incidence of multiple skeletal variations involving ossification sites at the</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Carney et al., 1999	Rat, CD, Sprague-Dawley, females. N=25/group time-mated rats	Developmental study (non-guideline, GLP compliant) Design: Treatment from GD 6 to 15. Necropsy on GD 21. Examinations: maternal body weight, organ weights (liver, kidney, gravid uterus), foetal external, visceral, skeletal abnormalities. Statistics:	Purity EG: 99.98% Concentration: 2500 mg/kg, by oral gavage Solution within 99-107% of target concentration Control: deionized water via gavage	Decreased maternal body weights on GD 12, 16, 21. Decreased body weight gain intervals (except the 0-6 pre-dosing period). Increased absolute and relative kidney weights. Increased liver weight, with absolute liver weight being significantly different than control.	cervical and thoracic region. Fatal body weight (g): 0 mg/kg: 5.53±0.30 2500 mg/kg: 3.75±0.58*, P=0.05 <u>External malformations</u> (% foetuses affected (%litters affected)): Meningoencephalocele: 3.5 (25.0)* Exencephaly: 5.2 (25.0)* Cleft lip: 3.5 (29.2)* Cleft palate: 2.8 (29.2)* Omphalocele: 9.8 (54.2)* Gastroschisis: 0.3 (4.2) Umbilical hernia: 1.7 (16.7) P=0.05 <u>Visceral malformations</u> (% foetuses affected (%litters affected)): Dilated cerebral ventricles: 15.9 (33.3)*	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		Bartlett's test, ANOVA, Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction, Fisher exact test		One dam exhibited vaginal bleeding on GD 19 and 20 and was found to have a completely resorbed litter on GD 21. Mean feed-consumption values were decreased by 6-13%.	Anophthalmia: 3.3 (16.7) Microphthalmia: 2.0 (12.5) P=0.05 <u>Skeletal malformations</u> (% fetuses affected (% litters affected)): Hemivertebrae: 60.0 (95.8)* Extra vertebrae: 5.9 (29.2)* Missing vertebrae: 24.4 (62.5)* Fused vertebrae: 36.3 (75.0)* Fused centra: 8.9 (33.3)* Fused ribs: 62.4 (95.8)* Extra ribs: 4.5 (20.8)* Missing ribs: 60.2 (91.7)* P=0.05 <i>Skeletal variations:</i> Increased incidence of delayed ossification of skull, lumbar vertebrae, cervical centra, thoracic	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					centra, lumbar centra, sternebrae, ossification of ribs skull (extra site of ossification), floating ribs, fused sternebrae, irregular pattern sternebrae ossification, extra site of sternebrae ossification, and total malformed fetuses	
Schuler et al., 1984 ⁴³	Mice, CD-1, females N=50/group	Developmental study (non-guideline, non-GLP) with a preparatory dose finding study. Design: Treatment of pregnant dams from GD 7 to 14. Pups were sacrificed 3 days after birth.	Purity: >99% Concentration: 11090 mg/kg bw/day, by oral gavage Vehicle: water Negative control: vehicle	Maternal mortality: - 5/50 versus 0/50 in controls (not significant)	- Decreased viable litters (litter with at least one viable pup): 15/37 versus 29/29 in controls (P<0.05) - Decreased number of live pups per litter: 2 versus 9 in controls (P<0.05). - Increased number of dead pups per litter: 1.5 versus 0.1 in controls (P<0.05). - Decreased pup postnatal survival: 40%	No clear cause for maternal mortality. Maternal toxicity is relatively high, but no statistical analysis was performed. Adverse effects were observed, but without a dose-response relationship.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>Examinations: number of live pups, pup weight, maternal body weight.</p> <p>Statistics: - Body weight: analysis of variance - Mice with viable litters: Fisher-Irwin - Number of live pups and survival: student's t-test</p>			<p>versus 100% in controls (P<0.05). - Decreased pup weight gain: 0.2g versus 7g in controls (P<0.05). - Decreased pup birth weight: 1.4g versus 1.7g in controls (P<0.05).</p>	
