

# Ethylene glycol

Evaluation of the effects on reproduction, recommendation for classification

To: the minister of Work and Participation

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# Samenvatting

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

## Gebruik van ethyleenglycol

Ethyleenglycol is een kleurloze vloeistof die vooral wordt gebruikt voor de productie van polyestervezels, polymeren en harsen van polyethyleentereftalaat (PET). Het wordt ook gebruikt als antivries- en koelvloeistof, bijvoorbeeld voor het vorstvrij maken van vliegtuigen en voorruitens van auto's. Verder wordt het gebruikt als oplosmiddel in verf en als weekmaker in plastics. Beroepsmatige blootstelling kan plaatsvinden via inademing, huid- of oogcontact. Dit komt bijvoorbeeld voor wanneer werknemers die betrokken zijn bij ontdooiprocedures op luchthavens worden blootgesteld

aan ethyleenglycoldampen en nevel die vrijkomen bij het vernevelen van ontdooimiddelen in de lucht.

## Classificeren naar bewijskracht

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

Een classificatievoorstel zegt iets over de bewijskracht voor de schadelijke eigenschappen van een stof, maar niet over de mate waarin mensen op de werkplek een gezondheidsrisico lopen. Dat hangt af van de mate waarin mensen op hun werk worden blootgesteld aan de stof. Daar heeft de commissie geen zicht op.



### Geraadpleegde onderzoeken

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

#### *Vruchtbaarheid*

Uit de beschikbare onderzoeken komt geen eenduidig effect van ethyleenglycol op de vruchtbaarheid naar voren. In onderzoek bij muizen werden na blootstelling aan ethyleenglycol kleinere nestgroottes en minder levende pups waargenomen. Er kunnen echter geen conclusies worden getrokken op basis van deze bevindingen, omdat het onduidelijk is of deze effecten toegeschreven kunnen worden aan de vruchtbaarheid of de ontwikkeling. Een studie in muizen en een studie in ratten toonden geen effect van ethyleenglycol op de vruchtbaarheid. Andere onderzoeken zijn van lage kwaliteit, of de onderzoeksopzet is niet geschikt om de effecten op de vruchtbaarheid te kunnen beoordelen.

De opzet van de studies die de voorplantingsorganen onderzochten is over het algemeen zeer beperkt. De commissie beoordeelt de beschikbare gegevens als beperkt, en van onvoldoende kwaliteit om een definitieve conclusie op te baseren.

#### *Ontwikkeling nageslacht*

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

#### *Lactatie*

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





### Advies aan het ministerie

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

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

### 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 kanker- verwekkende, 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.



# Executive summary

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

## Use of ethylene glycol

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

## Classification based on strength of evidence

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

A recommendation for classification reflects the strength of evidence for the hazardous properties of a substance, but it does not reflect the health risk for workers. The health risk is based on the level of exposure to the substance in the workplace. The committee does not have sufficient data on these exposure levels.



## Reviewed literature

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

### *Fertility*

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

### *Development*

Studies in rats and mice clearly showed that exposure to ethylene glycol caused adverse effects on development. The effects were mainly related to foetal body weights, skeletal malformations, post-implantation loss and lower numbers of live fetuses per litter. No adverse effects were found in rabbits up to dose levels causing maternal toxicity. This could be because of differences between rodents and rabbits in foetal accumulation of

ethylene glycol and its metabolite glycolic acid, which is the primary toxicant causing developmental toxicity. However, the committee did not find conclusive evidence that shows that the rabbit model would be a more appropriate model for evaluating the effects of ethylene glycol in humans. Therefore, the rodent studies were not excluded from the evaluation. The committee concludes that based on the clear evidence for an adverse effect on development, classification in Category 1B is warranted.

### *Lactation*

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



## Recommendations to the ministry

Based on the available scientific data, the committee recommends:

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



**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.



# 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 Substances Toxic to Reproduction 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.

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

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

In 2026, the president of the Health Council released a draft of the report for public review. The committee has taken the comments received into account in deciding on the final version of the report. These comments, and the replies by the committee, can be found on [healthcouncil.nl](https://healthcouncil.nl).

**1.3 Labelling for lactation**

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The criteria define that substances which are absorbed by women and have been shown to



interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, should be classified and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects on or via lactation is based on a risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

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

## 1.4 Data

The committee evaluated the literature on reproductive toxicity of EG. A literature search was performed using PubMed and Scopus. Only reproductive animal studies were selected. No relevant cohort studies, case-control studies, cross-sectional studies, or human experimental studies were available. The committee excluded studies on ethylene glycol monomethyl ether, ethylene glycol monoethyl ether, ethylene glycol

monobutyl ether, ethylene glycol monophenyl ether, ethylene glycol monohexyl ether and ethylene glycol diethyl ether, as these were beyond the scope of the current literature summary.

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



# 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 <sub>2</sub> H <sub>6</sub> O <sub>2</sub>                                                                                                                                                    |
| Structural formula                                                        |                                                                                                                                                                                                 |
| SMILES notation (if available)                                            | OCCO                                                                                                                                                                                            |
| Molecular weight or molecular weight range                                | 62.07                                                                                                                                                                                           |

## 2.2 Physicochemical properties

The physicochemical properties of EG are presented in Table 2.

**Table 2** Summary of physicochemical properties of ethylene glycol<sup>1</sup>

| 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 <sup>3</sup> (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)                     |

## 2.3 International classifications

EG currently has a harmonized classification in Annex VI of the CLP-Regulation (EC) 1272/2008 for:

Acute tox. 4 (H302: harmful if swallowed)



A NTP evaluation of EG did not demonstrate carcinogenicity.<sup>2</sup> Therefore, EG is not listed as carcinogen by the NTP<sup>1</sup>

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

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

EG is currently not included in the list of substances NIOSH considers to be potential occupational carcinogens.<sup>5</sup>

EG has the following classification in Australia:<sup>6</sup>

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

EG has the following classification in Japan:<sup>7</sup>

- Acute tox. 5 (H303: may be harmful if swallowed)
- Skin corrosion/irritation 3 (H316: causes mild skin irritation)
- Serious eye damage/eye irritation 2B (H320: Causes eye irritation)
- Repro. 1B (H360: may damage fertility or the unborn child)
- STOT SE 1 (H370: causes damage to organs; central nervous system,

kidney, heart, respiratory system)

- STOT RE 1 (H372: cause damage to organs through prolonged or repeated exposure; central nervous system, respiratory system, heart)



# 3 Manufacture and uses

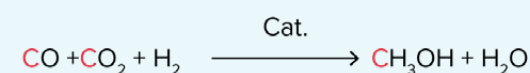
## In brief

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.

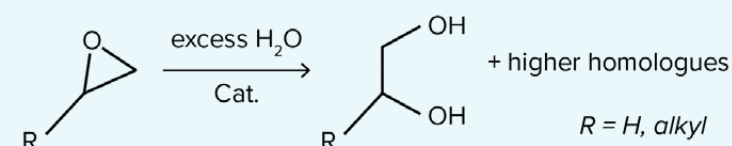
## 3.1 Manufacture

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

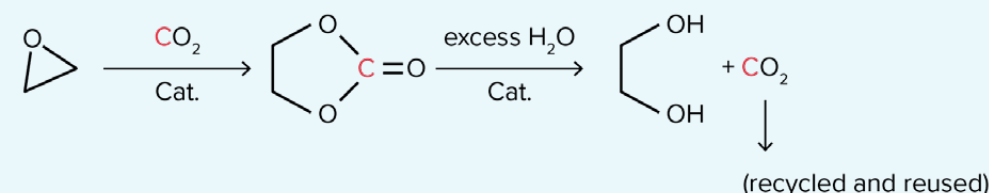
### a Industrial methanol production route



### b Hydration of epoxide to glycol



### c Industrial shell OMEGA process to produce ethylene glycol



**Figure 1** The initial steps of each industrial route for the production of ethylene glycol. The subsequent reaction steps are described in the accompanying text.<sup>8</sup>

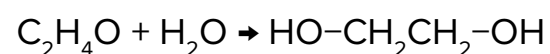
The first process is an industrial production route from carbon monoxide (Figure 1 (a)). It is used in countries producing large quantities of coal. The oxidative carbonylation of methanol to dimethyl oxalate provides an approach to the production of C1-based EG. Furthermore, dimethyl oxalate can be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



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

The second production process is from ethylene oxide (Figure 1 (b)).

The reaction with water allows the production of EG in accordance with the following chemical equation:



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

The third method of producing EG was developed by Shell. The equation is shown in Figure 1 (c). The ethylene oxide is first converted with carbon dioxide (CO<sub>2</sub>) to ethylene carbonate.<sup>9</sup> The latter is then hydrolysed in a second step to produce EG. The carbon dioxide is released again in this step and can be fed back into the production process circuit. The carbon dioxide partly results from ethylene oxide production, where a part of the ethylene is completely oxidized. According to Shell, the conversion efficiency of this process is over 99%.

### 3.2 Identified uses

EG is mostly used for the production of polyester fibres, polymers, and polyethylene terephthalate (PET) resins. It is also used for its antifreeze and

coolant properties, for example as a de-icing fluid for windshields and aircrafts. Additionally, it can be used as a desiccant for natural gas production as well as in hydraulic brake fluids, electrolytic condensers, as solvent in paints and plastics, in inks and toners, washing and cleaning products, dyes, as a softening agent in cellophane, and in the manufacture of plasticisers, solvents, polishes and waxes, for non-metal-surface treatment products and other coating products, leather treatment products, adhesives and sealants, and biocides (e.g. disinfectants, pest control products).<sup>10-12</sup>

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

It is estimated that workers are exposed mostly through inhalation, and skin and eye contact. Inhalation is an occupational hazard, for example for workers involved in airport de-icing operations by spraying the de-icing formulation through the air, generating EG vapour and mist. Dermal exposure to EG in de-icing fluids may also be hazardous, especially for workers who do not wear adequate skin protection during or after application.<sup>10</sup>



# 4 Toxicokinetics

## In brief

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.<sup>14</sup> 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.<sup>15</sup> 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.<sup>15</sup>

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.<sup>14</sup> 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.<sup>10</sup>



## 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.<sup>14</sup>

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

EG crosses the placenta and enters the developing foetus in rats and rabbits after oral administration to pregnant dams. Unchanged EG concentrations in yolk sac and embryos were 14-20% of maternal concentrations in a toxicokinetic study in rabbits.<sup>16</sup> Extraembryonic fluid levels of EG were 2-fold higher in rats than in rabbits after a single oral administration at gestation day (GD) 10.<sup>10</sup>

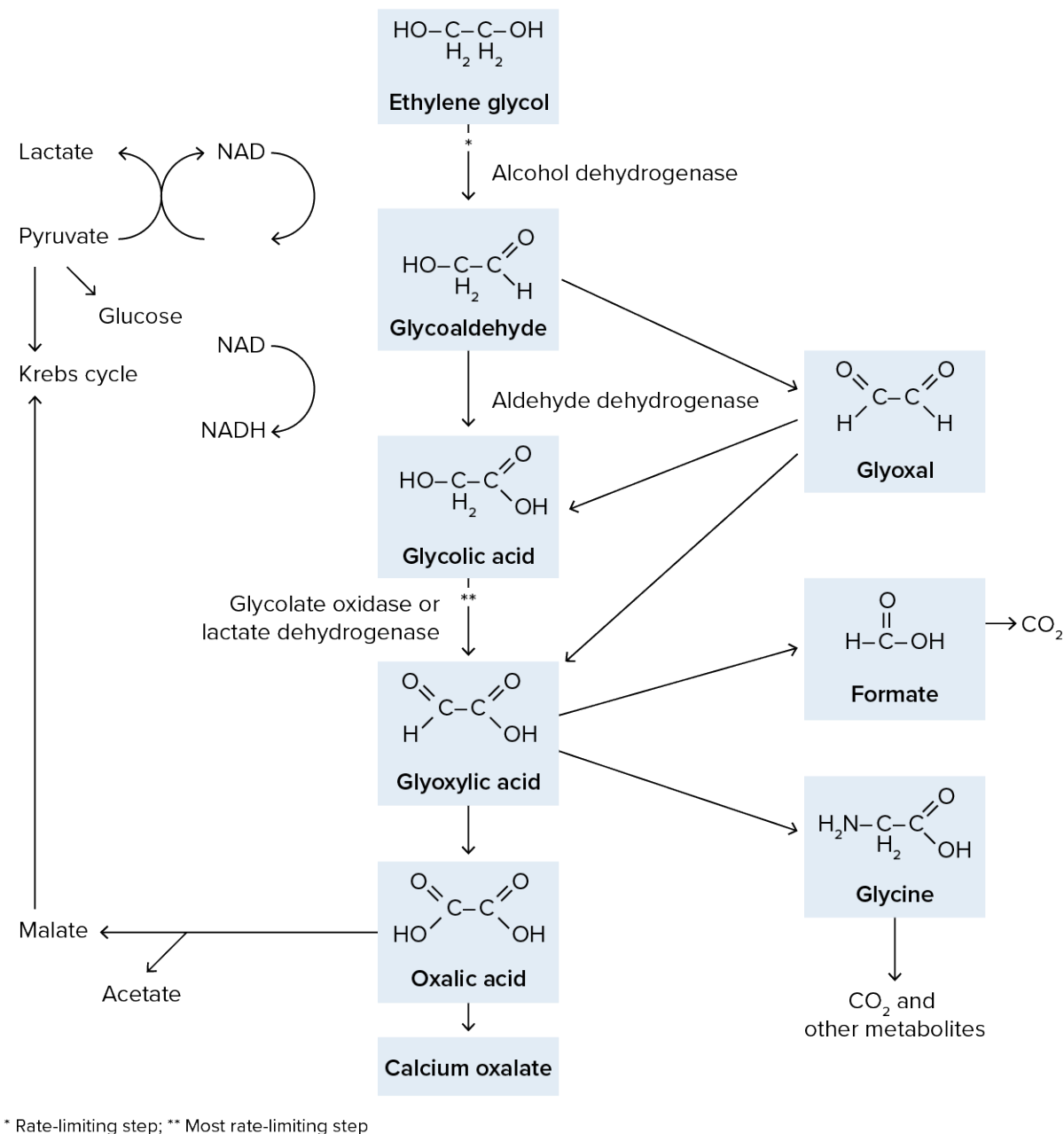
After dermal exposure, levels of EG are the highest in pelt, namely 5-6% of the applied dose or 8-15% of the applied dose when carcass and pelt are combined.<sup>10</sup>

## 4.3 Metabolism

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

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





**Figure 2** Metabolic pathway for ethylene glycol (source:<sup>10,17,18</sup>)

GA was the major metabolite in the plasma of orally exposed male rats (single dose). During the first 12 hours after dosing, at 100 and 1000 mg/kg, glyoxylate and glyoxal as well as trace levels of glycoaldehyde were detected and at 10 mg/kg glyoxylate levels exceeded glycolate levels. Plasma glycolate levels peaked 6h or 4 hours after a single dose in rats or dogs respectively where EG peaked after 2 hours in both species.<sup>10</sup>

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

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



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

Male rats exposed to  $\pm 1000$  mg/kg EG in drinking water for 21 days had urinary oxalate levels equivalent to 1.18% conversion of EG to oxalate.

In another study, male Wistar rats were treated with EG up to 400 mg/kg/day for 12 months. From  $>300$  mg/kg/day, GA levels in the kidney, blood and urine were increased compared to control. Oxalate levels were increased at  $>300$  mg/kg/day in the kidney but were similar across all doses in blood and urine. It is suggested that the metabolism of GA occurs at doses between 150 and 300 mg/kg/day in Wistar rats.<sup>10</sup>

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

One study suggested that pregnancy did not alter the pharmacokinetics in maternal blood and urine when groups of pregnant and nonpregnant rats were treated with a single dose at GD 10 (for the pregnant rats). However, a single time point is considered a too narrow exposure window to observe

pregnancy-related changes in metabolism. Foetal levels of EG or metabolites were not measured. In another study in rats, levels of GA in embryos and extraembryonic fluid paralleled maternal levels but were 1.4-4-fold higher than maternal levels. It is reported that there are species-specific differences in the transfer of GA from maternal blood to the conceptus.<sup>10</sup> These are further discussed in Chapter 5.

#### 4.4 Elimination

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

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

Rats exposed to EG vapour (32 mg/m<sup>3</sup>, 30 min) or aerosol (184 mg/m<sup>3</sup>, 17 min) excreted 63% (over 4 days) or 75% (over 6 days) as CO<sub>2</sub>. Urinary excretion was 20% or 12% of the initial body burden after vapour and



aerosol exposure respectively, while faecal excretion was 3% and 1%, respectively.<sup>10</sup>

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

In male and female rats receiving a single oral dose between 10 and 1000 mg/kg, the major excretory routes up to 96 hours of exposure were CO<sub>2</sub> exhalation (27-48% of radioactivity), urine (21-43%) and faeces (2-4%).

