Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS). This Scheme was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with the Australian Government Department of the Environment, Water, Heritage and the Arts, which carries out the environmental assessment for NICNAS.

NICNAS has two major assessment programs: the assessment of human health and safety and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focusing on the assessment of chemicals already in use in Australia, in response to specific concerns about their health/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia.

This assessment of the chemical diethylene glycol (DEG) was initiated in 2007 after DEG was found to be present in Australia in certain brands of imported toothpaste. Due to concerns over the potential health effects of ingestion of DEG, the Australian Competition and Consumer Commission issued recall notices for some brands of toothpaste containing DEG, and issued Consumer Protection Notices banning the supply of toothpaste containing > 0.25 percent of DEG, effective 3 August 2007 for a period of 18 months. According to information on the website of the ACCC, a permanent ban was subsequently declared on 4 March 2009.

NICNAS conducted a call for information from industry on the extent of use of DEG in oral cosmetic products in August 2007 to assist in determining the risk to the public. In April 2008 NICNAS made a submission to the National Drugs and Poisons Schedule Committee (NDPSC) recommending controls considered necessary to protect the public from the risk of injury from oral exposure to DEG from its inappropriate use in oral cosmetic products. The NDPSC considered the scheduling of DEG at their meeting of June 2008 and decided to include diethylene glycol for use in toothpastes and mouthwashes in Appendix C of the Standard for the Uniform Scheduling of Drugs and Poisons, with an exemption cut-off of 0.25 per cent.

For the purposes of Section 78(1) of the Act, copies of assessment reports for new and existing chemical assessments are freely available from the web. Hardcopies are available from NICNAS from the following address:

NICNAS  
GPO Box 58  
Sydney, NSW 2001  
AUSTRALIA  
Tel: +61 (2) 8577 8800  
Fax: +61 (2) 8577 8888
Other information about NICNAS (also available on request and on the NICNAS web site) includes:

- NICNAS Service Charter;
- Information sheets on NICNAS Company Registration;
- Information sheets on the Priority Existing Chemicals and New Chemical assessment programs;
- Safety information sheets on chemicals that have been assessed as Priority Existing Chemicals;
- Details for the NICNAS Handbook for Notifiers; and
- Details for the Commonwealth Chemical Gazette.

More information on NICNAS can be found at the NICNAS web site:

http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the web site of Safe Work Australia:

# Table of Contents

PREFACE ................................................................. III

ACRONYMS AND ABBREVIATIONS ................................. VI

SUMMARY ................................................................. VIII

1. INTRODUCTION .................................................. 1
   1.1 Chemical identity .................................. 1
   1.2 Regulatory information ............................ 1
   1.3 Physical properties of DEG ......................... 3
   1.4 Uses of DEG ............................................. 3

2. TOXICOLOGY ....................................................... 4
   2.1 Toxicokinetics ....................................... 4
   2.2 Acute toxicity ........................................ 5
   2.3 Irritation and sensitisation ....................... 5
   2.4 Repeated dose toxicity ............................. 7
   2.5 Mutagenicity and genotoxicity .................... 10
   2.6 Carcinogenicity ..................................... 11
   2.7 Toxicity to reproduction ............................ 11

3. CONCLUSIONS ..................................................... 14

BIBLIOGRAPHY ....................................................... 16

APPENDIX ............................................................... 22
   1. Short-term toxicity ................................... 22
   2. Long-term toxicity ................................... 25
   3. Episodes of human ingestion of DEG ............... 26
   4. Carcinogenicity ........................................ 27
   5. Toxicity to reproduction ............................. 30
# Acronyms and Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-HEAA</td>
<td>2-(hydroxyethoxy)acetic acid</td>
</tr>
<tr>
<td>ACCC</td>
<td>Australian Competition and Consumer Tribunal</td>
</tr>
<tr>
<td>AICS</td>
<td>Australian Inventory of Chemical Substances (NICNAS)</td>
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<tr>
<td>ASAT</td>
<td>aspartate aminotransferase activity</td>
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<tr>
<td>bw</td>
<td>bodyweight</td>
</tr>
<tr>
<td>Ca</td>
<td>calcium</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>cm</td>
<td>centimetre</td>
</tr>
<tr>
<td>CO₂</td>
<td>carbon dioxide</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
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<td>DEG</td>
<td>diethylene glycol</td>
</tr>
<tr>
<td>DEN</td>
<td>diethylnitrosamine</td>
</tr>
<tr>
<td>EC</td>
<td>European Community, or European Commission</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>F</td>
<td>female</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GD</td>
<td>gestational day</td>
</tr>
<tr>
<td>GIT</td>
<td>gastrointestinal tract</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HSIS</td>
<td>Hazardous Substances Information System (Safe Work Australia)</td>
</tr>
<tr>
<td>IC(NA) Act</td>
<td><em>Industrial Chemicals (Notification and Assessment) Act 1989</em> (Cwlth)</td>
</tr>
<tr>
<td>i.p</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IUPAC</td>
<td>International Union of Pure and Applied Chemistry</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LC50</td>
<td>median lethal concentration</td>
</tr>
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</table>
LD50  median lethal dose
LOAEL  lowest-observed-adverse-effect level
LOEL  lowest-observed-effect level
M  male
m³  cubic metre
mg  milligram
mg/kg bw/d  milligram per kilogram bodyweight per day
mL  millilitre
mo  month
µmol  micromole
NDPSC  National Drugs and Poisons Schedule Committee
NE  not established
NICNAS  National Industrial Chemicals Notification and Assessment Scheme
NOAEL  no-observed-adverse-effect level
NOHSC  National Occupational Health and Safety Commission
OECD  Organisation for Economic Cooperation and Development
PEG  polyethylene glycol
PND  postnatal day
ppm  parts per million
ppb  parts per billion
s.c  subcutaneous
SIDS  Screening Information Dataset
SUSDP  Standard for the Uniform Scheduling of Drugs and Poisons
TGA  Therapeutic Goods Administration
TWA  time-weighted average
USEPA  United States Environmental Protection Agency
v/v  volume per volume
w/v  weight per volume
w/w  weight per weight
wk  week
Summary

Diethylene glycol (DEG) is a widely used chemical in industrial and household applications. It is also used in cosmetics for topical use. In 2007, it was identified in use in certain brands of imported toothpastes.

Potential health concerns over ingestion of DEG led to withdrawal of toothpaste containing DEG from Australian and overseas markets in May-August 2007. Severe adverse effects including deaths have been documented in humans from inadvertent ingestion of DEG used as a glycerine substitute or as a contaminant in medicinal preparations.

DEG is rapidly and almost completely absorbed via the oral route and slowly and incompletely absorbed via the skin. DEG is of low acute oral toxicity in animals. Calculation of lethal doses in humans (median doses of approximately 1.4 g/kg bw) indicates a higher sensitivity to toxic effects compared to animals. Acute or chronic exposure to DEG can affect the nervous system, the kidney and, to a lesser extent, the liver. Lethal doses are associated with renal failure and uraemic coma.

DEG produces minimal skin or eye irritation and no evidence of sensitisation in animals.

From repeat dose toxicity studies in animals, mild renal effects (increases in urine volume) have been observed in experimental animals at doses of 230 mg/kg bw/d, with renal hydropic degeneration at 1.6 g/kg bw/d. Reproductive and developmental effects have also been observed in rodents but at significantly higher doses. Similar data for repeated exposure to the chemical are not available for humans.

As a result of concerns about the potential risks of injury from oral exposures to DEG, especially in oral cosmetic products such as toothpastes and mouthwashes, in 2008 the National Drugs and Poisons Schedule Committee listed DEG in the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). In the SUSDP, DEG for use in toothpastes or mouthwashes except in preparations containing 0.25 per cent or less of diethylene glycol is now listed in Appendix C. Appendix C comprises substances of such danger to health as to warrant prohibition of sale, supply and use.
1. Introduction

This hazard assessment of DEG uses information from the OECD SIDS Initial Assessment Report on the Ethylene Glycols Category (OECD, 2004) and the Dutch Expert Committee on Occupational Standards report - Health-Based Recommended Occupational Exposure Limit for Diethylene Glycol (Health Council of the Netherlands, 2007). The OECD report is a category assessment that includes data not only on DEG but also on several other ethylene glycols. Literature searches conducted up to December 2007 provided relevant supplementary studies.

In this report, references marked with an asterisk denote a secondary citation from key review articles, while references not marked with an asterisk denote an original article examined for this assessment.

1.1 Chemical identity

Common name: Diethylene glycol (DEG)

Structural formula:

\[
\text{HO} \quad \text{O} \quad \text{OH}
\]

Molecular formula: \( \text{C}_4\text{H}_{10}\text{O}_3 \) or \((\text{CH}_2\text{CH}_2\text{OH})_2\text{O}\)

Molecular weight: 106.1

CAS number: 111-46-6

IUPAC chemical name: 2,2’-oxybisethanol

AICS chemical name: Ethanol, 2,2’-oxybis-

1.2 Regulatory information

DEG is listed in:

- the Australian Inventory of Chemical Substances (AICS);
- the Adopted National Exposure Standards for Atmospheric Contaminants in the Occupational Environment [NOHSC:1003(1995)] with a time weighted average (TWA) of 100 mg/m\(^3\);
- the Hazardous Substances Information System (HSIS) in accordance with the Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)] 3rd Edition. The classification and labelling details are: Xn: Harmful (cut-off ≥ 25%); R22: Harmful if swallowed; S2: Keep out of the reach of children, and S46: If swallowed, seek medical advice immediately and show this container or label.
• the Standard for the Uniform Scheduling of Drugs and Poisons, No. 23, Amendment No. 2 – Effective Date 1 January 2009. Diethylene Glycol for use in toothpastes or mouthwashes except in preparations containing 0.25% or less of diethylene glycol, is listed in Appendix C. Appendix C comprises substances of such danger to health as to warrant prohibition of sale, supply and use.