Price et al., 1985 ³⁹	Mice, CD-1, females N=10/group/replicate (so total of 20/group)	<p>Developmental study (NTP study, GLP compliant, blinded).</p> <p>Design: Treatment of pregnant mice from GD6 through 15. 2 replicates of the teratology</p>	<p>Purity: >99% Vehicle: water</p> <p>Concentrations: 0, 750 (low), 1500 (mid) or 3000 (high) mg/kg bw/day, via oral gavage</p>	<p>- Decreased weight gain during treatment in mid and high dose (P<0.05 for both groups*): 0 mg/kg: 11.24±0.49g</p>	<p>Developmental toxicity: - Reduction in the number of implantation sites per litter in the mid dose (and not high dose) (11.57±0.70) compared to control (13.32±0.44), P<0.05. Note: since this reduction was not dose-related, it was assumed unlikely to</p>	<p>The doses selected were based on a preliminary toxicity study performed using timed-pregnant rats and mice (Price et al., 1984a, b).</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		evaluation were conducted. Caesarean section was performed on GD 17. Examinations: maternal body weights on GD 0, 6-15 and 17 maternal organ liver and gravid uterine weights, uterine contents (number of implantation sites, resorptions, dead foetuses, and live foetuses) and visceral, skeletal and morphological abnormalities. Statistics:	Negative control: vehicle Actual dose levels were within 10% of the calculated levels.	750mg/kg: 11.58±0.39g 1500mg/kg: 8.54±0.84* 3000mg/kg: 8.42±0.54* - Decreased absolute (but not relative) maternal liver weight in mid and high dose groups (P<0.05 for both groups*): 0 mg/kg: 2.72±0.05g 750mg/kg: 2.63±0.04g 1500mg/kg: 2.49±0.06* 3000mg/kg: 2.47±0.06* - Decreased gravid uterine weight in mid	be related to EG treatment. - Dose-related increasing trends in post-implantation loss per litter or at one or more site, but no significant pairwise comparisons. - Dose-related decrease in live litter size, with significant decrease in the high dose group (9.83±0.56) compared to control (11.88 ±0.49), P<0.05. - Dose-related decrease in average foetal body weight per litter. Low dose (0.882±0.017g), mid dose (0.787±0.024g) and high dose (0.712±0.022g) were decreased compared to control (0.974±0.013g), P<0.01 for all groups.	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Data are presented as mean \pm SEM - Dose response: test for linear trend - ANOVA with post hoc tests - test for linear trend on proportions and chi-square test (nominal data). One tailed- fisher exact test for pairwise comparison. 		<p>and high dose (P<0.05 for both groups*):</p> <p>0 mg/kg: 17.13\pm0.62</p> <p>750mg/kg: 15.46\pm0.55</p> <p>1500mg/kg: 12.17\pm0.93*</p> <p>3000mg/kg: 11.58\pm0.59*</p>	<ul style="list-style-type: none"> - Dose-related increase in the percentage of malformed foetuses per litter. Low dose (10.0\pm1.96%), mid dose (37.77\pm6.30%) and high dose (56.54\pm6.80%) compared to control (0.25\pm0.25%), P<0.01 for all groups. - Dose-related increase in the percentage of litters or with one or more malformed foetus. Low dose (66.67%), mid dose (81.82%) and high dose (96.65%) compared to control (4.0%), P<0.001 for all groups. <p>Malformations:</p> <ul style="list-style-type: none"> - External malformations in 8 litters (high dose) versus 0 in the controls, P<0.05, including exencephaly, 	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					<p>meningoencephalocele, cleft palate, cleft lip, facial cleft.</p> <p>- Dose response related increase in visceral malformations.</p> <p>Significant in high dose (7 litters) versus controls (0 litters), $P < 0.05$.</p> <p>Visceral malformations are among others aortic stenosis, hydronephrosis, hydroureter, retrotracheal or retrooesophageal pulmonary artery.</p> <p>- Dose response related increase in skeletal malformations.</p> <p>Significant in low dose (15 litters), mid dose (17 litters) and high dose (22 litters) versus control (1 litter), $P < 0.01$ for all groups. Malformations include malformed ribs (short, missing, branched</p>	