Female mice that were exposed similarly as the rats excreted EG as exhaled CO<sub>2</sub> (22-55%) and exhaled volatile organic compounds (3-11%), while excreting 24-56% via urine and 5-6% in faeces. The majority was eliminated within 12 hours after dosing. An increase in urinary excretion was evident between 10-100 mg/kg in female mice, between 10 and 400 mg/kg in female rats and between 800 and 1000 mg/kg in male mice. This probably resulted from saturation of enzymes that metabolize GA leading to increased excretion of this metabolite via urine.<sup>10</sup>

Monkeys given a single oral dose ( $\pm$ 1000 mg/kg) excreted 24% of the dose as the parent compound in urine within 48 hours. Dogs excreted about

50% of a single administered oral dose (173 mmol/kg) via urine within 72 hours.<sup>10</sup>

Rats and mice were treated with dermal application of 10-1000 mg/kg undiluted or 1000 mg/kg 50% aqueous solution of radiolabelled EG under occlusion for 6 hours. When measured over 96 hours after dosing, rats expired 6-14% of the administered dose, 4-8% was excreted in urine and 1% in faeces. Female mice exhaled volatile organic compounds (21-34%) and CO<sub>2</sub> (10-16%). No dose-related shift in excretory patterns was observed suggesting that metabolic pathways were not saturated with dermal exposure.<sup>10</sup>

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



# 5 Mechanism of action and mechanism of reproductive toxicity

## In brief

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.<sup>1,3-5,8</sup> Exposure to GA in rats produced effects matching the dysmorphogenesis seen after EG exposure in vivo in rodents at relevant concentrations.<sup>2</sup> 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 ( $\leq 12.5$  mM) did not show effects, while in reported rat WEC studies, effects were seen at much lower concentrations of GA ( $\geq 3$  mM).<sup>6</sup> 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.<sup>1</sup> 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 crystals<sup>1</sup>.

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 acidosis can contribute to GA developmental toxicity. Developmentally toxic effects of GA can occur in the absence of metabolic acidosis, but the effects are enhanced in the presence of metabolic acidosis.<sup>19,20</sup> The metabolic acidosis and hyperosmolarity resulting from EG exposure in rats were suspected to contribute to an increased occurrence of foetal abnormalities and reduced foetal body weight.<sup>19</sup>

## 5.3 PBK model for humans

A Physiologically based kinetic (PBK) model was developed to compare rat and human kinetics of EG.<sup>30</sup> The model was validated by comparing internal dose surrogates in rats and humans and by the use of human clinical case reports. The authors concluded that humans are unlikely to achieve blood levels of GA associated with developmental toxicity in rats.<sup>30,31</sup>

The committee agrees that the PBK model may be useful in risk

assessment to translate from rat to human and make a prediction at the maternal level by translating the external exposure scenarios into internal exposure scenarios in the form of maternal blood levels of EG and GA. It should be noted that based on these models alone, no conclusion can be drawn regarding the percentage of EG or GA accumulating in the rat or human embryo, as the models do not include an embryonic compartment. Importantly, no PBK model was developed that would allow a comparison between rabbits and humans. It is not clear to what extent the rabbit model would be relevant for extrapolation to human systemic and compartmental exposure scenarios as a rabbit PBK model is lacking.



# 6 Adverse effects on sexual function and fertility

## In brief

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

As follow-up of Lamb et al. (1985), a non-guideline continuous breeding study was performed by Gulati et al. (1986) in CD-1 mice.<sup>33</sup> Similarly, 20 animals per sex per group were dosed at 0.5, 1.0, and 1.5% EG in drinking

water, equal to 0, 897, 1798 and 2826 mg/kg bw/day. A control group consisted of 40 animals per sex. Mice were exposed during the premating period (7 days), during the breeding period (98 days), the segregation period (21 days), offspring was killed except for the last litters (F1), where exposure continued throughout the life of the offspring. Weaned animals of the last litters from the high dose and controls were mated in a cross-over design. Additionally, weanlings (F1) of all treatment groups were mated with partners of the same treatment group. In both the cross-over and the second-generation studies females were allowed to litter. Exposure occurred during premating, cohabitation, segregation and throughout the life of offspring. In the continuous breeding study, 2 control animals (1 male and 1 female) and 4 animals at 1.5% EG (3 males and 1 female) died during either the pre-cohabitation or cohabitation phase. Moreover, statistically significant lower body and liver weights were observed in P<sub>0</sub> parental male mice in the 1.5% exposed group. No effects on body weight or treatment-related lesions in kidney, liver, ovary, uterus, or vagina were observed in female mice. The fertility index (number of fertile / number of cohabited pairs) was 88% in the 1.5% exposure group (16 cohabited) and 100% in the other exposure groups (20 cohabited) and controls (38 cohabited). The number of live pups in the consecutive litters (9.99 vs. 11.81 live pups per litter in controls) and their weight (1.46 vs. 1.57 g in controls for combined weight) were significantly decreased. The proportion of pups born alive was unaffected by treatment with EG. In the cross-over study, the mating index (no. with copulatory plugs/no. cohabited) was higher in EG



treated groups and the fertility index (No. fertile/No. cohabited) was 50% in all groups including controls that were mated with controls. In the cross-over mating of control females with males exposed to 1.5% EG, live pups per litter and the proportion of pups born alive were unaffected. In males at 1.5% EG, sperm motility and density (not statistically significant) were lower compared to control and the percentage of abnormal sperm was higher compared to control. Histologic changes were observed in the testes from many of the male animals, consisting of degeneration of seminiferous tubules, loss of spermatozoa, spermatids, spermatogonia and spermatocytes and vacuolization of epithelial cells. Histologic changes were also noted in the kidneys of treated males. EG treatment did not interfere with oestrous cycle. No differences in organ weights were noted in both sexes after correction for body weight at necropsy. In addition, the reproductive performance of the F<sub>1</sub>-animals was evaluated by mating 20 F1 generation animals/sex of all treatment groups around day 74 of age. Mating was continued until a copulatory plug was found or for 7 days. No effects on body weight were noted, but 18% (i.e. 9 out of 49) weaned males at 1.5% EG died prior to cohabitation (vs. 0, 0 and 3% in the control, 0.5, 1.0% EG groups). No female pups died in any of the treatment groups.

No statistically significant changes were noted in mating index (85, 90, 95 and 75% at 0, 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% EG). Moreover, EG treatment did not affect the number of live pups per litter, the proportion of pups born alive and the sex ratio, although pup body weight was reduced at all 3 dose levels (without a dose

relationship). No differences in organ weights were noted in both sexes after correction for body weight at necropsy, with the exception of testis and epididymis weights for males exposed to 1.0 and 1.5% EG. The groups exposed to 0.5% and 1% EG showed a significant reduction in sperm density and the 1% and 1.5% dose groups showed a significant reduction in sperm motility in F1 mice. Although not statistically significant, the percentage of abnormal sperm was higher in males treated at 1.0 and 1.5% EG. In females, EG treatment did not interfere with oestrous cycle.<sup>33</sup>

The committee notes excessive toxicity (18% mortality) in the F<sub>1</sub> generation at 1.5% EG. Although for the F<sub>0</sub> generation the fertility index in controls was 100%, ideally the exposed groups would have been compared to historical control data to see whether the effects in the high dose were due to exposure or whether these occurred within the range of normal biological variation. In the F<sub>2</sub> generation, no statistically significant changes were noted in mating and fertility indices and these indices were generally more variable (as compared to 100% in most groups of the F<sub>0</sub>-generation).

DePass et al. 1986 performed a non-guideline three generation reproduction study.<sup>34</sup> Fisher 344 Rats (10 males and 20 females per group) were treated with 40, 200, and 1000 mg/kg bw/day EG in diet (calculated exposures based on dietary intake were 40-50, 200-300 and 1000-1300 mg/kg/day for males and 40-60, 200-300 and 900-1200 mg/kg/day for females). Rats were exposed starting 7 weeks of age, during a 7-week premating period and throughout breeding. The F<sub>1</sub> and F<sub>2</sub> offspring (10



males and 20 females per group randomly chosen from the litters) was exposed throughout life and subsequently mated at approximately 100 days of age. No treatment related effects on mortality, diet intake or body weight changes were observed. No treatment related effects on fertility index, gestation index, gestation survival index or days from first mating to litter were observed for all 3 generations. Moreover, pup survival indices (postnatal day (PND) 0-4, 4-14 and 4-21) were unaffected, as well as pup body weights.<sup>34</sup> Effects on developmental toxicity described within this study are discussed in Chapter 7.2. The committee concludes that this study did not show effects of EG on fertility up to the limit dose of 1000 mg/kg/day.

Harris et al. (1992) performed a non-guideline 21-day developmental toxicity study in which also a few fertility parameters were examined.<sup>35</sup> CD-1 mice (10/sex/group) were exposed to 0, 250, 700, and 2500 mg/kg/day EG in water by oral gavage. Proven fertile males were exposed from study day 3 to 7 and during cohabitation (day 8 to 13) and killed on day 20. Females were exposed from study day 0 to day 21. No treatment related effects on clinical signs, body weight, liver and kidney histopathology, testes weight or sperm number or mobility were observed in male mice. Continuous exposure of females did not result in clinical signs or mortality and the number of pregnant females was unaffected. At the highest dose (2500 mg/kg/day), EG exposure resulted in fewer live (7.4 vs. 10.0 in control) and more dead implants (1.4 vs. 0.4 in control). Although not statistically

significant, the total number of implantation sites in females at 2500 mg/kg/day was lower, i.e. 8.9 vs. 10.4 in control.<sup>35</sup> The committee evaluates the evidence for an effect on fertility as not convincing. Mice were exposed during a relatively short period, not covering the entire spermatogenic cycle (males). Nevertheless, exposure at the high dose level resulted in a lower number of live implantations and a higher number of dead implantations and although not statistically significant, a lower number of implantation sites was noted in exposed females. Despite the short exposure scenario, the data could be indicative of an effect on fertility.

#### *Studies on reproductive organs*

A non-guideline study was performed by Hong et al. (1988) with male and female B6C3F1 mice.<sup>36</sup> EG was administered via oral gavage for 4 consecutive days at doses of 0, 200, 400, and 1000 mg/kg bw/day (7/sex/group). No mortality occurred. Statistically significant reduction in body weight was observed for only female mice in the 1000 mg/kg bw/day group. No histological evidence of organ or tissue damage and no significant effects on testicular weight were observed.<sup>36</sup> The committee considers an exposure duration of 4 days as too short to be able to draw firm conclusions. The committee does not take this study into consideration in its evaluation for effects on fertility.

Bolon et al. (1997) studied ovary samples of mice from 18 NTP continuous breeding studies in an additional evaluation of the data generated by



Gulati et al. (1986).<sup>37</sup> CD1 mice (20/sex/group) were exposed to 1% EG in drinking water (equal to 1798 mg/kg bw/day). The study also included 0% controls (40/sex). Mice were exposed during the premating period (7 days), during the breeding period (98 days), the segregation period (21 days) and throughout the life of the offspring. Offspring (20/sex/group) was dosed with 0.5%, 1% or 1.5% EG in drinking water (equal to 897, 1798 and 2826 mg/kg bw/day). General toxicity parameters were not reported in the Bolon paper. No statistically significant changes in ovarian follicle counts were noted in both the F0 and F1 generation.<sup>37</sup> The committee considers the study design to be of limited value to deliver conclusive information.

### 6.3 Evaluation of the data

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

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

### Overall fertility

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

In the follow-up continuous breeding study by Gulati et al. (1986) it was also unclear whether the lower number of live pups could be attributed to an effect on fertility or development, since also in this study, the study design does not allow for assessment of the number of implantation sites.<sup>33</sup> In this study the dosages were higher than in the study by Lamb et al. and general toxicity was observed at the highest dose for both generations (up to excessive toxicity in the F1-generation at 1.5% EG in drinking water; equal to



2826 mg/kg bw/day), especially in male mice.<sup>33</sup> Effects were reported on testes histopathology, testis and epididymis weights, sperm motility and density and abnormal sperm, but the fertility and mating indices were not affected.<sup>33</sup>

No fertility effects were observed in a well performed three-generation study in rats (although not performed according to the current guidelines) administering EG via diet with dosages up to 1000 mg/kg bw/day.<sup>34</sup>

No treatment related effects on general toxicity endpoints were observed in terms of mortality, diet intake or body weight changes. No treatment related effects on fertility index, gestation index, gestation survival index or days from first mating to litter were observed for all 3 generations.<sup>34</sup>

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

### Reproductive organ parameters

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

Two other studies in mice did not report effects on testes and sperm parameters, but the exposure durations were too short to be able to draw firm conclusions with regards to the testes and sperm parameters.<sup>35,36</sup>

The studies that specifically evaluated mice female reproductive parameters also had limitations in design and exposure duration, making it difficult to draw firm conclusions.<sup>36,37</sup>

## 6.4 Conclusion

All studies described have limitations. Overall, 2 mice studies reported lower litter sizes and lower number of live pups at high dose levels for which it is unclear whether they can be attributed to fertility or development because the number of implantation sites could not be specified in these studies.<sup>32,33</sup> One three-generation study showed no fertility effects in rats.<sup>34</sup> A lower number of live uterine implants was observed in a developmental study in mice. However, this study was not optimally designed to



assess fertility endpoints.<sup>35</sup> The studies on reproductive organs were overall very limited in design. One well-designed study in mice reported effects on the histopathology of the testes and sperm parameters, although no functional fertility effects were observed.<sup>33</sup>

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

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



# 7 Adverse effects on development

## In brief

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.<sup>38</sup> 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 GD 21. 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).<sup>38</sup> 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.

A non-guideline developmental toxicity study was performed by Price et al. (1985).<sup>39</sup> The study was GLP compliant and performed by the NTP. The authors performed a study in rats and mice (described later). Female CD rats were treated with EG at 0, 1250, 2500 or 5000 mg/kg/day in water via oral gavage from GD 6 through 15. C-section was performed on GD 20. Two replicates of the study were performed making the total number of animals per group 20. All treated rats showed piloerection and treated rats at the mid and the high dose showed an increased water consumption compared to control. There was a dose-related decrease in all maternal body weight parameters and gravid uterine weight. Maternal weight gain during treatment (GD 6-GD 15) was statistically significantly lower in all treatment groups. Maternal liver weight (high dose) and relative kidney weight (mid and high dose) were significantly decreased compared to controls. There was a decrease in the number of live foetuses per litter at mid dose ( $11.90 \pm 0.60$ ) and high dose ( $11.04 \pm 0.79$ ) vs  $13.54 \pm 0.28$  in the control), and in the average foetal body weight at mid dose ( $2.916 \pm 0.056$  g) and high dose ( $2.388 \pm 0.089$  g) compared to the control ( $3.404 \pm 0.052$  g). The following developmental toxicity parameters were affected in a dose-

related manner: an increase in post-implantation loss per litter (high dose only,  $21.34 \pm 5.24\%$  vs  $4.70 \pm 1.23\%$  in control), an increase in the percentage of malformed live foetuses per litter (mid dose ( $25.11 \pm 4.84\%$ ) and high dose ( $75.53 \pm 6.42\%$ ) compared to the control ( $1.37 \pm 0.97\%$ ), and the percentage of litters with one or more malformed live foetuses at the low (39.29%), mid (68.97%) and high dose (96.15%). For all morphological defects, a dose response relationship was observed. External malformations were significantly increased at the high dose (15 vs 0 in control), visceral malformations were increased at the low (6 vs 0) and high dose (8 vs 0), and skeletal malformations and the mid (19 vs 2) and high dose (24 vs 2). The authors reported malformations which included, amongst others, cleft palate, cleft lip, anophthalmia, meningoencephalocele, gastroschisis, exencephaly, anomalies of great vessels and malformations of ribs, arches and centra.<sup>39</sup> The committee points out that a dose-response relationship was only found for skeletal malformations. Anomalies of the great vessels, hydroureter and hydronephrosis that are generally considered variations were reported incorrectly as malformations.

A non-guideline three generation reproduction study was performed by DePass et al. in 1986.<sup>34</sup> Fisher 344 Rats (n=10 males and 20 females/group) were treated with 0.04, 0.2, and 1.0 g/kg/day EG (purity >99.9%) in diet. Rats were exposed during a 7-week premating period and throughout breeding. Offspring (n=10 males and 20 females/group randomly chosen from the litters) were exposed throughout life and breeding was started at



100 days of age. No treatment related effects on mortality, diet or body weight changes were observed. One second generation female and one second generation male in the high dose group showed mild focal interstitial nephritis but this condition was also observed in 2 control group pups. One third generation female in the high dose group showed mild focal tubular hyperplasia but this condition was also observed in 2 control male pups. Neonatal body weights at day 4, 14, or 21 postpartum were not affected after treatment with EG.<sup>34</sup> The committee considers the available data too limited to allow a conclusion for developmental related effects.

A non-guideline developmental study was performed by Price et al. (1988) (GLP compliant; performed by the NTP).<sup>40</sup> Female CD rats (N=4-5/group/replicate, multiple replicates to generate at least 20 F1 litters per dose group) were treated with EG at 0, 250, 1250 and 2250 mg/kg bw/day via oral gavage during GD 6-20. Dams were euthanized on PND 1. Pups were fostered by untreated dams as of post-natal day 1. Litters were culled to 8 pups per litter. A reduced maternal body weight, reduced gestational weight gain, increased kidney weight and reduced uterine weight were observed in the high dose group. Statistically significantly longer gestational periods were observed in the 1250 ( $21.58 \pm 0.07$  vs  $21.26 \pm 0.07$  in control) and 2250 mg/kg bw/day ( $21.84 \pm 0.09$  vs  $21.26 \pm 0.07$ ) exposure groups. A reduced live litter size and increased mortality per litter was observed in the high dose group ( $11.87 \pm 0.48$  vs  $13.67 \pm 0.33$  in control). Offspring showed reduced body weight on PND 1 and 22 in the high dose

groups. A reduced kidney and brain weight was observed on PND 22 and 63 in the high dose groups. Exposure to 2250 mg/kg bw/day also resulted in a reduced time to achieve wire grasping ( $9.71 \pm 0.18$  days vs  $11.98 \pm 0.78$  days in control). Hydrocephalus (n=6 of 88 pups examined at PND 22) was observed in the high dose. EG exposure did not result in external or visceral defects but did show an increase in skeletal malformations in the high dose group, such as malformed ribs, centra and sternbrae. In this group, 30% of the male and 60% of the female offspring showed malformations, compared to 0% in control groups.<sup>40</sup> The committee notes that selectively downsizing the litters could have caused bias. It is unclear how the findings on hydrocephalus relate to the historical control data.

