Propylene glycol (1,2-Propanediol)

Health-based recommended occupational exposure limit

Gezondheidsraad **Voorzitter**
Health Council of the Netherlands

Aan de Minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Propylene glycol (1,2-Propanediol)*
Uw kenmerk : DGV/MBO/U-932542
Ons kenmerk : U-1153/EP/pg/459-I55
Bijlagen : 1
Datum : 17 oktober 2007

Geachte minister,

Graag bied ik u hierbij het advies aan over de beroepsmatige blootstelling aan propyleenglycol. Het maakt deel uit van een uitgebreide reeks, waarin gezondheidskundige advieswaarden worden afgeleid voor concentraties van stoffen op de werkplek. Dit advies over propyleenglycol is opgesteld door de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS) van de Gezondheidsraad en beoordeeld door de Beraadsgroep Gezondheid en Omgeving.

Ik heb dit advies vandaag ter kennisname toegezonden aan de minister van Volksgezondheid, Welzijn en Sport, de minister van Volkshuisvesting, Ruimtelijk Ordening en Milieu en de staatssecretaris van Sociale Zaken en Werkgelegenheid.

Hoogachtend,

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Health-based recommended occupational exposure limit

to:

the Minister of Health, Welfare and Sport

No. 2007/02OSH, The Hague, 17 October 2007

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Preferred citation:
Health Council of the Netherlands. Propylene glycol (1,2-Propanediol); Health-based recommended occupational exposure limit. The Hague: Health Council of the Netherlands, 2007; publication no. 2007/02OSH.

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ISBN: 978-90-5549-664-8

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Samenvatting

Vraagstelling

Op verzoek van de minister van Sociale Zaken en Werkgelegenheid leidt de Commissie Gezondheid en Beroepsmatige Blootstelling aan Stoffen (GBBS, voorheen WGD) van de Gezondheidsraad gezondheidskundige advieswaarden af voor stoffen in de lucht op de werkplek waaraan werknemers beroepsmatig kunnen worden blootgesteld.

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan propyleenglycol en presenteert zij een gezondheidskundige advieswaarde voor deze stof. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór september 2006 zijn verschenen.

Fysische en chemische eigenschappen

Propyleenglycol (CAS-nummer 57-55-6) is een synthetische heldere kleurloze vloeistof met wateraantrekkende eigenschappen, een smeltpunt van –60 °C, een kookpunt van 188 °C en een dampspanning van 0,011 kPa bij 20 °C. De stof is vrijwel smaak- en geurloos. Propyleenglycol wordt gebruikt bij de productie van polyesters en als antivriesmiddel. Daarnaast wordt het gebruikt in remvloeistoffen, in hydraulische vloeistoffen, in koelvloeistoffen, als voedseladditief, als weekmaker in hars en papier, als middel om kleding en tabak vochtig te houden, en als oplosmiddel voor geurstoffen, kleurstoffen, medicijnen, cosmetica, verf en

plastics. Propyleenglycol wordt ook gebruikt om kunstmatige nevel te maken bij brandbestrijdingsoefeningen en bij diverse muziek- en theaterevenementen.

Blootstelling

Individuele blootstelling kan plaatsvinden bij gebruik van geneesmiddelen die zijn opgelost in propyleenglycol en bij gebruik van cosmetica, voedsel en andere producten waarin de stof is verwerkt.

Er zijn onvoldoende gegevens beschikbaar over de blootstelling van werknemers aan propyleenglycol. Contact met ogen en huid kan optreden bij het bereiden van bijvoorbeeld hydraulische vloeistoffen of koelvloeistoffen. Dampen van propyleenglycol worden nauwelijks ingeademd omdat de stof bij kamertemperatuur een lage dampspanning heeft. Bij gebruik van propyleenglycol bij hogere temperaturen kan meer damp ontstaan en worden ingeademd.

Een belangrijke toepassing van propyleenglycol is het maken van kunstmatige rook (nevels) bij oefeningen van brandweer en bij diverse muziek- en theaterevenementen. Zowel bezoekers als werknemers kunnen dan nevels van propyleenglycol inademen.

Monitoring

Voor de meten van propyleenglycol in lucht zijn gedeeltelijk gevalideerde methoden beschikbaar (NIOSH-methode 5523 en OSHA-methode PV2051). Deze zijn gebaseerd op gaschromatografie met vlam-ionisatiedetectie. Ook bestaan er methoden voor de meting van propyleenglycol in bloedplasma, bloedserum en urine.

Grenswaarden

Er is geen wettelijke grenswaarde in Nederland voor de inhalatoire blootstelling aan propyleenglycol. Er is ook geen norm vastgesteld door de Europese Commissie. In het Verenigd Koninkrijk is een grenswaarde (gemiddeld over een achturige werkdag) vastgesteld van 474 mg/m^3 voor propyleenglycol (som van deeltjes en damp in de lucht) en een grenswaarde van 10 mg/m^3 voor deeltjes. In de Verenigde Staten heeft de American Industrial Hygiene Association een Workplace Environmental Exposure Limit van 10 mg/m^3 (gemiddeld over een achturige werkdag) voor de inademing van aërosoldeeltjes van propyleenglycol.

Kinetiek en toxisch werkingsmechamisme

Er zijn geen gegevens over de opname van propyleenglycol na inademing van deeltjes of dampen van deze stof. Na orale inname wordt propyleenglycol snel en volledig opgenomen. Over de opname van propyleenglycol door de huid zijn geen kwantitatieve biologische gegevens beschikbaar. Enkele kwalitatieve gegevens uit proefdieronderzoek wijzen op beperkte (ca 1% gedurende 24 uur) opname via de huid. Berekening met het model SkinPerm laat zien dat de maximale huiddoordringing van propyleenglycol 0,20 mg/cm^2/uur bedraagt.

Na opname vindt een snelle verdeling plaats van propyleenglycol over het lichaamswater. Binnen 48 uur scheiden de nieren 20 tot 45 procent van de opgenomen hoeveelheid onveranderd uit. De rest wordt omgezet door oxidatie in de lever tot D,L-lactaat.

Het toxisch werkingsmechanisme is niet bekend. Men veronderstelt dat zowel propyleenglycol als de metabolieten ongewenste effecten op de gezondheid kunnen veroorzaken.

Effecten bij mensen

Propyleenglycol heeft nauwelijks irriterende of sensibiliserende werking op de huid. De stof prikkelt de ogen en de luchtwegen. Blootstelling aan nevel van propyleenglycol geeft acute kortdurende klachten zoals pijnlijke droge ogen, droge keel en hoesten.

Propyleenglycol heeft bij mens en dier een geringe acute toxiciteit. De dodelijke orale dosis voor mensen is vermoedelijk groter dan 15 gram per kilogram lichaamsgewicht. Na acute inname en intraveneuze toediening vertonen zowel mensen als proefdieren een aantal effecten waaronder verstoring van het evenwicht, vermindering van het bewustzijn en veranderingen in het bloed, de lever en de nieren. Bij hoge doses en bij langdurige blootstelling (oraal en intraveneus) kan metabole acidose ontstaan. In ernstige gevallen kan men het bewustzijn verliezen en kunnen epileptische aanvallen en hartritmestoornissen optreden.

Er zijn geen aanwijzingen dat propyleenglycol kankerverwekkend is voor de mens.

Effecten bij proefdieren

Geconcentreerd propyleenglycol veroorzaakt milde irriterende effecten op de huid en in de ogen van proefdieren. Het kan niet worden uitgesloten dat propyleenglycol een sensibiliserende werking heeft op de huid.

De acute orale en dermale toxiciteit is gering. Effecten na orale toediening zijn vertraging van de ademhaling en vermindering van de pijngewaarwording. Bij hoge doses ziet men stuiptrekkingen, bewusteloosheid, en eventueel de dood van het proefdier. Ook zijn bloedingen gezien in de dunne darm.

Langdurige orale inname van hoge doseringen (> 2,0 gram per kilogram lichaamsgewicht) veroorzaakte bij honden en ratten relatief kleine veranderingen in het bloedbeeld. De hemoglobineconcentratie nam af en ook het aantal rode bloedcellen en het volume van de rode bloedcellen.

Inademing van nevel van propyleenglycol (concentratie 160, 1000 en 2200 mg/m^3) gedurende negentig dagen, zes uur per dag en vijf dagen per week, veroorzaakte bij ratten neusbloedingen vanaf de tweede week van de blootstelling tot aan het einde van het onderzoek. Bij inademing van 160 mg/m^3 verminderde bij de vrouwtjes het aantal dieren met neusbloedingen na enkele weken en waren neusbloedingen na de vierde week nog maar bij vier procent van de dieren zichtbaar. Zowel bij vrouwtjes als bij mannetjes zag men daarnaast bij blootstelling aan 1000 en 2200 mg/m^3 propyleenglycol een verdikking van het luchtwegepitheel met toename van het aantal bekercellen en hun slijminhoud. Op basis van dit onderzoek is 160 mg/m^3 de ‘No Observed Adverse Effect Level’ voor de toename van het aantal bekercellen in de luchtwegen. Deze waarde heeft de commissie gebruikt bij de risicobeoordeling.

Ratten die gedurende dertig dagen via hun voer 2,9 gram propyleenglycol per kilogram lichaamsgewicht kregen toegediend, vertoonden een significante verlaging van het aantal leukocyten, van de hoeveelheid glutathion per milliliter bloed en van de bezinkingssnelheid van rode bloedcellen. Daarnaast nam de hoeveelheid eiwit in de rode bloedcellen toe en ook de activiteit van enkele membraangebonden enzymen. In ander onderzoek kregen ratten iedere dag 2,5 gram propyleenglycol per kilogram lichaamsgewicht toegediend gedurende twee jaren. Bij hen werden geen veranderingen gezien in het bloedbeeld, in de nierfunctie, in het gewicht van de organen en ook geen veranderingen bij macroscopisch en microscopisch onderzoek van de organen. Ook bij honden zijn geen effecten gevonden wanneer ze gedurende twee jaar iedere dag 2,0 gram propyleenglycol per kilogram lichaamsgewicht kregen toegediend. Bij 5,0 gram per kilogram lichaamsgewicht per dag nam onder andere het aantal rode bloedcellen

af en het totaal biluribine in het bloed toe. Deze veranderingen waren omkeerbaar en er waren geen aanwijzingen voor schade aan het beenmerg of de milt.

De ‘International Agency for Research on Cancer’ heeft propyleenglycol niet geëvalueerd. Uit onderzoek blijkt dat propyleenglycol niet kankerverwekkend is. Er zijn ook geen relevante effecten waargenomen op de voortplanting en het nageslacht.

Evaluatie en advies

Propyleenglycol heeft ongewenste effecten op de luchtwegen. Er is geen geschikt onderzoek bij mensen naar de schadelijke gezondheidseffecten als gevolg van chronische blootstelling. Uit proefdieronderzoek concludeert de commissie dat het kritisch effect van chronische inademing van propyleenglycol het effect is op de slijmbekercellen. In een onderzoek waarbij ratten (obligate neusademhaler) gedurende negentig dagen een nevel van propyleenglycol inademden, bleek het aantal dieren met veranderde slijmbekercellen toe te nemen bij 1000 en 2200 mg/m^3, maar nog niet bij 160 mg/m^3. De commissie concludeert hieruit dat de ‘No Observed Adverse Effect Level’ (NOAEL) voor de toename van het aantal dieren met veranderde slijmbekercellen 160 mg/m^3 is. Deze waarde heeft de commissie als uitgangspunt gekozen voor de afleiding van de ‘Health-Based Recommended Occupational Exposure Limit’ (HBROEL).

Voor locale effecten is geen onzekerheidsfactor aangewezen. Voor variatie in individuele gevoeligheid (intraspecies) past de commisie een onzekerheidsfactor van drie toe. Met deze factor berekent de commissie een HBROEL van 50 mg/m^3, gemiddeld over een achturige werkdag. De commissie merkt hierbij op dat deze advieswaarde betrekking heeft op de som van de concentratie propyleenglycol in dampvorm en als aërosol.

Volgens de commissie kan blootstelling aan een aërosol effecten veroorzaken die vergelijkbaar zijn met de effecten van blootstelling aan inhaleerbaar en respirabel stof. Daarom is de commissie van mening dat gezondheidskundige advieswaarden voor de beroepsmatige blootstelling aan inhaleerbaar en respirabel stof ook moeten gelden voor een aërosol van propyleenglycol.

Er zijn geen gegevens over kankerverwekkende effecten van propyleenglycol op de mens. Ook is niets bekend over effecten op de vruchtbaarheid en de ontwikkeling van het nageslacht van de mens. In onderzoek met proefdieren bleek propyleenglycol niet kankerverwekkend te zijn en geen schadelijke effecten te hebben op de voortplanting en het nageslacht.

Berekening met het model SkinPerm laat zien dat propyleenglycol door de huid dringt met een maximale snelheid van 0,20 mg/cm^2/uur. Hieruit volgt dat

blootstelling van de huid aan propyleenglycol belangrijk kan bijdragen aan de interne belasting. Toch beveelt de commissie geen huidnotatie aan omdat propyleenglycol een geringe systemische toxiciteit heeft.

Gezondheidskundige advieswaarde

De commissie GBBS van de Gezondheidsraad adviseert een gezondheidskundige advieswaarde van 50 mg/m^3, gemiddeld over een achturige werkdag (8-uurs tijdgewogen gemiddelde). Deze advieswaarde heeft betrekking op de som van de concentratie propyleenglycol in dampvorm en als aërosol.

Daarnaast adviseert de commissie dat gezondheidskundige advieswaarden voor de beroepsmatige blootstelling aan inhaleerbaar en respirabel stof ook moeten gelden voor een aërosol van propyleenglycol. De commissie beveelt geen huidnotatie aan voor propyleenglycol.

Executive summary

Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in the air in the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards (DECOS).

In this report, the committee discusses the consequences of occupational exposure to propylene glycol and recommends a health-based occupational exposure limit. The committee's conclusions are made on scientific papers published before September 2006.

Physical and chemical properties

Propylene glycol (CAS no. 57-55-6) is a synthetic clear colourless viscous liquid substance that absorbs water, has a melting point of –60 °C, a boiling point of 188 °C and a vapour pressure of 0.011 kPa at 20 °C. It may exist in air as a vapour, although it must be heated or briskly shaken to produce a vapour, and as an aerosol. Propylene glycol is practically odourless and tasteless. Propylene glycol is used in the production of polyester compounds and it is used in de-icing and anti-freeze fluids, in brake and hydraulic fluids, and in coolants. It is also used as a food additive and plasticizer for resins and paper, and as a humectant in

textiles and tobacco. In addition, it is a solvent for food colours, flavours, pharmaceuticals, cosmetics, and in the paint and plastics industries. Propylene glycol is also used to create artificial smoke or mist during fire-fighting trainings, in discotheques, and in movie, television, and theatre productions.

Exposure

Common routes of exposure to propylene glycol are ingestion and dermal contact of propylene glycol containing products (pharmaceutical preparations, cosmetics, food). People attending theatres and discotheques may be exposed to propylene glycol when it is being used to generate artificial mist.

In occupational settings, workers may be exposed by dermal contact during the manufacturing or use of propylene glycol containing products (antifreeze, coolants, de-icing fluids, brake fluids, solvents), in particular in operations involving heating or spraying of these products. Inhalation of vapours of propylene glycol at room temperature is minimal due to its low vapour pressure. People working in the entertainment industry (theatre, discotheque, television, movie) and those attending emergency trainings may be exposed to propylene glycol when it is being used as an artificial mist.

Monitoring

The National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) recommend sampling tubes containing a glass fibre filter and XAD-7 absorbent for sampling, and capillary gas chromatography with flame ionisation detection for detection and quantification of propylene glycol (NIOSH method 5523 and OSHA method PV2051).

Several methods exist for the measurement of propylene glycol in blood plasm, blood serum, and urine. These are based on gas chromatography with flame ionisation, electron capture or mass detection, and most require derivatisation of propylene glycol before chromatography.

Limit values

Currently, there is no occupational exposure limit for propylene glycol in the Netherlands, nor at the European level. The United Kingdom has set an occupational exposure limit of 474 mg/m^3 for total propylene glycol (vapour and particulate material) and a limit value of 10 mg/m^3 for particulate material. No specific

short-term exposure limit was established. The American Industrial Hygiene Association has a Workplace Environmental Exposure Limit of 10 mg/m^3 (8-hour time-weighted average) for propylene glycol as an aerosol. There is no threshold limit value for propylene glycol set by the American Conference of Governmental Industrial Hygienists.

Kinetics and mechanism of action

No information is available on the inhalatory retention or absorption of propylene glycol vapours and aerosols. Propylene glycol is rapidly absorbed from the gastrointestinal tract in humans and animals after oral exposure. Animal experimental data indicate a limited absorption of propylene glycol through the skin. However, quantitative biological data on the rate of absorption through the intact skin are lacking. According to the model SkinPerm, the maximal skin permeation is 0.20 mg/cm^2/hour.

After absorption, propylene glycol is distributed into the total body water. The kidneys eliminate 20-45% of the propylene glycol unchanged within 48 hours. The remainder is metabolised, mainly by oxidation to D,L-lactate in the liver. The L-lactate produced may undergo further oxidation in the tricarboxylic acid cycle or contribute to glycogen formation in the glycolytic pathway.

The mechanism of action of propylene glycol is not well understood. Both propylene glycol itself and its metabolites have been suggested to cause adverse health effects.

Effects – human data

With regard to humans, propylene glycol appears to have mild irritating and sensitising effects to the skin. Immediate transitory stinging, blepharospasm and lacrimation was experienced after application of propylene glycol to the eye. No residual discomfort or injury was reported.

Short inhalation exposure (1 min) to propylene glycol mist (mean 309 mg/m^3; range 176-851 mg/m^3) from artificial smoke generators caused acute ocular and upper airway irritating effects in healthy subjects. A few individuals also reacted with cough and slight airway obstruction as indicated by a slightly (5%) reduced Forced Expiratory Volume in 1 second (FEV_1). Propylene glycol in a pharmaceutical formulation (20%) caused nasal burning, stinging, and throat irritation in patients with allergic rhinitis after inhalation exposure for 1-4 weeks.

The lethal oral dose of propylene glycol in humans is probably over 15 g/kg bw. Case reports show that high oral and intravenous doses of propylene glycol

may be associated with serious toxic effects such as unconsciousness, epileptic seizures, metabolic acidosis with elevated osmol gap and anion gap, tachypnea, diaphoresis, and cardiac arrhythmias. Repeated seizures were observed after oral exposure to 4-8 g propylene glycol per day for 13 months. Increased incidence of seizures was found in low birth weight infants after daily intravenous administration for 19 months of 10 ml as compared to 1 ml of a multivitamin solution containing 300 mg/ml propylene glycol.

There is no evidence that propylene glycol is carcinogenic in humans.

Effects - animal data

Concentrated propylene glycol is mildly irritating to the skin and the eyes. Propylene glycol is not sensitising to animal skin.

Propylene glycol has a low acute oral and dermal toxicity in animals. Effects that occurred after oral administration were depression of respiration, analgesia, and at high doses seizures, coma and eventually death. Haemorrhagic areas could be seen in the small intestines and minimal changes in the kidneys with nuclear pyknosis and vacuolar degeneration of the cytoplasm. The liver showed slight congestion and hyperaemia. In rats, haematological effects were observed after exposure to 730 mg/kg bw once by gavage.

In a 90-day (6 hours per day and 5 days per week) nose-only inhalation study in rats, nasal haemorrhaging occurred in all exposed groups (propylene glycol aerosol concentrations 160; 1,000; and 2,200 mg/m^3). The nasal bleedings at the level of 160 mg/m^3 were transient in the females and the number of females with bleedings dropped to less than 4% after the fourth week of exposure. Ocular discharge also occurred in all exposed groups. Although there were no treatment-related gross pathology changes, light microscopy revealed thickening of respiratory epithelium with an increased number of goblet cells and mucin content, in both males and females receiving the medium and high propylene glycol dose. The lowest concentration tested in this study (160 mg/m^3) was the No Observed Adverse Effect Level (NOAEL) for the increase of the number of goblet cells. This NOAEL was used for the risk assessment.

Significant reductions were found in the erythrocyte sedimentation rate, total leukocyte count and glutathione concentration in rats after a daily oral dose of 2.9 g/kg bw/day for 30 days. In addition, increased protein content of the erythrocytes and increased activity of several membrane-bound erythrocyte enzymes were found. In other studies, rats exposed up to 2.5 g propylene glycol/kg bw/day in the diet for up to two years did not show effects on haematological parameters, kidney function, weights of the major organs, or the macroscopic

and microscopic appearance of tissues from a wide range of organs. No effects were observed at the dose level of 2.0 g/kg bw/day in dogs after two years of exposure. A dose of 5.0 g/kg bw/day induced changes in haematological parameters, increases in the total bilirubin level and decreases in the number of erythrocytes. This dose level was insufficient to produce any irreversible change and the authors reported that there was no evidence of damage to bone marrow or spleen.

Propylene glycol is not genotoxic. In studies on its carcinogenicity, there was no evidence of carcinogenic potential in rats by inhalation administration, in rats and dogs by oral administration, and in mice and rabbits by dermal application or injection. The International Agency for Research on Cancer has not evaluated propylene glycol.

No effects on fertility were reported when mice were exposed up to 10 g/kg bw/day in a 14-week continuous breeding experiment. No developmental toxicity was observed after oral administration of 10 g/kg bw/day propylene glycol to pregnant mice (days 8-12 of gestation), 6.2 g/kg bw/day to pregnant rats (days 10-12 and day 14 of gestation), and 1.2 g/kg bw/day to pregnant rabbits (days 6-18 of gestation).

Evaluation and recommendation

Propylene glycol has adverse health effects on the respiratory tract. Chronic human studies are not available. From animal studies, the committee identifies the increase of the number of goblet cells as the critical effect of propylene glycol aerosol inhalation. In a 90-day nose-only inhalation study in rats, the number of goblet cells increased at exposure levels of 1,000 and 2,200 mg/m^3, and not at 160 mg/m^3. Therefore, the committee concludes that 160 mg/m^3 is the NOAEL for the increased number of goblet cells and uses this value as the starting-point for the derivation of the HBROEL. For the extrapolation to a HBROEL, an interspecies factor for local effects is not indicated. For intraspecies variation, the committee applies a factor of three and calculates an inhalatory HBROEL of 50 mg/m^3 as an 8-hour time-weighted average concentration. This value of 50 mg/m^3 applies to the sum of propylene glycol existing as a vapour and as an aerosol.

According to the committee, exposure to an aerosol can have effects that are comparable to the effects of exposure to inhalable and respirable dust. Therefore, it is the committee's opinion that health-based occupational exposure limits for inhalable and respirable dust must be applied to aerosols of propylene glycol.

Human data on carcinogenic effects and reproduction (fertility and development) are not available. Animal data indicate that propylene glycol is not carci-

nogenic and has no toxic effects on reproduction. The permeation data obtained with SkinPerm indicate that dermal exposure may substantially contribute to the body burden of propylene glycol. The committee decided, however, not to recommend a skin notation because of propylene glycol's low systemic toxicity.

Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit of 50 mg/m^3 as an eight-hour time-weighted average concentration (8-hour TWA), applying to the sum of the concentrations of propylene glycol existing as a vapour and as an aerosol.

The committee also recommends to apply health-based occupational exposure limits for inhalable and respirable dust to aerosols of propylene glycol. The committee does not recommend a skin notation for propylene glycol.

Chapter 1

Scope

1.1 Background

At the request of the Minister of Social Affairs and Employment (annex A), the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, performs scientific evaluations of the toxicity of substances that are used at the workplace. The purpose of these evaluations is to recommend health-based occupational exposure limits for concentrations in the air, provided the database allows the derivation of such values. In the Netherlands, these recommendations serve as the basis for setting public occupational exposure limits by the Minister.