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					and/or fused), malformed arches (enlarged, small, fused and/or missing) and malformed centra (misaligned, unilateral ossification, off centre, fused and/or missing).	
Neeper-Bradley et al., 1995 ⁴²	Mice, CD-1, females N=30/group	Developmental study (non-guideline, GLP compliant) Design: Females were treated daily from GD5 through GD15. Dams were euthanized on GD18. Examinations: - Maternal liver, kidney and gravid uterine weight. Kidney histology.	Purity: >99.9% Vehicle: water Concentrations: 0 (vehicle), 50, 150 (low), 500(mid) and 1500 (high) mg/kg bw/day, via oral gavage. Dosing solutions were 92-107% of nominal concentrations.	Maternal toxicity: One death occurred in control group, 2 deaths/group occurred at 50, 500 and 1500 mg/kg bw/day (not significant). Slight reductions in weight gain and body weight at 1500 mg/kg bw/day (not significant).	- Foetal body weights/litter were reduced at 1500 mg/kg bw/day (1.156±0.11 g versus 1.325±0.09 g in controls), P<0.01. Malformations external/soft tissue: - Exencephaly noted in 2 litters (500 mg/kg bw/day) and 3 litters (1500 mg/kg bw/day), not significant. Skeletal variations (500 mg/kg bw/day): - Extra rib 14, first lumbar arch, bilateral (17/24	The malformations in soft and visceral tissues are so incidental that they are not considered treatment related effects. However, the increase in skeletal malformations is considered an adverse effect.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Corpora lutea, number of live and dead foetuses, resorptions. - Sex of foetuses, variations and malformations. <p>Statistics:</p> <ul style="list-style-type: none"> - Data presented as mean \pmSd - Continuous data: ANOVA and t-tests with Bonferroni post-hoc test. - Non-parametric data: Kruskal-Wallis followed by Mann Withney U when appropriate. - Incidence data: Fisher exact test 			<p>versus 4/19 control litters), P<0.05.</p> <p>Skeletal malformations (1500 mg/kg bw/day):</p> <ul style="list-style-type: none"> - 2-12 thoracic arches fused (8/21 versus 0/19 control litters), P<0.01. - 2-12 ribs fused (15/21 versus 1/19 control litters), P<0.01. - Extra 14 thoracic centrum and arches (10/21 versus 0/19 control litters), P<0.01. - Extra rib 14, thoracic arch 14: bilateral (10/21 versus 0/19), P<0.01. <p>Skeletal variations: Increased incidence of multiple skeletal variations involving ossification sites at cervical, thoracic and</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Lamb et al., 1985 ³²	Mice, COBS CrI:CD1, (ICR)BR outbred albino N=20/sex/group Controls: 40/sex	Reproductive study (non-guideline, non-GLP). Fertility assessment by continuous breeding with a preparatory dose range finding study. Design: - Premating period of 7 days - 98 days cohabitation and breeding (male+female), all pups born during this period were killed - 21 days segregation period, pups born	Purity: 99.6% Concentrations: 0, 0.25, 0.5 and 1.0% (weight/volume), oral via drinking water. According to the authors, this was equal to 410, 840 and 1640 mg/kg bw/day, based on daily water consumption per pair of mice for 2 weeks and mean body weight data. Dosage solutions were within 98 – 107% of intended concentrations.	No general toxicity, i.e. clinical signs, effects on body weight or water consumption in parental animals. Some deaths occurred in all groups. One death in the 0.5% group could be treatment related according to the authors (oxalate crystals in renal tubules).	lumbar region as well in the head. Offspring defects: - Live pup weight decreased at high dose (1.53±0.02 g) versus control (1.63±0.02 g), P<0.01. - A pattern of skeletal defects (affecting skull, sternbrae, ribs and vertebrae) in treated mice, not in controls (not quantified). Defects included shortened facial bones, fused ribs, abnormally shaped or missing sternbrae, abnormally shaped vertebrae and twisting of spine.	A limitation of this study is the number and selection of animals used.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>during this period were kept alive</p> <ul style="list-style-type: none">- Exposure occurred during pre mating, cohabitation, segregation and throughout the life of offspring.- High dose and control pups (N=20/sex/group) born during segregation were mated. <p>Examinations:</p> <ul style="list-style-type: none">- Fertility and reproductive performance of the adults and F1 generation. <p>Viability, sex and weight of the pups.</p>				