The reduced kidney and brain weight in the offspring were reported as absolute values, whereas these are likely secondary to the effects seen on the lower body weight of the pups. Overall, clear developmental effects were present, even at late postnatal age.

A non-guideline developmental study was performed by Marr et al. (1992) and was a follow-up to the studies by Price et al. (1985, 1988).<sup>41</sup> Pregnant Sprague-Dawley rats (n=7 per group) were treated with 2500 mg/kg/day EG via oral gavage from GD 6-15. A vehicle control group was included. Dams were euthanized on GD 18, 20 or PND 1, 4, 14, 21 or 63. The focus of the study was on skeletal malformations and ossification and whether these effects could be outgrown over time. Maternal gestational weight gain was statistically significantly 3-6% lower compared to control at GD 11,



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

A non-guideline developmental study in rats and mice was performed by Neeper-Bradley et al. (1995).<sup>42</sup> The study in mice will be described later in this document. Per group, 25 rats were treated once daily with EG from GD 5 through GD 15 at dose levels of 0, 150, 500, 1000 and 2500 mg/kg bw/day via oral gavage. Dams were euthanized on GD 21. Maternal toxicity was observed at 2500 mg/kg bw/day. Body weight gain was significantly lower at GD 6-9 and GD 15-18 and the final body weight was also lower compared to controls. During treatment, water consumption was significantly increased. Gravid uterine weight was decreased compared to controls and absolute and relative kidney weights as well as relative liver weights were increased at 2500 mg/kg bw/day. In addition, the relative liver weight was increased at 1000 mg/kg bw/day, but whether this effect is adverse remains uncertain in absence of any histopathology. In foetuses, most malformations occurred at a dose of 2500 mg/kg bw/day. A number of external and soft tissue malformations were observed such as gastroschisis (7/21 vs 0/24 in control litters), hydrocephaly (10/21 vs 0/24), severe lateral ventricle dilation (6/21 vs 0/24), umbilical hernia (5/21 vs 0/24) and foetal atelectasis (19/21 vs 12/24). Moreover, increased incidences of 31 skeletal malformations were observed primarily in the thoracic region, for example missing ribs (20/21 vs 0/24) or thoracic arches (17/21 vs 0/24). At 500 mg/kg bw/day an increased incidence in poorly ossified supraoccipital was observed (8/22 vs 2/24). At 1000 mg/kg bw/day, there was one foetus with gastroschisis (non-significant effect), and increased incidences of skeletal malformations were observed, including extra 14 thoracic centrum



and arches (8/23 vs 1/24), missing rib (8/23 vs 0/24), and missing thoracic arches (6/23 vs 0/24).<sup>42</sup> The committee concludes that maternal toxicity is evident in the high dose but is considered unrelated to the observed developmental effects. The committee considers the maternal NOAEL to be 1000 mg/kg bw/day. The foetal NOAEL would be 500 mg/kg bw/day, a dose where some developmental toxicity was observed. Overall, the committee considers this study as robust.

Carney et al., 1999 performed a non-guideline, GLP compliant developmental toxicity study to investigate the toxicokinetics of EG.<sup>20</sup> Female Sprague-Dawley rats (25/group) were exposed to a single dose of 2,500 mg/kg/day EG or distilled water (control) via gavage on GD 10 and necropsy took place on GD 21. In treated dams, an increase in kidney weight and liver weight was observed, while a decrease in maternal body weight was observed on GD 12, 16, and 21. No effects were observed on pregnancy rate, litters with viable foetuses, corpora lutea/dam, implantations/dam, % preimplantation loss/dam, foetuses/litter, and foetal sex ratio (M:F). Only a significant difference was observed in the percentage resorbed implantations (15.9 at 2,500 mg/kg vs 2.5 at 0 mg/kg). Significant increased external malformations were found at 2,500 mg/kg/day, including meningoencephalocele (3.5% foetuses affected (25.0% litters affected)), exencephaly (5.2 (25.0)), cleft lip (3.5 (29.2)), cleft palate (2.8 (29.2)), and omphalocele (9.8 (54.2)), as well as visceral malformations, including dilated cerebral ventricles (15.9 (33.3)). Also skeletal malformations,

including 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)), skeletal variations, and a decrease in foetal bodyweight ( $3.75 \pm 0.58$  g vs  $0.553 \pm 0.30$  g in control) were found at 2500 mg/kg/day.<sup>20</sup>

The committee notes that only one dose was tested and was administered at a single timepoint, so no dose-response relationship could be assessed.

#### *Mice studies*

Schuler et al. (1984) performed a non-guideline developmental study with female CD-1 mice.<sup>43</sup> First, a dose range-finding study was performed to determine the lethal dose. For the developmental study, pregnant dams (n=50) were treated with 11090 mg/kg (LD10) EG via oral gavage from GD 7 to GD 14. A vehicle control group was included (n=50). Pups were sacrificed 3 days after birth. Mortality in treated pregnant dams (5/50) was higher than in controls (0/50), but not statistically significant. The number of viable litters was decreased in treated dams compared to the controls (15/37 vs 29/29 in control) and the number of dead pups per litter was increased (1.5 vs 0.1). The number of live pups per litter (2 vs 9), the pup postnatal survival (40% vs 100%), pup weight gain (0.2 g vs 7 g) and pup birth weight (1.4 vs 1.7) were all statistically significant decreased compared to controls.<sup>43</sup> It is unclear to the committee when or why maternal animals died. Although no statistics were performed on mortality of the dams, the committee assessed this as high, since the observed mortality is reaching the limit of



common practice (10%). The number of viable litters was low, also in the control group. Nonetheless, the dose group showed lower number of litters, and a lower survival compared to controls. Since only one dose was tested, no dose-response relationship could be assessed.

A non-guideline GLP-compliant developmental toxicity study was performed by Price et al. (1985, NTP study) in rats (described earlier) and mice.<sup>39</sup> Female CD-1 mice were treated with EG at 0, 750, 1500 or 3,000 mg/kg/day via oral gavage from GD 6 through GD 15. C-section was performed on GD 17. Two replicates of the study were performed making the total number of animals per group 20. Dams of the mid and high dose group showed a significantly decreased weight gain during treatment, a decreased liver weight and decreased gravid uterine weights. There was an increase in post-implantation loss per litter reaching statistical significance at the high dose level. In addition, a dose-related decrease in live litter size was observed in the high dose group ( $9.83 \pm 0.56$  vs  $11.88 \pm 0.49$  in control), and a dose-related decrease in average foetal body weight per litter was observed in all dose groups (low ( $0.882 \pm 0.017$ g), mid ( $0.787 \pm 0.024$ g) and high dose ( $0.712 \pm 0.022$ g) compared to control ( $0.974 \pm 0.013$ g)). There was a dose-related increase in the percentage of malformed foetuses per litter (low dose ( $10.0 \pm 1.96$ %), mid dose ( $37.77 \pm 6.30$ %) and high dose ( $56.54 \pm 6.80$ %) compared to control ( $0.25 \pm 0.25$ %)). Furthermore, the percentage of litters with at least one malformed foetus was increased in all dose groups (low (66.67%), mid

(81.82%) and high dose (96.65%) compared to control (4.0%)). External malformations, including exencephaly, meningoencephalocele, cleft palate, cleft lip, and facial cleft were significantly increased in the high dose group compared to the control (8 litters vs 0 in control), as well as visceral malformations, including aortic stenosis, hydronephrosis, hydroureter, retro-tracheal or retrooesophageal pulmonary artery (7 vs 0). Skeletal malformations, including malformed ribs, malformed arches, and malformed centra were significantly increased at all dose levels (low dose (15 litters), mid dose (17), and high dose (22) vs control (1)).<sup>39</sup> According to the committee, this study in mice is more convincing than the rat study by the same authors. The reported malformations were not always malformations but in some cases variations (see also summary on rats). Nonetheless, clear developmental effects of EG were observed in terms of skeletal malformations at dose levels where no maternal toxicity occurred. Since the reduction in the number of implantation sites per litter was not dose-related, it was considered unlikely to be related to EG treatment.

Neeper-Bradley et al. (1995) performed a non-guideline developmental study on female CD-1 mice ( $n=30$ /group).<sup>42</sup> They were treated once daily with EG from GD 5 through GD 15 at dose levels of 0, 50, 150, 500, and 1500 mg/kg bw/day via oral gavage. Dams were euthanized on GD 18. Although not statistically significant, some deaths occurred in treatment groups 50, 500 and 1500 mg/kg bw day. There was a slightly lower maternal weight gain and body weights at 1500 mg/kg bw/day (not



statistically significant). Foetal body weights per litter were reduced at 1500 mg/kg bw/day ( $1.156 \pm 0.11$  g vs  $1.325 \pm 0.09$  g in controls). Exencephaly was noted in 2 litters at 500 mg/kg bw/day and 3 litters at 1500 mg/kg bw/day, however this was not statistically significant. Some skeletal variations were observed at 500 mg/kg bw/day (Extra rib 14, first lumbar arch, bilateral (17/24 vs 4/19 control litters)). At 1500 mg/kg bw/day, skeletal malformations occurred, including fused thoracic arches (8/21 vs 0/19), fused ribs (15/21 vs 1/19), extra thoracic centrum and arches (10/21 vs 0/19), and extra bilateral rib and thoracic arches (10/21 vs 0/19).<sup>42</sup> The committee concluded that maternal toxicity at the high dose was considered minimal. Developmental effects were found at the high dose and included decreased foetal weight and skeletal malformations.

A non-guideline fertility assessment by continuous breeding was performed by Lamb et al. in 1985, who also reported some developmental effects (see also Chapter 6.2).<sup>32</sup> This study was a dose-range finding study for the study of Gulati et al. 1986. CD-1 mice (20/sex/group) were dosed at 0.25, 0.5 and 1% EG in drinking water (equals to 410, 840 and 1640 mg/kg bw/day). A control group of 40 mice per sex was present. Exposure occurred during a premating period, cohabitation and breeding, segregation and throughout the life of the offspring. No general toxicity occurred in the parental animals, but some deaths occurred in all groups. Regarding developmental effects, a lower live pup weight and lower number of live pups per litter were observed in the high dose compared to controls. Also,

the number of live pups per litter and live pup weights were lower (not significant). A pattern of skeletal defects was observed in the offspring in treated mice only, although this was not quantified. Skull, sternbrae, ribs and vertebrae were affected in both males and females.<sup>32</sup> The committee points out that since this is a fertility study, the data on development are limited. However, the effects on number of live pups and pup weight together with the reported skeletal defects provide an indication that developmental toxicity becomes apparent at the high dose where minimal parental toxicity was observed.

As a follow-up to the study of Lamb et al. (1985), a non-guideline continuous breeding study was performed by Gulati et al. in 1986 (also described in Chapter 6.2).<sup>33</sup> CD-1 mice were dosed at 0.5, 1.0 or 1.5% EG in drinking water, vehicle controls were included (equals to 0, 897, 1798 and 2826 mg/kg bw/day). The animals were exposed during premating, breeding, segregation, and throughout the life of the offspring. The pup numbers per litter were lower at 1.5% as compared to controls ( $p < 0.05$ ). Both absolute and adjusted pup weights (male, female, and combined) were also adversely affected in a dose-related pattern at all 3 dose levels ( $p < 0.05$ ). A significant number of pups delivered by breeding pairs in the 1.0 and 1.5% EG dose groups showed distinct facial deformities, including in some cases cleft palate and ablepharon. Weanlings of the last litter of the continuous breeding phase were mated on PND  $74 \pm 10$ . A significant number of these weanlings showed facial abnormalities during the early



post-partum phase. There were no effects on body weight, kidney and liver weights in the males and females of the parental generation. In males no effects were noted on seminal vesicle, right cauda and prostate gland, but the relative weight of the right testis and right epididymis were significantly lower in the 1.0 and 1.5% groups. EG treatment had no significant effect with respect to the number of live pups per litter (male, female, and/or combined), proportion of pups born alive, and the sex ratio. The adjusted live pup weight (males and females combined) was minimally reduced ( $p < 0.05$ ) at all 3 dose levels.<sup>33</sup> The committee notes that the study is primarily intended to assess fertility rather than development. In the continuous breeding phase, there were some indications for developmental effects (facial abnormalities), but the effects on development (i.e. pup weight) in the second generation were considered minimal.

A non-guideline 21-day developmental toxicity study was performed by Harris et al. in 1992.<sup>35</sup> CD-1 mice (10 per group) were exposed to 0, 250, 700, and 2500 mg/kg/day EG in drinking water. Females were treated between GD 8 and 14. No adverse clinical signs were observed. The 2500 mg/kg/day EG exposure group showed a significant decrease in mean litter weight.<sup>35</sup> The committee considers this screening study as limited.

A decrease in mean litter weight was observed only in the high-dose group. However, it is not clear how many litters were included. Compared to the control, this may or may not be considered an adverse effect.

#### *Rabbit studies*

Tyl et al. (1993) studied New Zealand White rabbits (N=23-24/dose) administered with EG by gavage on GD 6-19 at doses of 0, 100, 500, 1000, and 2000 mg/kg bw/day.<sup>44</sup> After exposure to 2000 mg/kg bw/day, 42.1% mortality, three early deliveries and one spontaneous abortion were observed. Maternal absolute kidney weight (but not relative weight) was slightly increased at 2000 mg/kg bw/day (to 106.3% of the control value of the right kidney and 107.6% of the control of the left kidney). This was accompanied by renal lesions, which were limited to the cortical renal tubules and included intraluminal crystals, epithelial necrosis, and tubular dilation and degeneration. The death of the dams at 2000 mg/kg bw/day was directly related to the acute renal failure from crystal deposition, consistent with third-stage toxicity, including the presence of oxalate crystals. No maternal toxicity occurred at  $\leq 1000$  mg/kg bw/day. There was no indication of developmental toxicity at any dose tested, including no effects on pre- or post-implantation loss, number of foetuses, foetal body weight, or sex ratio (% male foetuses) per litter, and no evidence of teratogenicity.<sup>44</sup> The committee assesses this study as negative for developmental toxicity. Exposure to a dose of 2000 mg/kg bw/day showed significant maternal toxicity in the kidney, without effects on the developing offspring. At the lower dose of 1000 mg/kg bw/day no abnormalities were observed.



### 7.2.2 Inhalation studies

A non-guideline developmental inhalation study was performed by Tyl et al. (1995a).<sup>45</sup> Female CD rats and CD-1 mice were exposed to EG for 6 hours per day from GD 6 through GD 15. EG was nebulized, and animals were exposed to 0, 150, 1000, 2500 mg/m<sup>3</sup> EG aerosols (2.4 µm) in inhalation chambers through whole body exposure. Depending on the lung retention and the level of grooming of the contaminated fur, the authors estimated a theoretical dose of 947 mg/kg/day corresponding to an exposure of 2500 mg/m<sup>3</sup>. No mortality was observed within this study. No effects on food or water consumption, body weight or weight gain were observed in rats. The highest exposure group in rats showed an increase in liver weight. No effects on gestational parameters or malformations were observed in rats. CD-1 mice showed a significant reduction in maternal body weight and uterus weight in the 1000 and 2500 mg/m<sup>3</sup> exposure groups. Moreover, mice showed a reduction in viable implants in the 2500 mg/m<sup>3</sup> exposure group. Also in mice, a statistically significant increase was observed in the incidences of a number of external, visceral and skeletal malformations in the 1000 and 2500 mg/m<sup>3</sup> exposure groups, such as cleft palate, exencephaly, misshapen nasopharynx, and protruding tongue. Wet fur due to deposition of EG was observed in all exposure groups. Based on the amount of EG in extract from fur, oral exposure was calculated to be the major route of exposure to the extent of 64-90%.<sup>45</sup> The committee notes that both rats and mice received the same exposure levels that may not have been in proportion to the species (see also Chapter 7.3). No effects

were observed in rats although potentially maternal toxicity effects in the liver were noted in the high exposure group. In mice, clear maternal toxicity effects and developmental effects were observed in the mid and high exposure groups. This difference may be explained by the wet fur and subsequent oral ingestion of EG in mice.

A non-guideline developmental inhalation study was performed by Tyl et al. in 1995b.<sup>46</sup> Female CD-1 mice were exposed to EG for 6 hours per day from GD 6 through GD 15. EG was nebulized, and animals were exposed to 0, 150, 1000, 2500 mg/m<sup>3</sup> EG aerosols (1.7 µm) through nose-only or to 0 or 2100 mg/m<sup>3</sup> EG aerosols through whole body exposure (positive control group). Mortality was observed in controls and the 2500 mg/m<sup>3</sup> EG nose-only exposure group, found to be due to asphyxiation related to the exposure method and not EG related. Nose-only EG exposure did not result in alterations in maternal body weight, weight gain or liver weight. Increased kidney weight was observed in the 1000 and 2500 mg/m<sup>3</sup> exposure groups. A non-significant reduction in live foetuses per litter was observed in the 1000 and 2500 mg/m<sup>3</sup> exposure groups and a significant reduction in foetal body weight was observed in the 2500 mg/m<sup>3</sup> exposure group. No treatment-related increases in external or visceral malformations were observed. A significant increase in the incidence of fused ribs (malformation) and skeletal variations was observed in the 2500 mg/m<sup>3</sup> exposure group.<sup>46</sup> The whole body exposure group exhibited maternal and developmental toxicity, including increased fetal skeletal



malformations and variations, confirming previous results. The committee concludes that skeletal malformations developed in both whole body and nose-only exposure routes.