From late May 2007, several countries, including Australia, issued safety warnings to consumers to avoid using certain toothpastes suspected of containing DEG (used as a solvent replacing or being mislabelled as glycerine). Major recalls of imported toothpastes containing DEG were also issued. Excel brand toothpaste was recalled and Mr Cool and Raven brands were withdrawn from sale after negotiations by the Australian Competition and Consumer Commission (ACCC). Subsequently, certain batches of Tri Leaf Spearmint toothpaste were also recalled on 14 August 2007 (ACCC, 2007a; ACCC, 2007b). With advice from the National Industrial Chemicals Notification and Assessment Scheme (NICNAS), the ACCC also banned the supply of toothpaste containing more than 0.25% w/w of DEG, effective 3 August 2007 for a period of 18 months (ACCC, 2007b; ACCC, 2007c). According to information published on the website of the ACCC, a permanent ban on toothpaste containing more than 0.25 per cent by weight of diethylene glycol (DEG) was declared on 4 March 2009 and published in Special Gazette no. S 41 of 12 March 2009 (ACCC, 2009).

In 2007, besides mandatory general labelling requirements for cosmetics under the Trade Practices Act 1974 and the temporary ACCC Consumer Protection Notice on maximum limits of DEG in toothpastes, no specific regulations existed for the use of DEG in cosmetic products, including oral cosmetic products, in Australia. In April 2008, NICNAS made a submission to the National Drugs and Poisons Schedule Committee (NDPSC) recommending the scheduling of DEG under the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). The NDPSC considered the scheduling of DEG at its 53rd meeting of 17-18 June 2008, and resolved to include DEG for use in toothpastes or mouthwashes except in preparations containing 0.25 per cent or less of diethylene glycol in Appendix C of the SUSDP (NDPSC, 2008). Overseas, both the USA and Canada took action (Import Alert IA6674) to prevent the importation of toothpaste containing DEG (FDA, 2007a; Health Canada, 2007). The Chinese General Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) also announced on 11 July 2007 that it had banned the use of DEG in toothpaste (FDA, 2007b). In Europe, the Italian and Spanish authorities ordered the seizure of tens of thousands of tubes including counterfeited well-known Western branded products and toothpaste samples handed out in hospitals, hotels and on airplanes on suspicion of being tainted with DEG (EC, 2007).

In Australia, chemicals in toothpastes are regulated either as cosmetics, by NICNAS, or as therapeutic goods, by the Therapeutic Goods Administration (TGA), depending on their characteristics and performance claims. The TGA regulates toothpastes when they are classed as medicines. Toothpaste is classed as a medicine if the benefits claimed to result from its use go beyond those normal claims made for toothpastes of improvements to oral hygiene or the use of fluoride for the prevention of tooth decay. There are currently no medicines, including toothpastes, containing DEG as an allowable ingredient approved for general sale in Australia.

DEG is not listed on the TGA’s list of substances that may be used in listed medicines in Australia.
DEG is not an approved food additive in Australia. However, DEG is allowable in food in Australia as an impurity in polyethylene glycol (PEG) used as a processing aid or miscellaneous food additive. PEG used for this purpose must contain no more than 0.25% w/w DEG. The amount of PEG used in food is limited only to the lowest level to perform its stated function.

1.3 Physical properties of DEG

DEG is an odourless, colourless, viscous and hygroscopic liquid with a sharply sweetish taste. It is miscible with water and reacts violently with strong oxidants. DEG has a density of 1.118 g/cm$^3$. It has a low vapour pressure (< 0.01 kPa at 25°C). It has a boiling point of 245°C, a melting point of -10°C, a flash point of 124°C (closed cup) and auto-ignition temperature of 229°C. Conversion factors for DEG at 20°C, 101.3 kPa are 1 mg/m$^3$ = 0.227 ppm or 1 ppm = 4.414 mg/m$^3$ (IPCS, 2004; OECD, 2004, Health Council of the Netherlands, 2007).

1.4 Uses of DEG

Worldwide, the largest use of DEG is as an intermediate in chemical syntheses (e.g. in the production of polyester resins, polyurethanes, the explosive diethylene glycol nitrate and other ethylene glycols). It is also used in cement grinding, as an anti-freeze agent, as a constituent of brake fluids, as a humectant for tobacco, glues and corks, as a solvent for paints, lacquers and cosmetics and as a plasticiser for paper, packaging materials and coatings (Health Council of the Netherlands, 2007; HSDB, 2003; OECD, 2004). DEG is also used in skin cosmetics (Bruckner and Warren, 2001; Health Council of the Netherlands, 2007).

Diethylene glycol has been used illegally as counterfeit glycerin and sold as a component of cough syrup and toothpaste (see Section 3.2 and Appendix, point.3).

NICNAS sought information on the Australian use of DEG in oral cosmetic products (e.g. toothpaste and mouthwash) from industry in August 2007. No manufacture or importation of oral cosmetic products containing DEG was reported by Australian companies. Data regarding the use of DEG in other types of cosmetic products such as skin creams and lotions in Australia are not available. However, DEG is listed as an ingredient in specific brands of foundations, acne treatments, facial powders and concealers on overseas cosmetic safety databases (Environmental Working Group, 2008).
2. Toxicology

2.1 Toxicokinetics

DEG is rapidly and almost completely absorbed via the oral route in laboratory animals. Up to 96% DEG was absorbed within 2 hours in rats after single gavage doses of 1 and 5 mL/kg bw (1.12 and 5.6 g/kg bw). A higher dose of 10 mL/kg bw (11.2 g/kg bw), was absorbed over 150-240 min (Heilmair et al., 1993*).

DEG is slowly absorbed through the skin. After 3 days, a cumulative total of 9% of a dermal dose of 50 mg DEG/12 m² skin in rats was found recovered in excreta (urine, faeces, and as exhaled CO₂) and 0.9% was found in tissues (Mathews et al., 1991*). Calculation with the dermal absorption model SkinPerm indicates a maximal skin permeation of 0.1 mg/cm²/h under steady-state conditions when skin absorption equals systemic delivery (Health Council of the Netherlands, 2007).

No studies on the absorption of DEG after inhalation exposure are available. However, because of its polar and hygroscopic characteristics, DEG in vapour or aerosol form is likely to be absorbed soon after it enters the upper respiratory passages (Health Council of the Netherlands, 2007).

Upon absorption, DEG is well distributed throughout the aqueous tissues of the body with lower concentrations in adipose tissues due to its high water solubility and low partition coefficient (log $K_{ow} = -1.98$, Verschueren, 1983*). After gavage dosing of $^{14}$C-DEG in rats, radioactivity was rapidly distributed from the blood into the organs and tissues in the order kidneys > brain > spleen > liver > muscle > fat (i.e. the same order as the blood flow) with the volume of distribution determined as 1 L/kg bw, indicating widespread distribution (Heilmair et al., 1993*).

In animals, the postulated pathway for metabolism of DEG is oxidation via alcohol dehydrogenases and aldehyde dehydrogenases (ADH/ALD). Identified DEG metabolites include CO₂, 2-(hydroxyethoxy)acetic acid (2-HEAA), and oxalic acid (Lenk et al., 1989*). In rats, oxalic acid is not a significant metabolite (Mathews et al., 1991*).

Dose-related increases in percent elimination of DEG and 2-HEAA in urine were noted for both gavage and drinking water dosing in rats (Mathews et al., 1991*). Depending on the dose administered, approximately 45-70% of the total DEG dose is excreted unchanged in the urine within 48 hours, with approximately 11-37% as 2-HEAA after oxidative metabolism (Health Council of the Netherlands, 2007). With increasing dose, the fraction oxidised to CO₂ also decreased from 1.3% to 0.3% (Lenk et al., 1989*). Winek et al. (1978*) found biological half-lives of 8 h and 12 h after oral doses in rats of 6 and 12 mL/kg bw (6.7 and 13.4 g/kg bw) DEG, respectively, indicating that the plasma half-life was dose-dependent and that the metabolism and/or elimination of DEG (either via urine or exhaled CO₂) may become saturated. Excretion in the faeces accounts for minor amounts, between 0.7%-2.2% of the total dose (Heilmair et al., 1993*; Mathews et al., 1991*).

In dogs, a larger portion (up to 92%) of the administered DEG was excreted in the urine unchanged (Mathews et al., 1991*). Repeated administration to dogs for a week did not lead to a consistent increase in urinary oxalate. However, the urinary oxalate was increased in rats maintained on water containing DEG (Mathews et al., 1991*).
Conclusion

In animals, absorption of DEG after oral administration is rapid and distribution occurs to all organs and tissues. In contrast, dermally administered DEG is slowly and incompletely absorbed. DEG and its metabolites are readily cleared from the blood and excreted in the urine. Depending on the dose administered, approximately 45%-70% of an oral dose is excreted unchanged in the urine within 48 hours, and 11%-37% as 2-HEAA after oxidative metabolism. Saturation of metabolism was observed at high doses. Metabolic breakdown of DEG into oxalate appears to be a minor route of elimination in laboratory animals.

2.2 Acute toxicity

Acute oral toxicity data in animals are available.

<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>LD50/LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Rat</td>
<td>15.6 – 30.1 g/kg bw; LD60 = 16.7 g/kg bw#</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>13.3 – 28.1 g/kg bw</td>
</tr>
<tr>
<td>Dermal</td>
<td>Rabbit</td>
<td>12.5 – 13.3 g/kg bw</td>
</tr>
<tr>
<td>Inhalation</td>
<td>Rat</td>
<td>&gt; 4600 mg/m³/4 h (aerosol)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>&gt; 130 mg/m³/2 h</td>
</tr>
</tbody>
</table>

# LD60: minimum lethal dose that kills 60% of the animals tested (Haag and Ambrose, 1937*). Source: OECD (2004); Health Council of the Netherlands (2007).

In animals, the acute oral, dermal and inhalational toxicity of DEG are low. Oral toxicity is similar for both rats and mice with LD50 values ranging 13-30 g/kg bw across both species. A single study of dermal toxicity in rabbits derived an LD50 value of 12.5 or 13.3 g/kg bw (value differs between review sources). Acute inhalational toxicity has also been tested in rats and mice. The 4-hour LC50 in rats was 4600 mg/m³.