1.2 Committee and procedure

This document contains the assessment of DECOS, hereafter called the committee, of the health hazard of propylene glycol. The members of the committee are listed in annex B. The draft document has been prepared by G. Schaafsma and C. de Heer of the Toxicology Division of TNO Quality of Life, Zeist, the Netherlands.

In 2006, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft are listed in annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

The committee's recommendations on the health-based occupational exposure limit of propylene glycol have been based on scientific data from generally available publications. Data were extracted from the online databases Toxline, Medline/Pubmed, Current Contents and Chemical Abstracts with the keywords propylene glycol and/or CAS no 57-55-6 in combination with the following key words: use, expos*, kinetic*, toxic*, animal, human, adverse effects. The literature from this search was selected based on titles and abstracts. The last search was performed in September 2006. Furthermore, relevant literature cited in the IUCLID dataset of propylene glycol was consulted.[1]

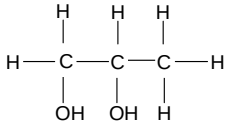
Chapter 2

Identity, properties and monitoring

2.1 Chemical identity

Chemical name	1,2-Propanediol
Synonyms	Propylene glycol; propane-1,2-diol; 1,2-dihydroxypropane; 1,2-propylene glycol; 2,3-propanediol; hydroxyl-propanol; alpha-propylene glycol; methyl glycol; methylethyl(ene) glycol; monopropylene glycol; trimethyl glycol; Dowfrost; PG 12; propylene glycol USP; Sirlene; Solar winter BAN; Solagard P; Ucar 35
CAS number	57-55-6
EEC number	-
RTECS number	TY2000000
EINECS number	200-338-0

2.2 Physical and chemical properties

Molecular formula	$C_3H_8O_2$
Structural formula	
Molar mass	76.10 g/mol
Melting point	-60 °C
Boiling point	188 °C
Relative density (water=1)	1.036 g/cm³ = 1036 kg/m³ (20 °C/4 °C)
Solubility	miscible with water, acetone, chloroform and alcohol; soluble in ether
Log $P_{octanol/water}$	-0.92
Vapour pressure	0.011 kPa at 20 °C; saturated vapour: 342 mg/m³ (110 ppm)
Relative vapour density (air=1)	2.6
Flash point	99 °C (closed cup); 107 °C (open cup)
Odour threshold (mg/m³)	practically odourless
Conversion factor (20 °C, 101.3 kPa)	1 mg/m³ = 0.316 ppm 1 ppm = 3.17 mg/m³

At ambient temperatures propylene glycol is a clear, colourless, almost odourless, viscous and hygroscopic liquid with a slightly acrid taste.[2]

2.3 EU Classification and labeling

Propylene glycol is not classified in the Annex I of Directive 67/548/EEC (as adapted to technical progress for the 29th time by Directive 2004/73/EC).[3]

2.4 Analytical methods

In this chapter, analytical methods are described for the detection and measurement of propylene glycol in biological samples and environmental media. Only well-established methods are given that are used as the standard methods of analysis.

2.4.1 Environmental monitoring

In ambient air at room temperature and atmospheric pressure, propylene glycol may exist as a vapour and as aerosol particles. For monitoring vapour and aerosol particles, the partially evaluated NIOSH method 5523 is available with a XAD-7 OVS tube as the sampling tube. For monitoring aerosol particles, the particle size should be determined first. Depending on the particle size, the inhalable aerosol fraction can be measured by the gravimetric sampling technique. In the Netherlands, personal inhalable particulates are usually sampled with the Dutch 'PAS6' sampling head mounted near the breathing zone of the worker.

Table 2.1 Analytical methods for determining propylene glycol in environmental samples.

Sample matrix	Sampler	Assay procedure	Limit of detection	Reference
Air (NIOSH method 5523)[a]	XAD-7 OVS tube containing two sections of XAD-7 adsorbent preceded by a glass fiber filter	GC-FID	LOD: 6 µg/sample	NIOSH, 1996[4]
Air (OSHA method PV2051)[a]	OSHA Versatile Sampler Tube containing two sections of XAD-7 adsorbent preceded by a glass fiber filter	GC-FID	LOQ: 0.035 mg/m^3	OSHA, 1999[5]
Air	Not reported	GC-FID	Not reported	OSHA, 1999[5]
Air	Not reported	GC-FID	Not reported	Andersson *et al.*, 1982[6]

GC-FID: gas chromatography with flame ionisation detection; LOD: limit of detection; LOQ: limit of quantitation; [a] partially evaluated method

Gas chromatography is the technique used to determine propylene glycol concentrations in environmental samples whether in air, water or other substances. Capillary gas chromatography with flame-ionisation detection for quantification gives good results down to the mg/l range with recoveries usually higher than 80%. The determination of propylene glycol in air requires trapping onto an adsorbent followed by extraction with an organic solvent. Table 2.1 gives a summary of the methods for detecting propylene glycol in air samples.

2.4.2 Biological monitoring

Table 2.2 gives a summary of commonly used methods for the measurement of propylene glycol in biological samples. The primary method is derivatisation followed by gas chromatography with flame-ionisation detection or mass spectrometry. Sample preparation and glycol derivatisation are needed to facilitate quantitative elution of the glycols and their acidic metabolites from the chromatographic columns. These steps proceed through acidification, esterification,

and extraction into an organic solvent. A rapid and simple method without derivatisation is also available.[7] The sample is directly injected into the gas chromatograph without prior solvent extraction and derivatisation.

Detection of propylene glycol in biological samples with gas chromatography is sensitive, with detection limits ranging from sub to low mg/l. The coefficient of variation varies with the concentration of propylene glycol used but typically ranges from 0.4% to 27% and is usually less than 10%. Propylene glycol is not associated with any specific effect markers.[8]

No information was found on detecting propylene glycol in faeces, adipose tissue, or human milk.

Table 2.2 Analytical methods for determining propylene glycol in biological samples.

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Plasma	Deproteinisation; derivatisation with butylboronic acid; and extraction	High resolution GC-MS	1 mg/l	Giachetti *et al.*, 1989[9]
Serum	Deproteinisation and extraction with p-bromophenyl boric acid	High resolution GC-ECD	0.38 mg/l	Needham *et al.*, 1982[10] and ATSDR, 1997[8]
Serum	No sample preparation	GC-FID on a high polar stationary phase (Nukol column)	Not reported	Edinboro *et al.*, 1993[7]
Blood	Deproteinisation with $HClO_4$	GC-MS	0.7 mg/l	Sisfontes *et al.*, 1986[11] and ATSDR, 1997[8]
Serum, urine	Derivatisation with phenylboronic acid	GC-FID	0.5 mg/l	Houze *et al.*, 1993[12]
Urine	Deproteinisation	GC- ECD	Not reported	Laitinen *et al.*, 1997[13]

GC: gas chromatography; MS: mass spectrometry; FID: flame-ionisation detection; ECD: electron-capture detection.

Chapter 3

Sources

3.1 Natural occurrence

Propylene glycol is not known to occur as a natural product.

3.2 Man-made sources

3.2.1 Production

Propylene glycol quantities in Europe range from 500 thousand to 1 million tonnes.[1] It is not clear whether this concerns import, production or both, and whether propylene glycol is produced in open or closed systems. Propylene glycol is produced via a non-catalytic reaction between propylene oxide and water (liquid-phase hydration at 100-200 °C). Excess water is removed by multistage evaporation. The formed crude glycol solution is subsequently fractionated by distillation into mono, di and tripropylene glycol. Propylene glycol may also be prepared from hydroxyacetone by yeast reduction.[14]

3.2.2 Use

Because of its physical properties and its low toxicity, propylene glycol is being used extensively in many industrial applications.[2,8,15-19] Propylene glycol is listed

as Generally Recognized As Safe (GRAS) by the U.S. Food and Drug Administration.

Propylene glycol is used:

- in the manufacture of unsaturated polyester resin production;
- in the manufacture (plasticizer) of paper;
- as a solvent in the paint and plastics industries;
- as a solvent for food colours and flavourings;
- as a base component of de-icing and anti-freeze fluids, brake and hydraulic fluids, and coolants in the airline and car industry;
- in heat exchangers;
- as a solvent/vehicle for various pharmaceuticals, cosmetics, food and tobacco products;
- as a emollient for various pharmaceuticals, cosmetics, food and tobacco products;
- as an anti-caking agent, antioxidant, dough strengthener, emulsifier, processing aid, stabilizer and thickener, surface active agent or texturiser in foods;
- in veterinary medicine, propylene glycol is used in oral medications for ruminants and as a solvent for various drugs;
- as a non-toxic antifreeze in breweries and dairy establishments;
- as an inhibitor of fermentation and mould growth;
- as an air sterilizer in hospitals and public buildings, and in veterinary applications to protect animals against the spread of airborne bacteria and influenza virus (vapour form);
- in mist generators to make artificial ‘smoke’ and mist.

Chapter 4

Exposure

4.1 General population

For the general population, common routes of exposure to propylene glycol are ingestion and dermal contact with propylene glycol containing products (pharmaceuticals, cosmetics, food and tobacco products). People attending theatres, concerts, parties, or fire fighting trainings may be exposed to propylene glycol if used to generate mist/smoke. Exposure may also occur during at-home servicing of the automobile coolant system when containing propylene glycol.

Propylene glycol has been detected in the water-soluble fraction of cigarette smoke and it has been found to migrate into a number of foods from regenerated cellulose films containing propylene glycol as a softening agent.[8] It was detected in chocolates at 20–1,460 mg/kg after 5.5 months of storage and at 25–1,890 mg/kg after 15 months, in fruit cakes at 10–154 mg/kg after 84–336 days of storage, in meat pies at <10 – 118 mg/kg after 3–7 days of storage, in toffee at <10–1,530 mg/kg after 168–450 days of storage, in madeira cake at < 10–365 mg/kg after 21–28 days of storage, and in boiled sweets at < 10–272 mg/kg after 168–450 days of storage.[20] Propylene glycol is a naturally occurring by-product in the fermentation of some beers and has been detected in concentrations of 1-51 mg/l in several commercially packaged beers.[21]

Individuals may be exposed to small amounts of propylene glycol released from newly installed carpet with polyvinyl backing. The quasi-steady-state spe-

cific emission rate of propylene glycol from these carpets was calculated to be 690 µg/m²/h at 24 hours and 193 µg/m²/h at 168 hours after carpet installation.[8]

4.2 Working population

In occupational settings, workers may be exposed by dermal contact and/or by inhalation during the manufacturing or use of propylene glycol containing products (antifreeze, coolants, de-icing fluids, brake fluids, solvents), in particular in operations involving heating or spraying of these products.[8] Oral exposure may occur when swallowing phlegm from droplets captured in the upper airways during inhalation of aerosols of propylene glycol. In hospitals and public buildings exposure may occur when propylene glycol based aerosols are used for disinfection purposes.[14]

In aircraft deicing workers, elevated concentrations of propylene glycol have been detected in post-shift urine samples.[22] For the exposed workers, the median pre-shift urine level was 1.49 mg/l (range 0.72–13.4 mg/l) and 1.67 mg/g creatinine (range 0.41–10.6 mg/g creatinine), and the median post-shift urine level was 2.07 mg/l (range 0.77–9.04 mg/l) and 2.46 mg/g creatinine (range 1.22–10.3 mg/g creatinine). Post-shift urine concentrations were higher in exposed workers than in unexposed controls (median, 1.35 mg/l and 1.18 mg/g creatinine).
In urine of Finnish motor servicing workers (n=10) frequently exposed to glycol-based cooling fluids, propylene glycol levels did not differ from urinary levels in unexposed male office workers.[23]

Norbäck et al (1995, 1996) measured propylene glycol exposure of Swedish painters during indoor application of water-based paints.[24,25] Propylene glycol was detected in personal air samples with a geometric mean of 0.35 mg/m³ (GSD 0.02 mg/m³), an arithmetic mean of 2.6 mg/m³, and peak values of 12.7 mg/m³ detected during roll painting on ceilings in a stair well, and 11.2 mg/m³ during wood painting.

Firemen and workers in the entertainment industry are likely to be exposed to relatively high concentrations of propylene glycol when it is used for smoke and mist simulation.[8] NIOSH detected propylene glycol in general area air samples from theatrical productions, at concentrations ranging from <0.01-1.9 mg/m³.[26] Wieslander *et al.* (2001) showed that acute exposure to propylene glycol mist can be high enough to produce acute irritating effects on the eyes and upper airways with cough, mild airway obstruction and mild dyspnoea.[19]

Chapter 5

Kinetics

5.1 Human

5.1.1 Absorption

Inhalation

No studies are available that describe propylene glycol absorption after inhalation of vapour or aerosol.

Dermal

No *in vivo* data are available for intact human skin. The rate of propylene glycol absorption through intact skin (origin not specified) has been estimated at 0.5 µM/cm²/hour, without further details given.[27] Calculation with the model SkinPerm[28] indicates that the maximal skin permeation is 0.20 mg/cm²/hour under steady state conditions when skin absorption equals systemic delivery. This model also predicts a significant latency period of approximately two hours after onset of skin exposure before systemic delivery starts to occur.

When there is no skin, propylene glycol is easily absorbed. In patients with severe burns, propylene glycol has been detected in serum when the wounds were covered with dressings or creams containing propylene glycol. These stud-

ies are described in the section *Distribution*. This absorption of propylene glycol through damaged/open skin is not relevant for occupational settings.

Propylene glycol enhanced the dermal absorption of 5-fluorouracil four-fold compared to saline in an *in vitro* study with human abdominal skin.[29]

Oral

After ingestion of 1 ml/kg bw (1.04 g/kg bw) propylene glycol, the maximum concentration in human blood was reached in about 30 minutes.[30] This concentration persisted for 4 hours, indicating delayed absorption keeping pace with elimination. The blood concentration of propylene glycol differed considerably (not further specified) between individuals. In another study, the maximum plasma concentration of propylene glycol in humans was reached within 1 hour after oral exposure (20.7 g or 41.4 g) during multiple (every 12 hr) oral-dosing regimens.[31]

5.1.2 Distribution

No data were found in the literature on the distribution of propylene glycol in humans after inhalation or dermal exposure. The volume of distribution after oral ingestion is 0.5-0.6 l/kg, approximating total body water.[31]

An 8-month old male infant was treated with topical silver sulfadiazine containing propylene glycol (9 g/kg bw /24 hours for a total of 70 hours) for a burn and complicating toxic epidermal necrolysis. The silver sulfadiazine covered approximately 78% of his total body surface area. Propylene glycol was absorbed through the damaged/open skin and a peak propylene glycol concentration of 10.6 g/l in serum was reported.[32]

Patients (n=45) with second and third degree burns over more than 20% of their total body surface were studied over a period of 30 months.[33] Dressings with silver sulfadiazine cream, containing propylene glycol (concentration not specified), were applied on the burned surfaces over a period of 3-7 days after admission to the hospital. Dressings were changed two or three times every 24 hours. Propylene glycol was detected in the serum of 24 of 45 patients (53%) and in the urine of 40 of 45 patients (90%). Average serum levels were 0.08 mg/ml, with a range of 0-1.3 mg/ml for patients who survived, and 0.82 mg/ml with a range of 0-9.8 mg/ml for patients who died. Propylene glycol levels correlated with total burn surface area and total third degree burn surface area.

5.1.3 Biotransformation

The major route of propylene glycol metabolism in mammals, including man, is to lactaldehyde and then lactate by alcohol dehydrogenase and aldehyde dehydrogenase, respectively, with methylglyoxal providing an alternative pathway (Fig. 1). From propylene glycol both D- and L-lactate can be formed. The formation of D-lactate in man has been confirmed recently in a case of severe D-lactate acidosis after propylene glycol ingestion.[34]

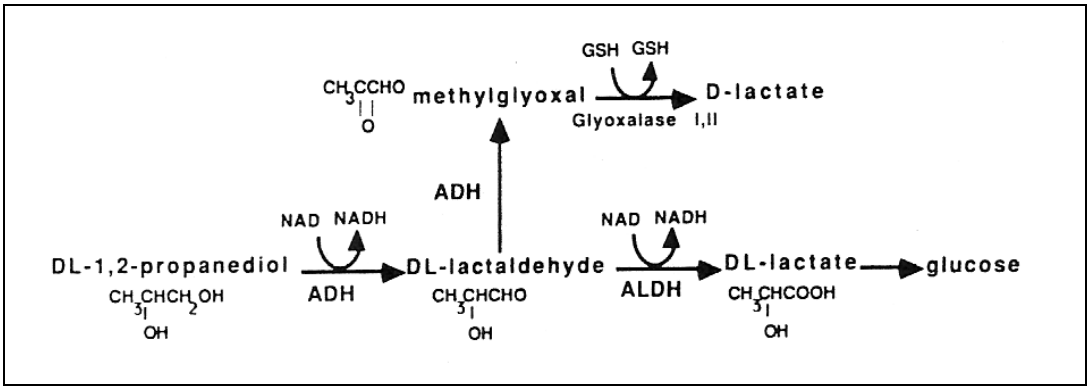


Figure 1 Propylene glycol metabolism in mammals.[35]

5.1.4 Elimination

No data on the elimination of propylene glycol in humans or animals after inhalation exposure were found in the literature.

In the 8-month old child with severe burns described above, the elimination of propylene glycol followed either zero-order kinetics at a rate of 135 mg/l/h or first-order kinetics with a half-life of 16.9 hours.[32]

In the study of Kulick *et al.* (1985)[33] described above, propylene glycol was detected in the urine of 40 of 45 patients treated with propylene glycol containing dressings for severe burns. Average urinary levels were 1.3 mg/ml, with a range of 0-17.9 mg/ml for patients who lived, and 2.9 mg/ml with a range of 0-23.0 mg/ml for patients who died.

In humans, 20-25% of the orally administered propylene glycol was eliminated via the urine in 10 hours after exposure to 1 ml propylene glycol/kg bw (1.04 g/kg bw).[30] The elimination kinetics of propylene glycol has also been studied in humans after oral administration by Yu and coworkers.[31] The elimination

followed apparent first-order kinetics. The terminal elimination half-lives after oral administration were 3.8 and 4.1 hours after administration of 20.7 and 41.4 g, respectively. After a minimum of 10 half-lives of maintenance dosing (20.7 and 41.4 g) on a fixed regimen, the accumulation of propylene glycol differed significantly among individuals because of variability in apparent clearance. The average apparent total body clearance is about 0.10 l/kg bw/hour and seems to be concentration dependent. It should be noted, however, that the presence of ethanol in the oral formulation may have led to prolonged elimination times and decreased clearance of propylene glycol because of competition for the enzyme alcohol dehydrogenase.[31]

Speth *et al.* (1987)[36] studied the pharmacokinetics of propylene glycol in six patients with confirmed malignancies. They were given a cytostatic drug by a 4-hour intravenous infusion of a solution containing 25 mg/ml propylene glycol. Subjects received 5.1-21.0 g propylene glycol/day. The pharmacokinetics of intravenously administered propylene glycol were nonlinear with a saturable clearance and an apparent terminal half-life varying from 1.4 hours at the dose of 5.1 g propylene glycol per day to 3.3 hours at the high dose of 21 g propylene glycol per day.

Kelner and Bailey (1985)[37] reported a serum half-life of 4.7 hours in a patient receiving propylene glycol intravenously at a dose of 267 mg/kg/day. Glasgow *et al.* (1983)[38] reported an average elimination half-life of propylene glycol of 19.3 hours (range 10.8-30.5 hours) in infants after intravenous propylene glycol exposure. Thus, premature infants treated intravenously had a much slower rate of propylene glycol removal than did adults as indicated by marked differences in the respective half-life values.

5.2 Animal

5.2.1 Absorption

Inhalation

No animal studies were found on the absorption of propylene glycol after inhalatory exposure.

Dermal

When applying a 5% molsidomine solution in propylene glycol to excised skin of SD-JCL male rats, mounted in a diffusion cell and in contact with receptor

saline solution, 95% of the administered propylene glycol remained on the skin after 6 hours.[39] Only 1% of the vehicle (1 g propylene glycol) permeated through the excised skin in the diffusion cell in 24 hours.[39]

Studies with rat abdominal stratum corneum indicate no penetration of propylene glycol into the dermis during the first two hours of treatment.[40,41] Fresh abdominal skin samples from male Wistar rats were mounted between the two compartments of the diffusion cell and the appearance of propylene glycol (as the control of oleic acid penetration) was measured by Fourier Transform Infrared/Attenuated Total Reflection spectroscopy of the surface of the dermis tissues. The skin surface area available for spectroscopy was 1.05 cm^2 and the peak corresponding to the C-O stretching of the alcoholic group was measured. Propylene glycol alone as the control did not cause any change in the spectral absorbance of the dermal tissue and the stratum corneum after two hours of treatment, indicating no detectable penetration into the skin in the first two hours.

Oral

Propylene glycol is rapidly and completely absorbed from the gastrointestinal tract in the rat, cat, rabbit and dog.[42-44]

Five groups of six rats each were given oral doses of 4.8-77 mmol of aqueous propylene glycol/kg bw (0.4-5.9 g/kg bw).[45] Blood was collected from these animals at times ranging from 5 min to 24 hours post dosing. Absorption after oral administration followed first-order kinetics and the appearance of blood propylene glycol was dose-related. The maximum blood concentration, 28 mmol/l (2.13 g/l), was found two hours after dosing.

In another study, cats (n=5) of both sexes were fed a diet containing 12% propylene glycol on a dry weight basis (1.6 g/kg bw/day) for 5 weeks.[35] Plasma concentrations of propylene glycol were 8.4 and 19.1 mmol/l (0.64 and 1.45 g/l), respectively, as measured in two cats on day 24 of ingestion.
In fasting dogs (n=4), the absorption and distribution of propylene glycol given by stomach tube was rapid. The maximum concentration in blood was reached in 30 minutes after a dose of 1.04 g/kg bw. After a dose of 4.1 and 6.2 g/kg bw the peak blood concentration was seen after 2-4 hours.[43]

5.2.2 Distribution

In rabbits, propylene glycol distributes into total body water without significant distribution to specific tissues. The apparent volume of distribution is 0.5-0.6 l/kg.[46]

5.2.3 Biotransformation

Propylene glycol is metabolised into D- and L-lactate.[8,35,47] The L-lactate produced is further oxidised in the tricarboxylic acid cycle or may contribute to glycogen formation through the glycolytic pathway. D-lactate is thought to be metabolised by a D-2-hydroxyacid dehydrogenase into pyruvate and CO_2 but does not significantly contribute to gluconeogenesis. In man, the metabolism of propylene glycol to D-lactate has been confirmed recently in a case of severe D-lactic acid acidosis after massive oral ingestion of propylene glycol.[34]

Yu *et al.* (1987)[46] studied the metabolism of propylene glycol in three groups of New Zealand White male rabbits (n=3/dose) given intravenous doses of 0.5; 1.0; and 2.0 g/kg bw propylene glycol in saline. Blood and urine were collected between 0 and 12 hours. As the plasma concentration of propylene glycol decreased, the metabolic clearance of propylene glycol increased proportionally, indicating saturation of metabolism at high concentrations

To study inhibition of propylene glycol metabolism, five groups of six rats each were given oral doses of 0-1 mmol of pyrazole, an alcohol dehydrogenase inhibitor, ten minutes before oral administration of 4.8-77 mmol propylene glycol/kg bw (0.4-5.9 g/kg bw).[45] Blood was collected from 5 minutes to 24 hours post dosing. The maximum metabolising capacity was 8.3 mmol propylene glycol/kg/h (0.64 g/kg bw/hr) in the rat which is equivalent to 1.06 kg/day for a 70-kg human being. The apparent K_M value was 17.9 mmol/kg bw (1.36 g/kg bw) on the basis of the elimination rate of propylene glycol. The apparent inhibition constant of pyrazole was 44 µmol/kg bw (3.3 mg/kg bw). The competitive inhibition of propylene glycol elimination by pre-administration of pyrazole suggests that the oxidation of propylene glycol by alcohol dehydrogenase is the major metabolic pathway.