### 7.2.3 Dermal studies

A non-guideline developmental dermal study was performed by Tyl et al. in 1995c.<sup>47</sup> Female CD-1 mice were exposed daily to EG from GD 6 through 15. EG was dissolved in water and applied in concentrations of 0%, 12.5%, 50% or 100% on the skin for 6 hours per day during which the skin was occluded and the mice were restrained. The concentrations correspond to 0, 404, 1677 and 3549 mg/kg bw/day. Oral EG (3000 mg/kg bw/day) was used as a positive control. Mice were euthanised on GD 18. There were no deaths in the dermal exposure groups and all clinical signs during treatment were associated with the restrain conditions. Gestational weight change increased significantly in high dose mice. Minimal-grade renal tubular lesions were observed in 3/30 mice in the high dose group.

The number of dams with fully resorbed litters was higher in all treatment groups compared to controls. There was a significantly reduced number of late resorptions per litter at 12.5% as well as a significantly reduced number of dead foetuses per litter. There were no increased incidences in malformations, but 2 variations occurred in the high dose group, namely a poorly ossified parietal skull bone and the majority of the intermediate phalanges of the hindlimb were unossified. Incidences of both variations were statistically significantly increased. Multiple signs of general and repro-

ductive toxicity occurred in the positive control group, such as maternal death, lower foetal body weights per litter and various soft tissue and skeletal malformations.<sup>47</sup> The committee concludes that dermal exposure did not cause detrimental effects that could lead to classification, since only variations were observed and no malformations.

## 7.3 Evaluation of the data

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

### *Rodent studies*

Developmental studies in rats and mice via oral gavage were published by Price et al. (1985).<sup>39</sup> Foetuses of rats exposed to EG doses exposed to 1250, 2500 and 5000 mg/kg bw/day were noted with dose-related increased skeletal malformations which were accompanied by effects on maternal and foetal body weights. Post-implantation loss was observed at 5000 mg/kg/day and there was a lower number of live foetuses per litter at doses where maternal toxicity also occurred. The studies in mice exposed to doses up to 3000 mg/kg bw/day showed even more convincing results



than the data observed in rats. Skeletal malformations caused by EG were observed at dose levels where no maternal toxicity occurred. EG exposure also resulted in increased post-implantation loss, a lower live litter size and lower average foetal body weights.<sup>39</sup>

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

Developmental toxicity studies in rats and mice via oral gavage were conducted according to EPA guidelines published by Neeper-Bradley et al. (1995).<sup>42</sup> Rats were dosed up to 2500 mg/kg bw/day and developmental effects were observed that were considered unrelated to maternal toxicity. External, soft tissue and skeletal malformations were observed. Overall, the committee considers this study as robust. In the mice study, doses up to 1500 mg/kg bw/day were administered. Maternal toxicity at 1500 mg/kg bw/day was minimal. At this dose, developmental effects were found, such as decreased foetal weights and skeletal malformations.<sup>42</sup>

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

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

As a follow-up of the studies by Price et al., Marr et al. 1992 studied whether or not skeletal variations can recover over time by administering rats one dose of 2500 mg EG/kg bw/day on GD 10.<sup>41</sup> The observed delayed ossifications were partly recovered later in life. Even though delayed ossification is reversible, this does not mean that a compound should not be classified as a hazard.

In 2 fertility studies in mice with EG in drinking water, some developmental endpoints were assessed as well, although the data were limited. In the first study, EG was dosed up to 1640 mg/kg bw/day and effects were



observed on number of live pups and pup weights together with skeletal malformations (craniofacial defects).<sup>32</sup> In the follow-up study EG was dosed up to 2826 mg/kg bw/day and minimal embryotoxicity was observed as well.<sup>33</sup> In the continuous breeding phase of this study, there were some indications of developmental effects (facial abnormalities) in the first generation, but the effects on development in the second generation were minimal. The question remains whether there were very few malformations or whether only a limited number of pups was included in the examination. The committee concludes there may be an effect on development in this study, but it is minimal.

Other mice studies had methodological limitations and were of less importance to the committee's conclusion. EG was administered by the oral route (drinking water or gavage). A study with only one dose group of 1400 mg/kg bw/day, described mortality of the dams.<sup>43</sup> The number of litters was low, also in the control group. Nonetheless, at 1400 mg/kg bw/day, a lower number of litters was noted and a lower survival compared to controls. A limited screening study with doses up to 2500 mg/kg bw/day showed minimal effects on litter weight and number of live pups, but no malformations were noted.<sup>35</sup> However, it was not clear how many litters were included in the evaluations.

Studies on inhalation or dermal exposure showed less convincing developmental effects than studies on oral exposure, and had

methodological limitations. Inhalation studies used analytical concentrations of 0, 119, 888, and 2090 mg/m<sup>3</sup> (2090 mg/m<sup>3</sup> equals to 606 mg/kg bw/day in rats, and 1074 mg/kg bw/day in mice. In rats, a respiratory volume of 0.8 l/min/kg and an exposure duration of 6 hours a day resulted in a conversion factor of 0.29 m<sup>3</sup>/kg/day. In mice, a respiratory volume of 1.43 l/min/kg and an exposure duration of 6 hours per day resulted in a conversion factor of 0.514 m<sup>3</sup>/kg/day).<sup>45</sup> The studies showed no developmental effects in rats at dose levels where no maternally toxic effects were observed, while in mice clear maternally toxic effects and developmental effects were observed in the 888 and 2090 mg/m<sup>3</sup> exposure groups.<sup>45</sup> This difference between rats and mice may be attributed to the much lower exposure expressed as mg/kg bw/day in rats as compared to mice. Additionally, the inhalation studies (whole body) also involved oral exposure because of ingestion of EG deposition during grooming of wet fur.

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



Rabbit studies

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

Species differences

The underlying causes for the interspecies discordance are important to be able to extrapolate in vivo developmental toxicity data to humans and conclude on potential human relevance of developmental effects observed in rodents. The differences can partly be explained by differences in distribution and accumulation of GA in maternal versus embryonal compartments. An abstract summarizing animal studies that compared pharmacokinetics and metabolism of EG in pregnant rats and rabbits showed that levels of GA in maternal blood and extraembryonic fluids (EEF) were 3 to 36-fold lower in rabbits than in rats.<sup>21</sup> In rats, concentrations of GA were higher in EEF than in maternal blood.<sup>21</sup> A study by Carney et al. that compared the administration of a teratogenic dose of EG to pregnant rats on GD 10 or rabbits on GD 9 showed similar maximum EG levels in maternal blood in rats and rabbits after a dose of 1000 mg/kg body weight (bw)/day (see Table 3 for specific concentrations). However, the maximum GA levels in embryos differed by a factor 10 between both species.<sup>16,22</sup>

The maximum concentration of GA in the rat embryo was 150% of that in the maternal blood, while in the rabbit embryo it was only 30% of the

maximum maternal blood concentration.<sup>16,22</sup> Maximal levels of GA in rabbit maternal blood were only 46% of the respective levels in rats.<sup>16,22</sup>

**Table 3** Overview of maximal concentrations of EG or GA in rats or rabbits as was studied by Carney et al. 2008 and summarised in the REACH registration dossier.<sup>16,23</sup>

| 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%       |                             |

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

However, other research has shown contradictory information regarding penetration of the rabbit embryo. In Carney’s study, GA levels in rabbit embryos remained below 50 µg/g and were approximately 30% of maximum maternal blood levels (see Table 3),<sup>12</sup> while in a publication by Moore et al. that studied EG (metabolite) maternal-foetal distribution in rats



and rabbits, a peak in rabbit embryonic GA values was seen that was comparable to the maximum maternal GA blood levels, and embryonic levels were higher than maternal blood levels during the elimination phase.<sup>16,24</sup> Differences in embryonic penetration between rat and rabbit embryos exist. However, the data by Moore et al. 2024 do not exclude the possibility of GA accumulation above maternal blood levels occurring in the rabbit embryo as well.

Other possible explanations for the differences in distribution between species reported in the literature are differences in visceral yolk sac biology and in the position of the carrier mediated transporters, located in the placenta, that are responsible for GA crossing the placental barrier.<sup>16,22,25</sup> The rat yolk sac completely surrounds the embryo, is incorporated into the developing placenta and is the major source of maternal-foetal exchange from GD 7-7.5 to ~GD 12.<sup>26</sup> The rabbit yolk sac is not closely apposed to the uterus during early organogenesis and does not completely enclose the embryo until relatively later in development (~GD 13).<sup>26</sup> During early development, the human yolk sac is connected to the midgut and later, in the first trimester, remains connected via the vitelline duct.<sup>26</sup> In short, the inverted yolk sac placenta exists in rodents but not in rabbits or humans which may have implications for GA distribution to the embryo in different species. This difference in yolk sac orientation between rabbits and rats may explain the differences in GA distribution between species. The early rabbit yolk sac might be a relatively inefficient transporter because of its

orientation and this was, according to the authors, supported by (unpublished) limited data for EG and GA given to GD 9 rabbits.<sup>26</sup>

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

A study by Moore investigated the distribution of GA between maternal blood and the conceptus at the beginning and end of placentation in both rats and rabbits (rat at GD 11 and GD 16, rabbit at GD 10 and GD 19). This study showed for the rat GA accumulation (GD 11) in EEF and embryo after



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

## 7.4 Conclusion

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

Developmental toxicity is observed in rats and mice, but not in rabbits up to dose levels causing maternal toxicity. The developmental effects reported in oral studies with rodents were mainly related to skeletal malformations, post-implantation loss, lower number of live foetuses per litter, and lower foetal body weights.<sup>32,33,38-42</sup> Additional studies in mice in which EG was administered by the oral route (gavage or drinking water) had methodological limitations and showed less pronounced developmental effects.<sup>35,43</sup> Inhalation studies in mice showed skeletal malformations after exposure via the whole-body and nose only exposure routes.<sup>45,46</sup> Whole-body inhalation

in rats and dermal exposure in mice showed no developmental effects.<sup>45,47</sup> This could be related to relatively low doses or limited uptake. Overall, developmental effects were observed in oral and inhalation exposure routes and in multiple species (rats and mice) and are serious and irreversible. Moreover, these effects are considered not to be a secondary non-specific consequence of other toxic effects, and thus directly related to EG treatment.

Based on the information on the mechanism of toxicity, the committee did not find conclusive evidence to exclude the rodent studies from the evaluation. Maternal-foetal distribution studies showed clear GA accumulation in rat embryos whereas contradictory evidence exists about GA levels in rabbit embryos relative to maternal blood concentrations. There is suggestive information on similarities between humans and rabbits related to yolk sac physiology and MCT orientation. Additionally, no maternal-foetal distribution studies in mice are available. However, the committee did not find conclusive evidence that shows that the rabbit model would be a more appropriate model for evaluating the effects of ethylene glycol in humans. Moreover, there is a lack of information on the mechanism of action, which further hampers the selection of the rabbit model as the more appropriate model for human effects of EG/GA.

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



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

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



# 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.<sup>34</sup> 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.<sup>32</sup> 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.<sup>33</sup>

## 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.



# 9 Conclusions on classification and labelling

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

## **Proposed classification for fertility**

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

## **Proposed classification for developmental toxicity**

Category 1B, H360D

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

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



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# Annexes



Fertility studies

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                                                                                                                                                                                                                                                                                                                                                                                                                                    |
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| Lamb et al., 1985 | Mice, COBS<br>Crl:CD1, (ICR)BR<br>outbred albino<br><br>N=20/sex/group<br>Controls: 40/sex | Reproductive study<br>(non-guideline, non-GLP). Fertility<br>assessment by continuous<br>breeding (NTP protocol) with a<br>preparatory dose range finding<br>study.<br><br><b>Design:</b> <ul style="list-style-type: none"><li>• Premating period of 7 days</li><li>• 98 days cohabitation and<br/>breeding (male+female), all<br/>pups born during this period<br/>were killed</li><li>• 21 days segregation period,<br/>pups born during this period<br/>were kept alive</li><li>• Exposure occurred during<br/>premating, cohabitation,<br/>segregation and throughout the<br/>life of offspring.</li><li>• High dose and control pups<br/>(N=20/sex/group) born during<br/>segregation were mated.</li></ul><br><b>Examinations:</b> <ul style="list-style-type: none"><li>• Fertility and reproductive<br/>performance of the adults and<br/>F1 generation. Viability, sex and<br/>weight of the pups.</li><li>• Organ weights of F1 offspring<br/>and skeletal examination of a<br/>proportion of F1 mice.</li><li>• Histology of the head of F1<br/>mice.</li></ul><br><b>Statistics:</b> <ul style="list-style-type: none"><li>• Results presented as Mean<br/>±SE.</li><li>• Tests used differ per parameter.<br/>Mostly Chi-Square approxima-<br/>tion to Kruskal Wallis is used<br/>for group comparisons and<br/>Mann-Withney U or Fisher exact<br/>test for pairwise comparisons.<br/>An ANOVA was carried out for<br/>assessment of body weight.</li></ul> | <b>Purity:</b><br>99.6%<br><br><b>Concentrations:</b><br>0, 0.25, 0.5 and 1.0% (w/v), oral<br>via drinking water. According<br>to the authors, this is equal to<br>410, 840 and 1640 mg/kg bw/<br>day, based on daily water<br>consumption and mean body<br>weight data.<br><br><b>F1 parents:</b><br>0, 1.0% (w/v), oral via drinking<br>water. This is equal to 1640<br>mg/kg bw/day, based on daily<br>water consumption and mean<br>body weight data.<br><br>Dosage solutions were within<br>98-107% of intended<br>concentrations. | <b>P0 Parents:</b><br>No general toxicity in<br>parental animals, i.e.<br>clinical signs, effects on<br>body weight or water<br>consumption. Some<br>deaths occurred in all<br>groups. One death in the<br>0.5% group could be<br>treatment related<br>according to the authors<br>(oxalate crystals in renal<br>tubules).<br><br><b>F1 parents:</b><br>No general toxicity<br>observed. | <b>P0 parents:</b> <ul style="list-style-type: none"><li>• Decreased number of litters per fertile pair<br/>at high dose (4.5±0.2) versus control<br/>(4.9±0.08), P&lt;0.01.</li><li>• Decreased mean number of live pups per<br/>litter high dose (10.2±0.3) versus control<br/>(10.8±0.5), P&lt;0.05.</li></ul><br><b>F1 parents:</b> <ul style="list-style-type: none"><li>• Fertility was lower in high dose animals<br/>(61%) compared to controls (81%), not<br/>significant.</li><li>• Number of live pups per litter and live pup<br/>weights were lower in high dose animals<br/>(not significant). es, and vacuolization of<br/>epithelial cells.</li></ul> | <b>High dose levels: above<br/>1000 mg/kg bw/day.</b><br><br>The authors measured<br>drinking water consumption<br>per pair of mice for 2 weeks.<br>This could be used to<br>calculate actual dose levels.<br>Furthermore, they reported a<br>decreased number of litters<br>per fertile pair of parental<br>animals (F0) in the highest<br>dose group.<br><br><b>However, no general toxicity<br/>was found at these dose<br/>levels.</b> |