Following oral administration of DEG, the clinical signs of toxicity are similar between animals and also resemble those reported for humans (see Section 4.4). Acute toxic doses exert their effect on the central nervous system, the kidney and, to a lesser extent, on the liver. Lethal doses are associated with renal failure with anuria, uraemic coma and death. Macroscopic and histopathological effects include hydropic degeneration of the kidney tubules and the centrilobular areas of the liver, with generalised oedema and haemorrhages.

2.3 Irritation and sensitisation

Irritation and sensitisation data for DEG available for animals and humans are summarised below. None of the animal studies were apparently performed according to OECD test guidelines.

Overall, available data indicate that DEG causes no or only minimal skin and eye irritation in laboratory animals. Respiratory depression was reported in mice although the characteristics were reported as not typical of a pure airway irritant (OECD, 2004). No other information on respiratory irritation was available.

DEG does not cause skin sensitisation in guinea pigs.
<table>
<thead>
<tr>
<th>Species/Study Procedure</th>
<th>Concentrations/Doses</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin irritation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit, Draize test</td>
<td>Unknown</td>
<td>No irritation</td>
<td>Deichmann, 1969* cited in Nordic Steering Group, 1998*</td>
</tr>
<tr>
<td>Rabbit, occlusive</td>
<td>10%-100%</td>
<td>No irritation</td>
<td>Guillot et al., 1982*</td>
</tr>
<tr>
<td>Rabbit, non-occlusive</td>
<td>10%</td>
<td>No irritation</td>
<td>Guillot et al., 1982*</td>
</tr>
<tr>
<td>Rabbit, 100 d 5 females</td>
<td>1:1 DEG:propylene glycol</td>
<td>No significant macroscopic or microscopic changes</td>
<td>Rantuccio et al., 1979*</td>
</tr>
<tr>
<td>Rat, 5/dose, semi-occlusive</td>
<td>100%</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Rat, 2 h; 2x2 h for 2 d</td>
<td>100%, 25 mL/kg</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Guinea pig, 2 h for 2 d; 2x2 h for 2 d; 2x2 h for 14 d</td>
<td>100%, 25 mL/kg</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Eye irritation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit, Draize test</td>
<td>100%</td>
<td>Minimal irritation</td>
<td>Anonymous, 1931* cited in ECB IUCLID, 2000</td>
</tr>
<tr>
<td>Rabbit</td>
<td>100%</td>
<td>No irritation</td>
<td>Carpenter and Smyth, 1946*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>100%</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10%</td>
<td>No irritation</td>
<td>Guillot et al., 1982*</td>
</tr>
<tr>
<td>Rat, Cat, Dog</td>
<td>100%</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Respiratory irritation</td>
<td></td>
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<tr>
<td>Mouse</td>
<td>Unknown</td>
<td>RD50 = 0.0116 g/L</td>
<td>WIL Research Laboratories, 2001*</td>
</tr>
<tr>
<td>Skin sensitisation</td>
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<tr>
<td>Guinea pig, Maximisation test</td>
<td>Unknown</td>
<td>No sensitisation</td>
<td>Bio/Dynamics, 1990*</td>
</tr>
<tr>
<td>Guinea pig, Maximisation test according to Directive 84/449/EEC, B.6</td>
<td>Unknown</td>
<td>No sensitisation</td>
<td>BASF, 1991* cited in DFG, 1995*</td>
</tr>
</tbody>
</table>

RD50: the dose responsible for a 50% decrease in the respiratory rate; h: hour; d: day.
**Human studies**

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Concentrations/Doses</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin irritation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2x2h forearm exposure</td>
<td>100%</td>
<td>No irritation</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>7 men, 6 women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Draize test, 3 d</td>
<td>0.122 g</td>
<td>Minimal irritation</td>
<td>Drill, 1976* cited in ECB IUCLID, 2000</td>
</tr>
<tr>
<td>Patch test, 48 h</td>
<td>20% (in petrolatum)</td>
<td>No irritation</td>
<td>Meneghini et al., 1971*</td>
</tr>
<tr>
<td>occlusive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 volunteers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary irritation patch test</td>
<td>Unknown</td>
<td>Minimal irritation</td>
<td>TKL Research, 1989a* cited in OECD, 2004</td>
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</tr>
<tr>
<td><strong>Skin sensitisation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patch test, 24 h</td>
<td>5%</td>
<td>Local reactions in the test</td>
<td>Newman, 1938* cited in BIBRA, 1993*</td>
</tr>
<tr>
<td>occlusive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 man</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patch test, 48 h</td>
<td>15% glycol mixture</td>
<td>No skin and allergic reactions</td>
<td>Meneghini et al., 1971*</td>
</tr>
<tr>
<td>occlusive</td>
<td>(DEG concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>480 eczematous</td>
<td>unspecified)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dermatitis patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeated insult patch</td>
<td>Unknown</td>
<td>No sensitisation</td>
<td>TKL Research, 1989b* cited in OECD, 2004</td>
</tr>
<tr>
<td>test</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

h: hour; d: day.


Similar to experimental animals, DEG causes no or only minimal skin irritation in humans. Data for eye irritation in humans were not available. In humans, there is a single case study reporting skin sensitisation 2-4 weeks after a man had started smoking a brand of cigarettes containing DEG. However, overall, available data indicate that DEG is not a skin sensitisier in humans.

2.4 **Repeated dose toxicity**

**Animal studies – short- and long-term**

Short-term animal repeated dose toxicity studies (approx. ≤ 90 days in duration) are available in the Appendix (see point.1). Early studies note predominantly kidney and liver damage. These data may be limited to the extent that DEG samples in very early studies may have been contaminated with other glycols (Health Council of the Netherlands, 2007).

In the most recent short-term studies, oral DEG administration was shown to result in tremor, lethargy, piloerection, increased serum ASAT (aspartate aminotransferase...
activity), increased blood coagulation time, decreased antibody response, kidney lesions, retinopathy (histopathological and electrophysiological), myocardium damage and death (Huber et al., 1986*; Rossa and Weber, 1987*; Freundt and Weis, 1989*; Williams et al., 1990*; and Ogbuihi et al., 1991*). Overall, data were insufficient to determine adequately the effects from short-term dermal and inhalation exposure.

Studies of long-term DEG exposure in animals are also available (Appendix, point 2). Effects from long-term exposure are seen predominantly in the kidney and to a lesser degree in the liver. Long-term oral studies before 1950 (Morris et al., 1942*; Fitzhugh and Nelson, 1946*; and Hanzlik et al., 1947*) revealed oxalate crystals, kidney and liver vacuolar degeneration, as well as bladder stones and bladder tumours (see Carcinogenicity section below) in rats fed DEG in the diet and drinking water.

Two well-conducted studies were identified from which effect levels from long-term oral DEG administration could be derived (OECD, 2004; Health Council of the Netherlands 2007). In these two studies by Gaunt et al. (1976*) using DEG doses in food of 0%-4% (0.3-3.7 g/kg bw/d) for 98 days and 0%-2% (0.05-1.5 g/kg bw/d) for 225 days in Wistar rats (10-15/sex/dose), kidney effects were reported consisting of oxalate crystalluria, increased urine volumes and histopathological evidence of hydropic degeneration and tubular necrosis.

For the crystalluria and increased urine volumes, there were inconsistent findings between male and female rats and questionable dose-response relationships. For example, the number of male rats with urinary oxalate crystals was not increased at the highest male dose of 1.2 g/kg bw/d in the 225 day study. In addition, the observed increase in urinary volumes was possibly caused by the osmotic diuretic effect of DEG and the oxalate crystalluria could not be explained in view of oxalic acid being a minor metabolite of DEG in rats. Therefore, the significance of elevated production of oxalate was regarded as unclear (Health Council of the Netherlands, 2007) and was viewed as a biomarker and not an indication of toxicity (OECD, 2004).

OECD (2004) identified a LOAEL for kidney effects of 230 mg/kg bw/d from the 225 day study based on increases in urine volume. The NOAEL was 100 mg/kg bw/d. Health Council of the Netherlands (2007) regarded a NOAEL based on renal histopathological findings as more relevant than a NOAEL based on increased urine volumes. From the 98 day study, a LOAEL based on renal hydropic degeneration was established at 1.6 g/kg bw/day with the NOAEL at 300 mg/kg bw/d (Health Council of the Netherlands, 2007).

**Human incidents – short-term toxicity**

Accidents in humans following acute and short-term DEG exposure have been recorded (Appendix, point 3; adopted from O’Brien et al., 1998 and Health Council of the Netherlands, 2007).

A large number of mass poisonings involving DEG ingestion have occurred within the last 70 years (1937-2006) with typical features of toxicity including metabolic acidosis and acute renal failure. Early mortality and morbidity are high in cases of human DEG toxicity, with most deaths occurring within the first 2 weeks post exposure. A small number of cases of neurologic impairment (encephalopathy, demyelinating neuropathy, optic neuritis, unilateral facial paralysis, cerebral oedema and haemorrhages) have been reported (Bowie and McKenzie, 1972*; Drut et al., 1994*; Hari et al., 2006; O’Brien et al., 1998). Neurological effects were also noted during severe intoxications after uptake of DEG in patients with burns. The patients developed acute anuric renal failure with
metabolic acidosis and concomitant severe neurological abnormalities progressing to coma and finally death (Cantarell et al., 1987*).

These incidents were attributed to the substitution of DEG for more expensive, non-toxic glycols in medicinal preparations. Typically, acetaminophen elixirs have been involved, explaining the preponderance of paediatric deaths. Large overlaps in ranges of lethal and non-lethal doses have been noted for adults (Calvery and Klump, 1939*) and children (O’Brien et al., 1998). After large-scale intoxication of Haitian children with a paracetamol syrup contaminated with DEG, it was estimated that a median dose of 1.49 g/kg bw DEG (range 0.25-4.9 g/kg bw) had caused acute renal failure (O’Brien et al., 1998). The ingested dose in the Haiti accident was estimated by multiplying the percentage of DEG in the bottle by the volume missing from the bottle. Ferrari and Giannuzzi (2005*) recently estimated an acute lethal dose of 0.014-0.17 g/kg bw for humans from the massive intoxication in Argentina in 1992. These were the lowest ever values reported in fatal accidents and considered prone to error since the estimation was based on the volumes of ingestion reported by family or relatives during interrogation (Health Council of the Netherlands, 2007).