5.2.4 Elimination

In rats, approximately 33% of orally administered propylene glycol was excreted unchanged by the renal pathway.[42] In dogs, 12-45% of orally administered propylene glycol (gavage doses of 1.04; 4.14 and 6.22 g propylene glycol/kg bw) was excreted unchanged in the urine.[43] Newman and Lehman (1937) found an excretion of 50% of the administered propylene glycol (intravenously and orally; dose not specified) in dog urine.[44]

Morshed *et al.* (1988)[45] found that the percentage excretion of propylene glycol in rats after oral administration of 19, 39 and 77 mmol/kg bw propylene gly-

col (respectively 1.5; 2.9 and 5.9 g/kg bw) was 2.3, 7.1 and 17% after 24 hours, respectively.

Three groups of New Zealand White male rabbits (n=3/dose) were given intravenous doses of 0.5, 1.0 and 2.0 g/kg bw propylene glycol in saline solution. Blood and urine were collected between 0 and 12 hours post dosing. In rabbits given the smallest dose, the plasma half-life was 64 min. For the 1 and 2 g/kg bw doses, the plasma half-lives were 76 and 75 min, respectively.[46]

5.3 Summary

No kinetic information is available on the absorption of propylene glycol after inhalatory exposure. The available data indicate limited (ca 1%) absorption of propylene glycol through intact rat skin during 24 hours when excised and placed in a diffusion cell. Quantitative biological data on skin absorption are lacking. Calculation with the model SkinPerm indicates that the maximal skin permeation is 0.20 mg/cm^2/hour under steady state conditions when skin absorption equals systemic delivery. Propylene glycol is rapidly absorbed from the gastrointestinal tract in humans and animals after oral exposure.

After absorption, propylene glycol is distributed into the body water with a distribution volume of 0.5-0.6 l/kg. The kidneys eliminate 20-45% of propylene glycol unchanged. The remainder is metabolised in the liver. The major route of metabolism in mammals is to lactaldehyde and then lactate via alcohol dehydrogenase and aldehyde dehydrogenase, with oxidation via methylglyoxal providing an alternative pathway. From racemic propylene glycol, L-lactate and D-lactate are formed. The L-lactate produced may undergo further oxidation in the tricarboxylic acid cycle or contribute to glycogen formation in the glycolytic pathway. The mean elimination half-life of propylene glycol is about 3-5 hours.

Chapter 6

Mechanisms of action

Propylene glycol is hygroscopic. It attracts water and this property is believed to cause transepidermal water transport and dehydration. This is probably the cause of the irritant properties of propylene glycol.[48] Several investigators have noted a seasonal variation in irritant reactions, with a higher percentage of irritant reactions observed in the cooler, less humid winter months. According to Kinnunen and Hannuksela (1989)[49] this trend is related to increased transepidermal water loss in winter time due to propylene glycol's hygroscopic properties.

Propylene glycol also has a diuretic effect and increases the urine volume at high doses.

Propylene glycol is metabolised into lactate. Oral ingestion or intravenous administration of propylene glycol may cause lactate acidosis with increased serum osmolality and increased anion gap.[8,14,34,37] The acidosis is accompanied by increased respiration and loss of carbon dioxide.

The central nervous system effects of propylene glycol (activity about one third that of ethanol) are probably caused by propylene glycol itself and not by its metabolites.[14] However, Morshed *et al.* (1988)[45] suggested that the metabolites lactaldehyde and other oxo-compounds contribute to the observed central nervous system effects because of the significant rate of propylene glycol metabolism.

Chapter 7

Effects

7.1 Observations in humans

The available human data on propylene glycol are summarised in Tables D-1 to D-5 of Annex D. The relevant studies are described in the following sections.

7.1.1 Irritation and sensitisation

Human data on skin and eye irritation and on sensitisation of propylene glycol are summarised in Table D-2 of Annex D.

Skin irritation and sensitisation

Propylene glycol produced skin irritating and sensitising effects in healthy subjects and in patients (see report of the Cosmetic Ingredient Review Expert Panel[50]). In the studies mentioned, test concentrations ranged from 1 to 100% propylene glycol. Reactions were observed at concentrations as low as 10% aqueous propylene glycol in predictive tests and as low as 2% aqueous propylene glycol in provocative tests.

In predictive patch tests, propylene glycol was studied in healthy subjects in concentrations ranging from 1 to 100% (see report of the Cosmetic Ingredient Review Expert Panel[50]). In one of the studies, mild skin irritation to 100% propylene glycol (but not to 50% propylene glycol) was observed under occlusive

patches: 11 of 35 subjects reacted with faint, patchy erythema, and 3 of 35 subjects reacted with erythema and oedema.[51] In another study with healthy subjects (n=24; closed patches; 1; 3; 10; and 30% aqueous propylene glycol), primary skin irritation was seen with 10% and 30% only. In a study with 204 subjects, neither skin irritation nor sensitisation was observed with 12% propylene glycol in a cream vehicle under occlusive patches. These studies indicate that in healthy subjects, propylene glycol is mildly irritating to the skin when tested under occlusive patches and that the ability of propylene glycol to irritate the skin is concentration dependent.[50] Based on the available data, the Cosmetic Ingredient Review Expert Panel[50] concluded that propylene glycol is safe for use in cosmetic products at concentrations up to 50%.

In provocative patch testing, allergic reactions were observed in 2 of 880 (0.2%) eczema patients patch tested with 2% aqueous propylene glycol, in 21 of 851 (2.5%) atopic patients patch tested with 5% aqueous propylene glycol, and in 13 of 330 (4%) patients patch tested with 10% aqueous propylene glycol. Thirty-three (3.9%) of the 851 atopic patients also had irritant/follicular reactions. In addition, the North American Contact Dermatitis Group observed 29 cutaneous reactions in a population of 399 patients with cosmetic-related dermatitis patch tested with 10% aqueous propylene glycol.[50]

Flares of propylene glycol dermatitis have been described following the ingestion of propylene glycol containing foods.[52]

After daily application of high amounts of 60% propylene glycol in aqueous cream topically (1.5-6.1 g/kg) for five days to patients with psoriasis (n=7; three female, four male; one patient was diabetic), three patients developed skin irritation, two of the remaining four patients had mild renal impairment, and most patients (number not stated) experienced desquamation. No effects were seen on serum electrolyte and lactate concentrations, and on serum osmolality.[53]

A commonly encountered problem in patch testing is reliably differentiating irritant effects from allergic effects. This is especially true with propylene glycol because skin reactions to propylene glycol are relatively weak. The concentration range used to detect sensitisation has been shown to produce low levels of irritant effects, too. This makes the interpretation of the findings difficult.[48,54] In several studies, the authors were unable to conclude whether propylene glycol gave irritation or true allergic sensitisation in their patients. The percentage of positive responses judged to represent allergy ranges from zero to 12.5% of the studied populations. In other studies, the subject group comprised eczema patients. In these patients, patch test results with propylene glycol 10% to 20% are even more difficult to interpret. Certainly, a significant number of reactions to propylene glycol represent a primary irritant effect.[48,54] An increase in the number of

irritant reactions is seen when propylene glycol is used in higher concentrations and especially when it is used under occlusion. In addition, several investigators noted a seasonal variation in irritant reactions, with the greater percentage of irritant reactions observed in the cooler, less humid winter months, presumably due to propylene glycol's hygroscopic properties.

Overall, propylene glycol appears to have weak effects on the skin. Recently, a similar conclusion was reached by Lessmann and co-workers[54]. In their paper on skin-sensitising and irritant properties of propylene glycol, they reviewed the medical literature and presented additional patch test data of 45138 patients tested with 20% propylene glycol in water between 1992 and 2002. Out of these, 1044 patients tested positively, 1083 showed a doubtful follicular or erythematous reaction, and 271 had explicit irritant reactions. They concluded that such a profile of patch test reactions is indicative of a slightly irritant preparation. No occupational exposures were identified with increased risk for sensitisation to propylene glycol. They further concluded on propylene glycol that 'despite obviously being endowed with a certain irritative property, it rarely causes sensitisation and would need further enhancing cofactors, such as increased individual susceptibility or pre-existing dermatitis'. In line with the literature data, their results indicate that 20% propylene glycol in water has mild irritant properties and a low sensitisation potential in humans. In summary, the committee concluded that, as it is difficult to differentiate between irritant and sensitising effects, sensitisation to propylene glycol can not be excluded.

Eye irritation

Propylene glycol applied to the human eye has been reported to induce immediate transitory stinging, blepharospasm and lacrimation. Discomfort lasts for several seconds until tears wash the substance away. This is followed by mild transient conjunctival hyperaemia. Residual discomfort or injury is not reported.[55]

7.1.2 Acute toxicity

Human studies and accidents with regard to acute and short-term exposure to propylene glycol are summarised in Table D-3 of Annex D.

Healthy non-asthmatic volunteers (22 man, 5 women; mostly pilots working in civil aviation) were exposed in an aircraft simulator to propylene glycol mist over 1 minute during realistic training conditions. Propylene glycol in the simulator was sampled by pumping the air for 1 minute at a rate of 200 ml/min on a

synthetic polymer tube (XAD-7; SKC 226-95). Personal air samples were not taken. The geometric mean concentration of propylene glycol was 309 mg/m^3 (GSD=1.7) and the arithmetic mean concentration of propylene glycol was 360 mg/m^3 (range 176-851 mg/m^3), with an arithmetic mean exposure of 220 mg/m^3 in the morning and 520 mg/m^3 in the afternoon. Tear film stability decreased, forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) was slightly decreased, dry eyes and dry throat symptoms were increased, and self-rated severity of dyspnoea was slightly increased. No effect was found for nasal patency, vital capacity (VC), FVC, nasal symptoms, dermal symptoms, smell of solvent, or any systemic symptoms. Nose bleedings were not reported. The subjects that were exposed to the higher concentrations in the afternoon had a more pronounced increase of throat symptoms, and a more pronounced decrease of tear film stability. In four subjects who reported development of irritative cough during exposure to propylene glycol, FEV_1 was decreased by 5%, but FEV_1 was unchanged among those who did not develop a cough. Those who developed a cough also had an increased perception of mild dyspnoea.[19] Women seemed to be more sensitive for throat symptoms, and atopic subjects for ocular and throat symptoms, respectively. The number of females (5 of 27) and the number of atopic subjects (8 of 27) were small and the statistical significance of the differences was not given by the authors.[19] In summary, short inhalation exposure to propylene glycol mist may induce acute ocular and upper airway effects, and some individuals may react with cough, mild dyspnoea, and slight airway obstruction as indicated by the 5% reduced FEV_1.

Acute and chronic effects of exposure to glycol-based fogs have also been found among workers in the entertainment industry.[56,57] Movie, television, theatre, music and other production sites were included in the study. Personal inhalable glycol-based aerosol concentrations were measured by gravimetry after sampling on 0.45-µm pore size Teflon filters with a 7-hole inhalable aerosol sampler. The filters were desiccated for 24 hours before triplicate weighing. The concentrations varied between 0.02 and 3.22 mg/m^3 with a geometric mean of 0.31 (SD 2.52) mg/m^3 and with an arithmetic mean of 0.49 (SD 0.63) mg/m^3 for the workers on all production sites together. Acute inhalation of glycol-based theatrical smoke induced dry cough or dry throat as local effects, and increased acute headache, dizziness, drowsiness, and tiredness as systemic effects. Unfortunately, the glycol-based aerosols were not further specified and no data were given on the type of glycol (propylene glycol or triethylene glycol) used for generation of the aerosols. Similarly, prevalence rates of chronic respiratory symptoms were for glycol-based and mineral-oil based aerosols combined. The committee noted that this study identifies a group of workers at possible health

risk. However, as this study does not contain quantitative data on propylene glycol exposure, the committee concluded that this study can not be used to establish a NOAEL.

The acute lethal dose for propylene glycol in humans is probably above 15 g/kg bw.[14] Case reports show that propylene glycol (orally or intravenously) can cause metabolic acidosis with increased osmolal gap, increased anion gap, elevated serum lactate level, diaphoresis and tachypnea, loss of consciousness, repetitive convulsions and cardiac arrhythmias.[17,58-65]

Neurological effects

Some reports on human incidents with propylene glycol (see Table D-3 of Annex D) describe acute neurological effects after oral or intravenous exposure. Acute effects include central nervous system depression, vertigo and nausea. Episodes of unconsciousness occurred in a 15-month old boy who received vitamin C suspended in propylene glycol for 8 days. The daily propylene glycol dose was 7.5 ml (7.7 g/day).[60]

7.1.3 Short-term toxicity

Human data on short-term exposure to propylene glycol are summarised in Table D-4 of Annex D. No data on dermal short-term exposure were found in literature.

In two 4-week trials (a double-blind, randomized, crossover study with 107 patients and a multi-centre, randomized, double-blind, parallel group study with 201 patients), the efficacy and tolerability of two formulations of flunisolide were studied in patients with seasonal or perennial allergic rhinitis. Both formulations contained 0.025% flunisolide, a glucocorticoid with anti-inflammatory activity. Flunisolide was sprayed into the nasal membranes by a metered-dose pump spray device. Each activation of the device resulted in the delivery of approximately 0.025 mg of flunisolide. The total dose was two sprays to each nostril two times a day (total of 0.2 mg of flunisolide per day). The existing formulation was an aqueous solution containing of 20% propylene glycol and 15% polyethylene glycol; the new formulation was an aqueous solution containing 5% propylene glycol, 20% polyethylene glycol and 2.5% polysorbate. Nasal burning and stinging with the two formulations was significantly different on severity ($p<0.001$), duration ($p<0.001$) and tolerability ($p<0.006$) in favour of the new product.[66,67] Overall efficacy was similar for both formulations.

A 15-year old boy was treated with a propylene glycol containing formulation of lorazepam, by continuous infusion for 42 days. With this formulation, the

boy received 2.25 ml of propylene glycol per hour, continuously. The total cumulative dose was 2.27 l or 2.35 kg. The boy developed increased serum osmolality (331 mosmol/kg) with an osmolal gap of 25 mosmol/kg, and developed hypotension and increased requirement for packed red blood cell transfusions.[68] After switching to another sedative without propylene glycol the patient's hyperosmolality resolved within 24 hours.

7.1.4 Long-term toxicity

Human data on long-term exposure to propylene glycol are summarised in Table D-5 of Annex D. No data on inhalation and dermal long-term exposure were found in literature.

Varughese *et al.* (2005) reported on chronic respiratory symptoms among Canadian entertainment industry workers exposed to artificial fogs.[57] Glycol-based fogs were associated with chronic adverse health effects including wheezing and chest tightness. The report did not contain exposure data on propylene glycol and is, therefore, not further described here. Case reports involving repeated oral and intravenous exposure to propylene glycol have been published. A 11-year old boy developed repeated seizures followed by unconsciousness lasting 20 to 30 minutes after ingestion of a vitamin D preparation containing propylene glycol (4-8 g propylene glycol/day) for 13 months.[58]

After intravenous administration of a multivitamin solution containing propylene glycol for 19 months to two groups of premature infants with low birth weight, the incidence of seizures among infants who received 3.0 g/day of propylene glycol (33%), compared with infants receiving 0.3 g/day of propylene glycol (14%), was significantly increased. In addition, the peak serum osmolality and the peak serum blood urea level were significantly higher among infants who received 3 g/day of propylene glycol.[59]

Carcinogenicity

No studies on human carcinogenicity have been found in the literature. The International Agency for Research on Cancer has not evaluated propylene glycol as a carcinogen.[69] The U.S. Environmental Protection Agency has not assigned propylene glycol a weight-of-evidence classification.[70]

Reproduction toxicity (fertility and development)

There are no data available on fertility and developmental effects of propylene glycol in humans. The use of propylene glycol in cryopreservation of early human embryos revealed no adverse effect on embryo survival.[14]

Immunological effects

No human studies or case reports with regard to immunologic effects were found in the literature.

Neurological effects

Two reports on human incidents with propylene glycol (see Table D-5 of Annex D) describe neurological effects after long-term exposure. Repetitive seizures and unconsciousness are described.

7.2 Animal experiments

The relevant animal data on the effects of propylene glycol are summarised in Tables E-1 to E-9 of Annex E. The key data are described below.

7.2.1 Irritation and sensitisation

Animal data on skin and eye irritation and on sensitisation of propylene glycol are summarised in Table E-2 of Annex E.

Skin irritation

Many skin irritation studies (some of which performed according to OECD or comparable guidelines) with propylene glycol were available.

In a skin thickness test (daily open patches) with guinea pigs (n=27) and rabbits (n=20), undiluted propylene glycol caused a significant increase in skin fold thickness (a measurement of skin irritation) in the guinea pigs on days 7-10.[71] There were no significant increases in skin fold thickness in rabbits. In another study, undiluted propylene glycol was slightly irritating.[72] In addition, daily application for six weeks of undiluted propylene glycol to the uncovered skin of three rabbits caused a very slight irritation. In an epicutaneous maximization test,

undiluted propylene glycol caused no irritation in guinea pigs.[73] In addition, no irritation potential of undiluted propylene glycol was observed in rabbits tested according to three different protocols.[74]

- the OECD guideline 404 (Huels AG, 1984 cited in IUCLID dataset 2000[1]; original report not available);
- a Draize test (BASF AG, 1979 cited in IUCLID dataset 2000[1]; original study report not available);
- skin irritation test method comparable to OECD guidelines.[75,76]

Hypertrophy, dermal inflammation and proliferation were observed at a concentration of 50% (w/v) propylene glycol.[77] Application of 20% propylene glycol to the uncovered skin of three rabbits daily for 6 weeks caused no irritation.[72] In a 24-hour skin irritation test involving nude mice (n=3), there were no reactions to 10% propylene glycol. Draize test results indicated that propylene glycol (not further specified) was, at most, a mild skin irritant when applied for 24 hours to abraded and intact skin of rabbits.[78]

In a repeated dose study, five female rabbits were exposed daily to a 0.5 ml mixture of equal parts of propylene glycol and diethylene glycol (semi-occlusive) for 100 days.[79] Exposure area was 100 cm^2; exposure time per day was not specified nor whether the substance was removed after each day. There were no macroscopic changes. Microscopic examination after 20-30 days showed a slightly thickened stratum granulosum and signs of proliferation in the stratum basale. Superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes. The collagen fibres were slightly fragmented and scattered. The findings remained unaltered in later stages.

The results taken together indicate that concentrated propylene glycol is mildly irritating to the skin in animals.

Eye irritation

In eye irritation studies, one performed in accordance with OECD guideline 405, with rabbits, undiluted propylene glycol was slightly irritating for the eyes with an acute ocular irritation index of 11.[72,80] Grant (1962)[55] reported that propylene glycol administered to the eye of rabbits caused only transient slight conjunctival hyperaemia. In the IUCLID dataset, it is reported that mild effects were observed in the eyes after application of 100 and 500 mg propylene glycol.[1] No further details or test scores are given. Other eye irritation data, some of which obtained according to 79/831/EEC guidelines and OECD guideline 405, show that propylene glycol was not irritating to the eyes with zero scores for cornea, iris, and

conjunctiva.[1] In an *in vitro* ocular irritancy test with a cultured corneal endothelial cell line, propylene glycol caused no irritation.[81]

The available studies showed that propylene glycol was slightly irritating for the eyes.

Sensitisation

In a ear swelling sensitisation test with male en female mice (n=19 in total) of various strains (Swiss, BALB-c, CBA, C56B1/6, DBA-2 and B6D2F1 strain), results for undiluted propylene glycol were negative.[82] The results of a maximization test (GPMT), an open epicutaneous test and a 48-hour chamber (Finn chamber) test indicated no sensitisation reactions to 70% propylene glycol.[83] In another epicutaneous maximization test, propylene glycol (concentration not specified) also caused no sensitisation.[73] The results of other guinea pig sensitisation tests (Magnusson and Kligman maximization test; Split adjuvant technique; Guinea pig optimisation test; Guillot/Brulos method; Freund's Complete Adjuvant test; Dossou and Sicard method; Open epicutaneous test) indicated that propylene glycol was not an allergen.[84] Furthermore, the results of 48 hours open and closed patch tests involving rabbits, guinea pigs and Gottingen swine and the results of 21 days open and closed patch tests involving Gottingen swine indicated no reactions to undiluted propylene glycol.[85] Overall, the available studies, some performed according to OECD or comparable guidelines, showed that propylene glycol is not sensitising to the skin of the animals investigated.

7.2.2 Acute toxicity

Acute toxicity studies are summarised in Table E-3 of Annex E.

In a study on the effects of propylene glycol on the tracheal epithelium, rabbits were exposed to an aerosol prepared from 10% propylene glycol at a flow rate of 10 l air/min, for 20 and 120 minutes (n=6/group).[86] The authors did not document exposure conditions, particle size or any other methodological conditions, and they did not give detailed information on the pathological changes they observed. Therefore, the committee decided not to use this study for the derivation of an HBROEL. The committee noted, however, that the effects of propylene glycol inhalation are on the respiratory epithelium and on the goblet cells, and that the investigators did not mention nose bleedings as an effect. After a 20-minute exposure period, the ultrastructure of the ciliated cells in the trachea showed changes indicative of a rapid, massive expulsion of mucus. The ciliary border

above the epithelium was regular and remained unchanged. After a 120-minute exposure period, the number of degenerated goblet cells had increased to 69% in the tracheal epithelium.[86]

In another study, seven dogs were exposed to 10% or 20% nebulized propylene glycol aerosol for 15 minutes.[87] No effects were reported. However, because this study was focused on haemolysis and haemodynamic effects, it is not clear from the study description whether other effects occurred.

In albino Wistar rats (n=6 females/dose) exposed to 0.73 or 2.94 g/kg bw once by gavage, propylene glycol caused a statistically significant progressive decrease of total blood haemoglobin, packed cell volume (mainly due to hyperosmolality of the plasma and altered morphology of erythrocytes), and red cell counts (ascribed to destruction of cells or enhanced removal from the blood), for a period of two days and returning to baseline values on day eight.[88] Reticulocyte counts, plasma haemoglobin and osmolality were increased (changes were more pronounced on day two) at both doses. The osmotic fragility of erythrocytes remained unaffected, whereas electron microscope revealed rough cell surface, ruptured membranes and increased cell adherence throughout the observation period. These features were not marked on day eight. According to the authors, these modified red cell surface characteristics promoted removal by the reticulo-endothelial system resulting in the significant increase in spleen weights in both groups.[88]

The acute oral toxicity of propylene glycol is low in experimental animals. Effects at high doses (> 2 g/kg bw) include respiratory depression, ataxia, tetany, analgesia, coma and finally death; haemorrhagic areas in the small intestine and minimal changes in the kidneys with nuclear pyknosis and vacuolar degeneration of the cytoplasm; the liver showed slight congestion and hyperaemia. The oral LD_{50} values for mice, rats, rabbits and guinea pigs are well above 2 g/kg bw. NIOSH mentions an oral LD_{50} value for rabbits of 20 g/kg bw.[89] However, the original study description is not available.

7.2.3 *Short-term toxicity*

Short-term toxicity studies are summarised in Table E-4 of Annex E.