| Reference           | Species                                                                         | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Dose and route                                                                                                                                                                                                                                                                                                   | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Effects on sexual function and fertility                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Remarks                                                                                                                                                                                  |
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| Gulati et al., 1986 | Mice, Crl:CD-1 (ICR)BR outbred albino<br><br>N=20/sex/group<br>Controls: 40/sex | Reproductive study (non-guideline, GLP-compliant).<br>Fertility assessment by continuous breeding<br><br><b>Design:</b><br><b>Task 2</b><br><ul style="list-style-type: none"> <li>Premating period of 7 days</li> <li>98 days cohabitation and breeding (1 male+1 female), all pups born during this period were killed</li> </ul> <b>Task 3</b> <ul style="list-style-type: none"> <li>Weaned animals of the last litter of task 2 from the high dose group (N=20/sex/group) and untreated males or females were mated.</li> <li>Unexposed cohabitation for max 1 week</li> <li>Females were allowed to litter, all pups born were killed.</li> </ul> <b>Task 4</b> <ul style="list-style-type: none"> <li>Weaned pups from the last litters of task 2 (F1)</li> <li>Exposure for 74 days, thereafter during mating and until weaning of the offspring (F2 generation)</li> </ul> <b>Examinations:</b> <ul style="list-style-type: none"> <li>Fertility (mating index+fertility index), viability and weight of pups/litters.</li> <li>body weight and organ weight</li> <li>sperm parameters</li> <li>external abnormalities in pups</li> </ul> <b>Statistics:</b><br>Data is displayed as mean ±SE. 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. | <b>Purity:</b><br>99.6%<br><br><b>Concentrations:</b><br>0, 0.5, 1, 1.5% (weight/volume)<br>0, 897, 1798 and 2826 mg/kg bw/day, oral in drinking water<br><br>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) | <b>Task 2</b><br>Parental animals:<br><ul style="list-style-type: none"> <li>No treatment-related effects on body weight, water consumption significantly increased in males at 1.5%</li> </ul> <b>Task 3</b><br>Parental animals:<br><ul style="list-style-type: none"> <li>No treatment-related effects on body, liver or kidney weight observed in female mice.</li> <li>Significant reduction in body (42.29±0.10g in 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.</li> <li>kidney degeneration, dilation, and regeneration of tubules and deposition of crystals (oxalate) in the tubules</li> </ul> <b>Task 4</b> Parental parents (F1):<br><ul style="list-style-type: none"> <li>No treatment-related effects on body weight and kidney weight (abs + rel), significant lower liver weight (abs, not rel) in females</li> <li>No treatment-related effects on body weight, sign decreased liver weight (1.93±0.05g in 1.5% group vs 2.11±0.07g in control group) in males</li> </ul> | <b>Task 2</b><br><ul style="list-style-type: none"> <li>Non-significant reduction in fertility index (14/16 (88%) in 1.5% ethylene glycol group vs 38/38 (100%) in control group).</li> <li>Reduced live pup count (11.8/litter in controls, 9.99/litter at 1.5%) (p&lt;0.05)</li> <li>Reduced pup weight (absolute and adjusted for litter size (&lt;10%, p&lt;0.05))</li> </ul> <b>Task 3</b> <ul style="list-style-type: none"> <li>No significant effect on mating and fertility index</li> <li>Significant effect (&lt;10%) on adjusted live pup weight in 1.5% group males/control females</li> <li>Increased % abnormal sperm at 1.5% (8.28±2.02 vs 5.06±0.53 in control groups, P&lt;0.05).</li> <li>Reduced sperm mobility (80.6±8.05 vs 94.3±0.51 in control group, P&lt;0.05)</li> <li>Degeneration of seminiferous tubules (85% 1.5% males and 57% of control), loss of spermatozoa, spermatids, spermatogonia, and spermatocyt</li> </ul> <b>Task 4</b> <ul style="list-style-type: none"> <li>No effects on mating and fertility index</li> <li>Significant effect (&lt;&lt;10%) on adjusted live pup weight in all dose groups 0.5%<br/>1.0%<br/>1.5%</li> <li>Reduced sperm density (801±61 compared to 1036±63 in control group, P&lt;0.05)</li> <li>Reduced sperm density (855±63) compared to 1036±63 in control group, P&lt;0.05)</li> <li>Reduced sperm motility (92.1±1.47 vs 94.6±0.89 in control groups, P&lt;0.05).</li> <li>Reduced testes weight (0.120±0.006 g vs 0.140±0.005 g in control group</li> <li>Reduced sperm motility (84.1±5.02 vs 94.6±0.89 in control groups, P&lt;0.05).</li> <li>Degeneration of seminiferous tubules in 60% of males at 1.5% vs 40% in controls</li> </ul> | <b>Follow-up from the study by Lamb et al. (1985), but higher dose tested.</b><br><br><b>Parental NOAEL 1.0%</b><br><br><b>Fertility NOAEL 0.5% based on effects on sperm in task 4.</b> |



| Reference           | Species                                                                | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Dose and route                                                                                                                                                                                     | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Effects on sexual function and fertility                                                                                                                                                                                                                                 | Remarks                                                     |
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| DePass et al., 1986 | Rats, Fisher 344<br><br>N=10 males/<br>group and 20<br>females/ group  | Three-generation reproduction study (non-guideline, non-GLP).<br><br><b>Design:</b><br>• Premating period of 7 days<br>• During breeding, period not mentioned.<br>• Offspring was exposed throughout life and started breeding on 100 days of age.<br><br><b>Examinations:</b><br>Fertility, behaviour and histopathology, kidney lesions.<br><br><b>Statistics:</b><br>Continuous data using Barlett's test. T-test for equal and unequal variance. Frequency data using chi-square and Fisher's exact tests.              | <b>Purity:</b><br>99.93%<br><br><b>Concentrations:</b><br>Approximately 0.04, 0.2 and 1.0 g/kg bw/day<br><br>Oral, diet<br><br>2 untreated control groups.                                         | No effects on mortality, diet consumption or body weight observed.                                                                                                                                                                                                                                                                                                                                                                                                                 | 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. |
| Carney et al., 1999 | Rat, CD, Sprague-Dawley, females.<br><br>N=25/group<br>time-mated rats | Developmental study (non-guideline, GLP compliant) in which also fertility parameters were examined.<br><br><b>Design:</b><br>Treatment from GD 6 to 15.<br><br>Necropsy on GD 21.<br><br><b>Examinations:</b><br>maternal body weight, organ weights (liver, kidney, gravid uterus), corpora lutea, implantations, pre-implantation loss, resorptions, litter size, sex ratio<br><br><b>Statistics:</b><br>Bartlett's test, ANOVA, Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction, Fisher exact test | <b>Purity EG:</b><br>99.98%<br><br><b>Concentration:</b><br>2500 mg/kg, by oral gavage<br><br>Solution within 99-107% of target concentration<br><br><b>Control:</b><br>deionized water via gavage | Decreased maternal body weights on GD 12, 16, 21.<br><br>Decreased body weight gain intervals (except the 0-6 pre-dosing period).<br><br>Increased absolute and relative kidney weights.<br><br>Increased liver weight, with absolute liver weight being significantly different than control.<br><br>One dam exhibited vaginal bleeding on GD 19 and 20 and was found to have a completely resorbed litter on GD 21.<br><br>Mean feed-consumption values were decreased by 6-13%. | 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).<br><br><b>% Resorbed implantations:</b><br>0 mg/kg: 2.5<br>2500mg/kg: 15.9*<br>P=0.05 |                                                             |



| Reference           | Species                          | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Dose and route                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | General toxicity                                                         | Effects on sexual function and fertility                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Remarks                                                                                                                                                          |
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| Harris et al., 1992 | Mice, Crl:CD-1<br>N=10/sex/group | <p>Short-Term Reproductive and Developmental Toxicity Screening study (non-guideline, non-GLP).</p> <p><b>Design 1:</b></p> <ul style="list-style-type: none"> <li>Treatment of pregnant female mice during GD 8-14</li> </ul> <p><b>Design 2:</b></p> <ul style="list-style-type: none"> <li>Treatment of males (SD3-20) and females (SD0-20)</li> <li>Cohabitation on study day 8</li> <li>Animals are sacrificed on study day 21.</li> </ul> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>Liver, kidney and testes histology in male mice.</li> <li>Fertility (impregnation, no. live implants per female, no. dead implants/female, total implants/female) and litter weight.</li> </ul> <p><b>Statistics:</b></p> <p>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>Purity not mentioned.</p> <p>Oral gavage</p> <p><b>Concentrations:</b></p> <p>0, 250, 700, and 2500 mg/kg/day, formulation were within 93 to 100% of target.</p> <p>This is equivalent to 0, 50, 140 and 500 mg/kg bw/day conform the CLP guidance.</p> <p><b>Positive control:</b></p> <p>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 conform the CLP guidance [19].</p> <p>Dosage solutions were between 93-100% of target concentrations.</p> | <p>No adverse clinical signs observed.</p> <p>No mortality observed.</p> | <p><b>Males:</b></p> <p>no effects on testes weight, sperm mobility or number observed.</p> <p><b>Females (study design 1):</b></p> <ul style="list-style-type: none"> <li>No effects on numbers of live or dead implants observed.</li> </ul> <p><b>Females (study design 2), EG in mg/kg/day:</b></p> <p>no. pregnant:</p> <p>0: 9/10<br/>250: 9/10<br/>700: 7/10<br/>2500: 9/10</p> <p>no. live implants/female:</p> <p>0: 10.0±0.9<br/>250: 10.7±0.4<br/>700: 11.1±0.5<br/>2500: 7.4±1.2*<br/>P&lt;0.05</p> <p>no. dead implants/female:</p> <p>0: 0.4±0.2<br/>250: 0.8±0.1<br/>700: 0.3±0.3<br/>2500: 1.4±0.4*<br/>P&lt;0.05</p> <p>total implants/female:</p> <p>0: 10.4±1.0<br/>250: 11.4±0.3<br/>700: 11.4±0.4<br/>2500: 8.9±1.1</p> <ul style="list-style-type: none"> <li>No effects on fertility observed.</li> </ul> <p><b>Positive control:</b></p> <ul style="list-style-type: none"> <li>Reduction in testes weight, sperm mobility and number (P&lt;0.05) in 250 and 700 mg/kg/day exposure groups.</li> <li>Study design 1: reduced number of live implants (P&lt;0.01) in 250 and 700 mg/kg/day groups.</li> <li>Study design 2: reduced impregnation (P&lt;0.05) in 700 mg/kg/day group.</li> </ul> | <p>Although no significant effect was observed on testes weight, sperm mobility or number, potential fertility effects cannot be excluded based on the data.</p> |



| Reference          | Species                                                                                | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Dose and route                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | General toxicity                                                                                                                                                                                                                                       | Effects on sexual function and fertility                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Remarks                                                                                                                                                      |
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| Hong et al., 1988  | Mice, B6C3F1, males and females<br><br>N=7/sex/group                                   | 4-day mouse study (non-guideline, non-GLP).<br><br><b>Histological examinations on day 1 post exposure:</b> lung, heart, liver, kidneys, adrenal glands, spleen, thymus, stomach, bone marrow, urinary bladder, small and large intestine, uterus (female) or testes (male).<br><br><b>Statistics:</b> Wilk-Shapiro test for normality, one-way analysis of variance and Dunnett's test for multiple comparison with control group.                                                                                                                                                                                                                                                                                                                                                                                                                                | <b>Purity:</b> 99.6%<br><br><b>Concentrations:</b> 0, 50, 100 or 250 mg/kg bw/day by oral gavage.<br><br><b>Positive control:</b> 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 ( $21.4\pm0.3$ g vs $23.4\pm0.2$ g in control groups).<br><br>No histological evidence of organ or tissue damage observed. | No effect on testicular weight observed in male mice.<br><br>Positive control: Significant ( $P<0.01$ ) decreases in testicular weight in 250 mg/kg bw/day group ( $3.3\pm0.1$ g vs $4.1\pm0.2$ g in control group)                                                                                                                                                                                                                                                                                                                                                                       | Short study duration of 4 days.<br><br><b>Positive control has a specific working mechanism and is therefore not directly comparable to ethylene glycol.</b> |
| Bolon et al., 1997 | Mice, Crl:CD-1 (ICR)BR outbred albino mice (CD1)<br>N=20/sex/group<br>Controls: 40/sex | Retrospective study (non-guideline, GLP compliant), ovaries obtained from 18 Reproductive Assessment by Continuous Breeding (RACB) bioassays in NTP archive.<br><br><b>Design:</b> <ul style="list-style-type: none"><li>• Premating period of 7 days</li><li>• 98 days cohabitation and breeding (male+female), all pups born during this period were killed</li><li>• 21 days segregation period, pups born during this period were kept alive</li><li>• Exposure occurred during premating, cohabitation, segregation and throughout the life of offspring.</li><li>• High dose and control pups (N=20/sex/group) born during segregation were mated.</li></ul><br><b>Examinations:</b> Ovarian follicle counts (N=10)<br><br><b>Statistics:</b> Group comparisons using Kruskal-Wallis non-parametric ANOVA and dose response trends using Mann-Witney U test. | <b>Concentrations:</b><br><b>Parental:</b> 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].<br><br><b>Offspring:</b> 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.<br><br>Purity not mentioned.<br><br><b>Positive control:</b> ethylene glycol monomethyl ether, 0, 0.03, 0.1, 0.2 and 0.3% (w/v) oral via drinking water. | Not mentioned.                                                                                                                                                                                                                                         | <b>Parental animals:</b> Non-significant increase in ovarian follicle count in 1.5% group.<br><br><b>Offspring:</b> 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.<br><br><b>Positive control:</b> <ul style="list-style-type: none"><li>• Parental: Non-significant reduction in ovarian follicle counts.</li><li>• Offspring: significant reduction (<math>P&lt;0.05</math>) in small, growing and antral ovarian follicle counts compared to controls.</li></ul> | Ovary samples used from Gulati et al., 1986.<br><br>Poor study design.                                                                                       |



Development studies

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                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
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| Maronpot et al., 1983 | Rat, Fischer<br>344, females.<br><br>N=20/group | <p>Prenatal development study (non-guideline, non-GLP).</p> <p><b>Design:</b><br/>Treatment of pregnant dams from GD 6 to 15. C-section on GD 21.</p> <p><b>Examinations:</b><br/>visceral (nasal cavity, eyes, brain) and skeletal alterations and malformations.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"><li>• Continuous data: F-test (with paired group F-max test or t-test)</li><li>• Non-parametric data: multiple sum of ranks test.</li><li>• Binominal data: Fischer exact test</li></ul> | <p><b>Purity:</b><br/>&gt;99.9%</p> <p><b>Concentrations:</b><br/>0, 40, 200 and 1000 mg/kg bw/day (targeted dose) in diet.</p> <p>Negative controls: regular diet.<br/>Positive control: hydroxyurea 500 mg/kg (ip) on GD 11.</p> | No effect on body weight gain (corrected for litter weight) and no clinical signs. | <p>No effect on foetal length, foetal weight, total implantations or litter size.</p> <p>1000 mg/kg bw/day:<br/>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.</p> <p>There was no increased incidence of major malformation in litters of ethylene glycol treated females.</p> <p>1000 mg/kg bw/day:<br/>Increased incidence of poorly ossified (14.2% versus 1.8%, P&lt;0.001) and unossified vertebral centra (26.0% versus 11.4%, P&lt;0.001) compared to control.</p> <p>Major malformations did occur in the positive controls.</p> | <p><b>Minimal embryotoxicity due to delayed foetal maturation (increased incidences of poorly ossified and unossified vertebral centra)</b></p> <p><b>Bodyweight only measured on day 6, 11, and 21, which was not synchronized with the treatment.</b></p> <p><b>Only targeted dose was reported.</b></p> <p><b>Treatment only started after implantations and no further resorptions occurred.</b></p> <p><b>Implantation loss is possibly non-related to the treatment.</b></p> |



| Reference          | Species          | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Dose and route                                                                                  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| Price et al., 1985 | Rat, CD, females | <p>Developmental study (NTP study, GLP compliant, blinded).</p> <p><b>Design:</b><br/>Treatment of pregnant rats from GD 6 through 15. 2 replicates of the teratology evaluation were conducted.</p> <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 fetuses, and live fetuses) and visceral, skeletal and morphological abnormalities.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data are presented as mean <math>\pm</math> SEM</li> <li>Dose response: test for linear trend</li> <li>ANOVA with post hoc tests</li> <li>Test for linear trend on proportions and chi-square test (nominal data). One tailed-fisher exact test for pairwise comparison.</li> </ul> | <p><b>Purity:</b><br/>&gt;99%</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Concentrations:</b><br/>0, 1250 (low), 2500 (mid) or 5000 (high) mg/kg bw/day, via oral gavage</p> <p><b>Negative control:</b><br/>vehicle</p> <p>Actual dose levels were within 10% of the calculated levels.</p> | <p><b>Maternal toxicity:</b><br/>No maternal deaths<br/>Piloerection in all treated groups but not in controls.</p> <ul style="list-style-type: none"> <li>Dose-related lower maternal body weight parameters and gravid uterine weight compared to control.</li> <li>Decreased weight gain during treatment (all <math>P &lt; 0.01</math>):<br/>Control: 42.03<math>\pm</math>1.96g<br/>1250 mg/kg: 34.81<math>\pm</math>1.73g<br/>2500 mg/kg: 29.45<math>\pm</math>1.38g<br/>5000 mg/kg: 20.68<math>\pm</math>1.93g</li> <li>Decreased weight on GD 20 (mid and high dose, <math>P &lt; 0.01</math>):<br/>Control: 366.06<math>\pm</math>3.94g<br/>2500 mg/kg: 345.69<math>\pm</math>5.86g<br/>5000 mg/kg: 324.99<math>\pm</math>6.64g</li> <li>Decreased weight gain during gestation (mid and high dose, <math>P &lt; 0.01</math>):<br/>Control: 129.50<math>\pm</math>3.06g<br/>2500 mg/kg: 108.26<math>\pm</math>4.06g<br/>5000 mg/kg: 90.17<math>\pm</math>6.35g</li> <li>Decreased gravid uterine weight (mid and high dose, <math>P &lt; 0.01</math>):<br/>Control: 73.04<math>\pm</math>1.63g<br/>2500 mg/kg: 58.00<math>\pm</math>2.97g<br/>5000 mg/kg: 46.61<math>\pm</math>3.75g</li> <li>Decreased absolute (but not relative) liver weight (high dose, <math>P &lt; 0.05</math>):<br/>Control: 15.47<math>\pm</math>0.26g<br/>5000 mg/kg: 13.70<math>\pm</math>0.35g</li> <li>Relative (but not absolute) kidney weight increased (mid and high dose, <math>P &lt; 0.05</math>):<br/>Control: 0.517<math>\pm</math>0.012g<br/>2500 mg/kg: 0.573<math>\pm</math>0.008g<br/>5000 mg/kg: 0.615<math>\pm</math>0.021g</li> <li>Increased water consumption during and after treatment (mid and high dose, <math>P &lt; 0.05</math>).</li> </ul> | <p><b>Developmental toxicity:</b></p> <ul style="list-style-type: none"> <li>Increased post-implantation loss (resorptions + dead fetuses) per litter at the high dose (21.34<math>\pm</math> 5.24%) compared to control (4.70<math>\pm</math> 1.23%), <math>P &lt; 0.05</math>.</li> <li>Higher percentage litter with post implantation loss at one or more sites at the high dose (74.1 vs 39.3 in control)</li> <li>Decreased number of live fetuses per litter at mid dose (11.90<math>\pm</math>0.60) and high dose (11.04<math>\pm</math>0.79), vs 13.54<math>\pm</math>0.28 in control, <math>P &lt; 0.05</math>.</li> <li>Decreased average foetal body weight per litter at mid dose (2.916<math>\pm</math>0.056 g) and high dose (2.388<math>\pm</math>0.089 g) compared to control (3.404<math>\pm</math>0.052 g).</li> <li>Increase in percentage live fetuses malformed per litter at mid dose (25.11<math>\pm</math>4.84%) and high dose (75.53<math>\pm</math>6.42%) compared to control (1.37<math>\pm</math>0.97%).</li> <li>Increase in percentage litters with one or more malformed live fetuses at low dose (39.29%, <math>P &lt; 0.01</math>), mid dose (68.97%, <math>P &lt; 0.001</math>) and high dose (96.15%, <math>P &lt; 0.001</math>).</li> </ul> <p><b>Morphologic defects:</b><br/>Dose response relationship observed for all types of malformations.</p> <ul style="list-style-type: none"> <li>External malformations in 15 litters at high dose compared to 0 control litters (<math>P &lt; 0.001</math>), amongst others cleft palate, cleft lip, anophthalmia, meningoencephalocele, gastroschisis, exencephaly.</li> <li>Visceral malformations in 6 litters at low dose (<math>P &lt; 0.05</math>), 3 litters at the mid dose (n.s.) 8 at high dose (<math>P &lt; 0.01</math>) compared to 0 control litters. Increase mainly due to anomalies of great vessels.</li> <li>Skeletal malformations in 19 litters at mid dose and 24 litters at high dose versus 2 control litters (<math>P &lt; 0.001</math>), 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).</li> </ul> | <p>The doses selected were based on a preliminary toxicity study performed using timed-pregnant rats and mice (Price et al., 1984a, b).</p> <p>The reduction in live litter size at the mid dose may have been related to the lower number of implantation sites per litter at that dose relative to controls.</p> |