When comparing the median lethal dose of 1.49 g/kg bw with reported lethal doses for animals species including rodents, humans appear to be 10 times more sensitive to DEG than animals for acute toxic effects (Health Council of the Netherlands, 2007).

**Mode of action**

The main reported health hazard of DEG is renal toxicity with renal failure being the most prominent cause of death in animal studies and human accidents.

Metabolism of a similar glycol, ethylene glycol (EG), yields oxalate ions that readily form calcium oxalate monohydrate crystals in the presence of calcium. Guo and Martin (2005*) found that these crystals, and not the oxalate ions, were responsible for the membrane damage and subsequent cell death observed in normal human and rat renal proximal tubule cells. Human case findings support the view of calcium oxalate crystals as the toxic agent of EG poisoning (Armstrong et al., 2006*). As DEG is fractionated from crude EG mixtures during production and the purity of DEG was poorly characterised in early studies, DEG toxicity could result also from oxidation of small amounts of EG present as a contaminant in DEG as well as from DEG itself (Health Council of the Netherlands, 2007).

DEG also produces a dose-dependent metabolic acidosis in animals and man. In rats, the acidosis indicated by transient accumulation of 2-HEAA was shown to resolve after 24-48 hours at doses up to 10 mL/kg bw (11.2 g/kg bw) (Heilmair et al., 1993*). At higher doses of DEG (> 12.5 mL/kg bw or 13.9 g/kg bw), higher concentrations of 2-HEAA were produced which overwhelmed blood buffering capacity (Heilmair et al., 1993*). Alcohol dehydrogenase inhibition with pyrazole pre-treatment in rats reduced the lethality of DEG, indicating that the oxidation of DEG and/or its metabolite 2-HEAA contributes to acute toxicity (Wiener and Richardson, 1989*).

DEG-related metabolic acidosis may be further enhanced by accumulation of lactate. In rats, metabolic acidosis is known to inhibit hepatic gluconeogenesis in vivo. The highest lactate concentration in blood was found 120 hours following DEG administration (Heilmair et al., 1993*). In humans, metabolic acidosis is not immediately seen in acute DEG poisoning but may develop within a day after ingestion (Alfred et al., 2005). This slow development of metabolic acidosis in humans has been
explained by the slower formation of ethylene glycol and 2-HEAA from DEG (Sangster, 1985*).

DEG also induces osmotic diuresis in animals. In rats, doses of 1 to 15 mL/kg bw (1.12 to 16.7 g/kg bw) produced a linear increase in the volume of urine excreted over 24 hours (Heilmair et al., 1993*), with doses of 16.7 and 19.5 g/kg DEG producing a 4-fold increase in the volume of the 24-hour urine as compared to that of control animals. In these studies, because of the narcotic effect of DEG, not all the rats were able to replenish the significant loss of body water. Hydropic degeneration of the tubuli, oliguria and anuria ensured after 24-48 hours and rats subsequently developed symptoms of uraemia with death occurring 1-2 days later from uraemic coma.

2.5 Mutagenicity and genotoxicity

In vitro

DEG was negative in Ames tests with Salmonella typhimurium strains TA97a, TA98, TA100, TA102, TA104, TA1535, TA1537 and TA1538, with and without metabolic activation at concentrations up to 100 mg/plate (NTP, 1982*; Pfeiffer and Dunkelberg, 1980*; Slesinski et al., 1986*; Yoshida et al., 1986*; Zeiger et al., 1987*). A weak mutagenic effect was detected in strain TA104 at 315 µmol/mL DEG in the presence of metabolic activation (Krug et al., 1986*). DEG (150 and 750 µmol/mL) did not induce gene conversion or mitotic crossing over or reverse mutation in Saccharomyces cerevisiae D7 and D61M. In an aneuploidy test with Saccharomyces cerevisiae D61M, an increase of the mitotic aneuploidy rate was observed in the absence of metabolic activation (Krug et al., 1986*). DEG (up to 50 mg/mL) was also negative with or without metabolic activation in a chromosome aberration, an HPRT-test, a sister chromatid exchange assay with Chinese hamster ovary cells (Slesinski et al., 1986*), and a SOS chromotest with Escherichia coli PQ37 (Hude et al., 1988*).

In vivo

DEG was tested in vivo for chromosome aberration in Chinese hamsters (Yoshida et al., 1986* - Japanese paper, only tabulated data in English). Groups of 100 cells was analysed for all concentrations in the various exposure scenarios. The background in the controls was one or two aberrations in 100 analysed cells for all scenarios, regardless the treatment time. Slight increases in the number of chromosome aberrations were observed, but in view of the Health Council of the Netherlands (2007) the results are difficult to interpret due to a lack of differentiation between chromosomal gaps and chromosomal breaks. With treatment times of 6, 24 and 48 hours, intraperitoneal injection induced chromosome aberrations at 1.25, 2.5, 5 g/kg (7.5 g/kg not tested) while oral dosing induced aberrations only at 7.5 g/kg. Exposure via the drinking water for 1-2 weeks resulted in an increase of aberrations at all dose levels (0.5, 1, 2%). After dietary exposure for 12 weeks at dose levels of 1.25%-5%, the number of chromosome aberrations was similar to the control (1-2 aberrations per 100 cells).

An increase in chromosome damage in the bone marrow cells was also reported after administration of 1/5 of the LD50 of DEG by gavage in hamster (Barilyak, 1985*). In rats, DEG administration (dose not reported) caused dominant lethal mutations (Barilyak, 1985*).

In a micronucleus test (species not reported), a single intraperitoneal injection of 60% of LD50 of DEG caused kidney damage such as tubular necrosis. This induction was
suppressed when the animals were pretreated during 7 days with a low daily dose of DEG (4% of the LD50) (Krug et al., 1986*).

**Conclusion**

DEG was shown to be negative in the majority of gene mutation and chromosome aberration studies in vitro. Some indications of chromosomal damage were seen in vivo only at high doses. Taken together, DEG is considered non-genotoxic.

2.6** Carcinogenicity**

Information on animal and human carcinogenicity studies for DEG are summarised in the Appendix (see point 4).

Urinary bladder calculus and tumour responses were recorded in some long-term oral studies in the rat. Bladder tumours were found associated with the formation of oxalate containing bladder stones in a 2-year feeding study by Fitzhugh and Nelson (1946*). On the other hand, Weil et al. (1965*, 1967*) found that DEG did not induce bladder tumours in rats unless a foreign body or lesion was present, such as an oxalate-containing bladder stone or a surgery-induced bladder lesion. These authors concluded that the bladder tumours seen were due to mechanical irritation by oxalate-containing bladder stones rather than the carcinogenic response to DEG. In more recent studies such as Ito et al. (1988*), Masui (1988*) and Hiasa et al. (1990* and 1991*), DEG did not demonstrate any evidence of carcinogenic effects after oral administration. Several studies in mice also showed that DEG is not carcinogenic after dermal application.

No information was found in the literature concerning the occurrence of bladder stones in humans after ingestion of DEG. Overall, although some human carcinogenicity information are available (Appendix), data are insufficient (e.g. lack of a quantitative estimate of DEG exposure and sound methodology) to evaluate the carcinogenic potential of DEG. The International Agency for Research on Cancer (IARC) has not evaluated DEG as a carcinogen.

2.7** Toxicity to reproduction**

Data for the reproductive toxicity of DEG are available in the Appendix (see point 5). DEG was evaluated for reproductive toxicity in Swiss CD-1 mice over two generations using a continuous breeding protocol (NTP, 1984*; Williams et al., 1990*). F0 mice (20 pairs/dose) were exposed to drinking water containing 0, 0.35, 1.75, and 3.5% w/v DEG (approx. 0, 0.61, 3.1, and 6.1 g/kg bw/d) for 14 weeks. While F0 body weight was unchanged during the mating period, the number of litters/pair and live pups/litter were reduced by 12% and 32% respectively at the high dose. There was also a significant increase in the cumulative days to litter and a significant decrease in the number of pairs producing the third, fourth, and fifth litters in the high dose group. After F1 weaning, necropsy of F0 mice showed no treatment-related change in male body or organ weight and histopathology, but a 7% decrease in female body weight after 6.1 g/kg bw/d DEG consumption. Relative organ weights were unchanged.

The F1 generation had decreased body weights at birth and exhibited poor postnatal survival. Body weight adjusted for litter size was reduced by nearly 12%. In the fifth or final litters, 12% of the liveborn pups and 95% of the pups found dead on postnatal day (PND) 0 had craniofacial malformations including exencephaly and cleft palate. At PND 2, 50% of the malformed pups had died. Similar malformations were also noted for live and dead pups in the other litters exposed to 6.1 g/kg bw/d DEG. At 3.1 g/kg
bw/d DEG, body weights of both sexes were depressed at weaning, at onset of mating, and at necropsy. However, no adverse effects on reproduction were observed. For F1 mating, mice exposed to 3.1 g/kg bw/d DEG were used because of insufficient mice available from the high dose group. After birth of F2 and oestrous evaluation of F1, necropsy of F1 mice showed decreased body weight in males (11%) and females (7%), but no effects on organ weight, sperm indices, pup weight or survival.

A crossover mating trial of the F0 mice to determine the affected sex was inconclusive, but suggested that offspring development was compromised in females exposed to 3.5% DEG. The NOAEL for fertility and developmental effects in this study was 3.1 g/kg bw/d (1.75%) with a LOAEL being 6.1 g/kg bw/d (3.5%) based on reductions in litters/pair, live pups/litter and live pup weight.