In an inhalatory experiment, young and healthy Sprague-Dawley rats were divided into four groups of 19 males and 19 females each.[90] One group served as the control and was exposed to humidified, filtered room air. Three groups were exposed by nose-only inhalation to target aerosol concentrations of 160; 1,000; and 2,200 mg/m^3 propylene glycol (mean daily aerosol exposure concentrations: 160 ± 40; 1,010 ± 110; and 2,180 ± 310 mg/m^3 propylene glycol), during 90 days

(6 hours/day; 5 days/week). The median aerodynamic diameters of the diluted aerosol were less than 2.22 and 1.96 µm for the medium and high concentration groups, respectively. The mean geometric diameter for the low concentration group was not obtainable, possibly due to evaporation which occurred with large quantities of diluting air. Particle size of the undiluted aerosol was not measured. The geometric standard deviations were 1.44 and 1.57, respectively for the medium and high dose groups. Nasal haemorrhaging occurred in all exposed groups of male and female rats. From week 2 to 13, the average incidences of nasal haemorrhaging in male rats was <1, 64, 74, and 75% in controls, low-exposure, medium-exposure, and high-exposure groups, respectively. In females, the average incidences were <1% in controls, 14% in the low-exposure group (and less than 4% after the fourth week of exposure), and 71% in the medium and high-exposure groups. Recovery from clinical signs of the nose bleedings occurred in the non-exposure weekend periods. Similar trends were observed for ocular discharge, with incidences of 16% in low-exposure males, 40% in medium- and high-exposure males and 5% in controls. There was generally less ocular discharge in females with incidences of 8% (controls), 14% (low-exposure group), 28% (medium-exposure group) and 35% (high-exposure group). A significant reduction in body weight of 5-7% starting on day 50 and continuing until the end of the study was observed in female rats receiving the highest dose of 2,200 mg/m^3 propylene glycol. Similar weight reduction was observed in the group receiving 1,000 mg/m^3 propylene glycol but occurred later in the study, from day 64 onwards. This body weight reduction correlated significantly with reduced food intake from study days 43 and 50 for the high- and medium-exposure females, respectively. Female rats exposed to 1,000 mg/m^3 and 2,200 mg/m^3 propylene glycol had significant decreases of white blood cells, lymphocytes and band neutrophils. At 2,200 mg/m^3, female rats had, additionally, a significant decrease of mean corpuscular haemoglobin concentration. Male rats in the medium and high dose groups showed significant decreases in serum sorbitol dehydrogenase and gamma-glutamyl transferase. A significant decrease in total serum protein was observed in male rats treated with high dose of propylene glycol while females treated with a medium dose of propylene glycol had an increase in total serum protein. These measurements of haematological and clinical chemical parameters did, however, not show consistent trends with respect to treatment group or sex and were not considered biologically significant. When organ weights were expressed relative to terminal body weight, high-exposure male spleen weights were significantly decreased. Although there were no treatment-related gross pathology changes, light microscopy revealed thickening of respiratory epithelium with increase in the number of goblet cells or increase in

the mucin content of the goblet cells in both female and male animals receiving medium (1,000 mg/m^3) and high (2,200 mg/m^3) propylene glycol dose. There was no significant effect on the goblet cells noted at the 160-mg/m^3 dose level. Minute volume, tidal volume, and respiratory rates were not significantly altered in rats exposed to 160; 1,000 and 2,200 mg/m^3 propylene glycol, suggesting that animals adapted to the exposure concentrations.
In an oral exposure experiment, adult male rats (n=6/group) received daily doses of 2.84 ml/kg bw/day (2.94 g/kg bw/day) propylene glycol for 30 days. The control group of rats received distilled water.[91] A significant reduction was found in the sedimentation rate of erythrocytes, the total leukocyte count and the glutathione content. Furthermore, increased protein content of the erythrocytes and increased activity of several membrane-bound erythrocyte enzymes were observed.

In other studies, Heinz body-induced acceleration of erythrocyte destruction developed in a dose-dependent manner in cats.[35,92-97] Heinz bodies consist of denaturated haemoglobin normally occurring in ageing erythrocytes. Heinz bodies are formed by changes in the tertiary structure of haemoglobin, ultimately leading to the collapse of the molecule and aggregation into Heinz bodies. An increase in Heinz bodies (28% increase compared to control value) was observed in cats (n=6/dose) which were exposed for 5 weeks to propylene glycol in food at a dose of 1.6 g/kg bw/day. Additional effects at this dose level included a significantly increased erythrocyte glutathione concentration, increased anion gap and D-lactate level (on a dose-dependent basis and positively correlated with anion gap), increased iron pigment concentration, and a decreased erythrocyte survival (18.8%) and L-lactate level. In cats (n=5) exposed to 8.0 g/kg bw/day for 22 days, there was an increase from 4.8% to 92% of red blood cells containing Heinz bodies. Other effects were decreased activity, slight to moderate ataxia after 2-3 days, hypercellularity, polyuria, polydipsia, decreased blood packed cell volume (accompanied by an increase in both punctate and aggregate reticulocytes), significant decrease in erythrocyte glutathione concentration, significant decrease in ATP, decrease in red blood cell survival (60%) and an increase in iron pigment.[35,92] The lowest dose level at which Heinz bodies were observed in cats was 443 mg/kg bw/day (exposure for up to three months).

When evaluating the studies with cats, it should be noted that cats can not form the glucuronide metabolite of propylene glycol. Propylene glycol glucuronide represents a substantial fraction of the propylene glycol recovered from the urine of several species other than cats. This lack of conjugation potential in cats may prolong the presence of propylene glycol in cat blood and enhance hae-

matoxicity in this species. Cats, though seen as the most sensitive species tested, are not viewed as being the most predictive of human responses to propylene glycol.[15]

In Charles River CD rats exposed orally to 0 or 2.5 g/kg bw/day for 15 weeks, no effects were observed on the blood, kidney function, weights of the major organs or the macroscopic and microscopic appearance of tissues from a wide range of organs.[98]

From the dermal exposure study of Rantuccio *et al.* (1979)[79] (see also section 7.2.1 under Skin irritation) no data are available on systemic effects.

Long-term toxicity

Animal data on long-term toxicity of propylene glycol are summarised in Table E-5 of Annex E.

In a primitive inhalation study from 1947, 20 rats were exposed to 170-350 mg/m^3 propylene glycol, 24 hours/day for 12-18 months.[99] The formation of propylene glycol vapour was changed during the study as it was initially not possible to control the mist formation well. The glycol level was described as 'continuous supersaturation' and a 'dense fog of condensed glycol droplets'. The control group consisted of 10 animals. The number of rats in each group was increased by birth of young. Breeding was controlled to produce about equal populations in the two groups. Comparative observations on the growth rates, blood counts, urine examination, kidney function tests, fertility and general condition of the test and control group, exhibited no essential differences between the groups with the exception that the rats in the glycol atmospheres exhibited consistently higher weight gains. At 12 months, the weights of the exposed animals were about 50% increased compared to the weights of the control rats. Microscopy of tissues from the major organs revealed no effects. The committee noted that an exact exposure level can not be derived from this study and that the authors did not record nose bleedings as an effect of propylene glycol inhalation.

Charles River CD rats exposed to 0; 0.31; 0.62; and 2.5 g/kg bw/day in the diet (n=30/sex/dose) for 2 years did not show any effects on blood chemistry, kidney function, weights of the major organs or the macroscopic and microscopic appearance of tissues from a wide range of organs. In addition, no convincing evidence of carcinogenicity was observed (a wide range of tissues were examined in detail).[98]

In another study, Beagle dogs were exposed to 0; 2.0; and 5.0 g/kg bw/day for 2 years (n=5/sex/dose). No effects were observed at the dose level of 2.0 g/kg

bw/day. At 5.0 g/kg bw/day, the number of erythrocytes decreased (increased erythrocyte haemolysis). Furthermore, the haemoglobin and haematocrit concentrations decreased with increases in anisocytosis, poikilocytes and reticulocytes, assuming to indicate erythrocyte destruction and compensation by the bone marrow. Slight increase in serum total bilirubin concentration, increased urinary output and decreased water intake were also observed. The dose level of 5.0 g/kg bw/day was insufficient to produce any irreversible change and the authors reported that there was no evidence of damage to bone marrow or spleen. Blood was however not examined for Heinz body formation. No tumours were observed at any dose level.[100]

Mutagenicity and genotoxicity

In Annex E, Table E-6, the available data with regard to *in vitro* genotoxicity are presented.

In the Ames test, propylene glycol (up to 10 mg/plate) was not mutagenic in the absence and presence of metabolic activation in *Salmonella typhimurium* strains TA92, TA94, TA98, TA100, TA1530, TA1535, TA1537, TA1538 and G46.[78,101-104] Furthermore, propylene glycol was negative in a gene mutation test with silk worms[104], in an *Escherichia coli* microsuspension assay with strains WP2, WP2uvrA, WP67, CM611, WP100, W3110polA$^+$ and p3478pola[105], in a Rec assay with *Bacillus subtilis*[104], in a host-mediated assay with *Salmonella typhimurium* G46 and TA1530 (resident in the peritoneal cavity of mice treated by gavage with doses up to 5 g/kg bw given singly or daily for five days) and in a host-mediated assay in which *Salmonella typhimurium* was resident in the blood of mice (mice received an intravenous injection of up to 2.7 g/kg bw propylene glycol)[106]. In another host-mediated assay with *Saccharomyces cerevisiae D3* (resident in the peritoneal cavity of mice treated by gavage with doses up to 5 g/kg bw given once or daily for five days) weak mutagenic activity in the absence of metabolic activation was reported. No further details are available.

Propylene glycol caused a weak dose-dependent increase in the frequency of sister chromatid exchanges in the Chinese hamster cell line Don-6, up to 1.5 times the control value: 6.6, 6.8, 9.6 and 10.0 chromatid exchanges/cell at propylene glycol concentrations of 0; 3.8; 7.6; and 22.8 mg/ml, respectively.[107] The authors assumed propylene glycol to be a weak but potential inducer of sister chromatid exchanges. Propylene glycol was, however, not mutagenic up to 22.8 mg/ml when tested in a sister chromatid exchange assay with the human fibroblast cell line HE2144.[107]

Chromosomal aberrations were induced by propylene glycol (32 mg/ml propylene glycol; 420 mM) in Chinese hamster fibroblast cells in the absence of metabolic activation.[101] However, in subsequent studies, propylene glycol did not show chromosome aberrations in Chinese hamster fibroblast cells, human embryonic lung cells and in human embryo fibroblasts in the absence and presence of metabolic activation up to 64 mg/ml propylene glycol.[103,104,108] In addition, propylene glycol up to 10 mmol/l was negative in an alkaline elution assay with Chinese hamster lung fibroblast V79 cells[109] and negative in concentrations ranging from 0.125-8% in a cell transformation assay with embryo cells from Syrian hamsters.[110]

In conclusion, three studies reported positive findings. Other studies which investigated the same indicators of mutagenicity and genotoxicity at the same or higher doses were negative. Therefore, propylene glycol is not considered to be genotoxic *in vitro*.

In Annex E, Table E-7, the available data with regard to *in vivo* genotoxicity are presented.

Propylene glycol (single or repeated doses up to 15 g/kg bw) was negative in micronucleus studies[111,112] and did not show genetic damage (single or repeated doses up to 10 g/kg bw) in sperm cells in a dominant lethal study[113]. Based on the available data it is concluded that propylene glycol is not mutagenic *in vivo*.

Carcinogenicity

Carcinogenicity studies of propylene glycol are summarised in Table E-8 of Annex E.

Propylene glycol was studied for carcinogenicity in experimental animals using different administration routes (inhalation, oral and dermal). In several tests, propylene glycol has been used as a negative control. There was no evidence of carcinogenic potential in rats by inhalation administration, in rats or dogs by oral administration or in mice and rabbits by dermal application or injection.

Reproduction toxicity (fertility and development)

Animal data on reproduction toxicity of propylene glycol are summarised in Table E-9 of Annex E.

In an early inhalation study, rats (initially groups of 10 rats animals per sex) reproduced normally after exposure to 170-350 mg/m^3 propylene glycol for up to 18 months.[99]

Propylene glycol was tested for reproductive toxicity in Swiss CD-1 mice according to the RACB protocol (14-week continuous breeding study).[114] Data collected on body weights, clinical signs, and food/water consumption during the dose-range-finding segment were used to set concentrations for the main study (n=20 mice/sex/dose) at 0.0; 1.0; 2.5; and 5.0% (w/v) propylene glycol in drinking water (equivalent to 1.82; 4.80; and 10.1 g/kg bw/day). Although water consumption in the F0 generation was consistently higher for all groups (by 6 to 15%), these increases were not statistically different from controls. There was no effect on body weights during either the continuous cohabitation portion of the study. All groups had more than or equal to 4.6 litters per pair, with more than or equal to 11.9 pups per litter. There was no treatment-related effect on pup weight adjusted for litter size. The viability and growth of the final litter was unaffected by propylene glycol consumption. Since there was no effect on fertility, a cross-over of the F1 animals was not conducted. At the time this study was conducted, the protocol called for no necropsy of F0 animals in the absence of a fertility effect, so the F0 mice were killed and discarded without necropsy. For the second generation, just the control and 10,118 mg/kg bw/day propylene glycol groups were evaluated. There was no treatment-related effect on mating, fertility, or on the number, weight, or viability of the F2 offspring. After delivery of the F2 pups, the F1 adults were killed. There was no effect on body or organ weights in males or females, no change in sperm endpoints, no change in oestrous cycle parameters and no effect on serum calcium levels in F1 mice. In summary, propylene glycol, under the conditions of this experiment, had no effect on fertility and development in either generation of Swiss mice up to 10.1 g/kg bw/day.

In mice, no effects on growth or viability of the offspring were observed when maternal animals were given propylene glycol up to 10 g/kg bw/day from day 8 to 12 of pregnancy.[115] In another study, no developmental effects (growth, viability, and percentage of litters with malformed foetuses) were observed after prenatal administration of propylene glycol by gavage to maternal mice in a dose of 5.2 g/kg bw/day from day 6 to 15 of gestation.[116] The only significant finding at the highest dose level of 10.4 g/kg bw/day was a 3% decrease in fetal body weight which was not considered biologically relevant by the authors due to the magnitude of the effect and the absence of a dose-response relationship.[116]

In other studies, no developmental effects were observed after oral administration of propylene glycol to maternal mice up to 1.6 g/kg bw/day on days 6-10

of pregnancy, to maternal rats up to 1.6 g/kg bw/day on days 6-15 of pregnancy, and to maternal rabbits up to 1.2 g/kg bw/day on days 6-18 of pregnancy (Food and Drug Research Laboratories 1973, cited in BIBRA 1996[117]; original study report not available). A study with rats given 6.2 g/kg bw/day on days 10, 11, 12 and 14 of pregnancy also gave negative results (Seidler 1970, cited in BIBRA 1996[117]; original study report not available).

Immunological effects

Some of the animal studies on acute and short-term oral exposure to propylene glycol (see Table E-3 and Table E-4 of Annex D) describe immunological effects.

Female CD-1 mice (n=30) exposed to oral doses of 1.25, 2.5 and 5 g/kg bw/day for 5 days on a daily basis showed no evidence of an effect on cell-mediated and humoral immune response.[118]

In contrast, female mice (n=5) exposed to 4 g/kg bw/day propylene glycol for 21 days showed a decrease in the cellularity and relative organ weight of the spleen. The number of T-helper cells and the number of B-lymphocytes were decreased in the animals. No marked differences were noted in hematological parameters. Microscopic examination of the spleen showed a minor depletion of lymphocytes.[119]

Neurological effects

Some of the animal studies on acute and short-term oral exposure to propylene glycol (see Table E-3 and Table E-4 of Annex D) describe neurological effects.

Acute oral lethal doses in rats, rabbits, guinea pigs and dogs caused loss of muscular control, profound respiratory depression, analgesia, sleeplessness and coma shortly after administration of the propylene glycol dose.

Singh *et al.* (1982)[120] exposed Swiss mice and Wistar rats (number not mentioned) orally and intraperitoneally to a single dose of 10 ml propylene glycol solution/kg at concentrations of 10, 20, 50 or 100% (1.1, 2.1, 5.2 and 10.4 g/kg bw). At lower concentrations (1.1-2.1 g/kg bw) propylene glycol did not show any significant neuropharmacological activity. Higher concentrations (5.2-10.4 g/kg bw) were found to have moderate to marked effects. There was a decrease in locomotor activity, body and limb tone, respiration, and rectal temperature. On the other hand, propylene glycol increased pentobarbital sleep, slightly increased D-amphetamine toxicity and produced analgesia and had anti-inflammatory activity. On isolated smooth muscle preparations, in 50-100% concentrations, a

dose-dependent non-specific blockade was observed against agonists like acetylcholine, histamine, serotonin and barium chloride.

Cats (n=5) exposed to 8 g/kg bw/day for 22 days were less active and showed slight ataxia after 2-3 days. D-lactate concentrations were as high as 7 mmol/l. These levels are associated with encephalopathy in man. D-lactate was also significantly increased in cats fed with 1.6 g propylene glycol/kg bw/day for 5 weeks.[35,92]

7.3 Summary and evaluation

Human data

A commonly encountered problem in patch testing is reliably differentiating irritant effects from allergic effects. This is especially true with propylene glycol because skin reactions to propylene glycol are relatively weak. Propylene glycol has mild irritating effects. Allergic sensitisation can not be excluded. Reliable data on human skin absorption are not available.

When applied to the eye, propylene glycol causes immediate transitory stinging, blepharospasm and lacrimation. No residual discomfort or injury has been reported.

Short inhalation exposure (1 min) to propylene glycol mist (mean 309 mg/m^3; range 176-851 mg/m^3; personal exposure not measured) from artificial mist generators caused acute irritation of the eyes and upper airways of healthy subjects. A few (4 out of 27) individuals reacted with additional cough and slight airway obstruction as observed by a 5% reduction of FEV_1. In patients with allergic rhinitis, inhalation for 1-4 weeks of a pharmaceutical formulation containing 20% propylene glycol caused nasal burning, stinging, and throat irritation. These effects were significantly less after changing the propylene glycol content in the formulation from 20% to 5%.

The oral lethal dose of propylene glycol in humans is probably above 15 g/kg bw. Case reports show that multiple doses of propylene glycol (4-8 g/day for 13 months orally or 3 g/day for 19 months intravenously) can cause serious systemic toxicity with unconsciousness, repetitive convulsions, metabolic acidosis with elevated serum osmolal gap and anion gap, diaphoresis and tachypnea; and cardiac arrhythmias.

There is no evidence that propylene glycol is carcinogenic in humans. No human data on fertility, development and immunological parameters are available. The use of propylene glycol in cryopreservation of early human embryos revealed no adverse effect on embryo survival.

Animal data

Concentrated propylene glycol is mildly irritating to the skin and the eyes of animals. Propylene glycol has no sensitising effects on the animal skin.

Rabbits exposed to 10% propylene glycol aerosol for 20 and 120 minutes by inhalation, showed mild changes of the ultrastructure of the ciliated cells in the trachea indicative of a rapid, massive expulsion of mucus. No changes of the regular ciliary border above the epithelium was found. After 120 minutes of exposure, the number of degenerated goblet cells had increased to 69% in the tracheal epithelium. Nose bleedings were not recorded in this experiment.[86]

The acute oral and dermal toxicity of propylene glycol is low in experimental animals. The oral LD_{50} values for mice, rats, rabbits and guinea pigs are well above 2 g/kg bw. Effects at high doses (>2 g/kg bw) include analgesia, ataxia, tetany, respiratory depression, convulsions, coma and death.

In a 13-week nose-only inhalation study in rats, nasal haemorrhaging began during the second week of exposure at all propylene glycol levels (160; 1,000; and 2,200 mg/m^3) in both males and females. Recovery from the clinical signs of the nasal bleedings occurred during the non-exposure weekend periods. Nasal bleedings were transient in the females exposed to 160 mg/m^3 and the number of females with nasal bleedings dropped to less than 4% after the fourth week of exposure. Ocular discharge also occurred in all exposed groups. Although there were no treatment-related gross pathology changes, light microscopy revealed thickening of respiratory epithelium with a significant increase in the number of goblet cells or an increase in the mucin content of the goblet cells in both male and female animals exposed to the medium (1,000 mg/m^3) and high (2,200 mg/m^3) propylene glycol dose levels. There was no significant effect on the goblet cells noted at the 160-mg/m^3 level.

In a primitive study with rats, inhalatory exposure to propylene glycol (170-350 mg/m^3) was not controlled well and changed during the study. Exposure was 6 hours/day for 12-18 months. This resulted in a consistently higher weight gain. At 12 months the weights of the exposed animals were about 50% increased compared to the weights of the control rats. No other effects, including nose bleedings, were reported. An exact exposure level can not be derived from this study.

In rats, significant reductions in the sedimentation rate of erythrocytes, the total leukocyte count and the glutathione concentration were observed at an oral dose of 2.94 g/kg bw/day (exposure for 30 days). In addition, increased protein content of the erythrocytes and increased activity of several membrane-bound erythrocyte enzymes were found. In another study with rats, dietary propylene

glycol (2,500 mg/kg bw/day for two years) had no effects on blood, kidney function, weights of the major organs or the macroscopic and microscopic appearance of tissues from a wide range of organs.

In studies with cats, an increase in Heinz body formation occurred from repeated doses. The lowest dose level at which Heinz bodies were observed in cats was 443 mg/kg bw/day for 21-101 days. However, because of the known sensitivity of cats compared to humans for these effects, the observations in cats are not considered relevant for the present risk assessment. No effects were observed at the dose level of 2.0 g/kg bw/day in dogs after 2 years of exposure. A dose of 5.0 g/kg/day induced changes in several blood parameters, increased total bilirubin level and decreased erythrocyte counts. This dose level was insufficient to produce any irreversible changes and the authors reported that there was no evidence of damage to bone marrow or spleen. Blood, however, was not examined for Heinz body formation.

Despite a few positive *in vitro* test results, it is concluded that propylene glycol is not a genotoxic substance.

Propylene glycol was studied for carcinogenicity in experimental animals using different administration routes (inhalation, oral and dermal). In several tests, propylene glycol has been used as a negative control. There was no evidence of carcinogenic potential in rats by inhalation administration, in rats or dogs by oral administration or in mice and rabbits by dermal application or injection.

No effects on fertility were reported when mice were exposed up to 10 g/kg bw/day in a 14-week continuous breeding experiment. No developmental toxicity was observed after oral administration of 10 g/kg bw/day propylene glycol to pregnant mice (days 6-15 of gestation), 6.2 g/kg bw/day to pregnant rats (days 10-12 and day 14 of gestation), and 1.2 g/kg bw/day to pregnant rabbits (days 6-18 of gestation).

Chapter 8

Existing guidelines, standards and evaluations

8.1 General population

No health-based limit values for the general population were retrieved for propylene glycol.

8.2 Working population

Currently, there is no occupational exposure limit for propylene glycol in the Netherlands and no limit value set by the European Commission (Table 8.1).

The Health and Safety Executive (HSE) from the United Kingdom states that exposure to propylene glycol may involve contributions from vapour and aerosol material.[121] The HSE referred to the study of Suber *et al.* (1989)[90] in which a concentration of 1,000 mg/m^3 was considered as a no-effect level for all health effects other than nasal and eye irritation. There was concern that the vapour limit should be set at a level below the saturated concentration, to ensure that a limit suitable for vapour would not allow uncomfortable or visibility impairing conditions if the substances were present as a mist. An occupational exposure standard was set at 474 mg/m^3 (8-hour TWA) for total propylene glycol (vapour and particulates). A concurrent occupational exposure standard was set at 10 mg/m^3 (8-hour TWA) for particulate material. No specific short-term limit was set.

The American Conference of Governmental Industrial Hygienists (ACGIH) has not specified a threshold limit value (TLV) for propylene glycol.[122] The National Institute for Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) have not set occupational exposure limit values for propylene glycol.[123] The American Industrial Hygiene Association (AIHA) has a Workplace Environmental Exposure Limit (WEEL) of 10 mg/m^3 (8-hour TWA) for propylene glycol as an aerosol.[124]

Table 8.1 Occupational exposure limits for propylene glycol in various countries.