| Reference           | Species                                                      | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Dose and route                                                                                                                                                           | General toxicity                                                   | Effects on development                                                         | Remarks |
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| DePass et al., 1986 | Fisher 344 Rats<br><br>N=10 males/group and 20 females/group | Three-generation reproduction study (non-guideline, non-GLP).<br><br><b>Design:</b> <ul style="list-style-type: none"><li>During 7-week premating period</li><li>During breeding, time period not mentioned.</li><li>Offspring was exposed throughout life and started breeding on 100 days of age.</li></ul> <b>Examinations:</b> neonatal body weight, histopathology, kidney lesions.<br><br><b>Statistics:</b> Continuous data using Barlett's test. T-test for equal and unequal variance. Frequency data using chi-square and Fisher's exact tests. | <b>Purity:</b> 99.93%<br><br><b>Concentrations:</b> Approximately 0.04, 0.2 and 1.0 g/kg bw/day in diet as calculated by the authors.<br><br>2 untreated control groups. | No effects on mortality, diet consumption or body weight observed. | No effects on observed on neonatal body weight at day 4, 14, or 21 postpartum. |         |



| Reference          | Species                                                                                                                                                                                                                                                          | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Dose and route                                                                                                                                                                                                                                                                                                           | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Remarks                                                                                                                                                                                                                 |
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| Price et al., 1988 | COBS CD (SD)BR outbred albino rats<br><br>N=4-5 females per dose group/replicate, as well as 3-6 sperm positive females to each associated group of untreated foster dams.<br><br>Multiple replicates to come to:<br><br>N=at least 20 F1 litters per/dose group | Developmental study (NTP study, GLP compliant).<br><br><b>Design:</b><br>Treatment of pregnant rats from GD 6-20<br><br>Dams were euthanised on PND 1. Pups were cross-fostered to untreated mothers on PND 1 and were euthanised PND 4, 22 or 63.<br><br><b>Examinations:</b><br>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.<br><br><b>Statistics:</b><br>• Data are presented as mean $\pm$ SEM<br>• General Linear Model analysis<br>• One-way ANOVA and pairwise comparisons<br>• Chi-square test (nominal data). One tailed- fisher exact test for pairwise comparison. | <b>Purity:</b><br>99.6%<br><b>Vehicle:</b><br>water<br><br><b>Concentrations:</b><br>0, 250, 1250 and 2250 mg/kg bw/day in water via oral gavage.<br><br>Dose formulations were analysed by gas chromatography and compared to an internal standard to verify concentration prior to administration to the test animals. | <b>Maternal toxicity:</b><br>• No effect on body weight GD 0-15, reduced body weight in high dose group on GD 20 ( $P<0.01$ , $352.0\pm4.8$ g compared to $367.8\pm4.3$ g in control group).<br>• Reduced gestational weight gain GD 6-20 ( $P<0.01$ ) in high dose group ( $84.3\pm3.5$ g compared to $106.2\pm3.0$ g in control group).<br>No effect on BW on PND 1 after littering.<br>• Increased kidney weight ( $P<0.01$ ) in high dose group ( $2.42\pm0.07$ g compared to $2.20\pm0.05$ g in control group).<br>• No significant effect on liver weight observed<br>• Reduced uterine weight on PND 1 ( $P<0.05$ ) in high dose group ( $2.08\pm0.14$ g compared to $4.62\pm0.14$ g in control group).<br>No effect on number of uterine implantation sites per dam. | <b>P0 parents:</b><br>• Longer gestational periods ( $P<0.01$ ) in high dose groups.<br>Control: $21.26\pm0.07$ days<br>1250 mg/kg: $21.58\pm0.07$ days<br>2250 mg/kg: $21.84\pm0.09$ days<br>• Reduced live litter size on PND 1 ( $P<0.01$ ) in high dose group ( $11.87\pm0.48$ compared to $13.67\pm0.33$ in control group).<br>• Increased mortality per litter PND 1-4 ( $P<0.01$ ) in high dose group ( $5.15\pm1.06\%$ compared to $2.07\pm1.12\%$ in control group)<br><br><b>F1 offspring:</b><br>• Reduced body weight in high dose group on PND 1 ( $P<0.01$ ) and PND 22 ( $P<0.05$ ).<br>• Reduced absolute kidney weight in high dose group ( $P<0.05$ ) on PND 22 and PND 63.<br>• Reduced absolute brain weight in high dose group ( $P<0.05$ ) on PND 22 and in females on PND 63.<br>• Relative kidney weight females at mid and high affected on PND 63<br>• Reduced time to achieve wire grasping in high dose group ( $P<0.05$ ) ( $9.71\pm0.18$ days compared to $11.98\pm0.78$ days in control group). No effects on other developmental landmarks<br>• No significant effect on external and visceral defects observed.<br>• 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 sternebrae. | All litters with fewer than eight surviving pups on PND 4, as well as those which could not be culled in accordance with the stated sex ratio requirements were also sacrificed. This could have led to potential bias. |



| Reference         | Species                                                        | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Dose and route                                                                                                     | General toxicity                                                                                                                                                                                                                                                                                                                                                                                             | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Remarks                                                                                                                                                                               |
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| Marr et al., 1992 | Rat, Sprague-Dawley, N=7/ dose group/ time point of euthanasia | <p>Developmental study (non-guideline, non-GLP).</p> <p><b>Design:</b><br/>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><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>• Foetus (GD 18 or 20) /pup weight (PND 1, 4, 7, 14, 21 and 63)</li> <li>• Examination of skeleton: The regions of the skeleton that were examined included the sternum, carpals, tarsals, phalanges, and vertebral centra.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>• Results are presented as mean <math>\pm</math> SEM</li> <li>• Continuous data: t-test or Mann-Whitney U test.</li> <li>• Categorical data: Fisher's Exact test</li> <li>• Proportions of ossification: general linear model (ANOVA) (with and without body weight as covariate)</li> </ul> | <p><b>Purity:</b><br/>&gt;99%</p> <p><b>Concentrations:</b><br/>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 <math>P &lt; 0.05</math>). 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 gestational day of delivery, i.e., gestational length,</p> | <ul style="list-style-type: none"> <li>• Foetal body weight was 26 and 24% lower than control at GD 18 and 20, respectively (<math>P &lt; 0.05</math> for both time points).</li> <li>• Pup body weight was 10% lower than control at PND 1 (<math>P &lt; 0.05</math>).</li> <li>• Pup body weight was 8-5% lower between PND 4 to 63 (not statistically significant)</li> </ul> <p><b>Skeletal malformations:</b></p> <ul style="list-style-type: none"> <li>• Increased percentage malformed per litter, exposed versus control:<br/>GD 18: 76.3<math>\pm</math>11.3% versus 0%, <math>P &lt; 0.05</math><br/>GD 20: 88.4<math>\pm</math>3.4% versus 1.1<math>\pm</math>1.1%, <math>P &lt; 0.05</math><br/>PND 1: 95.2<math>\pm</math>4.8% versus 0%, <math>P &lt; 0.05</math><br/>PND 4: 83.4<math>\pm</math>6.1% versus 1.0<math>\pm</math>0.5%, <math>P &lt; 0.05</math><br/>PND 14: 77.5<math>\pm</math>16.5% versus 0%, <math>P &lt; 0.05</math><br/>PND 21: 87.5<math>\pm</math>7.2% versus 0%, <math>P &lt; 0.05</math><br/>PND 63: 28.2<math>\pm</math>8.3% versus 7.5<math>\pm</math>7.5%, not significant</li> <li>• Percentage of litters with malformations was 100% at GD 18, GD 20, PND 1, PND 21 (all <math>P &lt; 0.05</math> compared to control) and 100% at PND 4 and 80% at PND 63 (not significant).</li> <li>• Agenesis of the ribs (incidence 24-41%) and short ribs (incidence 6-34%) were common.</li> <li>• Bipartite cartilage and bipartite ossification centres, incidence peaks at PND 1 and 21.</li> <li>• Increase in variations in exposed group. Rudimentary rib on lumbar arch I was most common.</li> </ul> <p><b>Ossifications (ANOVA without foetal body weight as covariate):</b></p> <p>GD 20:</p> <ul style="list-style-type: none"> <li>• Percent total ossification lowered (24.85<math>\pm</math>1.46% versus 37.42<math>\pm</math>1.19%, <math>P &lt; 0.05</math>)</li> <li>• Percent sternebrae, centra, forelimb distal phalanges and metatarsals ossified were lowered (<math>P &lt; 0.05</math>).</li> </ul> <p>PND 1:</p> <ul style="list-style-type: none"> <li>• Percent total ossification lowered (66.49<math>\pm</math>1.33% versus 78.64<math>\pm</math>0.45%, <math>P &lt; 0.05</math>).</li> <li>• Percent sternebrae and centra ossified lowered (<math>P &lt; 0.05</math>).</li> </ul> <p>PND 4:</p> <ul style="list-style-type: none"> <li>• Percent total ossification lowered (84.32<math>\pm</math>0.95% versus 92.58<math>\pm</math>0.98%, <math>P &lt; 0.05</math>).</li> <li>• Percent sternebrae, centra, forelimb middle phalanges ossified were lowered (<math>P &lt; 0.05</math>).</li> </ul> <p>PND 14:</p> <ul style="list-style-type: none"> <li>• Percent total ossification lowered (94.08<math>\pm</math>0.54% versus 99.75<math>\pm</math>0.13%, <math>P &lt; 0.05</math>)</li> <li>• Percent sternebrae and centra ossified lowered (<math>P &lt; 0.05</math>).</li> </ul> <p>PND 21:</p> <ul style="list-style-type: none"> <li>• Percent total ossification lowered (95.22<math>\pm</math>0.50 versus 100<math>\pm</math>0.0%, <math>P &lt; 0.05</math>).</li> <li>• Percent sternebrae and centra ossified lowered (<math>P &lt; 0.05</math>).</li> </ul> <p>PND 63:</p> <ul style="list-style-type: none"> <li>• Percent sternebrae ossified lowered (<math>P &lt; 0.05</math>)</li> </ul> | <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> |



| Reference                   | Species                        | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | Dose and route                                                                                                                                                                                                                             | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Remarks                                                                                                                                                                                                                           |
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| Neeper-Bradley et al., 1995 | Rat, CD, females<br>N=25/group | <p>Developmental study (EPA TSCA testing guideline, GLP compliant).</p> <p><b>Design:</b><br/>Females were treated daily from GD 5 through 15. Dams were euthanized on GD 21.</p> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>Maternal liver, kidney and gravid uterine weight. Kidney histology.</li> <li>Corpora lutea, number of live and dead fetuses, resorptions.</li> <li>Sex of fetuses, variations and malformations.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data presented as mean <math>\pm</math>SD</li> <li>Continuous data: ANOVA and t-tests with Bonferroni post-hoc test.</li> <li>Non-parametric data: Kruskal-Wallis followed by Mann Withney U when appropriate.</li> <li>Incidence data: Fisher exact test</li> </ul> | <p><b>Purity:</b><br/>&gt;99.9%</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Concentrations:</b><br/>0 (vehicle), 150, 500, 1000 and 2500 mg/kg bw/day, via oral gavage.</p> <p>Dosing solutions were 103-108% of nominal concentrations</p> | <p><b>Maternal toxicity:</b><br/>1000 mg/kg bw/day:</p> <ul style="list-style-type: none"> <li>Increased relative liver weight (<math>P&lt;0.05</math>).</li> </ul> <p>2500 mg/kg bw/day:</p> <ul style="list-style-type: none"> <li>Body weight gain was reduced at GD 6-9 (<math>P&lt;0.05</math>) and GD 15-18 (<math>P&lt;0.01</math>).</li> <li>During treatment, water consumption was increased (<math>P&lt;0.01</math>).</li> <li>Reduced final body weight (<math>365.08\pm33.0</math>g versus <math>382.17\pm30.6</math>g in controls), <math>P&lt;0.01</math>.</li> <li>Reduced gravid uterine weight (<math>74.042\pm24.0</math>g versus <math>98.013\pm30.6</math>g in controls), <math>P&lt;0.01</math>.</li> <li>Increased absolute and relative kidney weight and relative liver weight (all <math>P&lt;0.01</math>).</li> </ul> | <p><b>Developmental effects:</b><br/>500 mg/kg bw/day:<br/>Skeletal variations:</p> <ul style="list-style-type: none"> <li>Increased incidence in poorly ossified supraoccipital, <math>P&lt;0.05</math></li> </ul> <p>1000 mg/kg bw/day:</p> <ul style="list-style-type: none"> <li>Reduced foetal body weight per litter (<math>4.981\pm0.31</math> g versus <math>5.245\pm0.26</math> g in controls), <math>P&lt;0.05</math>.</li> </ul> <p><b>Malformations external/soft tissue:</b></p> <ul style="list-style-type: none"> <li>Gastroschisis in one foetus, not significant.</li> </ul> <p><b>Malformations skeletal:</b></p> <ul style="list-style-type: none"> <li>Extra 14 thoracic centrum and arches (8/23 versus 1/24 control litter), <math>P&lt;0.05</math>.</li> <li>Missing rib (8/23 versus 0/24 control litters), <math>P&lt;0.01</math>.</li> <li>Missing thoracic arches (6/23 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> </ul> <p><b>Variations skeletal:</b><br/>Increased incidence of multiple skeletal variations involving ossification sites at the cervical and thoracic region.</p> <p>2500 mg/kg bw/day:</p> <ul style="list-style-type: none"> <li>Reduced foetal body weight per litter (<math>4.033\pm0.40</math>g versus <math>5.245\pm0.26</math>g in controls), <math>P&lt;0.01</math>.</li> </ul> <p><b>Malformations external/soft tissue:</b></p> <ul style="list-style-type: none"> <li>Gastroschisis (7/21 versus 0/24 litters in controls), <math>P&lt;0.05</math>.</li> <li>Hydrocephaly (10/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> <li>Lateral ventricle dilated (6/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> <li>Umbilical hernia (5/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> <li>Atelectasis (19/21 versus 12/24 control litters), <math>P&lt;0.01</math>.</li> </ul> <p><b>Skeletal malformations:</b></p> <ul style="list-style-type: none"> <li>Cervical arch 7 missing (6/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> <li>Extra 14 thoracic centrum and arches (18/21 versus 1/24 control litter), <math>P&lt;0.01</math>.</li> <li>Lumbar centra skewed (11/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> <li>Rib missing (20/21 versus 0/24 control litters), <math>P&lt;0.01</math>.</li> <li>Ribs fused (17/21 versus 0/24 control litters), <math>P&lt;0.01</math>.</li> <li>Thoracic arch missing (17/21 versus 0/24 control litters), <math>P&lt;0.01</math>.</li> <li>Thoracic arches fused (13/21 versus 0/24 control litters), <math>P&lt;0.01</math>.</li> <li>Thoracic centrum and arch missing (5/21 versus 0/24 control litters), <math>P&lt;0.05</math>.</li> </ul> <p><b>Variations skeletal:</b><br/>Increased incidence of multiple skeletal variations involving ossification sites at the cervical and thoracic region.</p> | 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 |
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| Carney et al., 1999 | Rat, CD, Sprague-Dawley, females.<br><br>N=25/group time-mated rats | Developmental study (non-guideline, GLP compliant)<br><br><b>Design:</b><br>Treatment from GD 6 to 15.<br><br>Necropsy on GD 21.<br><br><b>Examinations:</b><br>maternal body weight, organ weights (liver, kidney, gravid uterus), foetal external, visceral, skeletal abnormalities.<br><br><b>Statistics:</b><br>Bartlett's test, ANOVA, Dunnett's test or Wilcoxon Rank-Sum test with Bonferroni's correction, Fisher exact test | <b>Purity EG:</b><br>99.98%<br><br><b>Concentration:</b><br>2500 mg/kg, by oral gavage<br><br>Solution within 99-107% of target concentration<br><br><b>Control:</b><br>deionized water via gavage | Decreased maternal body weights on GD 12, 16, 21.<br><br>Decreased body weight gain intervals (except the 0-6 pre-dosing period).<br><br>Increased absolute and relative kidney weights.<br><br>Increased liver weight, with absolute liver weight being significantly different than control.<br><br>One dam exhibited vaginal bleeding on GD 19 and 20 and was found to have a completely resorbed litter on GD 21.<br><br>Mean feed-consumption values were decreased by 6-13%. | Fatal body weight (g):<br>0 mg/kg: 5.53±0.30<br>2500 mg/kg: 3.75±0.58*, P=0.05<br><br><b>External malformations</b> (% fetuses affected (%litters affected)):<br>Meningoencephalocele: 3.5 (25.0)*<br>Exencephaly: 5.2 (25.0)*<br>Cleft lip: 3.5 (29.2)*<br>Cleft palate: 2.8 (29.2)*<br>Omphalocele: 9.8 (54.2)*<br>Gastroschisis: 0.3 (4.2)<br>Umbilical hernia: 1.7 (16.7)<br>P=0.05<br><br><b>Visceral malformations</b> (% fetuses affected (%litters affected)):<br>Dilated cerebral ventricles: 15.9 (33.3)*<br>Anophthalmia: 3.3 (16.7)<br>Microphthalmia: 2.0 (12.5)<br>P=0.05<br><br><b>Skeletal malformations</b> (% fetuses affected (%litters affected)):<br>Hemivertebrae: 60.0 (95.8)*<br>Extra vertebrae: 5.9 (29.2)*<br>Missing vertebrae: 24.4 (62.5)*<br>Fused vertebrae: 36.3 (75.0)*<br>Fused centra: 8.9 (33.3)*<br>Fused ribs: 62.4 (95.8)*<br>Extra ribs: 4.5 (20.8)*<br>Missing ribs: 60.2 (91.7)*<br>P=0.05<br><br><b>Skeletal variations:</b><br>Increased incidence of delayed ossification of skull, lumbar vertebrae, cervical centra, thoracic 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 |         |