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>- Organ weights of F1 offspring and skeletal examination of a proportion of F1 mice.</p> <p>- Histology of the head of F1 mice.</p> <p>Statistics:</p> <p>- Results presented as Mean \pmSE.</p> <p>- Tests used differ per parameter. Mostly Chi-Square approximation to Kruskal Wallis is used for group comparisons and Mann-Whitney U or Fisher exact test for pairwise comparisons. An ANOVA was carried out for</p>				

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		assessment of body weight.				
Gulati et al., 1986	Mice, Crl:CD-1 (ICR)BR outbred albino (CD1)	<p>Continuous breeding study (non-guideline, GLP-compliant).</p> <p>Design: Task 2 - Premating period of 7 days - 98 days cohabitation and breeding (male+female), all pups born during this period were killed</p> <p>Task 3 - weaned animals of the last litter of task 2 from the high dose group (N=20/sex/group) and untreated</p>	<p>Purity: 99.6%</p> <p>Concentrations: 0, 0.5, 1, 1.5% (weight/volume), oral in drinking water.</p> <p>0, 897, 1798 and 2826 mg/kg bw/day</p> <p>Dosage solutions were within 94 and 104% of intended concentration at all timepoints (task 2 4 times, task 3 once and three times task 4)</p>	<p>Task 2 Parental animals: - No treatment-related effects on body weight, water consumption significantly increased in males a 1.5%</p> <p>Task 3 parental animals: No treatment-related effects on body, liver or kidney weight observed in female mice. - Significant reduction in body (42.29±0.10g in</p>	<p>Task 2: - Reduced live pup count (11.8/litter in controls, 9.99/litter at 1.5%) (p<0.05) - Reduced pup weight (absolute and adjusted for litter size (<10%, p<0.05) - Significant increase of distinct facial deformities in pups at 1.0 and 1.5%, including cleft palate 17/4 and 28/9 (pups/litters) at 1.0 and 1.5% and ablepharon 19/16 and 21/15 (pups/litters) at 1.0 and 1.5% (no data included in tables of the report)</p> <p>Task 3: -Significant effect (<10%) on adjusted live pup</p>	<p>NTP study</p> <p>The total number of offspring examined for deformities is unclear.</p> <p>Parental NOEL: 1.0%</p> <p>Development NOEL: 0.5%</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>males or females were mated.</p> <p>-unexposed cohabitation for max 1 week</p> <p>- Females were allowed to litter, all pups born were killed.</p> <p>Task 4</p> <p>- weaned pups from the last litters of task 2 (F1)</p> <p>-Exposure for 74 days, thereafter during mating and until weaning of the offspring (2nd generation)</p> <p>Examinations:</p> <p>- Fertility, viability and weight of litters.</p>		<p>1.5% group vs 46.45±1.04g in control group) and liver weight (1.93±0.05g in 1.5% group vs 2.11±0.07g in control group) observed in male mice.</p> <p>- kidney degeneration, dilation, and regeneration of tubules and deposition of crystals (oxalate) in the tubules</p> <p>Task 4 Parental parents:</p> <p>- No treatment-related effects on body weight and kidney</p>	<p>weight in 1.5% group males/control females</p> <p>-2/2 (pups/litters) with ablepharon and 1 pup with possible cleft lip/palate in 1.5% group males/control females</p> <p>Task 4:</p> <p>- Significant effect (<<10%) on adjusted live pup weight in all dose groups</p> <p>0.5%</p> <p>- Reduced sperm density (801±61 compared to 1036±63 in control group, P<0.05)</p> <p>1.0% - Reduced sperm density (855±63) compared to 1036±63 in control group, P<0.05)</p> <p>- Reduced sperm motility (92.1±1.47 vs 94.6±0.89 in control groups, P<0.05).</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>- Organ weight and skeletal deformities in offspring.</p> <p>Statistics: - Data is displayed as mean \pmSE. - Cochran-Armitage or Jonckheere test for trends. Statistics differ per parameter, mostly used are Chi-Square, Kruskal-Wallis, Wilcoxon rank-sum and Fisher's exact test.</p>		<p>weight (abs+rel), significant lower liver weight (abs, not rel) in females - No treatment-related effects on body weight, sign decreased liver weight (1.93\pm0.05g in 1.5% group vs 2.11\pm0.07g in control group) in males</p>	<p>1.5% - Reduced testes weight (0.120\pm0.006 g vs 0.140\pm0.005 g in control group - Reduced sperm motility (84.1\pm5.02 vs 94.6\pm0.89 in control groups, P<0.05). - Degeneration of seminiferous tubules in 60% of males at 1.5% vs 40% in controls</p>	
Harris et al., 1992	Mice, Crl:CD-1 N=10/sex/group	Short-Term Reproductive and Developmental Toxicity Screening study (non-guideline, non-GLP).	Purity not mentioned. Concentrations: 0, 250, 700, and 2500 mg/kg/day	No adverse clinical signs observed. No mortality observed.	2500 mg/kg/day exposure during gestation resulted in a significant (P<0.05) decrease in total litter weight on PND1 (15.3 \pm 0.9 g vs 19 \pm 1.1 g	Screening study A decrease in mean litter weight was observed only in the high-dose