Country	occupational exposure limit (mg/m^3)	time-weighted average	type of limit
The Netherlands			
- Ministry of Social Affairs and Employment[125]	-		
United Kingdom			
- HSE[126]	474 (vapour and particulate material)	8 hour	WEL
	10 (particulate material)	8 hour	WEL
Denmark[127]	-		
Germany[128,129]	-		
Sweden[130]	-		
European Union			
- SCOEL[131]	-		
USA			
- ACGIH[122]	-		
	10 (particulate material)	8 hour	WEEL
- AIHA[124]	-		
- OSHA[123]	-		
- NIOSH[123]			

Chapter 9

Hazard assessment

9.1 Hazard identification

In humans, acute intake of high doses of propylene glycol have resulted in metabolic acidosis with high lactate levels requiring restoration of acid-base balance. No fatal intoxications with propylene glycol have been described in the literature.

9.1.1 Effects on the skin

Human and animal data indicate that propylene glycol does have weak irritating effects to the skin. In man, irritative reactions were only observed under occlusion. For example, in one study involving healthy subjects, mild skin irritation was observed with 10% and 30% propylene glycol under occlusion, yet in another study no irritation was found with 50% propylene glycol under occlusion.

In healthy human subjects and in animals, propylene glycol was not sensitising to the skin. Nevertheless, in provocative patch tests with 10% propylene glycol allergic reactions were seen in about 4% of patients with eczema. Cutaneous reactions with 10% propylene glycol under occlusion were also found in patients with dermatitis related to the use of cosmetics. Overall, the committee concluded that propylene glycol has weak irritating properties and can provoke allergic

reactions in some patients with allergic skin disease, and that allergic sensitisation after skin application can not be excluded.

No data are available on the absorption of propylene glycol through the intact human skin. In rats, approximately 1% of propylene glycol permeated through intact skin in 24 hours when placed in a diffusion cell. Calculation with the model SkinPerm[28] indicates that the maximal skin permeation is 0.20 mg/cm^2/hour under steady state conditions when skin absorption equals systemic delivery. These results indicate that dermal exposure may substantially contribute to the body burden of propylene glycol. This model also predicts a significant latency period of approximately two hours after onset of skin exposure before systemic delivery starts to occur.

9.1.2 Effects on the eyes and the airways

Application of propylene glycol to the human eye resulted in acute but transient stinging, blepharospasm and lacrimation, followed by transient conjunctival hyperaemia. Residual injury or discomfort was not reported. Similar results have been obtained in animal studies with undiluted propylene glycol applied to the eye. From this, the committee concluded that propylene glycol has mild, transient irritating effects to the eyes.

Three human studies are available on the (sub)acute inhalatory effects of exposure to a propylene glycol containing aerosol, one study by Wieslander *et al.* (1989)[19] and two clinical trials by Greenbaum *et al.* (1988)[66] and by Meltzer *et al.* (1990)[67], respectively. Wieslander *et al.* (2001)[19] showed that a 1-minute exposure to 309 mg/m^3 (geometric mean; range 176-851 mg/m^3) propylene glycol caused acute ocular (dry eyes) and upper airway (dry throat) irritating effects in healthy individuals. In addition, a few reacted with cough, mild dyspnoea, and slight airway obstruction as indicated by a 5% reduced forced expiratory volume in 1 second (FEV_1). From these acute inhalatory effects and from the fact that application of propylene glycol to the eyes gives acute stinging, blepharospasm and lacrimation, the committee concludes that a short-term exposure limit (STEL) is warranted. However, personal air measurements were not carried out and air concentrations of propylene glycol varied considerably between morning and afternoon sessions. Therefore, the committee can not derive a STEL from this study.

In the two clinical trials, the efficacy and tolerability of the anti-inflammatory glucocorticoid flunisolide was studied in a spray containing 0.025% flunisolide, 5% propylene glycol, 20% polyethylene glycol and 2.5% polysorbate, and compared to the original formulation containing 0.025% flunisolide, 20% propylene

glycol and 15% polyethylene glycol only.[66,67] The total dose was two sprays to each nostril two times a day during the study period of four weeks. Each activation of the device provided approximately 0.1 ml of the spray. Patients with allergic rhinitis showed significantly less nasal burning, stinging and throat irritation when the propylene glycol concentration in the nasal flunisolide spray was reduced from 20% to 5%. Some patients rated the severity of nasal burning still as mild to moderate with the 5% propylene glycol spray.

Konradova *et al.* (1978)[86] exposed rabbits to a propylene glycol aerosol for 20 and 120 minutes, respectively. The authors presented no more details on the aerosol composition than that the aerosol was prepared from 10% propylene glycol at a flow rate of 10 l air/minute. The size of the droplets and density of the aerosol were not given. The committee noted that the acute effect of propylene glycol inhalation in this study is on the respiratory epithelium and the goblet cells. Konradova *et al.* (1978)[86] did not report the appearance of nose bleedings in the rabbits after 120 minutes of exposure.

9.1.3 *Carcinogenicity and reproduction toxicity*

Human data on carcinogenic effects and reproduction (fertility and development) are not available. Animal data show that propylene glycol is not mutagenic and not carcinogenic. The International Agency for Research on Cancer has not evaluated propylene glycol.

Decreased fertility was not observed at dose levels of 10 g/kg bw/day propylene glycol given in drinking water in a 14-week continuous breeding study in Swiss CD-1 mice. Propylene glycol did not show developmental toxicity at dose levels up to 10 g/kg bw/day when given by gavage to Charles River CD-1 mice from day 6-15 of pregnancy.

9.1.4 *Effects after long-term exposure*

There are no adequate human studies available.

Chronic intravenous administration of drugs formulated in propylene glycol have led to metabolic acidosis with high lactate levels requiring restoration of acid-base balance. Cases of estimated cumulative exposure to 2.3 kg propylene glycol over a period of 13 months and 19 months, respectively, have been described. Patients fully recovered after discontinuation of the intravenous infusion of the propylene glycol containing medication.

Suber *et al.* (1989)[90] studied the effects of propylene glycol in a 90-day nose-only inhalation experiment with Sprague-Dawley rats. Local effects included

nasal haemorrhages, thickening of the respiratory epithelium and increase in the number of goblet cells. Systemic effects, occurring in the females only, included body weight reduction and changes in leucocyte profile (Table 9.1). Nasal haemorrhaging occurred in the male and female rats at all aerosol concentrations tested, but in the female rats the nasal bleedings at 160 mg/m^3 were transient. At this latter level, the percentage of females showing signs of nasal haemorrhaging dropped to less than 4% after the fourth week of exposure. At all levels in both males and females, the clinical signs of the nasal bleedings disappeared during the non-exposure weekend periods. Light microscopy revealed thickening of respiratory epithelium with an increased number of goblet cells or an increase in the mucin content of the goblet cells in both male and female rats exposed to the medium (1,000 mg/m^3) and high (2,200 mg/m^3) propylene glycol levels, but not at the exposure level of 160 mg/m^3. Female rats had a significant reduction in body weight starting on day 50 and continuing until the end of the study at the highest dose level of 2,200 mg/m^3 propylene glycol, and at 1,000 mg/m^3 propylene glycol from day 64 onwards. This body weight reduction correlated significantly with reduced food intake. Female rats exposed to 1,000 mg/m^3 and 2,200 mg/m^3 propylene glycol had significant decreases of white blood cells, band neutrophils and lymphocytes. These systemic effects on body weight and leucocyte profile have not been found consistently in other studies. The results might indicate gender differences in susceptibility to propylene glycol's adverse effects in the rat, but other studies do not provide additional evidence for this.

In an 18-month inhalation study by Robertson *et al.* (1947)[99], rats were continuously exposed to condensed propylene glycol droplets (170-350 mg/m^3 propylene glycol, 'continuous supersaturation'). This resulted in higher weight gain: at 12 months, the weights of the exposed animals were about 50% increased compared to the weights of the control group. Nose bleedings were not reported in this study. An exact exposure level can not be derived from this study because the formation of the propylene glycol vapour was not controlled well and changed during the study.

Table 9.1 Effects of propylene glycol exposure on airways, eyes, body weight, and white blood cells in Sprague-Dawley rats.[90]

Propylene glycol concentration in aerosol[a] (mg/m^3)	Findings
160	nasal haemorrhaging and ocular discharge only in the males and not in the females after the fourth week of exposure;
1,000	nasal haemorrhaging and ocular discharge; thickening of respiratory epithelium with increased number of goblet cells and mucin content; decreased body weight and food consumption in the females; decreased number of white blood cells, band neutrophils and lymphocytes in the females;
2,200	nasal haemorrhaging and ocular discharge; thickening of respiratory epithelium with increased number of goblet cells and mucin content; decreased body weight and food consumption in the females; decreased number of white blood cells, band neutrophils and lymphocytes in the females;

a Exposure was by nose-only inhalation for 13 weeks, 5 days/week, and 6 hours/day; 19 males and 19 females per dose level.

9.2 Recommendation of the health-based occupational exposure limit

Human studies on the adverse effects of chronic inhalatory exposure to propylene glycol have not been found in the literature. Acute inhalatory exposure resulted in acute irritative effects on the upper respiratory tract. In acute inhalation, nose bleedings have never been reported in humans.

The animal data indicate that inhalatory propylene glycol has adverse effects on the respiratory tract. In the rat nose-only inhalation study by Suber *et al.* (1989)[90], local effects included nasal haemorrhaging, thickening of the respiratory epithelium and increase in the number of goblet cells in both male and female rats. In two other inhalation studies, one with acute exposure (20 and 120 minutes exposure to 10% propylene glycol aerosol)[86] and one with long-term exposure (18 months continuous exposure to condensed propylene glycol droplets)[99], nasal haemorrhaging was not reported. Apparently, nose bleedings have only been found in the nose-only inhalation study by Suber *et al.* (1989).[90] As the rat is an obligatory nose breather, the nose-only setup may have contributed to the increased number of animals with nose bleedings because of mechanical injuries.

The committee decided not to use the nose bleedings in the rat as the starting point for deriving the health-based recommended occupational exposure limit (HBROEL) for several reasons. First of all, the interpretation of the nose bleeding results is hampered by the absence of a dose-response relationship and by the transient character of the bleedings in the females exposed to 160 mg/m^3. Next to this, nose bleedings have not been reported in other long-term animal studies, and finally, it is unknown whether the nose-only setup of the experiment by

Suber *et al.* (1989)[90] has contributed to the nose bleedings because of mechanical injuries.

Because the effects of propylene glycol on the goblet cells are consistent in both male and female rats in the study of Suber *et al.* (1989)[90], and because goblet cell changes have also been found by Konradova *et al.* (1978)[86], the committee has decided to take the No Observed Adverse Effect Level (NOAEL) for the goblet cell changes as the starting point for the derivation of the HBROEL. In the study by Suber *et al.* (1989)[90], the number of goblet cells was increased at the dose level of 1,000 mg/m^3, but not at 160 mg/m^3. Therefore, the committee concludes that 160 mg/m^3 propylene glycol is the NOAEL for the increased number of goblet cells.

For the extrapolation to the HBROEL, the following aspects are taken into account: intraspecies and interspecies variation, and the difference between the experimental conditions and the exposure pattern of the worker. For local upper-respiratory effects an interspecies factor is not indicated. For intraspecies variation the committee applies a total factor of three. With this factor, the committee calculates an inhalatory HBROEL of 50 mg/m^3 as an 8-hour time-weighted average concentration. This value of 50 mg/m^3 applies to the sum of the concentrations of propylene glycol existing as a vapour and as an aerosol.

According to the committee, exposure to an aerosol can have effects that are comparable to the effects of exposure to inhalable and respirable dust. Therefore, it is the committee's opinion that health-based occupational exposure limits for inhalable and respirable dust must be applied to aerosols of propylene glycol.*

Human data on carcinogenic effects and reproduction (fertility and development) are not available. Animal data indicate that propylene glycol is not carcinogenic and has no toxic effects on reproduction.

The skin permeation data obtained with SkinPerm indicate that dermal exposure may substantially contribute to the body burden of propylene glycol. The committee, however, does not recommend a skin notation because of propylene glycol's low systemic toxicity.

9.3 Groups at extra risk

Groups at extra risk were not identified.

* In the Netherlands, MAC values for inhalable and respirable dust existed until January 2007. At the moment, the Dutch Expert Committee on Occupational Standards is re-evaluating the scientific literature in order to recommend health-based occupational exposure limits for inhalable and respirable dust.

9.4 Health based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards recommends a health-based occupational exposure limit for propylene glycol of 50 mg/m^3 as an 8-hour time-weighted average, applying to the sum of the concentrations of propylene glycol existing as a vapour and as an aerosol.

The committee also recommends to apply health-based occupational exposure limits for inhalable and respirable dust to aerosols of propylene glycol. The committee does not recommend a skin notation.

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A Request for advice

B The committee

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Annexes

Annex **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request

for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Annex **B**

The committee

- G.J. Mulder, *chairman*
  emeritus professor of toxicology; Leiden University, Leiden
- R.B. Beems
  toxicologic pathologist; National Institute for Public Health and the Environment, Bilthoven
- P.J. Boogaard
  toxicologist; Shell International BV, The Hague
- J.J.A.M. Brokamp, *advisor*
  Social and Economic Council, The Hague
- D.J.J. Heederik
  professor of risk assessment in occupational epidemiology; Institute for Risk Assessment Sciences, Utrecht University, Utrecht
- L.A.L.M. Kiemeney
  professor of cancer epidemiology; University Medical Centre St Radboud, Nijmegen
- H. van Loveren
  professor of immunotoxocology; Maastricht University, Maastricht, and National Institute for Public Health and the Environment, Bilthoven
- T.M. Pal
  occupational physician; Netherlands Center for Occupational Diseases, Amsterdam

- A.H. Piersma
  professor of reproductive toxicology; National Institute for Public Health and the Environment, Bilthoven
- H.P.J. te Riele
  professor of molecular biology; VU University Amsterdam, Amsterdam
- I.M.C.M. Rietjens
  professor of toxicology; Wageningen University and Research Centre, Wageningen
- H. Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, The Hague
- T. Smid
  occupational hygienist - epidemiologist; KLM Health Services, Schiphol, and professor of working conditions, VU University Amsterdam, Amsterdam
- G.M.H. Swaen
  epidemiologist; Dow Benelux N.V., Terneuzen
- R.A. Woutersen
  toxicologic pathologist; TNO Quality of Life, Zeist
- P.B. Wulp
  occupational physician; Labour Inspectorate, Groningen
- E.J.M. Pennings, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **C**

Comments on the public review draft

A draft of the present report was released in 2006 for public review. The following organisations and persons have commented on the draft report:

- A. Aalto, Ministry of Social Affairs and Health, Finland
- E. González-Fernández, Ministerio de Trabajo y Asuntos Sociales, Spain
- T. Scheffers, Maastricht, The Netherlands
- P. Teheux, CEFIC, Belgium
- R.D. Zumwalde, National Institute for Occupational Safety and Health, USA

Annex **D**

Human data

Table D-1 Human *in vitro* studies with propylene glycol.

Human cell type	Procedure	Concentration tested	Effects	Reference
KB cells	-	0.15, 0.3, 0.5 and 0.8 M	Dose dependent inhibition of cell growth; the ID50 (inhibitory concentration of a compound in growth medium that caused a 50% reduction in cell number after 72 hours of incubation) of propylene glycol was 0.31 M.	Mochida and Gomyoda, 1987[132]
Human natural killer cells	Natural killer cytotoxicity assay; cultured K562 erythroleukemia cells were used as the target cells; cytotoxicity measured by % ^{51}Cr release.	Three concentrations of effector cells were incubated with propylene glycol in phosphate-buffered saline (PBS) diluted to final concentrations of 0.01, 0.1 and 1%. PBS alone was used as a control.	The cytotoxicity of human natural killer cells was decreased significantly when cells were incubated with 1% propylene glycol.	Denning and Webster, 1987 cited in Anonymous, 1994[50]
Human isolated neutrophils	1-ml aliquots of cells were placed in cuvettes for 30 min and incubated with propylene glycol; latex particles coated with IgG were added and chemiluminescence measured.	The cells were incubated with propylene glycol diluted to final concentrations of 0, 0.1, 0.5 or 1.0% propylene glycol.	The neutrophil chemiluminescence was significantly decreased in the cells incubated with 0.5 and 1% propylene glycol.	Denning and Webster 1987 cited in Anonymous, 1994[50]

Table D-2 Human studies and incidents with regard to skin and eye irritation, and skin sensitisation properties of propylene glycol.

Humans involved/ No. of humans	Procedure	Dose or concentration tested	Results	References
Humans (not further specified) / n=not reported	not reported	not reported	When applied to the tongue of humans, propylene glycol caused a temporary burning sensitisation without apparent injury to this organ or to the oral mucosa.	Seidenfeld and Hanzlik, 1932 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Humans (not further specified) / n= not reported	7 days	500 mg	Slightly irritating	IUCLID dataset, 2000[1]
Six human volunteers	Pads with propylene glycol fixed to the forearm for 2 hrs; observation time 7 days	propylene glycol	No irritation was observed	Kimmerle, 1967 cited in IUCLID dataset, 2000[1]
Humans (not further specified) / n= not reported	not reported	propylene glycol	Moderate to slightly irritating; EC classification: not irritating	Drill and Lazar, 1977 cited in IUCLID dataset, 2000[1]
Human volunteers / n=not reported	Single 5-15 min exposure to 1 ml of propylene glycol (open and under occlusion)	100% propylene glycol	Weak erythema at test site (under occlusion); no reactions (open)	Wahlberg and Nilsson, 1984[71]
Human volunteers / n= not reported	Repeated open exposures to 1 ml propylene glycol for 12 days	100% propylene glycol	No irritation	Wahlberg and Nilsson, 1984[71]
Human volunteers / n= not reported	Repeated open exposures to propylene glycol for 36 days	100% propylene glycol	No increase in skinfold thickness	Wahlberg and Nilsson, 1984[71]
Sixteen adult white male volunteers	Single application (0.5 ml) to the skin and covered with a patch gauze; exposure time 24 hr	0.5 ml undiluted propylene glycol	The average irritation index was 0.19, which indicates that propylene glycol is not irritating to rabbit skin	Philips *et al.*, 1972[75]
Twelve adult white male volunteers (2/concentration)	21-Day continuous closed patch test; patch was removed every 24 h (when the test site was read) and refreshed by a new one	0.2 ml propylene glycol at concentrations of 1, 10, 20, 40, 60 and 80%	No relevant skin reactions were reported	Philips *et al.*, 1972[75]
Eight adult white male volunteers	21-Day continuous closed patch test; patch was removed every 24 h (when the test site was read) and refreshed by a new one	0.2 ml 10% propylene glycol	No irritation was noted	Philips *et al.*, 1972[75]
Adult white male volunteers (2/concentration)	Once a day for 21 days 0.02 ml propylene glycol was applied unoccluded to the test site	50% and undiluted propylene glycol	No irritation was noted	Philips *et al.*, 1972[75]

Humans (not further specified) / n= not reported	Chamber-scarification test; propylene glycol was applied to normal and scarified skin on the forearm once a day for 3 days;	0.1 ml propylene glycol	Propylene glycol was scored to 1.5-2.4 (moderately irritating)	Frosch and Kligman, 1977 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
480 eczematous dermatitis patients	Patch test (48 h, covered)	15% glycol mixture that contained unspecified proportions of diethylene glycol, ethylene glycol and propylene glycol in petrolatum	No skin reactions were reported	Meneghini *et al.*, 1971 cited in BIBRA, 1996[117]
20 patients (not further specified)	Propylene glycol was applied to the trunk and arms, twice daily for 2 weeks	50% propylene glycol in water	Two patients complained of a slight burning sensitisation after application of propylene glycol.	Faergemann and Frederikson, 1980 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
98 eczema out-patients at a skin clinic (no indication of a possible allergic contact dermatitis to PG could be found in the history of any of these patients)	48 h patch test	100% propylene glycol	11/98 patients were scored positive 48 hours after the application. Before testing, none of the patients had any indication of allergic contact dermatitis to propylene glycol. In view of this, the reaction was considered to be of a primary irritating nature.	Nater, 1977 cited in Nordic-chemicals-group; Mortensen B, 1993[2] and in Anonymous, 1994[50]
866 dermatologic patients	48-h patch test (closed or covered patches)	2.5, 10 and 100% propylene glycol	Skin irritant: 138 patients showed a positive reaction to 100% propylene glycol; 10% propylene glycol: 5/23 positive responses; 2.5% propylene glycol: 1/3 positive responses; no sensitisation was observed.	Warshaw and Herrmann, 1952 cited in Fisher 1978[52]
One patient with contact dermatitis developed from intolerance to a cream containing propylene glycol.	Patch test	10% and 100% propylene glycol	Positive reactions to 10% (+) and to 100% (+++) propylene glycol	Shore and Shelley, 1974 cited in Fisher 1978[52]
1556 patients with eczema	Patch test (20-24 hr)	3.2, 10, 32 and 100% propylene glycol	Positive reactions in 12.5% of the subjects; Of these, 70% (8.7% of total) were irritating reactions and 30% (3.8% of total) were allergic reactions; results for 3 groups of 42 positive patients patch tested with propylene glycol: 3.2% propylene glycol (9 positive reactions), 10% propylene glycol (12 positive reactions) and 32% propylene glycol (20 positive)	Hannuksela *et al.*, 1975[133]

38 patients with allergic-type epicutaneous test reactions to propylene glycol	Simultaneous 20 -24 h Finn chamber applications; per-oral challenge dose of 2% propylene glycol	2, 10, 32 and 100% propylene glycol	Allergic (n=11) and other types of eczema (n=16) during initiation; extensive exanthema in 15 patients; 8 of 10 patients with a positive epicutaneous reaction to 2% propylene glycol and 7 of the other 28 patients with a positive epicutaneous reaction to 10-100% propylene glycol developed exanthema 3-16 h after ingestion of 2-15 ml propylene glycol. In all but one case, the rash disappeared within 24-48 h without any medication. In one case, the exanthema was treated with prednisone for 4 days and symptoms disappeared gradually in 6 days. Allergic-type reaction to 10-100% propylene glycol seemed to be type IV hypersensitivity to propylene glycol. No local reactions after oral administration in 20 people without dermal response.	Hannuksela and Forstrom, 1978[134]
84 eczema patients from the Department of Dermatology, University of Oregon; 248 consecutive eczema patients from the Department of Dermatology, Gentofte Hospital	Patch test and oral provocation	100% propylene glycol 100%, 20%, and 2% propylene glycol in water	Five of 12 patch test-positive patients had allergic reactions while seven had irritant reactions. Two of five patients with positive reactions to patch tests showed an itchy eczematous eruption after oral provocation with 15 ml propylene glycol.	Andersen and Storrs, 1982 cited in BIBRA, 1996[117]
8 patients with allergic contact dermatitis; 11 normal subjects	5 simultaneous 20 µl applications per patient; repeated applications over 7-day period in normal subject; at end of exposure, benzoic acid applied to evaluate non-immunologic contact reactions (NICRs) to benzoic acid	50% propylene glycol	Propylene glycol had irritant properties; it significantly increased transepidermal water loss and increased cutaneous blood flow in both groups; NICRs were not significantly different from controls.	Kinnunen and Hannuksela, 1989 cited in Anonymous, 1994[50]
823 patients with histories of contact dermatitis	48 h occlusive application	30% propylene glycol	Erythema in 7.4% of patients; erythema and oedema in 3.8% of patients	Kinnunen and Hannuksela, 1989 cited in Anonymous, 1994[50]
22 humans (22 of the patients with erythema and edema in the study described in the row above)	48 h occlusive application	1, 2, 10 and 30% propylene glycol	Positive reactions: 1% propylene glycol (1 patient), 30% propylene glycol (3 patients)	Kinnunen and Hannuksela, 1989 cited in Anonymous, 1994[50]
183 patients with eczema	Patch test	38% propylene glycol	23 allergic reactions	Huriez *et al.*, 1966 cited in Anonymous, 1994[50]
86 contact dermatitis patients	24 h occlusive patch applications (Finn chambers) for 7 days	30% aqueous propylene glycol	Allergic reactions in 19 patients	Hannuksela and Salo, 1986 cited in Anonymous, 1994[50]