| Reference                          | Species                               | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Dose and route                                                                                                                                                                     | General toxicity                                                                                                                     | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Remarks                                                                                                                                                                                                                      |
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| Schuler et al., 1984 <sup>43</sup> | Mice, CD-1, females<br><br>N=50/group | <p>Developmental study (non-guideline, non-GLP) with a preparatory dose finding study.</p> <p><b>Design:</b><br/>Treatment of pregnant dams from GD 7 to 14. Pups were sacrificed 3 days after birth.</p> <p><b>Examinations:</b><br/>number of live pups, pup weight, maternal body weight.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>• Body weight: analysis of variance</li> <li>• Mice with viable litters: Fisher-Irwin</li> <li>• Number of live pups and survival: student's t-test</li> </ul> | <p><b>Purity:</b><br/>&gt;99%</p> <p><b>Concentration:</b><br/>11090 mg/kg bw/day, by oral gavage</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Negative control:</b><br/>vehicle</p> | <p><b>Maternal mortality:</b></p> <ul style="list-style-type: none"> <li>• 5/50 versus 0/50 in controls (not significant)</li> </ul> | <ul style="list-style-type: none"> <li>• Decreased viable litters (litter with at least one viable pup): 15/37 versus 29/29 in controls (P&lt;0.05)</li> <li>• Decreased number of live pups per litter: 2 versus 9 in controls (P&lt;0.05).</li> <li>• Increased number of dead pups per litter: 1.5 versus 0.1 in controls (P&lt;0.05).</li> <li>• Decreased pup postnatal survival: 40% versus 100% in controls (P&lt;0.05).</li> <li>• Decreased pup weight gain: 0.2g versus 7g in controls (P&lt;0.05).</li> <li>• Decreased pup birth weight: 1.4g versus 1.7g in controls (P&lt;0.05).</li> </ul> | <p>No clear cause for maternal mortality.</p> <p>Maternal toxicity is relatively high, but no statistical analysis was performed.</p> <p><b>Adverse effects were observed, but without a dose-response relationship.</b></p> |



| Reference                        | Species                                                                | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Dose and route                                                                                                                                                                                                                                                                             | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Remarks                                                                                                                              |
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| Price et al., 1985 <sup>39</sup> | Mice, CD-1, females<br><br>N=10/group/replicate (so total of 20/group) | <p>Developmental study (NTP study, GLP compliant, blinded).</p> <p><b>Design:</b><br/>Treatment of pregnant mice from GD 6 through 15. 2 replicates of the teratology evaluation were conducted.</p> <p>Caesarean section was performed on GD 17.</p> <p><b>Examinations:</b><br/>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 fetuses, and live fetuses) and visceral, skeletal and morphological abnormalities.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data are presented as mean <math>\pm</math> SEM</li> <li>Dose response: test for linear trend</li> <li>ANOVA with post hoc tests</li> <li>Test for linear trend on proportions and chi-square test (nominal data). One tailed-fisher exact test for pairwise comparison.</li> </ul> | <p><b>Purity:</b><br/>&gt;99%</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Concentrations:</b><br/>0, 750 (low), 1500 (mid) or 3000 (high) mg/kg bw/day, via oral gavage</p> <p><b>Negative control:</b><br/>vehicle</p> <p>Actual dose levels were within 10% of the calculated levels.</p> | <ul style="list-style-type: none"> <li>Decreased weight gain during treatment in mid and high dose (<math>P &lt; 0.05</math> for both groups*):<br/>0 mg/kg: 11.24<math>\pm</math>0.49g<br/>750mg/kg: 11.58<math>\pm</math>0.39g<br/>1500mg/kg: 8.54<math>\pm</math>0.84*<br/>3000mg/kg: 8.42<math>\pm</math>0.54*</li> <li>Decreased absolute (but not relative) maternal liver weight in mid and high dose groups (<math>P &lt; 0.05</math> for both groups*):<br/>0 mg/kg: 2.72<math>\pm</math>0.05g<br/>750mg/kg: 2.63<math>\pm</math>0.04g<br/>1500mg/kg: 2.49<math>\pm</math>0.06*<br/>3000mg/kg: 2.47<math>\pm</math>0.06*</li> <li>Decreased gravid uterine weight in mid and high dose (<math>P &lt; 0.05</math> for both groups*):<br/>0 mg/kg: 17.13<math>\pm</math>0.62<br/>750mg/kg: 15.46<math>\pm</math>0.55<br/>1500mg/kg: 12.17<math>\pm</math>0.93*<br/>3000mg/kg: 11.58<math>\pm</math>0.59*</li> </ul> | <p><b>Developmental toxicity:</b></p> <ul style="list-style-type: none"> <li>Reduction in the number of implantation sites per litter in the mid dose (and not high dose) (11.57<math>\pm</math>0.70) compared to control (13.32<math>\pm</math>0.44), <math>P &lt; 0.05</math>. Note: since this reduction was not dose-related, it was assumed unlikely to be related to EG treatment.</li> <li>Dose-related increasing trends in post-implantation loss per litter or at one or more site, but no significant pairwise comparisons.</li> <li>Dose-related decrease in live litter size, with significant decrease in the high dose group (9.83<math>\pm</math>0.56) compared to control (11.88<math>\pm</math>0.49), <math>P &lt; 0.05</math>.</li> <li>Dose-related decrease in average foetal body weight per litter. Low dose (0.882<math>\pm</math>0.017g), mid dose (0.787<math>\pm</math>0.024g) and high dose (0.712<math>\pm</math>0.022g) were decreased compared to control (0.974<math>\pm</math>0.013g), <math>P &lt; 0.01</math> for all groups.</li> <li>Dose-related increase in the percentage of malformed fetuses per litter. Low dose (10.0<math>\pm</math>1.96%), mid dose (37.77<math>\pm</math>6.30%) and high dose (56.54<math>\pm</math>6.80%) compared to control (0.25<math>\pm</math>0.25%), <math>P &lt; 0.01</math> for all groups.</li> <li>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%), <math>P &lt; 0.001</math> for all groups.</li> </ul> <p><b>Malformations:</b></p> <ul style="list-style-type: none"> <li>External malformations in 8 litters (high dose) versus 0 in the controls, <math>P &lt; 0.05</math>, including exencephaly, meningoencephalocele, cleft palate, cleft lip, facial cleft.</li> <li>Dose response related increase in visceral malformations. Significant in high dose (7 litters) versus controls (0 litters), <math>P &lt; 0.05</math>. Visceral malformations are among others aortic stenosis, hydronephrosis, hydroureter, retrotracheal or retrooesophageal pulmonary artery.</li> <li>Dose response related increase in skeletal malformations. Significant in low dose (15 litters), mid dose (17 litters) and high dose (22 litters) versus control (1 litter), <math>P &lt; 0.01</math> for all groups. Malformations include 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).</li> </ul> | The doses selected were based on a preliminary toxicity study performed using timed-pregnant rats and mice (Price et al., 1984a, b). |



| Reference                                 | Species                           | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Dose and route                                                                                                                                                                                                                                              | General toxicity                                                                                                                                                                                                                                      | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Remarks                                                                                                                                                                                                          |
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| Neeper-Bradley et al., 1995 <sup>42</sup> | Mice, CD-1, females<br>N=30/group | <p>Developmental study (non-guideline, GLP compliant)</p> <p><b>Design:</b><br/>Females were treated daily from GD 5 through GD 15. Dams were euthanized on GD 18.</p> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>Maternal liver, kidney and gravid uterine weight. Kidney histology.</li> <li>Corpora lutea, number of live and dead fetuses, resorptions.</li> <li>Sex of fetuses, variations and malformations.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data presented as mean <math>\pm</math> Sd</li> <li>Continuous data: ANOVA and t-tests with Bonferroni post-hoc test.</li> <li>Non-parametric data: Kruskal-Wallis followed by Mann Withney U when appropriate.</li> <li>Incidence data: Fisher exact test</li> </ul> | <p><b>Purity:</b><br/>&gt;99.9%</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Concentrations:</b><br/>0 (vehicle), 50, 150 (low), 500 (mid) and 1500 (high) mg/kg bw/day, via oral gavage.</p> <p>Dosing solutions were 92-107% of nominal concentrations.</p> | <p><b>Maternal toxicity:</b><br/>One death occurred in control group, 2 deaths/group occurred at 50, 500 and 1500 mg/kg bw/day (not significant).</p> <p>Slight reductions in weight gain and body weight at 1500 mg/kg bw/day (not significant).</p> | <ul style="list-style-type: none"> <li>Foetal body weights/litter were reduced at 1500 mg/kg bw/day (<math>1.156 \pm 0.11</math> g versus <math>1.325 \pm 0.09</math> g in controls), <math>P &lt; 0.01</math>.</li> </ul> <p><b>Malformations external/soft tissue:</b></p> <ul style="list-style-type: none"> <li>Exencephaly noted in 2 litters (500 mg/kg bw/day) and 3 litters (1500 mg/kg bw/day), not significant.</li> </ul> <p><b>Skeletal variations (500 mg/kg bw/day):</b></p> <ul style="list-style-type: none"> <li>Extra rib 14, first lumbar arch, bilateral (17/24 versus 4/19 control litters), <math>P &lt; 0.05</math>.</li> </ul> <p><b>Skeletal malformations (1500 mg/kg bw/day):</b></p> <ul style="list-style-type: none"> <li>2-12 thoracic arches fused (8/21 versus 0/19 control litters), <math>P &lt; 0.01</math>.</li> <li>2-12 ribs fused (15/21 versus 1/19 control litters), <math>P &lt; 0.01</math>.</li> <li>Extra 14 thoracic centrum and arches (10/21 versus 0/19 control litters), <math>P &lt; 0.01</math>.</li> <li>Extra rib 14, thoracic arch 14: bilateral (10/21 versus 0/19), <math>P &lt; 0.01</math>.</li> </ul> <p><b>Skeletal variations:</b><br/>Increased incidence of multiple skeletal variations involving ossification sites at cervical, thoracic and lumbar region as well in the head.</p> | <p>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.</p> |



| Reference                       | Species                                                                               | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | Dose and route                                                                                                                                                                                                                                                                                                                                                                 | General toxicity                                                                                                                                                                                                                                                           | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Remarks                                                                 |
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| Lamb et al., 1985 <sup>32</sup> | Mice, COBS Crl:CD1, (ICR) BR outbred albino<br><br>N=20/sex/group<br>Controls: 40/sex | <p>Reproductive study (non-guideline, non-GLP). Fertility assessment by continuous breeding with a preparatory dose range finding study.</p> <p><b>Design:</b></p> <ul style="list-style-type: none"> <li>• Premating period of 7 days</li> <li>• 98 days cohabitation and breeding (male+female), all pups born during this period were killed</li> <li>• 21 days segregation period, pups born during this period were kept alive</li> <li>• Exposure occurred during premating, cohabitation, segregation and throughout the life of offspring.</li> <li>• High dose and control pups (N=20/sex/group) born during segregation were mated.</li> </ul> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>• Fertility and reproductive performance of the adults and F1 generation. Viability, sex and weight of the pups.</li> <li>• Organ weights of F1 offspring and skeletal examination of a proportion of F1 mice.</li> <li>• Histology of the head of F1 mice.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>• Results presented as Mean <math>\pm</math>SE.</li> <li>• 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 assessment of body weight.</li> </ul> | <p><b>Purity:</b><br/>99.6%</p> <p><b>Concentrations:</b><br/>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.</p> <p>Dosage solutions were within 98-107% of intended concentrations.</p> | <p>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).</p> | <p><b>Offspring defects:</b></p> <ul style="list-style-type: none"> <li>• Live pup weight decreased at high dose (<math>1.53 \pm 0.02</math> g) versus control (<math>1.63 \pm 0.02</math> g), <math>P &lt; 0.01</math>.</li> <li>• A pattern of skeletal defects (affecting skull, sternebrae, ribs and vertebrae) in treated mice, not in controls (not quantified). Defects included shortened facial bones, fused ribs, abnormally shaped or missing sternebrae, abnormally shaped vertebrae and twisting of spine.</li> </ul> | A limitation of this study is the number and selection of animals used. |



| Reference           | Species                                     | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Dose and route                                                                                                                                                                                                                                                                                                     | General toxicity                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Remarks                                                                                                                                                                           |
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| Gulati et al., 1986 | Mice, Crl:CD-1 (ICR)BR outbred albino (CD1) | <p>Continuous breeding study (non-guideline, GLP-compliant).</p> <p><b>Design:</b></p> <p><b>Task 2</b></p> <ul style="list-style-type: none"> <li>Premating period of 7 days</li> <li>98 days cohabitation and breeding (male+female), all pups born during this period were killed</li> </ul> <p><b>Task 3</b></p> <ul style="list-style-type: none"> <li>Weaned animals of the last litter of task 2 from the high dose group (N=20/sex/group) and untreated males or females were mated.</li> <li>Unexposed cohabitation for max 1 week</li> <li>Females were allowed to litter, all pups born were killed.</li> </ul> <p><b>Task 4</b></p> <ul style="list-style-type: none"> <li>Weaned pups from the last litters of task 2 (F1)</li> <li>Exposure for 74 days, thereafter during mating and until weaning of the offspring (2nd generation)</li> </ul> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>Fertility, viability and weight of litters.</li> <li>Organ weight and skeletal deformities in offspring.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data is displayed as mean <math>\pm</math>SE.</li> <li>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.</li> </ul> | <p><b>Purity:</b><br/>99.6%</p> <p><b>Concentrations:</b><br/>0, 0.5, 1, 1.5% (weight/volume), oral in drinking water.<br/>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><b>Task 2 Parental animals:</b></p> <ul style="list-style-type: none"> <li>No treatment-related effects on body weight, water consumption significantly increased in males a 1.5%</li> </ul> <p><b>Task 3 parental animals:</b></p> <p>No treatment-related effects on body, liver or kidney weight observed in female mice.</p> <ul style="list-style-type: none"> <li>Significant reduction in body (42.29<math>\pm</math>0.10g in 1.5% group vs 46.45<math>\pm</math>1.04g in control group) and liver weight (1.93<math>\pm</math>0.05g in 1.5% group vs 2.11<math>\pm</math>0.07g in control group) observed in male mice.</li> <li>Kidney degeneration, dilation, and regeneration of tubules and deposition of crystals (oxalate) in the tubules</li> </ul> <p><b>Task 4 Parental parents:</b></p> <ul style="list-style-type: none"> <li>No treatment-related effects on body weight and kidney weight (abs+rel), significant lower liver weight (abs, not rel) in females</li> <li>No treatment-related effects on body weight, sign decreased liver weight (1.93<math>\pm</math>0.05g in 1.5% group vs 2.11<math>\pm</math>0.07g in control group) in males</li> </ul> | <p><b>Task 2:</b></p> <ul style="list-style-type: none"> <li>Reduced live pup count (11.8/litter in controls, 9.99/litter at 1.5%) (p&lt;0.05)</li> <li>Reduced pup weight (absolute and adjusted for litter size (&lt;10%, p&lt;0.05)</li> <li>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)</li> </ul> <p><b>Task 3:</b></p> <ul style="list-style-type: none"> <li>Significant effect (&lt;10%) on adjusted live pup weight in 1.5% group males/control females</li> <li>2/2 (pups/litters) with ablepharon and 1 pup with possible cleft lip/palate in 1.5% group males/control females</li> </ul> <p><b>Task 4:</b></p> <ul style="list-style-type: none"> <li>Significant effect (&lt;&lt;10%) on adjusted live pup weight in all dose groups 0.5%</li> <li>Reduced sperm density (801<math>\pm</math>61 compared to 1036<math>\pm</math>63 in control group, P&lt;0.05)</li> <li>Reduced sperm density (855<math>\pm</math>63) compared to 1036<math>\pm</math>63 in control group, P&lt;0.05)</li> <li>Reduced sperm motility (92.1<math>\pm</math>1.47 vs 94.6<math>\pm</math>0.89 in control groups, P&lt;0.05).</li> <li>Reduced testes weight (0.120<math>\pm</math>0.006 g vs 0.140<math>\pm</math>0.005 g in control group</li> <li>Reduced sperm motility (84.1<math>\pm</math>5.02 vs 94.6<math>\pm</math>0.89 in control groups, P&lt;0.05).</li> <li>Degeneration of seminiferous tubules in 60% of males at 1.5% vs 40% in controls</li> </ul> | <p><b>NTP study</b></p> <p><b>The total number of offspring examined for deformities is unclear.</b></p> <p><b>Parental NOAEL: 1.0%</b></p> <p><b>Development NOAEL: 0.5%</b></p> |