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>Design: Treatment of pregnant female mice during GD8-14.</p> <p>Examinations: - Liver, kidney and testes histology in male mice. - Fertility and litter weight.</p> <p>Statistics: Cochran-Armitage test for linear trend followed by Fisher's exact test. Kruskal-Wallis analysis of variance for dose group comparison and Jonckheere's test for dose-responses. Mann-</p>	<p>in water via oral gavage.</p> <p>Dosing solutions within 93-100% of target concentrations.</p>		<p>in control group) and PND4 (26.6±1.5 g vs 31.7±1.3 g in control groups).</p>	<p>group. However, not clear how many litters were included.</p> <p>Compared to the control, this may or may not be considered an adverse effect.</p>

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		Whitney U test for pairwise comparisons.				
Tyl et al., 1993 ⁴⁸	Rabbits, New Zealand white, females, N=23-24/group	<p>Developmental study (non-guideline, GLP compliant).</p> <p>Design: Treatment of pregnant rabbits from GD6 through 19. Pregnant rabbits were euthanized on GD 30.</p> <p>Examinations: - Maternal liver, kidney and intact uterine weight. Kidney histology. - Number of corpora lutea, implantations, live and dead</p>	<p>Purity: 98%</p> <p>Vehicle: water</p> <p>Oral gavage</p> <p>Concentrations: 0, 100, 500, 1000 or 2000 mg/kg bw/day</p>	<p>At 2000 mg/kg bw/day;</p> <p>- 42.1% mortality</p> <p>- three early deliveries</p> <p>- one spontaneous abortion</p> <p>- absolute kidney weight slightly increased (106.3% right kidney; 107.6% of the control of the left kidney), accompanied with renal lesions limited to the cortical renal tubules</p>	<p>No indication of developmental toxicity at any dose tested.</p>	<p>NTP study</p> <p>Exposure to a dose of 2000 mg/kg bw/day showed significant toxicity in the kidney. However, at the lower dose of 1000 mg/kg bw/day no abnormalities were observed.</p>