In a developmental toxicity study, DEG was administered by gavage to timed-pregnant Swiss CD-1 mice (26-31/dose) on gestational days (GD) 6-15 at dose levels of 0, 1.25, 5, 10 g/kg bw/d (NTP, 1991*). Animals were examined daily and at necropsy (GD 17) for maternal body and organ weights, implant status, foetal weight, sex, and morphological development. Food and water consumption and body weights were determined on GD 0, 3, 6, 9, 12, 15, and 17. Maternal body weights did not differ significantly at any doses. At ≥ 5 g/kg bw/d, relative water intake was significantly increased over control for every interval starting at GD 6. Necropsy on GD 17 showed significantly increased absolute and relative kidney weights. At 10 g/kg bw/d, relative food consumption was significantly decreased from GD 6 to 12. Necropsy and histopathologic examination of one high dose animal in extremis on GD 10 revealed evidence of DEG-related renal degeneration and morbidity. Renal tubular degeneration was found in 3/28 of the pregnant high dose females versus 0/20 of the pregnant control females. No effects of DEG were observed on pre- or post-implantation loss. The mean foetal body weight on GD 17 decreased linearly (99%, 96%, and 85% of the control from low to high dose) with a statistical significance seen at the high dose. Examination of the foetuses for external, visceral and skeletal malformations did not reveal any significant effects between dose groups. The 5 g/kg bw/d DEG dose produced significant maternal toxicity, but no clear evidence of developmental toxicity. Hence, the developmental NOAEL was considered to be 5 g/kg bw/d and the LOAEL 10 g/kg bw/d based on decreases in foetal body weight.

In another study, timed-pregnant CD-1 mice and CD rats were dosed daily by gavage with undiluted DEG over GD 6-15 (Neepher-Bradley et al., 1992*; Ballantyne and Snellings, 2005*). Mice received 0 (distilled water), 0.6, 2.8, 11.2 g/kg bw/d, and rats 0, 1.1, 4.5, 8.9 g/kg bw/d. Animals were examined daily and at necropsy (GD 18) for gross pathology, maternal body and organ weights, gravid uterus and implant status, foetal weight, sex, and morphological development.

With mice, maternal toxicity was present at 2.8 g/kg bw/d (increased water consumption) and at 11.2 g/kg bw/d (mortality 6/30 mice, increased water consumption). Implantations were comparable across all groups. Foetal body weights were significantly reduced at 11.2 g/kg bw/d without increases in variations or malformations, either total, by category or individually.

With rats, maternal toxicity was present at 4.5 g/kg bw/d (increased water consumption) and at 8.9 g/kg bw/d (mortality 3/25 rats, reduced body weight and food consumption, increased water consumption, kidney and liver weights, and renal histopathology). There were no treatment-related effects on corpora lutea or implantations. Foetal body weights were reduced at 8.9 g/kg bw/d. There were no significant effects with respect to
total or individual external or visceral variations. Individual skeletal variations were significantly increased at 8.9 g/kg bw/d (poorly ossified interparietal, thoracic centra number 10 and 13, and bilobed thoracic centrum number 10) and at 4.5 g/kg bw/d (split anterior arch of atlas and bilobed thoracic centrum number 10), which are consistent with reduced foetal body weight. No malformations were observed at any dose groups. Thus, under the conditions of this study, the NOAEL was 0.6 g/kg bw/d with the mouse and 1.1 g/kg bw/d with the rat for maternal toxicity, and 2.8 g/kg bw/d with the mouse and 1.1 g/kg bw/d with the rat for developmental toxicity.

Human data

No human data or case reports on reproductive and developmental effects of DEG are available.

Conclusion

In oral studies, adverse effects on fertility were seen in mice and foetal abnormalities occurred in rats and mice. Inhalation and injection studies in rabbits and hamsters also revealed foetal abnormalities and other adverse effects on the foetus. However, reduced fertility was observed only at high doses of DEG, up to 6.1 g/kg bw/d in mice with maternal toxicity. With regard to developmental toxicity, a significant decrease in mean foetal body weight in mice was seen at 10 g/kg bw/d in the presence of maternal toxicity. In addition, at an oral dose of 6.1 g/kg bw/d in a 2-generation study in mice, craniofacial malformations, including exencephaly and cleft palate, and related mortality were observed in the presence of maternal toxicity. In rats, a decreased foetal body weight with increased skeletal variations was seen at 4.5 g/kg bw/d in the presence of maternal toxicity. Foetal malformations were not observed at dose levels up to 8.9 g/kg bw/d. From these studies, the NOAEL for fertility and developmental effects is established at 3.1 g/kg bw/d with a LOAEL of 6.1 g/kg bw/d based on reductions in litters/pair, live pups/litter and live pup weight.
3. Conclusions

DEG is an industrial chemical with widespread usage. It is also reported to be used in cosmetic creams. It is a clear syrup liquid and totally miscible with water. In animals, DEG is readily absorbed via the oral route and distributed throughout the whole body in the order kidneys > brain > spleen > liver > muscle > fat. Dermal absorption is slow and limited, at approximately 9% after a 3-day application. DEG and its metabolites are rapidly cleared from the blood and excreted in the urine. Small amounts are exhaled as CO₂. In animals, depending on the dose administered, approximately 45%-70% of the oral dose is excreted unchanged in the urine within 48 hours, and approximately 11%-37% as 2-HEAA after oxidative metabolism. Saturation of metabolism occurs at high doses. Breakdown of DEG into oxalate appears to be a minor route in laboratory animals.

DEG produces minimal eye and skin irritation. DEG causes respiratory depression in mice, although the characteristics were not typical of a pure airway irritant. DEG does not cause skin sensitisation in animals. A single case of skin sensitisation to DEG was identified in a man who had been smoking cigarettes containing DEG.

In laboratory animals, DEG has relatively low acute toxicity. The oral LD50 values for mice and rats are in the range of 13-30 g/kg bw, and the dermal LD50 for rabbits is 12-13 g/kg bw.

Following single or repeated oral administration, the clinical signs of toxicity are similar between animals and resemble those reported for humans although humans appear about 10 times more sensitive to DEG for acute toxic effects. The target sites were the kidney, liver and nervous system. A large number of acute human DEG poisonings attributable to the substitution of DEG for more expensive, non-toxic glycols in medicinal preparations have occurred over the last 70 years (1937-2006) with typical features of toxicity including metabolic acidosis and acute renal failure.

In humans, mortality and morbidity are high in cases of inadvertent DEG ingestion, with most deaths occurring within the first 2 weeks post exposure. Neurological impairments observed after exposure include encephalopathy, demyelinating neuropathy, optic neuritis, facial paralysis, cerebral oedema and haemorrhages. Acute anuric renal failure with metabolic acidosis and concomitant severe neurological abnormalities progressing to coma and finally death were also noted during severe intoxications after uptake of DEG in patients with burns. A median lethal oral dose of 1.49 g/kg bw DEG (range 0.25-4.9 g/kg bw) was estimated from large-scale intoxication of Haitian children with a paracetamol syrup contaminated with DEG. However, large overlaps in ranges of lethal and non-lethal doses have been observed for adults and children.

Chronic toxicity from prolonged and repeated exposure to DEG are associated with kidney, and to a lesser degree, liver effects. From two studies by Gaunt et al. (1976*) in Wistar rats, the LOAEL for increased urine volumes is 230 mg/kg bw/d and the NOAEL 100 mg/kg bw/d. The LOAEL for renal hydropic degeneration is 1.6 g/kg bw/d and the NOAEL 300 mg/kg bw/d.

Available data indicate that DEG is negative in in vitro genotoxicity tests. Some positive results were obtained in in vivo genotoxicity studies, however, only at high toxic doses of DEG. Overall, DEG is considered non-genotoxic.
Urinary bladder calculus and tumour responses were recorded in some long-term oral studies in the rat. These are considered to result from chronic irritation of the bladder wall by DEG-induced stones. Human data are insufficient to evaluate the carcinogenic potential of DEG. The IARC has not evaluated DEG as a carcinogen.

Several animal reproductive toxicity studies indicate that DEG induces adverse effects on fertility and development, but at much higher doses than those associated with kidney and liver toxicity. From these studies, the LOAEL for fertility and developmental effects based on reductions in litters/pair, live pups/litter and live pup weight was established at 6.1 g/kg bw/d. The NOAEL was 3.1 g/kg bw/d.
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Telegina KA, Mustaeva NA, Sakaeva SZ, & Boiko VI (1971) [Health of persons handling diethyleneglycol in the industry producing aromatic hydrocarbons from crude oil]. Gig Tr Prof Zabol, 15(9):40-1. In Russian.