19 patients with allergic reactions (from the study as described in the row above)	24 h occlusive patch applications of 1, 10 and 30% propylene glycol (Finn chambers) for 7 days; for 5% PF, repeated open application test (0.1 ml applications twice daily for 7 days)	1, 10 and 30% propylene glycol in water; 5% in cream	Positive reactions: 1% propylene glycol (5 subjects with + or ++ reactions); 10% propylene glycol (2 with + or ++), 30% propylene glycol (19 with + or ++); incidence of + reactions to 5% propylene glycol in preceding groups: 5/5 (1% propylene glycol); ½ (10% propylene glycol); and 4/12 (30% propylene glycol)	Hannuksela and Salo, 1986 cited in Anonymous, 1994[50]
78 patients (not further specified)	Patch test	10% propylene glycol	3 allergic reactions	Braun, 1969 cited in Anonymous, 1994[50]
100 patients with allergic eczematous contact dermatitis	Patch test	10% propylene glycol	2 allergic reactions	Fisher *et al.*, 1971 cited in Anonymous, 1994[50]
399 patients with cosmetic-related contact dermatitis	48 h patch test using Finn chambers	10% aqueous propylene glycol	29 cutaneous reactions	Adams *et al.*, 1985 cited in Anonymous, 1994[50]
330 patients with eczematous lesions	Patch test	10% propylene glycol	13 allergic reactions	Blondeel *et al.*, 1978 cited in Anonymous, 1994[50]
1450 patients with eczema	Patch test	5 and 10% propylene glycol	15 positive reactions	Romaguera *et al.* 1981, cited in Anonymous, 1994[50]
3364 patients (not further specified)	Patch test	5% aqueous propylene glycol	27 positive (allergic) reactions	Angelini *et al.*, 1985 cited in Anonymous, 1994[50]
400 patients with eczematous contact dermatitis	Patch test	20% aqueous propylene glycol	6 positive (allergic) reactions	Angelini *et al.*, 1981 cited in Catanzaro and Smith, 1991[48]
851 atopic patients	Finn chamber application for 2 days	5% aqueous propylene glycol	2.5% of patients had allergic reactions; 3.9% had irritant/follicular reactions	Lammintausta *et al.* 1992 cited in Anonymous, 1994[50]
880 eczema patients	Epicutaneous chamber test; chambers applied for 20-24 hr	2% aqueous propylene glycol	2 allergic reactions	Hannuksela, 1976 cited in Anonymous, 1994[50]
Patients suspected of having contact dermatitis due to topical medications constituted the bulk of the study group / n= not reported	Patch test (48 hr; closed)	5, 10, 20 and 100% propylene glycol	The incidence of positive reactions was found to be 18.1%, 16.9%, 5.8% and 2.5%, respectively. The reactions were mainly irritant rather than allergic in nature	Bajaj *et al.*, 1986[135]
40 metal workers with dermatitis	Patch test	5% propylene glycol	None of the subjects was sensitised to propylene glycol	De Boer *et al.*, 1989 cited in Nordic-chemicals-group; Mortensen B, 1993[2]

51 healthy male volunteers	Patch test of propylene glycol applied to the skin (30 µl/cm²)	concentration: 50% in water to 16 persons; undiluted to 35 persons	None of the 16 subjects treated with 50% propylene glycol had any irritation. Fourteen of the 35 subjects treated with undiluted propylene glycol had mild to moderate irritation.	Willis *et al.*, 1988 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Ten adult male volunteers	48 h patch testing using Finn chamber	100% propylene glycol	No significant skin irritation	Willis *et al.*, 1989 cited in Anonymous, 1994[50]
Ten healthy non-atopic male volunteers	48 h patch testing using Finn chamber	100% propylene glycol	Mild to moderate erythema	Willis *et al.*, 1990 cited in Anonymous, 1994[50]
50 healthy Japanese male adults	48 h patch test; 0.05 ml application	100% propylene glycol	No irritation under open conditions; occlusive conditions produced severe response with erythema, oedema and vesicles.	Motoyoshi *et al.*, 1984[85]
24 healthy American male adults	Repeated closed patch applications (0.05 ml) for 21 days	1, 3, 10 and 30% propylene glycol	Primary irritation with 10 and 30% propylene glycol	Motoyoshi *et al.*, 1984[85]
269 volunteers with or without eczema (propylene glycol was tested for its possible irritant effect in ointments)	Duhring chamber method	10% propylene glycol under occlusion	Very few irritations; no allergic sensitisation reaction was observed against propylene glycol.	Bäurle *et al.*, 1985[136]
14 patients with auxiliary dermatitis due to an antiperspirant containing 90% propylene glycol	Patch test	0.01-10% propylene glycol solution in water	Six patients had positive response to 10% propylene glycol, but because the 10% concentration may be irritating in some patients, the patch test was repeated with a 1% solution. Only one patient reacted positively to 1% and 0.01% propylene glycol.	Agren-Jonsson and Magnusson, 1976 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
7 patients with an allergic eczema due to the same antiperspirant as described in the row above	Patch test	propylene glycol	2/7 had positive response to a 2% propylene glycol solution.	Hannuksela, 1975 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
293 healthy humans	Draize test; after 2 weeks, re-challenge with 12% propylene glycol patch for 72 hours	204 subjects were treated with 12% and 89 subjects with 60% propylene glycol in petrolatum.	No allergic responses were seen in any of the subjects.	Marzulli and Maibach, 1974 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
204 normal human test subjects	10 successive 48 or 72 h occlusive patches (induction); 72 h occlusive challenge patch	12% propylene glycol in cream vehicle	No reactions (no irritation and no sensitisation)	Marzulli and Maibach, 1973 cited in Anonymous, 1994[50]
25 healthy volunteers	Repeated exposures under occlusive patches for 21 days	propylene glycol (concentration not stated)	Total cumulative irritation score of 72	Patel *et al.*, 1985 cited in Anonymous, 1994[50]

10 healthy subjects	Repeated exposures; 0.2 ml under occlusive patches for 21 days	propylene glycol	1 subject with equivocal reaction	Trancik and Maibach, 1982 cited in Anonymous, 1994[50]
22 volunteers	Patch test; 24 h covered contact	propylene glycol	Non-irritating	Davies *et al.*, 1972 cited in BIBRA, 1996[117]
817 dermatitis patients	Patch test; 24 h covered contact	propylene glycol	Irritant response was observed in 23/817 patients to 10% aqueous solutions.	Iden and Schroeter, 1977 cited in BIBRA, 1996[117]
106 healthy individuals 63 healthy individuals 63 healthy individuals	Patch test; 48 h covered contact	propylene glycol	Irritant response was observed in 13/106 patients to 50% aqueous solutions. Irritant response was observed in 4/63 patients to 30% aqueous solutions. Irritant response was observed in 1/63 patients to 10% aqueous solutions.	Eun and Lee, 1985 cited in BIBRA, 1996[117]
203 healthy volunteers	Modified Draize sensitisation test; 10 0.2 applications under occlusive patches (induction); 48 to 72 h challenge two weeks later	propylene glycol	13 positive reactions (if these subjects were sensitised their responsiveness was, however, low since none reacted when their skin was in open contact with 100% propylene glycol daily for 7 days); 6 equivocal reactions	Trancik and Maibach, 1982 cited in Anonymous, 1994[50]
19 (subjects with positive reactions in preceding study)	Provocative use test; 0.1 ml applications twice daily for 7 days	propylene glycol	Cutaneous reactions noted in preceding study confirmed as irritant reactions	Trancik and Maibach, 1982 cited in Anonymous, 1994[50]
A 78-year old woman developed a pruritic exacerbation of her methotrexate-dependent psoriasis after applying topical calcipotriene ointment.	Patch tests with both the calcipotriene and the excipients provided by the pharmaceutical company	1% propylene glycol	A positive reaction at 72 hours to 1% propylene glycol in white petrolatum and to no other substance in the medication. Questioning revealed that the patient had tried in the past a variety of topical compounds, both for psoriasis and as general skin care products, and found herself intolerant to many.	Fisher, 1997[137]
61-year old man treated with Rifocine as topical antibiotic for an infection	Patch test with the European standard series and topical Rifocine	0.5% aqueous propylene glycol	Eczema; topical antibiotic had been used for 10 years before, without any local reaction. Patient had past history of atopic eczema in childhood. Patch testing showed a strong reaction to Rifocine at day 3. Complementary tests showed a strong reaction to 0.5% aqueous propylene glycol at day 2 (no reaction to 5% propylene glycol in petrolatum, illustrating the importance of the vehicle in patch testing).	El Sayed *et al.*, 1995[138]
A 30-year old woman	-	-	Allergic to propylene glycol. Propylene glycol exposure occurred by a 5% ibuprofen gels (to treat a sprained ankle) and by a brassiere containing padding made of a gel insert. The gel insert was made from 100% propylene glycol.	Lamb *et al.*, 2003[139]

A 49-year old women who had developed facial dermatitis to cosmetics	Patch test	2% propylene glycol	Strongly positive response to 2% propylene glycol. Avoidance of cosmetics containing propylene glycol brought improvement in facial condition; unexplained periodic flares of dermatitis were seen, until it was found that she was ingesting propylene glycol in her salad dressing.	Fisher, 1978[52]
30-year old woman with persistent housewife's eczema	Patch test	2 and 5% propylene glycol	Strongly positive reaction to Keri lotion, which contains propylene glycol, and to 2 and 5% propylene glycol. When the woman stopped using the Keri lotion, the hand dermatitis improved considerably	Fisher, 1978[52]
45-year old man with pruritic contact dermatitis to Keralyt gel, superimposed on psoriasis	Patch test	2, 5 and 60% propylene glycol	An open test to Keralyt gel, containing 60% propylene glycol, and closed patch tests to 2 and 5% propylene glycol gave strongly positive results.	Fisher, 1978[52]
17-year old man had applied Vaseline Intensive Care lotion for several weeks to a dry, pruritic area on his legs. A pruritic, erythematous eruption ensued.	Patch test	2% propylene glycol	Strongly positive patch test reactions were obtained to Vaseline Intensive Care lotion, containing propylene glycol, and to 2% propylene glycol but not to the other ingredients of the lotion.	Fisher, 1978[52]
40-year old woman	-	-	The woman acquired an allergic contact dermatitis due to propylene glycol from its presence in Spectra 360 Gel (Parker) used in connection with transcutaneous electrical nerve stimulation.	Fisher, 1978[52]
One cardiac patient	-	-	The patient acquired an allergic reaction due to propylene glycol from its presence in Spectra 360 Gel (Parker) used for electrocardiography.	Fisher, 1978[52]
9-year old child who acquired an allergic contact dermatitis superimposed upon atopic eczema from the use of Cetaphil lotion.		2% propylene glycol	Positive patch test reactions were obtained to the lotion, containing propylene glycol, and to 2% propylene glycol.	Fisher, 1978[52]
A 60-year old man who developed contact dermatitis from propylene glycol in ECG electrode gel	Patch test Blind repeated open application test (ROAT)	1%, 5% and 10% aqueous propylene glycol 20% aqueous propylene glycol	Negative patch test reactions after 1%, 5% and 10% aqueous propylene glycol; contact sensitisation was diagnosed on an unequivocally positive (controlled, blind) repeated open application test with 20% aqueous propylene glycol; concordant patch test results; flare-up of distant sites on re-exposure; possible sensitisation by repeated shaving prior to application of electrodes. Irritation as the sole cause of both clinical dermatitis and patch test reactions could not be ruled out; mild irritation may have contributed to the development of dermatitis and facilitation of sensitisation.	Uter and Schwanitz, 1996[140]

52-year old man with herpes zoster of the left upper chest was treated with Zovirax cream 4 times daily;	Patch test with the European standard test series and constituents of Zovirax cream	1% aqueous propylene glycol	few days after use of the cream, acute itchy vesicular eczema appeared; the patient reacted only to Zovirax cream and to 1% aqueous propylene glycol; it was concluded that the patient was sensitive to propylene glycol in the cream.	Kim and Kim, 1994[141]
51-year old woman	-	-	Hypersensitivity to Cycloviran cream (to treat recurrent herpes simplex labialis infection) due to the vehicle propylene glycol; vesicular dermatitis on the buttock simultaneously to vesicular dermatitis on the face; close chemical similarity between propylene glycol in the antiviral cream and hydroxylpropyl cellulose in the reservoir of the transdermal plaster suggested cross-reactivity.	Corazza *et al.*, 1993[142]
26-year old woman who applied minoxidil to an area of diffuse alopecia located above the nape of her neck; after six weeks of application, a pruritic popular eruption was noted in the treated area and nape of the neck.	Patch test to minoxidil solution, 5% aqueous propylene glycol and 2% minoxidil solution in which glycerin was the solvent	5% propylene glycol	Patch test to minoxidil solution and 5% aqueous propylene glycol were positive	Fisher, 1990 cited in Anonymous, 1994[50]
A man who had applied minoxidil for 1 month on the scalp developed a pruritic, macular and popular eruption of this area.	Patch test to minoxidil solution, 5% aqueous propylene glycol and 5% minoxidil solution with glycerin as solvent	5% propylene glycol	Patch test to minoxidil solution and 5% aqueous propylene glycol were positive; the eruption recurred when 5% propylene glycol was rubbed into a small area of the scalp;	Fisher, 1990 cited in Anonymous, 1994[50]
52-year old woman suffering from chronic otitis media for 2 years; she had used various ear drops and ointments; in the last 2 weeks of the 2-year episode, the patient used eardrops with 2.5% hydrocortisone in propylene glycol:water 1:1.	Patch test	10, 50 and 100% propylene glycol	No reaction was observed to 10% aqueous propylene glycol; oral challenge with 5 ml of propylene glycol caused a pruritic macular rash on the abdomen, flare-up of the external ear dermatitis and flare-up of the patch test reaction on the back in 20 h; Patch test 1 week after oral challenge gave a + reaction to 50% propylene glycol and a ++ reaction to undiluted propylene glycol after 72 hours.	Frosch *et al.*, 1990[143]
Eye irritation				
Humans / n= not reported	not reported	-	No irritation of the eyes by propylene glycol; Acute effects of a drop of propylene glycol applied to the human eye: stinging, blepharospasm, lacrimation, with discomfort lasting for the few seconds; mild conjunctival redness, but no residual discomfort or injury.	Grant, 1962[55]

Table D-3 Human studies and incidents with regard to acute exposure to propylene glycol.

Humans involved/No. of humans	Procedure	Dose or concentration tested	Effects	Reference
Inhalation				
27 non-asthmatic volunteers	Exposure to propylene glycol mist in an aircraft simulator for 1 minute; medical examination before and after exposure (within 15 minutes); it included measurement of tear film stability break-up time, nasal patency by acoustic rhinometry, dynamic spirometry, and symptoms questionnaire	Geometric mean concentration of propylene glycol was 309 mg/m^3 (GSD=1.7); arithmetic mean concentration of propylene glycol was 360 mg/m^3 (range 176-851 mg/m^3), with an arithmetic mean exposure of 220 mg/m^3 in the morning and 520 mg/m^3 in the afternoon.	Tear film stability decreased; ocular and throat symptoms increased; forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) slightly reduced; self-rated severity of dyspnoea slightly increased; no effect on nasal patency, vital capacity (VC), FVC, nasal symptoms, dermal symptoms, smell of solvent, or any systemic symptoms. Those exposed to the higher concentrations in the afternoon had a more pronounced increase of throat symptoms and a more pronounced decrease of tear film stability. In four with irritative cough during exposure, FEV_1 decreased by 5%; FEV_1 was unchanged in those without irritative cough. Those with cough had increased perception of mild dyspnoea and slight airway obstruction .	Wieslander *et al.*, 2001[19]
Patients with airflow disorders	Treatment with an aerosol prepared from solution of 1.0 ml isoproterenol-HCl and 7.0 ml of a physiological saline solution containing 40% propylene glycol by volume; 8 ml were added to a reservoir and heated to 185 °F; patients inhaled the mist through a face mask (15min at 115 -124 °F)	40% propylene glycol by volume	Inhalation of the mist was well-tolerated, and no adverse clinical reactions were reported, prompting the authors to recommend propylene glycol as an appropriate vehicle for route administration of bronchodilator drugs.	Cohen and Crandall, 1964 cited in Lakind *et al.*, 1999[15]
Oral				
2-year old child	Accidental ingestion of a high amount of hair gel	Gel contained 1.75-2.25% propylene glycol	Central nervous system depression and severe metabolic acidosis. Initial assessment: elevated serum anion gap, increased serum osmolality, normal osmolal gap, lactate acidosis.	Glover and Reed, 1996[17]
A child of 15 months	Ingestion of a vitamin C preparation containing propylene glycol for 8 days	7.7 g/day of propylene glycol	Irregular apical heart rate; tachycardia, tachypnoea, diaphoresis; unconsciousness; no further episodes after discontinuation of vitamin C preparation.	Martin and Finberg, 1970[60]
39-year old woman with long-standing history of uncontrollable epilepsy	Propylene glycol intoxication which occurred from fruit juice as well as antiepileptic drugs		High plasma osmolal gap on admission, metabolic acidosis, and cerebral depression. Serum pyruvate and lactate concentrations 16 hours after hospital admission were within the normal reference range. After discharge from hospital, no further seizures.	Lolin *et al.*, 1988[61]

Three human subjects	Propylene glycol was administered after 12 h of fasting	50 cc (51800 mg) in a 50% aqueous solution	Propylene glycol had no demonstrable impacts on the basal metabolic rate.	Hanzlik *et al.*, 1939 cited in Lakind *et al.*, 1999[15]
Two-year old toddler	Chewing disposable cleansing towels		The child was found in the morning by his parents in his cradle, lethargic, responsive only to sharp pain. On admission, vital signs were temperature 38.5 °C, lethargy, polypnea; propylene glycol intoxication through disposable cleansing towels chewing was ascertained by anamnesis and blood urine analyses which revealed metabolic acidosis and serum propylene glycol.	Guillot *et al.*, 2002[62]
58 individuals	Ingestion of a single dose of propylene glycol	2.1-15.7 g propylene glycol	Nausea, vertigo, curious sensations and other vague symptoms.	Hannuksela and Forstrom, 1978[134]
Ten volunteers	Ingestion of propylene glycol (given every 5 h for 48 hr)	5.1 g propylene glycol	No effect on gross liver function (as measured by antipyrine clearance)	Nelson *et al.*, 1987 cited in BIBRA, 1996[117]
16 outpatients of a neurology clinic 6 outpatients of a neurology clinic	Oral formulation of phenytoin containing propylene glycol 400 ml/l (414 g/l), ethanol 145 ml/l, flavour, fructose, glycerol (100 ml/l) and water	21 g propylene glycol every 8 hr for at least 3 d; 42 g propylene glycol every 12 h for at least 3 d	Variable degree of CNS toxicity in patients receiving an oral formulation of phenytoin in propylene glycol with additional ethanol; no clear relationship between plasma propylene glycol and CNS toxicity. Comment: results biased by the presence of ethanol in the oral formulation.	Yu *et al.*, 1985[31]
58-year old man with chronic schizophrenia and azotemic renal disease	Exposure prior to hospital admittance	not reported	Stupor on hospital admittance; metabolic acidosis with blood pH of 7.25; anion gap 28 mmol/l; bicarbonate 11 mmol/l; lactate 18 mmol/l; propylene glycol 0.7 g/l; urine propylene glycol 0.6 g/l. Full recovery	Cate and Hedrick, 1980[63]
61-year old man	Co-ingestion of ethanol and a propylene glycol containing antifreeze	Unknown amount	Minimally depressed sensorium with normal vital signs; initial serum ethanol 1.7 g/l, osmol gap 120 mosmol/kg; 7 hours post-ingestion: ethanol 1.45 g/l (during i.v. ethanol), propylene glycol 4.7 g/l, osmol gap (corrected for ethanol) 75 mosmol/kg. Uneventful clinical course.	Brooks and Wallace, 2002[65]
49-year old alcoholic man	Ingestion of ‘homemade’ alcoholic beverage	Unknown amount	Lethargic on hospital admittance; metabolic acidosis with blood pH of 7.10 with serum anion gap 18 mmol/l, osmol gap 35 mosmol/kg, ethanol 0.76 g/l, lactate 5.6 mmol/l, and propylene glycol 0.12 g/l. Full recovery after i.v. ethanol and haemodialysis.	Doty and Sahn, 2003[64]

5-year old girl with Angelman Syndrome (Chromosome 15-related developmental disorder with metabolic defects)	Accidental ingestion	Large amount of gel air freshener containing propylene glycol 6%, perfume 5%, ethoxylate 13% and water 77%;	Unresponsive on hospital admittance; Initial arterial pH 7.0; osmolality 364 mosmol/kg; lactate 29.9 mmol/l; ammonia 0.30 mmol/l; propylene glycol 0.66 g/l (pre-dialysis); acetone, isopropanol, methanol and ethylene glycol not detected. Died despite haemodialysis. Comment: cause of death uncertain due to co-exposure and incomplete evidence of case involving co-existing metabolic disorder.	White *et al.*, 2002[144]
3 male fatalities	Accidental/suicidal co-ingestions of propylene glycol with pentobarbital (case 1), ethylene glycol (case 3) and possibly propylene oxide (case 2).	Unknown amounts	Lack of evidence for propylene glycol as the cause of fatalities: case 1 involved PG, pentobarbital, doxylamine, diazepam, and ethanol; case 2 involved propylene glycol and acute renal failure; case 3 involved propylene glycol and ethylene glycol with oxalate crystals seen in the kidney tubules.	deRoux *et al.*, 2005[145]
Dermal				
7 inpatients with psoriasis (one patient was diabetic)	Topical daily application of 60% propylene glycol for 5 days	1.5-6.1 g/kg/24 h	Skin irritation (3 patients); two of the remaining four patients had mild renal impairment, and most patients experienced desquamation; no effects in serum osmolality and lactate	Commens, 1990[53]
8-month old boy	Treatment with topical silver sulfadiazine for a burn and complicating toxic epidermal necrolysis involving 78% of his total body surface area	Estimated exposure was around 9 g/kg bw/24 h for 70 hr	Transdermal absorption of propylene glycol from the silver sulfadiazine produced hyperosmolality with an increased osmolal gap. Peak propylene glycol concentration: 1,059 mg/dL (10.6 g/l). Elevated concentrations of propylene glycol possibly contributed to the patient's cardio-respiratory arrest. Hypoxic encephalopathy remained.	[32] and BIBRA, 1996[117]
Other routes				
Premature infants	Propylene glycol in a vitamin preparation given i.v. for periods of at least 5 days	Around 3 g of propylene glycol	Hyperosmolality	Glasgow *et al.*, 1983[38]
Five patients who received intravenous medications containing propylene glycol as a vehicle	Intravenously administration of different preparations containing propylene glycol	57 to 771 mg propylene glycol/kg/day	The lactate concentration increased both in serum and cerebrospinal fluids. Propylene glycol may be an important cause of lactic acidosis	Kelner and Bailey, 1985[37]
16-year old boy	Intravenous administration of high doses of pentobarbital and phenobarbital, both of which solubilised with propylene glycol		Acute renal failure; a renal biopsy showed proximal renal tubular cell swelling and vacuole formation. The data from this case suggest that the reversible acute renal failure caused by propylene glycol is attributable to proximal renal tubular cell injury.	Yorgin *et al.*, 1997[18]

One patient with hypoxemic respiratory failure	Therapy with lorezapam infusion with propylene glycol as the drug vehicle for 5 days	Over the entire cause of therapy, the total propylene glycol load was 540 g	Osmolar gap metabolic acidosis; after the lorezapam was discontinued, the anion and osmolar gaps returned to normal within 72 hours	Arbour and Esparis, 2000[146]
A patient with a poor kidney function	Intravenous administration of propylene glycol for 8 days	340 g (daily dose)	Severe hyperosmolality, lactic acidosis and CNS depression. For a brief period during the second day of treatment, propylene glycol and blood transfusion were given down the same intravenous line, resulting in haemolysis of the red blood cells.	Demery *et al.*, 1984 cited in BIBRA, 1996[117]
Child	Intravenous administration of propylene glycol for a number of days		Hyperosmolality	Huggon *et al.*, 1990 cited in BIBRA, 1996[117]
28 patients with acute myocardial ischemia or preload reduction (16 male and 12 female; aged 36-72 years)	Intravenous NTG, containing propylene glycol, for acute myocardial ischemia or preload reduction		Hyperosmolality, haemolysis and lactic acidosis	Demey *et al.*, 1988[147]
A 46-year old morbidly obese man, requiring mechanical ventilation and high dose sedation	Sedation by continuous infusion of lorazepam; man developed pneumonia which was treated with intravenous trimethoprim-sulfamethoxazole. Each of these drugs were formulated in propylene glycol.	Several hundred mg/ml of propylene glycol	On day 17 of hospital course (3 days after starting the trimethoprim-sulfamethoxazole), the patient developed acute renal failure consistent with acute tubular necrosis; propylene glycol toxicity was suspected and drugs containing propylene glycol were discontinued. Laboratory analysis: marked osmol gap, metabolic acidosis. Renal toxicity was attributed to continuous and high intermittent doses of i.v. propylene glycol.	Hayman *et al.*, 2003 cited in HSDB, 2004[14]
One patient who received etomidate infusion for the treatment of postoperative seizures and prevention of brain edema	Propylene glycol was present at 35% (v/v) in the undiluted etomidate; intravenous infusion for 24 h	480 g propylene glycol	Lactic acidosis and hyperosmolality	Bedichek and Kirschbaum, 1991 cited in BIBRA, 1996[117]

Table D-4 Human studies and incidents with regard to short-term exposure to propylene glycol.