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>foetuses, and resorptions counted.</p> <ul style="list-style-type: none">- Foetus weight, foetus abnormalities, variations and malformations. <p>Statistics:</p> <ul style="list-style-type: none">- General Linear Trend Models procedures applied for ANOVA of maternal and foetal parameters.- Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data		<p>The death of the pregnant females at 2000 mg/kg bw/day was directly related to the acute renal failure.</p>		

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		- Bartlett's test for homogeneity of variance was performed on all data to be analysed by ANOVA.				

1
2

Table 3 inhalation studies on effects of ethylene glycol on development

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Tyl et al., 1995a	Rat, COBS CD (SD)BR outbred albino N=25/sex/group	Developmental toxicity study (non- guideline, GLP compliant). <u>Design:</u> Whole-body exposure during GD days 6 to 15 for 6 hours per day.	<u>Test item:</u> Respirable aerosol of ethylene glycol (2.3 µm) in inhalation chambers. <u>Purity:</u> >99.9% <u>Target concentrations:</u> 0, 150, 1000, 2500	- Significant increase in liver weight in 2500 mg/m ³ exposure groups (15.001±1.31g vs 13.841±1.72g in control group) - No effects on food, water	No effect on gestational parameters or malformations.	Ethylene glycol measured in fur indicates oral exposure, calculated to be the major route of exposure (64-90%).

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p><u>Examinations:</u> body weight, organ weight, gestational parameters, developmental defects.</p> <p><u>Statistics:</u> - Data displayed as mean \pmSD. - Continuous variables using Levene's test for equal variances, ANOVA, and t-tests with Bonferroni probabilities. Non-parametric data using Kruskal-Wallis test followed by</p>	<p>mg/m³</p> <p><u>Analytical concentrations:</u> 0, 119\pm13, 888\pm149, and 2090\pm244 mg/m³</p>	consumption, body weight, or weight gain observed.		