## Appendix

### 1. Short-term toxicity

**Animal studies**

<table>
<thead>
<tr>
<th>Species/Study Procedure</th>
<th>Concentrations/Doses (g/kg bw/d)</th>
<th>NOAEL/LOAEL (g/kg bw/d) &amp; Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat, 2-8 d, 3/d</td>
<td>1.68, 7.56, 10.08, 20.07</td>
<td>1.68 / 7.56 Thirst, diuresis, kidney failure, coma, mortality after 2-5 d, tubular degeneration and necrosis</td>
<td>Geiling et al., 1937* and Cannon, 1937* cited in DFG, 1995*</td>
</tr>
<tr>
<td>Rat, 8 d, 2/d</td>
<td>0.56, 2.2</td>
<td>0.56 / 2.2 All died after a total dose of 15.6-20 g with symptoms matching those described after single oral doses</td>
<td>Geiling et al., 1937* cited in Hesser, 1986*</td>
</tr>
<tr>
<td>Rat, 20 d</td>
<td>3.1</td>
<td>3.1/ NE No accumulative effects</td>
<td>Plugin, 1968* cited in Cavender and Sowinski, 1994*</td>
</tr>
<tr>
<td>Rat Wistar, 28 d</td>
<td>0.038, 0.188, 0.75, 3</td>
<td>0.75 / 3 Oxalate accumulation in both sexes, oxalate stones observed in males, oxalate excretion was reversible on treatment withdrawal</td>
<td>BASF, 1988* cited in BG Chemie, 1990*</td>
</tr>
<tr>
<td>Rat, 1-35 d</td>
<td>1.12, 3.36, 8.4, 11.2, 28</td>
<td>NE / 1.12 Kidney lesions, tubular degeneration and necrosis, liver oedema</td>
<td>Harris, 1949* cited in DFG, 1995*</td>
</tr>
<tr>
<td>Rat, 40 d</td>
<td>5.9</td>
<td>NE / 5.9 Mortality not reported</td>
<td>Weatherby and Williams, 1939* cited in BG Chemie, 1990*</td>
</tr>
<tr>
<td>Rat, 11-50 d, 1-2/d</td>
<td>8.4</td>
<td>NE / 8.4 All died, kidney and liver vacuolar degeneration</td>
<td>Weatherby and Williams, 1939*</td>
</tr>
<tr>
<td>Rat, 50 d, 5/wk</td>
<td>0.112, 0.56, 1.12, 2.24</td>
<td>2.24 / NE</td>
<td>Loeser et al., 1954*</td>
</tr>
<tr>
<td>Rat Wistar, 60 d</td>
<td>8.4</td>
<td>NE / 8.4 All died, kidney and liver vacuolar degeneration</td>
<td>Weatherby and Williams, 1939* cited in DFG, 1995*</td>
</tr>
<tr>
<td>Rat, 63 d 5 males</td>
<td>0.125, 0.25, 0.5, 1, 2, 4, 5, 10, 20%</td>
<td>0.125% / 0.25% 0.25%: ↓ body weight gain 1%: slight myocardium damage</td>
<td>Holck, 1937* cited in DFG, 1995*</td>
</tr>
<tr>
<td>Species, Duration</td>
<td>Dose</td>
<td>Mortality</td>
<td>Effects</td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>Rat, 11-90 d</td>
<td>1-2 NE / 1-2</td>
<td>4%: mortality, GIT irritation</td>
<td>Kidney and liver damage</td>
</tr>
<tr>
<td>gavage, drinking water</td>
<td></td>
<td>≥ 5%: mortality within 2 wk</td>
<td></td>
</tr>
<tr>
<td>Rat, 90 d 6/sex/dose drinking water</td>
<td>1, 2, 5, 10, 20%</td>
<td>1%/2%</td>
<td>≥ 5%: rapid weight loss, stupor, weakness, haemoglobinuria, severe diuresis and necrosis Microscopy: ↓ liver cells size and more densely packed, kidney vacuolar degeneration and sporadic necrosis</td>
</tr>
<tr>
<td>Rat SD, 90 d 8 females drinking water</td>
<td>0.2</td>
<td>0.2 / NE</td>
<td>No change in renal function (single gavage dose ≥ 0.7: transient renal impairment)</td>
</tr>
<tr>
<td>Rat, 1-174 d 17-35/dose drinking water</td>
<td>0.3: 17 rats for 33-124 d 0.6: 30 rats for 33-174 d 3.5: 25 rats for 15-95 d 6.0: 35 rats for 1-6 d</td>
<td>0.6 / 3.5 3.5: 14/25 died after 5-56 d 6.0: 9/35 died after 1-6 d</td>
<td>Tubular epithelium lesions, urine retention, ↑ residual nitrogen and uraemia, liver and adrenal cortex vacuolar degeneration</td>
</tr>
<tr>
<td>Rat, 175 d 10/sex/dose drinking water</td>
<td>0.59, 1.94</td>
<td>NE / 0.59</td>
<td>2 rats/group died within 100 d. No mortality in later 75 d</td>
</tr>
<tr>
<td>Rat, 180 d 2/wk 45/sex/dose gavage</td>
<td>2.5, 5</td>
<td>5 / NE</td>
<td></td>
</tr>
<tr>
<td>Mouse, 14 d 4/sex/dose drinking water</td>
<td>2.6, 6.5, 13, 19.5, 26</td>
<td>6.5 / 13</td>
<td>13: ↓ body weight gain, water consumption and dehydration in females 19.5: all above plus piloerection, tremor, lethargy and mortality in 3/4 males 26: all above plus ataxia, hyperactive and mortality in 3/4 males and 2/8 females</td>
</tr>
<tr>
<td>Mouse NMRI, 98-120 d 20/dose drinking water</td>
<td>0.05, 0.5, 5 (after 1 wk, mice were immunised with tetanus toxoid, vaccinia virus, and human erythrocytes; after 4 mo, mice were inoculated with Streptococcus pyogenes)</td>
<td>NE / 0.05 0.5: ↑ ASAT after 2.5 mo, ↑ streptococcus induced mortality after 4 mo ≥ 0.05: dose-dependent ↑ coagulation time, ↓ immunity after 3.5 mo</td>
<td></td>
</tr>
<tr>
<td>Rabbit, 9 d 1-2/sex/dose gavage</td>
<td>1.68, 33.6</td>
<td>NE / 1.68</td>
<td>Weakness, ↑ breathing, anuria, kidney failure, coma, death</td>
</tr>
<tr>
<td>Rabbit, 28 d 6/dose</td>
<td>1-4</td>
<td>NE / 1</td>
<td>1/6 died after 7 d (kidney damage, pulmonary oedema), 4/6 killed</td>
</tr>
</tbody>
</table>
**drinking water**

<table>
<thead>
<tr>
<th>Animal, Duration, Dose</th>
<th>NE / Dose</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit NZ, 90 d 4/dose drinking water</td>
<td>7</td>
<td>Kidney lesions (vacuolar degeneration, tubular calcification and necrosis), with 2 of them also exhibited liver lesions (vacuolar degeneration)</td>
</tr>
</tbody>
</table>

**Retinopathy**

- **Rosa and Weber, 1987***
  - Guinea pig, 2-11 d 9 males 1.2 (0.4% ethylene glycol)
  - NE / 1.2
  - Myocardium microscopic changes: coagulative myocytolysis and loss of myofibrils, including mitochondrial swelling, pleomorphism and hyperplasia with an associated distension of interfibrillary spaces and a displacement, distortion and rupture of adjacent myofibrils

**Hamster, 21 d 4/dose drinking water**

- YEH et al., 1990*
  - Hamster, 2-12 d 5/dose 2.2
  - NE / 2.2
  - Kidney lesions in 5/5 and liver lesions in 2/5 animals

**Dog, 13 d 3/dose gavage**

- **Weatherby and Williams, 1939*** cited in DFG, 1995*
  - Dog, 13 d 3/dose 8.4
  - NE / 8.4
  - Mortality, kidney and liver damage

**Dog, 18 d 5/dose**

- **Weatherby and Williams, 1939*** cited in Hesser, 1986*
  - Dog, 18 d 5/dose 5.9
  - NE / 5.9
  - All died after a total dose of 23.4-105.4 g within 4-18 d

**Dermal**

<table>
<thead>
<tr>
<th>Animal, Duration, Dose</th>
<th>NE / Dose</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Rabbit, 30 d, 1 h/d 3/dose | 0.18, 0.36 | 0.18 / 0.36
  - All died after 21-25 d |

**Rabbit, 100 d 5 females 1:1 DEG:propylene glycol**

- Rantuccio et al., 1979*
  - NE / 2.8
  - No significant macroscopic or microscopic changes. Systemic effects were not reported.

**Mouse, 60 d**

- **Marchenko, 1973*** cited in BG Chemie, 1990*;
  - Mouse, 60 d 2.8
  - NE / 2.8
  - Oedema and hyperaemia in the brain and spinal cord, localised brain tissue bleeding, neurons destruction with compensatory outgrowth of glial cells.

**Inhalation**

<table>
<thead>
<tr>
<th>Animal, Duration, Route</th>
<th>NE / Dose</th>
<th>Notes</th>
</tr>
</thead>
</table>
| Rat, 9 d 0.001 g/L (aerosol) | 0.001 / NE | Kilgour, 2001*
  - No further information |

---

NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; NE: not established; GIT: gastrointestinal tract; ASAT: aspartate aminotransferase activity; d: day; wk: week; mo: month; ↓: decreased; ↑: increase.


## 2. Long-term toxicity

### Animal studies

<table>
<thead>
<tr>
<th>Species/Study Procedures</th>
<th>Concentrations/ Doses (g/kg bw/d)</th>
<th>NOAEL/LOAEL (g/kg bw/d) &amp; Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat, 3-7 mo Gavage</td>
<td>0.3</td>
<td>NE / 0.3 Oedema and hyperaemia in the brain and spinal cord, localised brain tissue bleeding, neuron destruction with compensatory outgrowth of glial cells Insufficient information on test method and results</td>
<td>Marchenko, 1973* cited in BG Chemie, 1990*; DFG, 1995*</td>
</tr>
<tr>
<td>Rat Wistar, 98 d 15/sex/dose diet</td>
<td>M-F: 0.3-0.4, 1.6-1.8, 3-3.7</td>
<td>M: 0.3 / 1.6 and F: 1.8 / 3.7 (based on renal histopathological effects) ≥ 0.4: oxalate crystalluria, mild renal function defects 3-3.7: mortality 6/15 male rats with renal damage. The survivors showed ↓ growth, ↑ water intake, ↑ urinary flow, haemoconcentration, enlarged kidneys, kidney and liver degeneration</td>
<td>Gaunt et al., 1976*</td>
</tr>
<tr>
<td>Rat Wistar, 225 d 10/sex/dose diet</td>
<td>M-F: 0.05-0.06, 0.1-0.13, 0.23-0.29, 1.2-1.5</td>
<td>M: 0.05 / 0.1 and F: 0.13 / 0.29 (based on oxalate crystalluria) M: 0.1 – F: 0.29: ↑ oxalate crystalluria in males (13-23%) and females</td>
<td>Gaunt et al., 1976*</td>
</tr>
<tr>
<td>Rat, 2 yr 4-6/sex/dose diet</td>
<td>1.3, 2.6</td>
<td>NE / 1.3 Oxalate crystals, kidney and liver degeneration</td>
<td>Morris et al., 1942* cited in BG Chemie, 1990*; DFG, 1995*</td>
</tr>
<tr>
<td>Rat Osborne-Mendel, 2 yr 12 males/dose diet</td>
<td>0.75, 1.5, 3</td>
<td>NE / 0.75 3: ↓ growth and survival, dose-related kidney and liver degeneration (less pronounced and dose-dependent at medium and low doses)</td>
<td>Fitzhugh and Nelson, 1946*</td>
</tr>
<tr>
<td>Rat, 2 yr 5 males drinking water</td>
<td>1.31, 2.56</td>
<td>NE / 1.31 1.13: Oxalate crystals, kidney and liver degeneration 2.56: mortality, ↓ body weight</td>
<td>Hanzlik et al., 1947* cited in DFG, 1995*</td>
</tr>
<tr>
<td>Rat Carworth-Farm-Nelson (weanling, 2 mo and 1 yr old),</td>
<td>1.5, 3 (0.03% ethylene glycol)</td>
<td>NE / 1.5 All male yearlings died after 1 yr of treatment. Bladder stones developed in 8/20 male rats fed 3 g/kg (sex-related</td>
<td>Weil et al., 1965* and 1967*</td>
</tr>
</tbody>
</table>
90 d - 2 yr
15-20/sex/dose diet