Humans involved/No. of humans	Procedure	Dose or concentration tested	Effects	Reference
Inhalation				
A total of 122 patients were enrolled in a 4-week trial; 18 patients withdrew from the trial prematurely; 10 patients were lost to follow-up; 8 withdrew due to side effects and/or inadequate therapeutic response	Double-blind, randomized, crossover study compared the incidence of nasal burning and stinging, as well as overall tolerability of the currently marketed formulation of Rhinalar (original formulation) to a new formulation of Rhinalar containing less propylene glycol. Rhinalar was reported to produce high incidence of transient local nasal burning and stinging, possibly due to a high propylene glycol content. Each patient received one formulation of Rhinalar for 2 weeks and then crossed over to receive the alternate formulation for an additional 2 weeks.	Rhinalar is formulated as a 0.025% solution in a vehicle containing a mixture of polyethylene glycol and propylene glycol. The new formulation contained a reduced amount of propylene glycol (5%) as compared with the original formulation which contains 20%.	Statistical comparisons of patient evaluations of nasal burning and stinging with the two formulations of Rhinalar: significant difference of severity ($p< 0.001$), duration ($p< 0.001$), and tolerability ($p=0.006$) in favour of the new formulation; reduction of severity of throat irritation with the new formulation ($p=0.006$). Nausea, headache, and other side effects including watery eyes, taste perversion, and runny nose were seldom reported with either test medication. Both formulations were shown to be equally effective in relieving the nasal symptoms of seasonal allergic rhinitis.	Greenbaum *et al.*, 1988[66]
More than 200 patients with symptoms of perennial allergic rhinitis	Multicenter, randomized, double-blind, parallel group study, symptomatic patients were treated with either the new or the original formulation of 0.025% solution of intranasal flunisolide for 4 weeks to provide 200 micrograms flunisolide daily.	In the new formulation, propylene glycol was decreased from 20 to 5%, polyethylene glycol was 15 to 20% and 2.5% polysorbate was introduced.	Both formulations were highly effective in decreasing symptom scores as evident from patient diary reports before and after treatment ($p<0.001$); nasal airflow improved with each treatment as measured by anterior rhinomanometry ($p<0.0002$); number of patients with nasal eosinophilia decreased ($p<0.01$); fewer patients using the new formulation reported nasal burning or stinging and the acceptability rating of the new formulation was higher ($p<0.001$)	Meltzer *et al.*, 1990[67]
Oral				
18 epileptic patients	20 days	62 g propylene glycol (and 22 ml ethanol)	Mild to moderate symptoms of nervous system toxicity (dizziness, fatigue, hot flushes)	Sawchuk *et al.*, 1982 cited in BIBRA, 1996[117]
Other routes				
15-year old boy		2.25 ml/h propylene glycol contained in an injection of 2-25 mg/h lorazepam administered for 42 days; concomitant therapy included morphine	On hospital day 54, the patient had increased serum osmolality (331 mOsmol/kg), increased osmol gap (25 mOsmol), hypotension requiring increased vasopressor support, increased requirement for packed red blood cell transfusion, and bilateral knee effusions. Lorazepam was discontinued and replaced with a midazolam infusion without propylene glycol. The dosage of morphine was increased. The patient's hyperosmolality resolved within 24 h (consistent with other case reports). Vasopressor and packed red blood cell requirements decreased and the knee effusions resolved.	Seay *et al.*, 1997[68]

Table D-5 Human studies and incidents with regard to long-term exposure to propylene glycol.

Humans involved/ No. of humans	Procedure	Dose or concentration tested	Effects	Reference
Oral				
11-year old boy	Ingestion of a vitamin D preparation containing propylene glycol for 13 months	4 – 8 g propylene glycol/day	Repeated seizures of convulsions followed by unconsciousness lasting 20 to 30 minutes. When propylene glycol intake was ended his seizures stopped.	Arulanantham and Genel, 1978[58]
Other routes				
Two groups of premature infants with low birth weight	Daily intravenously administration of 1 ml multivitamin solution containing propylene glycol for a 19 month period	multivitamin solution containing respectively 300 mg and 3 g of propylene glycol	The incidence of seizures among infants who received 3 g/day of propylene glycol (33%), compared with infants receiving 300 mg/day of propylene glycol (14%) was significantly increased. Peak serum osmolality and blood urea level were significantly higher in infants receiving 3 g/day of propylene glycol.	MacDonald *et al.*, 1987[59]

Annex **E**

Animal data

Table E-1 Animal *in vitro* studies with propylene glycol.

Animal cell type	Procedure	Dose or concentration tested	Effects	Reference
Rabbit muscles	Glycerol-extracted fibers of rabbit psoas muscle were cut into 50 mm bundles. Half of each bundle was incubated with 0.2 M KCl/0.05 M Tris (the incubation mixture), and 6.6*10-5 M Mg-ATP was added to initiate a reaction. The second half of the fiber bundle was incubated with the incubation mixture and propylene glycol.	16, 33 and 66 mg/ml propylene glycol.	At al the propylene glycol doses, shortening of the test muscle fibers, as well as development of tension within the test fibers occurred.	Kaldor *et al.*, 1971 cited in Anonymous, 1994[50]
Guinea pig smooth muscle samples	Pieces of the ileum from a guinea pig were placed in an organ bath and 0.5 ml of different propylene glycol concentrations were added for 3 min. Contractions were simulated in the muscles by addition of acetylcholine chloride, histamine hydrochloride or barium chloride.	10, 20, 50 or 100% propylene glycol	At concentrations above 20%, a nonspecific, dose-dependent inhibition of contraction was noted.	Singh *et al.*, 1982[120]
Rat smooth muscle samples	Uterine samples were immersed in organ baths and propylene glycol was added. Contractions were induced in the smooth muscle samples by addition of 5-hydroxy-tryptamine.	10, 20, 50 or 100% propylene glycol	All propylene glycol concentrations produced a nonspecific dose-dependent inhibition of contractions. Propylene glycol did not produce any paralytic effects, analgesic activity or hypnotic effects in mice or rats at any of the concentrations tested.	Singh *et al.*, 1982[120]

B6D2F1 mouse zygotes	The effects of propylene glycol on embryonic development, membrane integrity and metabolism on B6D2F1 mouse zygotes in the pronuclear stage were evaluated; exposure of zygotes to propylene glycol after incubation of the zygotes in either fluorescein diacetate (FDA) or 5 µmol acridine orange (AO). AO fluoresces yellow-green within the physiological pH range (7.4). At lower pH, the yellow-green colour is lost.	0, 1.5 and 3 M	In controls and the group treated with 1.5 M propylene glycol, 78% of the zygotes developed into 2-cell embryos; with 3 M propylene glycol, the proportion of 2-cell embryos was 7% ($p<0.05$).	Damien *et al.*, 1989[148]
		0, 1.5, 3 and 6 M	FDA-induced fluorescence was retained in 100% of the control and 98% of the zygotes treated with 1.5 M propylene glycol. Exposure to 3.0 and 6.0 M propylene glycol reduced the percentage of zygotes with FDA-induced fluorescence to 81% ($p<0.05$) and 5% ($p<0.05$), respectively. After AO exposure, 95% of the control zygotes and 95% of the zygotes exposed to 1.5 M propylene glycol possessed yellow-green fluorescence, indicating a normal cellular pH. Treatment with 3.0 and 6.0 M propylene glycol caused a shift in the fluorescence such that 93% ($p<0.05$) and 100% ($p<0.05$) of the zygotes lost yellow-green fluorescence. The results demonstrate that a 20-min exposure to 1.5 M propylene glycol does not affect embryonic development; treatment with 3 M propylene glycol caused both cell membrane damage and pH changes, which were associated with a decrease in embryonic development.	
B6D2F1 mouse zygotes	The effects of propylene glycol on membrane integrity in relation to embryonic development in mouse zygotes were evaluated. Prior to treatment, mouse zygotes were incubated with acridine orange (AO) a fluorescent dye which fluoresces within a physiological pH range. The zygotes were then perifused with propylene glycol and zygote volume and intracellular pH monitored.	Perifusing at rates of 0, 0.18 mol/min, 0.36 mol/min and 1.0 mol/min propylene glycol	Zygotes exposed to propylene glycol at a rate of 1 mol/min decreased in volume and lost their AO fluorescence by 10 min. The volume of the zygotes perifused at less than or equal to 0.36 mol/min was not altered. However, only 25% of the zygotes perifused at 0.36 mol/min maintained fluorescence at 10 min and all lost fluorescence by 15 min. At 0.18 mol/min 95% of the zygotes maintained fluorescence at 10 min and 49% at 15 min. All of the propylene glycol-exposed zygotes lost their fluorescence by 20 min. Although 2-cell development was not affected by 3.0 M propylene glycol for 2.5 min, blastocyst development was reduced compared with controls (P less than 0.05). Longer exposures resulted in a significant decrease in both 2-cell and blastocyst development. These data demonstrate that a 2-7 minutes exposure to greater than or equal to 2.5 M propylene glycol alters both the intracellular pH and developmental potential. Since these detrimental effects are independent of volume changes and therefore intracellular propylene glycol concentrations, it is postulated that propylene glycol mediates its toxic action by directly altering the cell membrane.	Damien *et al.*, 1990[149]

Mouse metaphase II (MII) oocytes	The objective of this study was to test the hypothesis that propylene glycol induces cytogenetic aberrations in mouse metaphase II (MII) oocytes that predispose zygotes to aneuploidy. Female ICR mice received 7.5 IU hCG and 5.0 IU hCG 48 h later. Propylene glycol was given 3 h post-hCG; controls received the solvent deionised water. Ovulated oocytes were collected 16 h after administration of propylene glycol and processed for cytogenetic analysis. For the one-cell zygote cytogenetic study, females were given propylene glycol and paired (1:1) with ICR males for 16 h. Females that mated were given 2 x 10(-3) M colchicine 22 h post-propylene glycol, and zygotes were collected 18 h later.	0, 1300, 2600, and 5200 mg/kg bw	Propylene glycol significantly ($p < 0.05$) increased both the proportion of MII oocytes with premature centromere separation (PCS) and the proportion of aneuploid one-cell zygotes. These results support the hypothesis that propylene glycol-induced PCS in MII oocytes predisposes zygotes to aneuploidy.	Mailhes *et al.*, 1997[150]
Chicken embryos	Exposure for 4-7 days	0.05 ml 100% propylene glycol injected onto the yolk sac	Propylene glycol was not found to be teratogenic after injection into the yolk sac; high mortality (90%) of the embryos when injected into the air sac on the fourth day of incubation; 21% of the surviving embryos developed unilateral micromelian.	Gebhardt, 1968 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Chicken embryos	Single exposure	0.2 ml 100% propylene glycol injected onto the yolk sac	No teratogenic effects were observed.	Landauer and Salam, 1972 cited in IUCLID dataset, 2000[1]

Table E-2 Animal studies with regard to skin and eye irritation and skin sensitisation properties of propylene glycol.

Species/Strain/No. per Sex per Group	Procedure	Dose or concentration tested	Results	Reference
Skin irritation and sensitisation				
Chinchillas / n=50	Fifty-five ears of 50 chinchillas were divided in three groups: 15 ears were treated with a single injection of 0.2 ml of undiluted propylene glycol; 30 ears were injected with 0.2 ml of undiluted propylene glycol once a day for 5 consecutive days and 10 ears were treated with 0.2 ml of normal saline once daily for 5 days.	0.2 ml undiluted propylene glycol	Histopathological observations: severe inflammation of the middle ear mucosa and tympanic membrane; papillary proliferation of the epidermis of the tympanic membrane and external auditory meatus; retraction and adhesion of the tympanic membrane. Findings suggested two types of acquired cholesteatoma, probably with different pathogenesis. In one type, the proliferated epidermal layer of the tympanic membrane penetrated into the middle ear cavity making tympanic perforations. In the other type, there was progressive retraction of the tympanic membrane forming a retraction pocket.	Imamura *et al.*, 1999[151]

Rabbits / n=20 Guinea pigs / n=27	Daily open patches; skin fold thickness test	100% propylene glycol	There was a significant increase in skin fold thickness (a measurement of skin irritation) in the guinea pigs on days 7-10. There were no significant increases in skin fold thickness in the rabbits. Most test sites in the rabbits had transient redness, which resolved spontaneously.	Wahlberg and Nilsson, 1984[71]
Five female rabbits	Daily treatment for 100 days with a mixture of equal parts of propylene glycol and diethylene glycol	mixture of equal parts of propylene glycol and diethylene glycol (0.5 ml)	No macroscopic changes; microscopic examination after 20-30 days: slightly thickened stratum granulosum; signs of proliferation in the stratum basale; superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes; collagen fibers slightly fragmented and scattered. The findings remained unaltered in later stages. Thus, the glycols seemed incapable of producing major changes in rabbit skin even after prolonged periods of continuous application.	Rantuccio *et al.*, 1979[79]
Albino rabbits (no information regarding strain) / 8 males	Skin irritation study very similar to the OECD guideline	0.5 ml propylene glycol was applied to the skin and covered by an impervious film; 24 h later the film was removed	No irritant reaction was reported	Weil and Scala, 1971[76]
New Zealand white rabbits / n=12	Skin irritation study very similar to the OECD guideline	0.5 ml propylene glycol was applied to the skin and covered with a patch gauze	The average irritation index was 0.39, which indicates that propylene glycol is not irritating to rabbit skin	Philips *et al.*, 1972[75]
New Zealand white rabbits / n=6	Skin irritation study; application of 0.5 g propylene glycol	propylene glycol (concentration not stated)	Mild to no irritation	Clark *et al.*, 1979[78]
New Zealand white rabbits / n=6	Cosmetic protocol (0.5 ml for 23 h under occlusive patches); AFNOR protocol (0.5 ml for 4 h under occlusive patches) and OECD protocol (0.5 ml for 4 h under semiocclusive patches)	propylene glycol	No irritation was observed	Guillot *et al.*, 1982[74]
Rabbits	Occlusive contact with the skin	20 and 100% propylene glycol	100% propylene glycol was slightly irritating	Guillot *et al.*, 1982[72]
Rabbits / n=3	Application of propylene glycol to the uncovered skin of rabbits daily for 6 weeks (2 ml)	20 and 100% propylene glycol	100% propylene glycol produced only slight irritation (EC classification: not irritating). No significant adverse reaction was macroscopically and histologically observed; a 20% aqueous solution was totally without irritant action in three rabbits	Guillot *et al.*, 1982[72]
New Zealand white rabbits	OECD Guideline 404	propylene glycol	No irritation was observed	Huels AG, 1984 cited in IUCLID dataset, 2000[1]

Rabbits	Draize test	propylene glycol	No irritation was observed	BASF AG, 1979 cited in IUCLID dataset, 2000[1]
Angora rabbits / n=6	48 h open and closed patch test; 0.1 ml propylene glycol	propylene glycol	No irritation was observed	Motoyoshi *et al.*, 1984[85]
Hartley guinea pigs / n=6	48 h open and closed patch test; 0.1 ml propylene glycol	propylene glycol	No irritation was observed	Motoyoshi *et al.*, 1984[85]
Guinea pigs / n=20	Maximisation test (GMPT)	70% propylene glycol	No sensitisation was observed	Kero and Hannuksela, 1980[83]
Guinea pigs / n=20	Open epicutaneous test	70% propylene glycol	No sensitisation was observed	Kero and Hannuksela, 1980[83]
Guinea pigs / n=20	Three 48 h chamber exposures	70% propylene glycol (20 µl)	No sensitisation was observed	Kero and Hannuksela, 1980[83]
Guinea pigs / n=20	Epicutaneous maximisation test; amount not specified	propylene glycol	No irritation and sensitisation were observed	Guillot and Gonnet, 1985[73]
Albino Dunkin-Hartley guinea-pigs / n=20	Magnusson and Kligman maximisation test; 0.5 ml	propylene glycol	No skin and sensitisation reactions were observed	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / n=10/sex/group	Split adjuvant technique; 0.1 ml	propylene glycol	Propylene glycol was found to be non-allergic	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / =10/sex	Guinea pig optimisation test; 0.1 ml	propylene glycol	Propylene glycol was found to be non-sensitising	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / =10/sex	Guillot/Brulos method; 0.5 ml	propylene glycol	Propylene glycol was found to be a non-significant or weak sensitiser	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / n=5/sex/group	Freund's Complete Adjuvant test; 0.1 ml	propylene glycol	Propylene glycol was found to be non-allergenic	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / =12/group	Dossou and Sicard method; 0.5 ml	propylene glycol	No irritation and sensitisation were observed	Guillot *et al.*, 1983[84]
Albino Dunkin-Hartley guinea-pigs / =8/group	Open epicutaneous test; 0.1 ml	propylene glycol	No irritation and sensitisation were observed	Guillot *et al.*, 1983[84]
Miniature Gottingen swine / n=2	48 h open and closed patch test; 0.1 ml propylene glycol		No irritation was observed	Motoyoshi *et al.*, 1984[85]
Swine / n=2	21 day open and closed patch test; 0.1 ml propylene glycol		No irritation was observed	Motoyoshi *et al.*, 1984[85]
Nude mice / n=3	24 h exposure in PVC cup (on the dorsal side of the mice)	10, 25 and 50% propylene glycol	In the animals exposed to 50% propylene glycol; hypertrophy, dermal inflammation and proliferation stimuli were noted, indicating that this concentration may cause skin irritation	Lashmar *et al.*, 1989[77]

Mice (Swiss, BALB-c, CBA, C56B1/6, DBA-2 and B6D2F1) / n=19	Mouse ear sensitisation assay	100% propylene glycol	No irritation and sensitisation were observed	Descotes, 1988[82]
Rats (Wistar) / males	*In vitro* histamine release assay	0.01, 0.10, 1.0 and 10% propylene glycol	Low skin irritation potential; only those cells exposed to a final concentration of 1.0% propylene glycol released significantly more histamine than control cells	Jacaruso *et al.*, 1985 cited in Anonymous, 1994[50]
Dogs (n=85)	Each of 85 dogs was injected, subcutaneously, with 5 different mixtures of propylene glycol and aqueous vehicles. Each dog was observed for immediate (within 15 minutes) and delayed and delayed (at 24, 48 and 72 hours) reactions	0, 25, 50, 75 and 100% propylene glycol	A mixture containing between 25% and 50% propylene glycol caused the fewest immediate reactions; the number of delayed reactions increased as the proportion of propylene glycol in the solutions increased. Viscosity of the mixture should be considered when selecting a vehicle.	Brown and Kasson, 1984[152]
Eye irritation				
New Zealand White rabbits / n=6	Eye irritation in accordance with OECD/EEC guidelines	0.1 ml of undiluted propylene glycol was instilled into one eye of each animal; the treated eye was not rinsed	The mean redness was scored to 0.40 and the mean corneal opacity was scored to 0. These results indicate that propylene glycol is not irritating to eyes	Jacobs *et al.*, 1988 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
New Zealand White rabbits / n=6	Eye irritation study which was very similar to the test recommended in the OECD guidelines	0.1 ml of undiluted propylene glycol (pH 8.8) was instilled into one eye of each animal; the treated eye was not rinsed	Undiluted propylene glycol was slightly irritating for the eyes, but caused no corneal opacity	Guillot *et al.*, 1982[80]
Albino rabbits (no information regarding strain) / 6 males	Eye irritation study very similar to the OECD guideline	0.1 ml of undiluted propylene glycol was instilled into one eye of each animal; the eyes were not washed following installation	No irritation was reported	Weil and Scala, 1971[76]
New Zealand white rabbits / n=6	Eye irritation study	0.1 ml of undiluted propylene glycol was instilled into one eye of each animal	No corneal damage was observed	Clark *et al.*, 1979[78]
New Zealand white rabbits	OECD Guideline 405	propylene glycol	No irritation was reported	Huels AG, 1984 cited in IUCLID dataset, 2000[1]
Rabbits	not reported	propylene glycol (100 and 500 mg)	Mild effects were observed	IUCLID dataset, 2000[1]
Rabbits	Eye irritation study	20 and 100% propylene glycol	100% propylene glycol was slightly irritating	Guillot *et al.*, 1982[72]
Rabbits (n=2)	One drop of propylene glycol in the conjunctival sac of the eye	propylene glycol	No irritation was reported	Kimerle, 1967 cited in IUCLID dataset, 2000[1]
Rabbits	Eye irritation study	propylene glycol	Transient slight conjunctival hyperaemia	Grant, 1962[55]

Rabbits	Eye irritation study	100% propylene glycol (0.5 ml)	No irritant effects were reported	Carpenter and Smyth, 1946[153]
Rabbits	It was reported that propylene glycol was not irritating for the eye and therefore propylene glycol was used as a solvent for other chemicals in eye irritation studies.	propylene glycol	Non-irritating	Smyth and Carpenter, 1944[154]
Cultured corneal endothelial cell line	*In vitro* ocular irritancy test	not reported	Propylene glycol was not irritant in this study	Douglas and Spilman, 1983[81]

Table E-3 Acute animal toxicity studies of propylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Dose or concentration tested	NOAEL	LOAEL	Critical effect	Reference
Inhalation						
Rabbits / n=6/group	20 and 120 min	10% propylene glycol aerosol	-	10% aerosol	Propylene glycol stimulated secretion of mucous from goblet cells in the trachea; 20-minute exposure: minimal alteration of the ultrastructure of the ciliated cells; no alteration of the regular ciliary border above the epithelium was observed; 120-minute exposure: pathological alteration of the cilial cells; increased (60%) number of degenerated mucous discharging goblet cells in the tracheal epithelium; both exposures produced alterations in the goblet cells.	Konradova *et al.*, 1978[86]
Dogs / n=7	15 min	10 and 20% nebulised propylene glycol	20% aerosol	-	No haemolysis or a haemodynamic effect; Possibly, different results if the administration time had been prolonged beyond 15 minutes.	MacCannell, 1969[87]
Oral						
Mice / n=20/dose	once by gavage	20-30 ml/kg (21-31 g/kg bw))	-	23.9 ml/kg bw (24.8 g/kg bw)	Mortality (LD_{50})	Laug *et al.*, 1939 cited in Lakind, 1999[15] and in Nordic-chemicals-group; Mortensen B, 1993[2]
Mice / n=4/dose	once	not reported	-	22 g/kg bw	Mortality (LD_{50}); depression of the respiration; death is apparently due to respiratory failure	Latven and Molitor, 1939 cited in Nordic-chemicals-group; Mortensen B, 1993[2]