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		Mann-Witney U test. Incidence data using Fisher's exact test. (p<0.05 2 tailed).				
Tyl et al., 1995a	Mice, CD-1 (CrI:CD-1 (1CR)BR) outbred albino N=25/sex/group	Developmental toxicity study (non-guideline, GLP compliant). Design: Whole-body exposure during GD days 6 to 15 for 6 hours per day. Examinations: Body weight, organ weight, gestational parameters, developmental defects.	Test item: Respirable aerosol of ethylene glycol (2.3 µm) in inhalation chambers. Purity: >99.9% Target concentrations: 0, 150, 1000, 2500 mg/m3 Analytical concentrations: 0, 119±13, 888±149, and	-Significant reduction in maternal body weight in 1000 mg/m3 (49.841±5.13g, P<0.05) and 2500 mg/m3 (47.391±5.46g P<0.001) compared to control groups (53.211 g±3.33) -Wet fur observed in all ethylene glycol exposure groups.	-Reduced gravid uterine weight in mg/m3 and 2500 mg/m3 groups (both P<0.001) -Reduction in viable implants in 2500 mg/m3 group (8.0±2.9 vs 10.7±1.8 in control group, P<0.001) - Increase in non-viable implants per litter in at 1000	Ethylene glycol measured in fur indicates that oral exposure was present, calculated to be the major route of exposure (64-90%). A significant increase in visceral, external and skeletal malformations was observed only in the mid- and high-dose groups.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>Statistics:</p> <ul style="list-style-type: none"> - Data displayed as mean \pmSD. - Continuous variables using Levene's test for equal variances, ANOVA, and t-tests with Bonferroni probabilities. - Non-parametric data using Kruskal-Wallis test followed by Mann-Witney U test. Incidence data using Fisher's exact test. ($p < 0.05$ 2 tailed). 	<p>2090\pm244 mg/m³</p>		<p>mg/m³ (2.9\pm2.0, $P < 0.01$) and 2500 mg/m³ (4.2\pm2.9, $P < 0.001$) compared to control groups (1.4\pm1.0)</p> <ul style="list-style-type: none"> - Decreased percentage live fetuses in 1000 mg/m³ ($P < 0.01$) and 2500 mg/m³ ($P < 0.001$) compared to control groups - Change in sex ratio in 1000 mg/m³ group compared to control group ($P < 0.01$) 	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					- Reduction (P<0.001) in foetal body weight in 1000 (1.07g± 0.14) and 2500 (0.94±0.14g) mg/m3 exposure groups compared to control groups (1.33±0.08g). - Increase (P<0.05) in incidence of external malformations in 1000 (30.4%) and 2500 (72.7%) mg/m3 groups compared to control group (4%).	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					- Increase (P<0.05) in incidence of visceral malformations in 1000 (34.8%) and 2500 (72.7%) mg/m3 groups compared to control group (%).	
					- Increase (P<0.05) in incidence of skeletal malformations in 1000 (100%) and 2500 (100%) mg/m3 groups compared to control group (72%).	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Tyl et al., 1995b ⁴⁹	Mice, CD-1 (CrI:CD-1 (1CR)BR) outbred albino N=30/sex/group	Developmental toxicity study (non-guideline, GLP compliant). Design: Whole-body or nose-only exposure on GD days 6 to 15 for 6 hours per day Examinations: clinical signs, water consumption, uterus, liver and kidney weight, foetus examination. Statistics:	Test item: Ethylene glycol (1.7 µm). <u>Nose-only:</u> - Target concentration: 0, 500, 1000 and 2500 mg/m3 - Analytical concentration: 0, 360, 779 and 2505 mg/m3 <u>Whole body: (positive control):</u> - Target concentration: 0, 2100 mg/m3 - Analytical concentration: 0, 2008 mg/m3	<u>Nose-only:</u> - Body weight, weight gain and liver weight unaffected by treatment. -Increased kidney weight at 1000 (0.46±0.046 g, P<0.05) and 2500 (0.472±0.034 g, P<0.01) mg/m3 compared to control group (0.431±0.040g) - Wet fur in nose-only group (around the head) at 2500 mg/m3	<u>Nose only:</u> - Non-significant and slight reduction of percentage live foetuses per litter in 1000 and 2500 mg/m3 groups (85.2±25.48 and 86.1±21.31 vs 93.3±7.09 in control group, respectively). - Reduction in foetal body weight observed in 2500 mg/m3 group (1.126±0.107g vs 1.289±0.126g in control	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<ul style="list-style-type: none"> - Data displayed as mean \pmSD. - Continuous variables using Levene's test for equal variances, ANOVA, and t-tests with Bonferroni probabilities. Non-parametric data using Kruskal-Wallis test followed by Mann-Witney U test. Incidence data using Fisher's exact test. (p<0.05, 2 tailed). 			<ul style="list-style-type: none"> group, P<0.001) - No increase in external or visceral malformations observed. - Increase in litters with at least one foetus with fused ribs in 2500 mg/m³ group (8/21 vs 1/22 in control group, P<0.05). <u>Whole body (positive control):</u> - Reduced gravid uterine weight (17.96\pm3.99 vs 	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					20.70±4.49 in control group, P<0.05). - Increase in non-viable implants per litter (P<0.01) and reduced percentage of live foetuses per litter (P<0.01). - Reduction in foetal body weight (1.13±0.11 vs 1.36±0.13 in control group, P<0.001). - Increase in skeletal malformations (96.3% vs 10.3	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					in control group, P<0.01), including fused arches and ribs.	