| Reference           | Species                              | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Dose and route                                                                                                                                                                            | General toxicity                                           | Effects on development                                                                                                                                                                                               | Remarks                                                                                                                                                                                                                                          |
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| Harris et al., 1992 | Mice, Crl:CD-1<br><br>N=10/sex/group | <p>Short-Term Reproductive and Developmental Toxicity Screening study (non-guideline, non-GLP).</p> <p><b>Design:</b><br/>Treatment of pregnant female mice during GD 8-14.</p> <p><b>Examinations:</b></p> <ul style="list-style-type: none"><li>• Liver, kidney and testes histology in male mice.</li><li>• Fertility and litter weight.</li></ul> <p><b>Statistics:</b><br/>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>Purity not mentioned.</p> <p><b>Concentrations:</b><br/>0, 250, 700, and 2500 mg/kg/day in water via oral gavage.</p> <p>Dosing solutions within 93-100% of target concentrations.</p> | 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±0.9 g vs 19±1.1 g in control group) and PND4 (26.6±1.5 g vs 31.7±1.3 g in control groups). | <p>Screening study</p> <p>A decrease in mean litter weight was observed only in the high-dose 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                                                                                                                                                                                    |
|--------------------------------|----------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Tyl et al., 1993 <sup>48</sup> | Rabbits, New Zealand white, females, N=23-24/group | <p>Developmental study (non-guideline, GLP compliant).</p> <p><b>Design:</b><br/>Treatment of pregnant rabbits from GD 6 through 19. Pregnant rabbits were euthanized on GD 30.</p> <p><b>Examinations:</b></p> <ul style="list-style-type: none"><li>• Maternal liver, kidney and intact uterine weight. Kidney histology.</li><li>• Number of corpora lutea, implantations, live and dead fetuses, and resorptions counted.</li><li>• Foetus weight, foetus abnormalities, variations and malformations.</li></ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"><li>• General Linear Trend Models procedures applied for ANOVA of maternal and foetal parameters.</li><li>• Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data</li><li>• Bartlett's test for homogeneity of variance was performed on all data to be analysed by ANOVA.</li></ul> | <p><b>Purity:</b><br/>98%</p> <p><b>Vehicle:</b><br/>water</p> <p>Oral gavage</p> <p><b>Concentrations:</b><br/>0, 100, 500, 1000 or 2000 mg/kg bw/day</p> | <p>At 2000 mg/kg bw/day;</p> <ul style="list-style-type: none"><li>• 42.1% mortality</li><li>• Three early deliveries</li><li>• One spontaneous abortion</li><li>• 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</li></ul> <p>The death of the pregnant females at 2000 mg/kg bw/day was directly related to the acute renal failure.</p> | No indication of developmental toxicity at any dose tested. | <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> |



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<br><br>N=25/sex/group | <p>Developmental toxicity study (non-guideline, GLP compliant).</p> <p><b>Design:</b><br/>Whole-body exposure during GD days 6 to 15 for 6 hours per day.</p> <p><b>Examinations:</b> body weight, organ weight, gestational parameters, developmental defects.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"><li>• Data displayed as mean ±SD.</li><li>• 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&lt;0.05 2 tailed).</li></ul> | <p><b>Test item:</b><br/>Respirable aerosol of ethylene glycol (2.3 µm) in inhalation chambers.</p> <p><b>Purity:</b><br/>&gt;99.9%</p> <p><b>Target concentrations:</b><br/>0, 150, 1000, 2500 mg/m<sup>3</sup></p> <p><b>Analytical concentrations:</b><br/>0, 119±13, 888±149, and 2090±244 mg/m<sup>3</sup></p> | <ul style="list-style-type: none"><li>• Significant increase in liver weight in 2500 mg/ m<sup>3</sup> exposure groups (15.001±1.31g vs 13.841±1.72g in control group)</li><li>• No effects on food, water consumption, body weight, or weight gain observed.</li></ul> | 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%). |



| Reference         | Species                                                            | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Dose and route                                                                                                                                                                                                                                                                                                                                                                  | General toxicity                                                                                                                                                                                                                                                                                                                                                                                      | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | Remarks                                                                                                                                                                                                                                                                    |
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| Tyl et al., 1995a | Mice, CD-1 (CrI:CD-1 (ICR)BR) outbred albino<br><br>N=25/sex/group | <p>Developmental toxicity study (non-guideline, GLP compliant).</p> <p><b>Design:</b><br/>Whole-body exposure during GD days 6 to 15 for 6 hours per day.</p> <p><b>Examinations:</b><br/>Body weight, organ weight, gestational parameters, developmental defects.</p> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Data displayed as mean <math>\pm</math>SD.</li> <li>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 (<math>p &lt; 0.05</math> 2 tailed).</li> </ul> | <p><b>Test item:</b><br/>Respirable aerosol of ethylene glycol (2.3 <math>\mu</math>m) in inhalation chambers.</p> <p><b>Purity:</b><br/>&gt;99.9%</p> <p><b>Target concentrations:</b><br/>0, 150, 1000, 2500 mg/m<sup>3</sup></p> <p><b>Analytical concentrations:</b><br/>0, 119<math>\pm</math>13, 888<math>\pm</math>149, and 2090<math>\pm</math>244 mg/m<sup>3</sup></p> | <ul style="list-style-type: none"> <li>Significant reduction in maternal body weight in 1000 mg/m<sup>3</sup> (49.841<math>\pm</math>5.13g, <math>P &lt; 0.05</math>) and 2500 mg/m<sup>3</sup> (47.391<math>\pm</math>5.46g <math>P &lt; 0.001</math>) groups compared to control groups (53.211 g<math>\pm</math>3.33)</li> <li>Wet fur observed in all ethylene glycol exposure groups.</li> </ul> | <ul style="list-style-type: none"> <li>Reduced gravid uterine weight in mg/m<sup>3</sup> and 2500 mg/m<sup>3</sup> groups (both <math>P &lt; 0.001</math>)</li> <li>Reduction in viable implants in 2500 mg/m<sup>3</sup> group (8.0<math>\pm</math>2.9 vs 10.7<math>\pm</math>1.8 in control group, <math>P &lt; 0.001</math>)</li> <li>Increase in non-viable implants per litter in at 1000 mg/m<sup>3</sup> (2.9<math>\pm</math>2.0, <math>P &lt; 0.01</math>) and 2500 mg/m<sup>3</sup> (4.2<math>\pm</math>2.9, <math>P &lt; 0.001</math>) compared to control groups (1.4<math>\pm</math>1.0)</li> <li>Decreased percentage live foetuses in 1000 mg/m<sup>3</sup> (<math>P &lt; 0.01</math>) and 2500 mg/m<sup>3</sup> (<math>P &lt; 0.001</math>) compared to control groups</li> <li>Change in sex ratio in 1000 mg/m<sup>3</sup> group compared to control group (<math>P &lt; 0.01</math>)</li> <li>Reduction (<math>P &lt; 0.001</math>) in foetal body weight in 1000 (1.07g<math>\pm</math>0.14) and 2500 (0.94<math>\pm</math>0.14g) mg/m<sup>3</sup> exposure groups compared to control groups (1.33<math>\pm</math>0.08g).</li> <li>Increase (<math>P &lt; 0.05</math>) in incidence of external malformations in 1000 (30.4%) and 2500 (72.7%) mg/m<sup>3</sup> groups compared to control group (4%).</li> <li>Increase (<math>P &lt; 0.05</math>) in incidence of visceral malformations in 1000 (34.8%) and 2500 (72.7%) mg/m<sup>3</sup> groups compared to control group (%).</li> <li>Increase (<math>P &lt; 0.05</math>) in incidence of skeletal malformations in 1000 (100%) and 2500 (100%) mg/m<sup>3</sup> groups compared to control group (72%).</li> </ul> | <p>Ethylene glycol measured in fur indicates that oral exposure was present, calculated to be the major route of exposure (64-90%).</p> <p>A significant increase in visceral, external and skeletal malformations was observed only in the mid- and high-dose groups.</p> |



| Reference                       | Species                                                            | Experimental period and design                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Dose and route                                                                                                                                                                                                                                                                                                                                                                    | General toxicity                                                                                                                                                                                                                                                                                                                                          | Effects on development                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Remarks |
|---------------------------------|--------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Tyl et al., 1995b <sup>49</sup> | Mice, CD-1 (CrI:CD-1 (1CR)BR) outbred albino<br><br>N=30/sex/group | Developmental toxicity study (non-guideline, GLP compliant).<br><br><b>Design:</b><br>Whole-body or nose-only exposure on GD days 6 to 15 for 6 hours per day<br><br><b>Examinations:</b><br>clinical signs, water consumption, uterus, liver and kidney weight, foetus examination.<br><br><b>Statistics:</b><br>• Data displayed as mean $\pm$ SD.<br>• 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). | <b>Test item:</b><br>Ethylene glycol (1.7 $\mu$ m).<br><br><b>Nose-only:</b><br>• Target concentration: 0, 500, 1000 and 2500 mg/m <sup>3</sup><br>• Analytical concentration: 0, 360, 779 and 2505 mg/m <sup>3</sup><br><br><b>Whole body: (positive control):</b><br>• Target concentration: 0, 2100 mg/m <sup>3</sup><br>• Analytical concentration: 0, 2008 mg/m <sup>3</sup> | <b>Nose-only:</b><br>• Body weight, weight gain and liver weight unaffected by treatment.<br>• Increased kidney weight at 1000 (0.46 $\pm$ 0.046 g, $P < 0.05$ ) and 2500 (0.472 $\pm$ 0.034 g, $P < 0.01$ ) mg/m <sup>3</sup> compared to control group (0.431 $\pm$ 0.040g)<br>• Wet fur in nose-only group (around the head) at 2500 mg/m <sup>3</sup> | <b>Nose only:</b><br>• Non-significant and slight reduction of percentage live fetuses per litter in 1000 and 2500 mg/m <sup>3</sup> groups (85.2 $\pm$ 25.48 and 86.1 $\pm$ 21.31 vs 93.3 $\pm$ 7.09 in control group, respectively).<br>• Reduction in foetal body weight observed in 2500 mg/m <sup>3</sup> group (1.126 $\pm$ 0.107g vs 1.289 $\pm$ 0.126g in control group, $P < 0.001$ )<br>• No increase in external or visceral malformations observed.<br>• Increase in litters with at least one foetus with fused ribs in 2500 mg/m <sup>3</sup> group (8/21 vs 1/22 in control group, $P < 0.05$ ).<br><br><b>Whole body (positive control):</b><br>• Reduced gravid uterine weight (17.96 $\pm$ 3.99 vs 20.70 $\pm$ 4.49 in control group, $P < 0.05$ ).<br>• Increase in non-viable implants per litter ( $P < 0.01$ ) and reduced percentage of live fetuses per litter ( $P < 0.01$ ).<br>• Reduction in foetal body weight (1.13 $\pm$ 0.11 vs 1.36 $\pm$ 0.13 in control group, $P < 0.001$ ).<br>• Increase in skeletal malformations (96.3% vs 10.3 in control group, $P < 0.01$ ), including fused arches and ribs. |         |



**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                                                                                                                                                                                                |
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| Tyl et al., 1995 <sup>c47</sup> | Mice, CD1, females | <p>Non-guideline developmental dermal study.</p> <p>Females exposed daily (6h occlusion of skin and restraining) on GD 6 through 15. On GD 18 mice were asphyxiated.</p> <p><b>Examinations:</b></p> <ul style="list-style-type: none"> <li>Maternal organ weights, corpora lutea, kidney microscopy status of implantation sites.</li> <li>Visceral and skeletal malformations and variations.</li> </ul> <p><b>Statistics:</b></p> <ul style="list-style-type: none"> <li>Continuous data: ANOVA and t-tests with Bonferroni post-hoc.</li> <li>Nonparametric data: Kruskal-Wallis and Mann-Whitney U.</li> <li>Incidence data: Fisher-exact test.</li> </ul> | <p><b>Purity:</b><br/>~100%</p> <p><b>Vehicle:</b><br/>water</p> <p><b>Dermal:</b><br/>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.</p> <p>Authors converse ethylene glycol dose by using the mean control body weights from GD 6-15.</p> <p><b>Oral gavage:</b><br/>(positive control): ethylene glycol 3000 mg/kg bw/day</p> <p>Actual dose levels were 99.2-101.2% of target levels.</p> | <ul style="list-style-type: none"> <li>No deaths in treatment groups.</li> <li>All clinical signs were associated with restrain conditions.</li> <li>Gestational weight change increased at 100% (5.044±2.055g) versus controls (3.310±1.796g), P&lt;0.05.</li> <li>Minimal-grade tubular lesions in 3/30 mice in high dose group.</li> </ul> <p><b>Oral gavage</b> (positive control):</p> <ul style="list-style-type: none"> <li>8 females died.</li> <li>Gestational weight change increased (5.283±3.172g) versus controls (3.310±1.796g), P&lt;0.05.</li> </ul> <p><b>Clinical signs:</b><br/>hypoactivity, cold extremities, hunched posture, urogenital discharge, and tissue on paperboard (from abortions).</p> <p><b>Increased incidence in kidney lesions:</b></p> <ul style="list-style-type: none"> <li>Tubular nephrosis (10/30) versus controls (0/30), P&lt;0.01.</li> <li>Tubular cell degeneration (7/30) versus control (0/30), P&lt;0.05.</li> <li>Autolysis (6/30) versus control (0/30).</li> </ul> | <ul style="list-style-type: none"> <li>Number of dams with fully resorbed litters was higher in all treatment groups compared to control.</li> <li>Reduced number of late resorptions/litter at 12.5% (0.0±0.20) versus control (0.3±0.54), P&lt;0.05.</li> <li>Reduced number of dead fetuses/litter at 12.5% (0.1±0.27) versus controls (0.4±0.71), P&lt;0.05.</li> </ul> <p><b>External variations in litters (%):</b><br/>0% EG: 17.4<br/>12.5% EG: 20.0<br/>50% EG: 0.0<br/>100% EG: 11.1<br/>Control: 6.2</p> <p><b>Visceral variations in litters (%):</b><br/>0% EG: 100.0<br/>12.5% EG: 100.0<br/>50% EG: 94.4<br/>100% EG: 100.0<br/>Control: 100.0</p> <p><b>Skeletal variations in litters (%)</b><br/>0% EG: 100.0<br/>12.5% EG: 100.0<br/>50% EG: 100.0<br/>100% EG: 100.0<br/>Control: 100.0</p> <p><b>Oral gavage</b> (positive control):</p> <ul style="list-style-type: none"> <li>Reduced foetal body weight per litter (0.990±0.207g) versus controls (1.241±0.110g), P&lt;0.001.</li> <li>Increased number of litters with soft tissue malformations (11/16) versus control (7/23), P&lt;0.05.</li> <li>Increased number of litters with skeletal malformations (16/16) versus control (12/23), P&lt;0.01.</li> </ul> <p><b>Visceral malformations:</b></p> <ul style="list-style-type: none"> <li>Dilation of lateral ventricle (9/16 litters) versus controls (1/23 litters), P&lt;0.01.</li> </ul> <p><b>Skeletal malformations:</b></p> <ul style="list-style-type: none"> <li>Fusion of 2-12 thoracic arches</li> <li>Fusion of lumbar arch (5/16 litters) versus controls (0/23 litters), P&lt;0.05.</li> <li>Fusion of lumbar centra (5/16 litters) versus controls (0/23 litters), P&lt;0.05.</li> <li>Fusion of sacral centra (4/16 litters) versus controls (0/23 litters), P&lt;0.05.</li> <li>Malaligned thoracic centra (7/16 litters) versus controls (0/23 litters), P&lt;0.01.</li> </ul> | <p><b>Multiple signs of toxicity in the positive control group.</b></p> <p>Increase in incidences of variations were observed in the dermal study, but no increase in incidences of malformations.</p> |



Committee and consulted expert<sup>a</sup>

Members of the Subcommittee on the Classification of Substances Toxic to Reproduction for the advisory report *Ethylene glycol*

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<sup>a</sup> Consulted experts are consulted by the committee because of their expertise. Consulted experts and observers are entitled to speak during the meeting. They do not have any voting rights and do not bear any responsibility for the content of the committee’s advisory report.



The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare and Sport, Infrastructure and Water Management, Social Affairs and Employment, and Agriculture, Fisheries, Food Security and Nature. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

This advisory report has been offered to the Minister of Work and Participation by Dr S.C. Tromp, vice chair of the Health Council.

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