Rat F344, 108 wk
50/sex/dose drinking water
1.2, 2.6 (< 3% ethylene glycol)
1.2 / 2.6
Mortality 19/50 male rats (vs 13/50 controls), ↑ water intake (males 25% vs females 17%), ↑ lactate dehydrogenase and ↓ urea nitrogen in males, ↑ creatinine phosphokinase and lung weight in both sexes. No urinary changes or bladder stones detected but no data available on oxalate measurements

Inhalation

<table>
<thead>
<tr>
<th>Route</th>
<th>DEG Conc.</th>
<th>DEG Source</th>
<th>Country</th>
<th>Deaths/DEG Poisoning Cases</th>
<th>DEG Vehicle</th>
<th>DEG Concentration/Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat, 6 mo, 5 d/wk</td>
<td>4-5, 20-30 mg/m³</td>
<td>No further information</td>
<td>Winek, 1979* cited in ECB IUCLID, 2000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat, Mouse, 3-7 mo</td>
<td>5 mg/m³</td>
<td>NE / 5</td>
<td>Oedema and hyperaemia in the brain and spinal cord, localised brain tissue bleeding, neurons destruction with compensatory outgrowth of glial cells. Insufficient information on test method and results</td>
<td>Marchenko, 1973* cited in BG Chemie, 1990*; BIBRA, 1993*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse, 6-7 mo, 2 h/d</td>
<td>4-5 mg/m³ (aerosol mist at 30-35°C)</td>
<td>NE / 4-5</td>
<td>Bronchitis, pneumonitis, kidney and liver damage. No data available on the control animals and analysis of the aerosol vapour mixture</td>
<td>Sanina, 1968* cited in BG Chemie, 1990*; BIBRA, 1993*; DFG, 1995*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; NE: not established; d: day; wk: week; mo: month; yr: year; ↓: decreased; ↑: increase; M-F: male-female; Ca: calcium.

### 3. Episodes of human ingestion of DEG

<table>
<thead>
<tr>
<th>Year (References)</th>
<th>Country</th>
<th>Deaths/DEG Poisoning Cases</th>
<th>Route</th>
<th>DEG Vehicle</th>
<th>DEG Source</th>
<th>DEG Concentration/Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1937 (Calvery and Klumpp, 1939*)</td>
<td>United States</td>
<td>105/353</td>
<td>Oral</td>
<td>Sulfanilamide elixir</td>
<td>DEG excipient</td>
<td>72% Mean fatal dose: 38 g/53 mL for children 71 g/99 mL for adults</td>
</tr>
<tr>
<td>1969 (Bowie and McKenzie, 1972*)</td>
<td>South Africa</td>
<td>7</td>
<td>Oral</td>
<td>Liquid sedatives</td>
<td>DEG replaced propylene glycol</td>
<td>Unknown</td>
</tr>
<tr>
<td>1985 (Anonymous - Lancet, 1985*)</td>
<td>Netherlands</td>
<td>21</td>
<td>Oral</td>
<td>White wine</td>
<td>DEG additive to improve the taste</td>
<td>1-10 g/L Highest conc.: 48 g/L</td>
</tr>
<tr>
<td>1985 (Cantarell et al., 1987*)</td>
<td>Spain</td>
<td>5</td>
<td>Topical</td>
<td>Sulfadiazine</td>
<td>DEG excipient</td>
<td>6.2-7.1 g/kg of substance</td>
</tr>
</tbody>
</table>
1986  
India  
(Pandya, 1988)  
14  
Oral  
Glycerin  
DEG-contaminated glycerin  
18.5%

1990  
Nigeria  
(Okuonghae et al., 1992*)  
47  
Oral  
Paracetamol syrup  
DEG replaced propylene glycol  
Unknown

1990-1992  
Bangladesh  
(Hanif et al., 1995)  
51/67  
Oral  
Paracetamol elixir  
DEG replaced propylene glycol/glycerol  
Unknown

1992  
Argentina  
(Drut et al., 1994*; Ferrari and Giannuzzi, 2005*)  
15/29 (7 with necropsy findings)  
Oral  
Propolis syrup  
DEG excipient  
65% w/v  
Lethal dose: 0.014-0.17 g/kg bw

1995-1996  
Haiti  
(O’Brien et al., 1998)  
99/109  
Oral  
Acetaminophen  
DEG-contaminated glycerin  
14.4%  
Toxic dose: 1.34 mL/kg or 1.49 g/kg

1998  
India  
(Hari et al., 2006)  
8/11  
Oral  
Paracetamol elixir  
DEG replaced propylene glycol  
15.4% w/w  
(range 2.3-23%)

1998  
India  
(Singh et al., 2001*)  
33/36  
Oral  
Acetaminophen  
DEG-contaminated glycerin  
17.5% v/v

2006  
Panama  
(Barr et al., 2007*; Bogdanich and Hooker, 2007*)  
365  
Oral  
Cough syrup  
DEG-contaminated glycerin  
Unknown


4. Carcinogenicity

Animal studies

<table>
<thead>
<tr>
<th>Species/Study Procedure</th>
<th>Concentrations/ Doses (g/kg bw/d)</th>
<th>NOAEL/LOAEL (g/kg bw/d) &amp; Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Rat, 2 d drinking water | 5.2 (or 5% DEG used as a negative control) | NE / 5.2  
No ↑ DEG-induced adenomatous hyperplasia. DEG showed a small but significant promoting effect | Hiasa et al., 1991* |
| Rat, 6 wk diet          | 10 (initiation with 0.2 g/kg DEN i.p., after 2 wk followed by DEG for 6 wk, partial hepatectomy at wk 3, necropsy at wk 6) | 10 / NE  
No ↑ in glutathione S-transferase placental form-positive (GST-P+) foci in the liver | Ito et al., 1988* |
| Rat F344, 30 wk         | 2.6 (with N-ethyl-N-hydroxyethyl-nitrosamine for 2 wk) | 2.6 / NE  
No renal promoting effect | Hiasa et al., 1990* |
<p>| Rat F344, 32 wk         | 20 | 20 / NE | Masui et al., |</p>
<table>
<thead>
<tr>
<th>Study Details</th>
<th>Animals</th>
<th>Diet</th>
<th>Dosage</th>
<th>Effects</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 males diet (with or without 0.05% N-butyln-(4-hydroxybutyl)nitrosamine)</td>
<td>Slightly ↑ urinary crystals in DEG treated rats but the incidence of bladder tumours not significantly different from the controls</td>
<td>1988*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat F344, 108 wk 50/sex/dose drinking water</td>
<td>1.2, 2.6 (&lt; 3% ethylene glycol)</td>
<td>2.6 / NE</td>
<td>Only one kidney carcinoma (1/100) and one nephroblastoma at a lower dose – no evidence of carcinogenic effects</td>
<td>Hiasa et al., 1990*</td>
<td></td>
</tr>
<tr>
<td>Rat Osborne-Mendel, 2 yr 12 males/dose diet</td>
<td>NE / 0.75</td>
<td>Bladder tumours (benign papillomas or malignancy) found in 5 and 6 animals at the high and medium dose respectively. Bladder stones (Ca oxalate concretions) detected at all doses</td>
<td>Fitzhugh and Nelson, 1946*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat Carworth-Farm-Nelson (weanling, 2 mo and 1 yr old), 90 d - 2 yr 15-20/sex/dose diet</td>
<td>1.5, 3 (0.03% ethylene glycol)</td>
<td>NE / 1.5</td>
<td>All male yearlings died after 1 yr of treatment. Bladder stones developed in 8/20 male rats fed 3 g/kg (sex-related effects). No bladder stones in rats fed 1.5 g/kg and in weanlings fed for 90 d. Bladder tumour found only in a high dose male weanling (which died after 362 d probably due to mechanical irritation). In this study, Ca oxalate stone, glass bead implant or sham operation similarly produced stones and tumours in rats that never received DEG.</td>
<td>Weil et al., 1965* and 1967*</td>
<td></td>
</tr>
</tbody>
</table>

**Dermal**

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Animals</th>
<th>Diet</th>
<th>Dosage</th>
<th>Effects</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, 3/wk (DEG added to cigarette tobacco as a humectant and applied on the dorsal skin)</td>
<td>0.8</td>
<td>0.8 / NE</td>
<td>The incidence of skin tumours not significantly different from the controls</td>
<td>Dontenwill et al., 1970*</td>
<td></td>
</tr>
<tr>
<td>Mouse, 2 yr, 3/wk 74 animals</td>
<td>3</td>
<td>3 / NE</td>
<td>Only one papilloma detected – no evidence of carcinogenic effects</td>
<td>Vasil’eva et al., 1971* cited in BIBRA, 1993*</td>
<td></td>
</tr>
<tr>
<td>Mouse NMRI, 106 wk, 1/wk 100 females s.c. injection</td>
<td>0.15, 0.5, 1.5 (in tricaprylin)</td>
<td>1.5 / NE</td>
<td>No tumours found locally or systemically. No data available on non-neoplastic lesions</td>
<td>Dunkelberg, 1987*</td>
<td></td>
</tr>
</tbody>
</table>