1
2

Table 4 dermal studies on effects of ethylene glycol on development

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
Tyl et al., 1995c ⁴⁷	Mice, CD1, females	Non-guideline developmental dermal study. Females exposed daily (6h occlusion of skin and restraining) on GD6 through 15. On GD18 mice were asphyxiated. Examinations: - Maternal organ weights, corpora lutea, kidney	Purity: ~100% Vehicle: water <u>Dermal:</u> Concentrations: 0% (vehicle), 12.5%, 50% and 100% (corresponds to 0, 404, 1677 and 3549 mg/kg bw/day) ethylene glycol, cutaneous in 0.1 mL/animal.	- No deaths in treatment groups. - All clinical signs were associated with restrain conditions. - Gestational weight change increased at 100% (5.044±2.055g) versus controls (3.310±1.796g), P<0.05.	- Number of dams with fully resorbed litters was higher in all treatment groups compared to control. - Reduced number of late resorptions/litter at 12.5% (0.0±0.20) versus control (0.3±0.54), P<0.05. - Reduced number of dead foetuses/litter at	Multiple signs of toxicity in the positive control group. Increase in incidences of variations were observed in the dermal study, but no increase in incidences of malformations.

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
		<p>microscopy status of implantation sites.</p> <p>- Visceral and skeletal malformations and variations.</p> <p>Statistics:</p> <p>- Continuous data: ANOVA and t-tests with Bonferroni post-hoc.</p> <p>- Nonparametric data: Kruskal-Wallis and Mann-Whitney U.</p> <p>- Incidence data: Fisher-exact test.</p>	<p>Authors converse ethylene glycol dose by using the mean control body weights from GD6-15.</p> <p><u>Oral gavage:</u> (positive control): ethylene glycol 3000 mg/kg bw/day</p> <p>Actual dose levels were 99.2-101.2% of target levels.</p>	<p>- Minimal-grade tubular lesions in 3/30 mice in high dose group.</p> <p><u>Oral gavage</u> (positive control):</p> <p>- 8 females died.</p> <p>- Gestational weight change increased (5.283±3.172g) versus controls (3.310±1.796g), P<0.05.</p> <p>Clinical signs: hypoactivity, cold extremities, hunched posture, urogenital discharge, and tissue on paperboard (from abortions).</p>	<p>12.5% (0.1±0.27) versus controls (0.4±0.71), P<0.05.</p> <p>External variations in litters (%): 0% EG: 17.4 12.5% EG: 20.0 50% EG: 0.0 100% EG: 11.1 Control: 6.2</p> <p>Visceral variations in litters (%): 0% EG: 100.0 12.5% EG: 100.0 50% EG: 94.4 100% EG: 100.0 Control: 100.0</p> <p>Skeletal variations in litters (%): 0% EG: 100.0 12.5% EG: 100.0 50% EG: 100.0</p>	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
				<p>Increased incidence in kidney lesions:</p> <ul style="list-style-type: none"> - tubular nephrosis (10/30) versus controls (0/30), P<0.01. - tubular cell degeneration (7/30) versus control (0/30), P<0.05. - Autolysis (6/30) versus control (0/30). 	<p>100% EG: 100.0 Control: 100.0</p> <p><u>Oral gavage</u> (positive control):</p> <ul style="list-style-type: none"> - Reduced foetal body weight per litter (0.990±0.207g) versus controls (1.241±0.110g), P<0.001. - Increased number of litters with soft tissue malformations (11/16) versus control (7/23), P<0.05. - Increased number of litters with skeletal malformations (16/16) versus 	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					control (12/23), P<0.01. Visceral malformations: - Dilation of lateral ventricle (9/16 litters) versus controls (1/23 litters), P<0.01. Skeletal malformations: - Fusion of 2-12 thoracic arches - Fusion of lumbar arch (5/16 litters) versus controls (0/23 litters), P<0.05. - Fusion of lumbar centra (5/16 litters) versus controls (0/23 litters), P<0.05.	

Version date: Monday 16 March 2026

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on development	Remarks
					- Fusion of sacral centra (4/16 litters) versus controls (0/23 litters), P<0.05. - Malaligned thoracic centra (7/16 litters) versus controls (0/23 litters), P<0.01	

1

PUBLIC



Gezondheidsraad

PUBLIC DRAFT