**Inhalation**

<table>
<thead>
<tr>
<th>Study Details</th>
<th>Animals</th>
<th>Diet</th>
<th>Dosage</th>
<th>Effects</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse, 6-7 mo, 2 h/d 16 animals</td>
<td>4-5 mg/m³ (aerosol mist at 30-35°C)</td>
<td>NE / 4-5</td>
<td>Tumours developed in 10/16 animals after 2.5 to 11 mo, including 1 lymphosarcoma, 1 smooth-cell, non-keratinising tumour of the mammary gland, 7 adenocarcinomas of the mammary</td>
<td>Sanina, 1968* cited in BG Chemie, 1990*; BIBRA, 1993*;</td>
<td></td>
</tr>
</tbody>
</table>
gland, and 1 solid tumour. No data available on the control animals and analysis of the aerosol vapour mixture

NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; NE: not established; d: day; wk: week; mo: month; yr: year; ↓: decreased; ↑: increase; s.c.: subcutaneous; DEN: diethylnitrosamine; Ca: Calcium,

**Human data**

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Concentrations/Doses</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhalation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retrospective study,</td>
<td>Unknown</td>
<td>No differences in the incidence of tumours of the skin, nervous system or internal organs. Inadequate methodology and no information on how long after the exposure the workers were studied</td>
<td>Telegina et al., 1971*</td>
</tr>
<tr>
<td>1-9 yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>56 men and 34 women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in the industry</td>
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<td></td>
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<tr>
<td>producing aromatic</td>
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<tr>
<td>hydrocarbons from</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>crude oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case-control study</td>
<td>Unknown</td>
<td>The risk of brain neoplasms due to exposure to DEG or tetraethylene glycol (4 cases) should be interpreted cautiously since there were wide confidence intervals around odds ratios and the association may be the result of multiple significance testing</td>
<td>Leffingwell et al., 1983*</td>
</tr>
<tr>
<td>17 cases of gliomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Each case was</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>matched with 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>controls for race, sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age, employment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>history, chemical</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>exposure history, and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>geographic data</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5. Toxicity to reproduction

*Animal studies*

<table>
<thead>
<tr>
<th>Species/Study Procedure</th>
<th>Concentrations/ Doses (g/kg bw/d)</th>
<th>NOAEL/LOAEL (g/kg bw/d) &amp; Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fertility</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Oral</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat, duration not reported drinking water</td>
<td>0.15, 0.3</td>
<td>NE / 0.15 ↓ fertility</td>
<td>Holck, 1937* cited in BIBRA, 1993*</td>
</tr>
<tr>
<td>• treated female rats were mated with untreated male rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• female and male rats were housed together</td>
<td>0.3</td>
<td>NE / 0.3 No pregnancies resulted</td>
<td></td>
</tr>
<tr>
<td>Rat, 12 wk (2-generation) 10/sex/dose</td>
<td>2.2</td>
<td>2.2 / NE No fertility or embryotoxicity</td>
<td>Wegner, 1953* cited in BIBRA, 1993* and Hellwig et al., 1995*</td>
</tr>
<tr>
<td>Rat SD, &gt; 73 d 30/sex/dose drinking water</td>
<td>0.15, 0.5, 1.5</td>
<td>1.5 / NE ↑ relative kidney weight in F0 and F1 male rats. No fertility or developmental toxicity</td>
<td>Rodwell et al., 1987*</td>
</tr>
<tr>
<td>Rat, 2 yr Diet</td>
<td>1, 1.7</td>
<td>1.7 / NE No changes in testicular histology. No other reproductive functions were analysed.</td>
<td>Morris et al., 1942* cited in Williams et al., 1990*</td>
</tr>
<tr>
<td>Mouse CD-1, 14 wk (2-generation continuous breeding) 20 pairs/dose drinking water</td>
<td>0.61, 3.1, 6.1</td>
<td>3.1 / 6.1 (for fertility and developmental effects) 6.1: F0 body weight unchanged during mating, ↓ litters/pair by 12%, ↓ live pups/litter by 32%, ↑ cumulative days to litter, ↓ pairs to produce the 3rd, 4th, 5th litters, ↓ pup weight (adjusted for litter size) by 12%. In the 5th or final litters, ↓ live pups and birth weights, 12% liveborn pups and 95% pups dead on PND 0 had craniofacial malformations including exencephaly and cleft palate. At PND 2, 50% malformed pups died. A cross-mating trial was inconclusive, but suggested that offspring development was compromised in females exposed to 6.1 g/kg bw/d. After F1 weaning, necropsy of F0 mice showed no treatment related change in male body or organ weight and histopathology, but 7% ↓ female body weight.</td>
<td>NTP, 1984*; Williams et al., 1990*</td>
</tr>
</tbody>
</table>
For F1 mating, mice exposed to 3.1 g/kg bw/d were used because of insufficient mice available from the high dose group. After birth of F2 and oestrous evaluation of F1, necropsy of F1 showed ↓ body weight in males (11%) and females (7%), but no effects on organ weight, sperm indices, pup weight or pup survival. 3.1: ↓ body weight in both sexes, but no adverse effects on reproduction.

### Inhalation

**Rat, 4 h/d during pregnancy**
- 10 females/dose
- 11, 46, 328 mg/m³
- 46 / 328
- ↓ viable animals
- Bariyak, 1989* cited in BIBRA, 1993*

### Intraperitoneal Injection

**Rabbit, 7 d**
- 2/dose
- 2.23 g/d/animal
- NE / 2.23 g/d/animal
- Degeneration of testis germinal epithelium
- Wiley et al., 1938* cited in Hardin, 1983*

### Development

**Oral**

**Rat, GD 0-20**
- 14/dose diet
- 0.140, 0.684, 3.556
- 3.556 / NE
- No developmental toxicity
- Kawasaki et al., 1984* cited in NTP, 1991*

**Rat, GD 0-20**
- Diet not reported
- NE / 3.3
- ↓ neonatal weight, musculoskeletal abnormalities

**Rat Wistar, GD 6-15**
- Gavage
- 0.2, 1, 5
- 5 / NE
- No maternal or embryotoxicity
- RCC, 1985* cited in ECB IUCLID, 2000

**Rat, GD 6-15**
- 38.21, 76.42
- NE / 38.21
- 38.21: musculoskeletal abnormalities
- USEPA, 1984* cited in ECB IUCLID, 2000

**Rat, multi-generation**
- 343
- NE / 343
- Altered sex ratio, foetotoxicity
- USEPA, 1984* cited in ECB IUCLID, 2000

**Rat CD, GD 6-15**
- 25/dose (necropsy on GD 21)
- 1.1, 4.5, 8.9
- 1.1 / 4.5
- Maternal toxicity at 4.5 (↑ water consumption) and at 8.9 (mortality 3/25, ↓ body weight and food consumption, ↑ water consumption, kidney and liver weights, and renal histopathology) ≥ 4.5: ↓ foetal body weight with skeletal variations. No treatment related effects on corpora lutea, implantations, external and visceral variations or malformations
- Neeper-Bradley et al., 1992*; Ballantyne and Snellings, 2005*

**Mouse CD-1, GD 6-15**
- 0.6, 2.8, 11.2
- 2.8 / 11.2
- Maternal toxicity at 2.8 (↑ water consumption) and at 11.2 (mortality)
- Neeper-Bradley et al., 1992*; Ballantyne and
<table>
<thead>
<tr>
<th>Study</th>
<th>Species</th>
<th>Dose (mg/kg or %)</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30/dose (necropsy on GD 18)</td>
<td>Mouse CD-1, GD 6-15</td>
<td>1.25, 5, 10</td>
<td>6/30, ↑ water consumption, △ foetal body weight without variations, malformations or implantation effects</td>
<td>Snellings, 2005*</td>
</tr>
<tr>
<td>Mouse CD-1, GD 6-15</td>
<td></td>
<td>5/10</td>
<td>Maternal toxicity at ≥ 5 (↑ relative water intake from GD 6, ↑ kidney weight at necropsy on GD 17) and at 10 (↓ food consumption from GD 6-12, renal histopathology in 3/28 vs 0/20 control) 10: no effects on pre- or post-implantation loss, linear ↓ mean foetal body weight on GD 17, with a statistical significance at high dose. No significant external, visceral and skeletal malformations at any doses</td>
<td>NTP, 1991*</td>
</tr>
<tr>
<td>Mouse CD-1, GD 7-14</td>
<td></td>
<td>11.8</td>
<td>NE / 11.8</td>
<td>Schuler et al., 1984*; Hardin et al., 1987*</td>
</tr>
<tr>
<td>Mouse CD-1, GD 7-14</td>
<td></td>
<td>2/50 dams died, pup weight gain after 3 d varied (0.6 g in the treated group vs 0.7 g in the control)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit, 7-19 d postinsemination Gavage</td>
<td></td>
<td>0.4, 1</td>
<td>NE / 1</td>
<td>BASF, 1987* cited in Hellwig et al., 1995*</td>
</tr>
<tr>
<td>Rabbit Himalayan, 7-19 d postinsemination</td>
<td></td>
<td>0.1, 0.4, 1</td>
<td>1 / NE</td>
<td>Hellwig et al., 1995*</td>
</tr>
<tr>
<td>Rabbit Dutch, GD 6-18</td>
<td></td>
<td>440, 1100, 1980 mg/m³</td>
<td>NE / 440</td>
<td>HSDB, 2003</td>
</tr>
<tr>
<td>Rabbit Dutch, GD 6-18</td>
<td></td>
<td>2.52, 2.80, 3.08, 3.36, 3.92, 4.48</td>
<td>NE / 2.52</td>
<td>Renwick and Cameron, 1992*</td>
</tr>
</tbody>
</table>

NOAEL: No Observed Adverse Effect Level; LOAEL: Lowest Observed Adverse Effect Level; NE: not established; GD: gestation day; PND: postnatal day; d: day; wk: week; mo: month; yr: year; ↓: decreased; ↑: increase; i.p.: intraperitoneal.