Mice	once	not reported	-	31.9 g/kg bw	Mortality (LD_{50})	Bornmann, 1954 cited in Lakind *et al.*, 1999[15]
Mice (Swiss) and rats (Wistar)	once	10 ml propylene glycol solution/kg bw at concentrations of 10, 20, 50 or 100% (i.e., 10.4 g/kg bw at 100% propylene glycol solution)	2.1 g/kg bw	5.2 g/kg bw	At lower concentrations (10-20%): no significant neuro-psychopharmacological effects; at higher concentrations (50-100%): moderate to marked effect with decreased locomotor activity, body and limb tone, respiration, rectal temperature and suppression of secondary conditioned responses; significant potentiation of pentobarbital hypnosis, slight increase in d-amphetamine toxicity in aggregated mice; pronounced analgesic and anti-inflammatory activity. On isolated smooth muscle preparations, a dose-dependent non-specific blockade was observed at 50-100% concentration; no effect on cardiac tissues.	Singh *et al.*, 1982[120]
Mice (CD-1) / n=30 females	5 days on a daily basis	1.25, 2.5 and 5 g/kg bw/day	5 g/kg bw/day	-	No evidence of an effect on immune function (cell-mediated and humoral immune response)	Gaworski *et al.*, 1994[118]
Rats	once	15-25 ml/kg (15.5-25.9 g/kg bw)	-	21 ml/kg bw (21.7 g/kg bw)	Mortality (LD_{50})	Laug *et al.*, 1939 cited in Lakind *et al.*, 1999[15]
Rats	once	not reported	-	21 g/kg bw	Mortality (LD_{50})	Sax, 1979 cited in Anonymous, 1994[50]
Rats / n=10/dose	once by gavage	15, 17.5, 20, 22.5 and 25 ml/kg bw (15.5, 18.1, 20.7, 23.3 and 25.9 mg/kg bw)	-	21 ml/kg bw (21.8 g/kg bw)	Mortality (LD_{50})	Laug *et al.*, 1939 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Rats (n=5/sex/dose)	once	Between LD_{16} (15.7 g/kg) and LD_{86} (42.7 mg/kg bw); propylene glycol was diluted in 0.9% NaCl solution	-	25.9 g/kg bw	Mortality (LD_{50})	Bartsch *et al.*, 1976[155]
Rats (Wistar) / n=10 males	once	not reported	-	26.4 g/kg bw	Mortality (LD_{50}); fatal or near fatal doses produced no narcosis but varying degrees of sluggish depressed functioning	Smyth *et al.*, 1941 cited in Lakind *et al.*, 1999[15]
Rats / n=5/dose	once	not reported	-	33.5 g/kg bw	Mortality (LD_{50})	Weatherby and Haag, 1938 cited in Lakind *et al.*, 1999[15]

Rats	once	not reported	-	27 g/kg bw	Mortality (LD_{50})	Layton *et al.*, 1987 cited in Anonymous, 1994[50]
Rats (Fischer 344) / n=5 females	once by gavage	not reported	-	20.9 g/kg bw	Minimum lethal dose: the 24 h LD_{50} was 22.8 g/kg bw; the lowest recorded 24 h lethal dose in this experiment was 20.9 g/kg bw and the lowest recorded 48 h lethal dose was 19.8 g/kg bw.	Clark *et al.*, 1979[78]
				23.5 g/kg bw	Acute haemorrhagic enteritis; extensive adrenocortical haemorrhage and widespread lymphocyte depletion (suggesting an acute stress reaction)	
Rats (Wistar) / females	1 ml oral doses	9.66, 19.32 and 38.64 mmol/kg bw	-	9.66 mmol/kg bw (735 mg/kg bw)	Significant decreases in the levels of fibrinogen, albumin and globulin in the plasma; propylene glycol affected the function of the liver in either the synthesis or the secretion of proteins in rats.	Saini *et al.*, 1987 cited in Anonymous, 1994[50]
Rats (albino Wistar) / n=6 females/dose	once by gavage	730 and 2940 mg/kg bw	-	730 mg/kg bw	Statistically significant and progressive decrease in haemoglobin, packed cell volume (mainly due to hyperosmolality of the plasma and altered morphology of erythrocytes) and red cell counts (ascribed to either the destruction of cells or to their enhanced removal from circulation) for 2 days, which returned to basal values on the day 8. Reticulocyte counts, plasma haemoglobin and osmolality was increased (more pronounced on day 2); the osmotic fragility of erythrocytes remained unaffected; electron microscopy revealed rough cell surface, ruptured membranes and increased cell adherence throughout the observation period, but these features were not marked on day 8.	Saini *et al.*, 1996[88]
Rats (Charles River) n=10/females	3 times a day for 3 days	0.75 ml/kg bw 100% propylene glycol; 1.50 ml/kg bw 100% propylene glycol; 3.0 ml/kg bw 100% propylene glycol; 3.0 ml/kg bw 75% propylene glycol and 3.0 ml/kg bw 50% propylene glycol	-	0.75 ml/kg bw 100% propylene glycol (0.78 g/kg bw)	One rat given 0.75 ml/kg bw 100% propylene glycol and two rats given 3.0 ml/kg bw 100% propylene glycol had slight hyperaemia of the gastro-intestinal tract; one rat given 1.50 ml/kg bw 100% propylene glycol had severe hyperaemia of the gastro-intestinal tract.	Staples *et al.*, 1967[156]
Rabbits	once	not reported	-	19.3 g/kg bw	Mortality (LD_{50})	Weatherby and Haag, 1938 cited in Lakind *et al.*, 1999[15]

Rabbits	once by gavage over a period of one hour	15.8, 18.9, 19.9 and 21 mg/kg bw (20% aqueous propylene glycol solution)	-	19.9 g/kg bw	Mortality (LD_{50}); death occurred within 18 to 36 hours	Braun and Cartland, 1936 cited in Lakind *et al.*, 1999[15]
Rabbits	once by gavage (1-h period)	13.7 – 21 mg/kg bw	-	13.7 g/kg bw	Increased respiratory rate; loss of equilibrium, profound depression, analgesia; coma; death (18 – 36 h)	Braun *et al.*, 1936 cited in Lakind *et al.*, 1999[15]
Guinea pigs / n=10	once	not reported	-	18.35 g/kg bw	Mortality (LD_{50}); fatal or near fatal doses produced no narcosis but varying degrees of sluggish depressed functioning	Smyth *et al.*, 1941 cited in Lakind *et al.*, 1999[15]
Guinea pigs / n=10	once by gavage	15-22.5 ml/kg	-	18.9 ml/kg bw (19.6 g/kg bw)	Mortality (LD_{50}); shortly after administration of high doses, the animals showed signs of loss of equilibrium, marked depression, analgesia, coma and finally death; no changes were seen in organs except for haemorrhagic areas in the small intestine and minimal changes in the kidneys with nuclear pyknosis and vacuolar degeneration of the cytoplasm; the liver showed slight congestion and hyperaemia with no fatty changes	Laug *et al.*, 1939 cited in Lakind *et al.*, 1999[15] and in Nordic-chemicals-group; Mortensen B, 1993.[2]
Dogs (mongrel) / n=1/dose	3 times a day for 3 days	0.75, 1.5 and 3.0 ml/kg propylene glycol	0.75 ml/kg bw	1.5 ml/kg bw (1.6 g/kg bw)	All stomach mucosa slightly slightly hyperaemic; duodenum was 'normal' except for several small, localised slightly hyperaemic areas.	Staples *et al.*, 1967[156]
Dogs / n=3		1036, 4144 and 6216 mg propylene glycol/kg	1036 g/kg bw	4144 g/kg bw	CNS effects (loss of muscular control, depression and sleeplessness); diuretic action (no liver and kidney injury)	Lehman and Newman, 1937[43]
Dogs		1036, 4144 and 6216 mg propylene glycol/kg			Dose-dependent increase in diuresis	Lehman and Newman, 1937[43]
Horses / n=4		2.25-4.5 litres via drinking	-	2.25 litres	Loss of muscular control and CNS depression. Recovery within 3 days	Myers and Userik, 1969 cited in BIBRA, 1996[117]
Horses / n=1		9 litres (16 g/kg bw)			Mortality; damage to GI-tract, kidney, brain and liver	
Dermal						
Rabbits	once	not reported	-	20.8 g/kg bw	Mortality (LD_{50})	NIOSH, 2003[89]
Other routes						
Mice (i.p.)/ n≥6	once	not reported	-	9.73 g/kg bw	Mortality (LD_{50}); slight kidney injury and inhibition of liver enzymatic activity	Karel *et al.*, 1947 and Zaroslinski *et al.*, 1971 cited in BIBRA, 1996[117]
Mice (NMRI) / n=6 males (i.p.)	once	Propylene glycol diluted in water to 10 ml/kg bw	-	10 ml/kg bw (10.4 g/kg bw)	Mortality (LD_{50})	Budden *et al.*, 1979 cited in Anonymous, 1994[50]

Mice (SPF-NMRI) / n=5/sex/dose (i.p.)	once	not reported	-	9.3 ml/kg bw (9.6 g/kg bw)	Mortality (LD_{50})	Bartsch *et al.*, 1976[155]
Mice (C3H) / n=5/sex (i.p.)	once	5 ml/kg bw	-	5 ml/kg bw (5.2 g/kg bw)	Signs of lack of coordination followed by deep narcosis; peritonitis; LD_{50} was 13.7 ml/kg bw (14.2 g/kg bw)	Hickman, 1965 cited in Anonymous, 1994[50]
Mice (Swiss) and rats (Wistar) (i.p.)	once	10 ml propylene glycol solution/kg bw at concentrations of 10, 20, 50 or 100% (i.e., 10.4 g/kg bw at 100% propylene glycol solution)	2.1 g/kg	5.2 g/kg bw	At lower concentrations (10-20%): no significant neuro-psychopharmacological effects; at higher concentrations (50-100%): moderate to marked effect with decreased locomotor activity, body and limb tone, respiration, rectal temperature and suppression of secondary conditioned responses; significant potentiation of pentobarbital hypnosis, slight increase in d-amphetamine toxicity in aggregated mice; pronounced analgesic and anti-inflammatory activity. On isolated smooth muscle preparations, a dose-dependent non-specific blockade was observed at 50-100% concentration; no effect on cardiac tissues.	Singh *et al.*, 1982[120]
Mice (SPF-NMRI) / males and females (i.v.)	once	Doses that supplied at least 3 values lying between the LD_{16} and LD_{84}	-	6.4 ml/kg bw (6.6 g/kg bw)	Mortality (LD_{50})	Bartsch *et al.*, 1976[155]
Mice (i.v.)	once	not reported	-	8.0 g/kg bw	Mortality (LD_{50})	Sax, 1979 cited in Anonymous, 1994[50]
Rats (i.p.)	3 days; twice a day	3-4 ml/kg bw; twice a day (6.2-8.3 g/kg bw/day)	-	6.2 g/kg bw/day	Change in a number of liver enzyme activities	Dean and Stock, 1974[157]
Rats (i.p.)	once	not reported	-	13 g/kg bw	Mortality (LD_{50})	Sax, 1979 cited in Anonymous, 1994[50]
Rats (SD) / males and females) (i.p.)	once	Doses that supplied at least 3 values lying between the LD_{16} and LD_{84}	-	13 ml/kg bw (13.5 g/kg bw)	Mortality (LD_{50})	Bartsch *et al.*, 1976[155]
Rats (SD) / males and females (i.v.)	once	Doses that supplied at least 3 values lying between the LD_{16} and LD_{84}	-	6.2 ml/kg bw (6.4 g/kg bw)	Mortality (LD_{50})	Bartsch *et al.*, 1976[155]
Rats (i.m.)	once	not reported	-	20 g/kg bw	Mortality (LD_{50})	Sax, 1979 cited in Anonymous, 1994[50]

Table E-4 Short-term animal toxicity studies of propylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Dose or concentration tested	NOAEL[a]	LOAEL[b]	Critical effect	Ref.
Inhalation						
Rats (SD) / n=19/sex per dose	90 day, nose-only inhalation; 6 hours/day; 5 days/week	0.16, 1.0 and 2.2 mg/l (160, 1000 and 2200 mg/m^3) propylene glycol; daily aerosol concentrations: 0.16 ± 0.04; 1.01 ± 0.11; 2.18 ± 0.31 mg/l. Median aerodynamic diameters of diluted aerosol were less than 2.22 and 1.96 µm for the medium and high concentration groups, respectively. Mean geometric diameter for the low concentration group was not obtainable. The geometric standard deviations were 1.44 and 1.57 for the medium and high dose groups.	-	160 mg/m^3	Nasal haemorrhaging in all exposed groups; in male rats (week 2 to 14), mean incidences <1, 64, 74, and 75% in controls, low-exposure, medium-exposure, and high-exposure groups, respectively; in females <1, 14, 71, and 71% in controls, low-exposure group, medium-exposure and high-exposure groups. Ocular discharge: 16% in low-exposure males, 40% in medium- and high-exposure males and 5% in controls; in females: 8% in controls, 14% in the low-exposure group, 28% in the medium-exposure group and 35% in the high-exposure group. Body weight reduction (5-7%) from day 50 till the end of the study in the female rats at the highest dose of 2.2 mg/l propylene glycol; and from day 64 in the females receiving 1.0 mg/l propylene glycol; body weight reduction correlated with reduced food consumption. Haematological parameters showed no consistent trends with treatment group or sex and were not considered biologically significant. Male spleen weights relative to terminal body weight significantly decreased in high-exposure groups. N treatment-related gross pathology changes; light microscopy revealed thickening of respiratory epithelium with increase in the number of goblet cells and their mucin content in males and females on medium and high propylene glycol dose. Minute volume, tidal volume, and respiratory rates were not altered in rats exposed to 0.16, 1.0 and 2.2 mg/l propylene glycol.	Suber *et al.*, 1989[90]

Rats (glycerin and propylene glycol are commonly used as humectant ingredients in manufactured cigarettes to control and maintain the moisture content of the cut tobacco filler. The potential of these added humectants to affect the toxicity of cigarette smoke was investigated).	Nose-only smoke inhalation study. Filtered test cigarettes contained glycerin (5.1% w/w), propylene glycol (2.2% w/w) or combinations of these humectants totaling 2.3%, 3.9%, and 7.2% w/w tobacco. Control rats were exposed to tobacco smoke without humectants, or to filtered air. Smoke exposures were: 1 hour/day, 5 days/week for 13 weeks, at target smoke particulates concentration of 350 mg/m^3.	See 'exposure duration'	See 'exposure duration'	-	All smoke-exposed groups had equivalent increases in blood carboxy-haemoglobin, serum nicotine, and serum cotinine relative to the air controls. Smoke-associated reductions in body weights and occasional increases in heart and lung weights were generally similar among the various exposure conditions at necropsy. Increases in serum alkaline phosphatase and decreases in serum glucose and cholesterol were observed among smoke-exposed females relative to air controls. However, no significant differences in these parameters were evident between the humectant-containing and reference cigarette smoke exposure groups. Assessments of respiration conducted after 3, 6, 9, and 12 wk of smoke exposure indicated an initial smoke-related but not humectant-related decrease in respiratory rate, tidal volume, and minute volume during the first 20 min of each smoke exposure. Respiratory-tract histopathology was consistent across sexes and smoke groups, comprising (1) diffuse and focal alveolar pigmented macrophages and chronic interstitial inflammation in the lung, (2) laryngeal epithelial hyperplasia, squamous metaplasia, and scab formation, and (3) epithelial hyperplasia in the anterior nose. Smoke-related histopathology resolved substantially during a 6-wk post-exposure recovery period. Addition of the tested humectants to cigarettes, singly or in combination, had no meaningful effect on the site, occurrence, or severity of respiratory-tract changes or on the measured indices of pulmonary function.	Heck *et al.*, 2002 cited in HSDB, 2004[14]
Oral						
Mice (CBA/J-BOM) / n=5 females	21 days	0 and 4 g/kg bw/day	-	4 g/kg bw/day	A decrease in the cellularity and relative organ weight of the spleen. The number of T-helper cells and the number of B-lymphocytes were decreased in the animals. No marked differences were noted in haematological parameters. Microscopic examination of the spleen showed a minor depletion of lymphocytes.	Lichtenberg *et al.*, 1994[119]
Rats (Wistar) / n=15	1 week to 3 months; gavage	Propylene glycol (15% (v/v) in drinking water	-	propylene glycol (15% (v/v) in drinking water	Enlarged mitochondria with well-developed cristae and a dense matrix in every hepatocyte. These changes were noted throughout the hepatic lobule, but were more distinct in hepatocytes of the peripheral zone of the hepatic lobule. Other ultrastructural changes in hepatocytes included proliferation of smooth-surfaced endoplasmic reticulum and an increase in the number of lysosomes and microbodies.	Wakabayashi *et al.*, 1991 cited in Anonymous, 1994[50]

Rats / n=6 adult male/dose	30 days; daily administration	0 and 2.84 ml/kg bw/day	-	2.84 ml/kg bw/day (2.94 g/kg)	Significant reduction in the rate of sedimentation of erythrocytes, total leukocyte count and glutathione content; increased protein content of the erythrocytes; increased activity of several membrane-bound erythrocyte enzymes.	Ahluwalia *et al.*, 1980[91]
Erythrocytes from rats (*in vitro* study)	once	propylene glycol concentrations of 100, 28.4, 2.84 and 0.284% v/v			Treatment of the erythrocytes with propylene glycol up to 28.4% concentration did not alter the activities of membrane-bound erythrocyte enzymes; on the other hand, addition of propylene glycol (100%) to a final concentration of 90% decreased the activity of some membrane-bound erythrocyte enzymes.	
Rats / n=6	30 days	0 and 284 µl	-	284 µl	A significant decrease in total lipids, cholesterol and pospholipids in the erythrocytes of the animals; the decrease in lipids altered the erythrocyte morphology of the animals.	Ahluwalia and Amma, 1984 cited in Anonymous, 1994[50]
Rats (SD) / n=10 males/dose group	30 days; gavage	0 and 4.3 ml/kg bw	-	4.3 ml/kg bw (4.5 g/kg bw)	Study focused on effects of propylene glycol on plasma and liver lipids. Liver total cholesterol was moderately, but significantly, increased (7%); questionable (according the to authors) significantly increase (26%) in free fatty acid concentration	Hoenig and Werner, 1980[158]
Rats / n=5 males/dose	100 days	0%, 0.1%, 0.3%, 1%, 3% and 10% propylene glycol in drinking water (0, 100, 400, 1300, 4000, 13200 mg/kg bw/day)	13200 mg/kg bw/day	-	No appreciable change in growth rate was observed and no significant changes compared to the control were found on microscopic examination of all internal organs	Weatherby and Haag, 1938 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Rats (Charles River CD) / n=15/sex/dose	15 weeks	0% and 50000 ppm propylene glycol in the diet (2.5 g/kg bw/day)	2.5 g/kg bw/day	-	No effects on the blood, kidney function, weights of the major organs or the macroscopic and microscopic appearance of tissues from a wide range of organs.	Gaunt *et al.*, 1972[98]
Rats / n=5/dose	140 days	0%, 1%, 2%, 5%, 10%, 25% and 50% propylene glycol in drinking water (1600, 3680, 7700 13200, 31300 and 62500 mg/kg bw/day)	7700 mg/kg bw/day	13200 mg/kg bw/day	Liver injuries, as a moderate degree of vacuolisation of the central lobular cells; mortality (within a few days) was observed in the two highest dose groups	Seidenfeld and Hanzlik *et al.*, 1932 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Rats / n=5/sex	20 weeks	0 and 5% propylene glycol in the diet	0 and 5% propylene glycol in the diet	-	No microscopic indications of toxicity (the range of tissues examined was not specified)	Guerrant *et al.*, 1947 cited in BIBRA, 1996[117]

Rats / n=1	5 months	24% propylene glycol in the diet	-	24% propylene glycol in the diet	Mortality; moderate liver cell degeneration and degenerative changes in the cells of the convoluted tubules in the kidneys	Hanzlik *et al.*, 1939[30]
Rabbits	50 days	Up to 3.2 mg/kg bw/day and 4.2 mg/kg bw/day by gavage	3.2 mg/kg bw/day	4.2 mg/kg bw/day	Loss of appetite	Braun and Cartland, 1936 cited in BIBRA, 1996[117]
Rabbits	8 weeks	5% solution of propylene glycol as the only drink fluid (8 g/kg bw/day)	5% solution of propylene glycol as the only drink fluid (8 g/kg bw/day)	-	Body weights were normal	Vaille *et al.*, 1971 cited in BIBRA, 1996[117]
Cats / n=3/dose	14 days; feed	0 and 12% propylene glycol in the diet	-	12% propylene glycol in the diet	Reticulocytosis; increased Heinz bodies; when RBC from cats fed propylene glycol were exposed to severe mechanical stress, their fragility were increased 2.2-2.8 times; decreased haptoglobin concentrations. These data suggest that intravascular lysis may be involved in the pathogenesis of propylene glycol-induced RBC destruction.	Weiss *et al.*, 1992[159]
Cats (mongrel) / n=6/dose	5 weeks;	12% propylene glycol in the diet (1600 mg/kg bw/day)	-	1600 mg/kg bw/day	Increase in Heinz body formation (28%); significant increase in GSH (erythrocyte glutathione concentration); decrease in RBC survival (18.8%); significantly increased anion gap; D-lactate levels increased dose-dependently and correlated positively with anion gap; significantly decreased L-lactate; increase in iron pigment.	Christopher *et al.*, 1989 and 1990[35,92,93]
Cats (mongrel) / n=5/dose	22 days;	40% propylene glycol in the diet (8000 mg/kg bw/day)		8000 mg/kg bw/day	At high dose, cats developed decreased activity, mental depression, depressed and slight to moderate ataxia after 2-3 days, consistent with intoxication by D-lactate. Furthermore, cats in this dose group developed moderate hypercellularity, polyuria and polydipsia. Other effects: increase in Heinz body formation (92%), decreased PCV (accompanied by an increase in both punctuate and aggregate reticulocytes); significant decrease in erythrocyte glutathione concentration; significant decrease in ATP; decrease in RBC survival (60%); increase in iron pigment.	
Cats / n=9	8 weeks; feed	0 and 8.3% propylene glycol in the diet (1.1 g/kg bw/day)	-	1.1 g/kg bw/day	Increase in the number of circulating Heinz bodies; administration of acetaminophen to the animals increased the methemoglobin concentrations in the 8.3% propylene glycol group. These data indicate that RBC from cats fed propylene glycol diets are more susceptible to oxidative stress.	Weiss *et al.*, 1990[160] and BIBRA, 1996[117]

Cats (kittens) / n=6/dose	12 weeks	5 and 10% (1100 and 2400 mg/kg/day)	-	1100 mg/kg/day	Increased Heinz bodies, with peak values for erythrocytes containing Heinz bodies: 28% for kittens of the 10% propylene glycol group; 20% for the kittens of the 5% propylene glycol group; normal growth rate; no apparent ill effects or clinical anemia; % methemoglobin was unchanged from the initial value.	Hickman *et al.*, 1990[95]
Cats (kittens) / n=2	12 weeks	a commercial soft-moist diet containing propylene glycol			Percentage of Heinz bodies recovered to pre-treatment values 6-8 weeks after discontinuation of diets containing propylene glycol. [51]Cromium-labeled erythrocytes were used to evaluate erythrocyte survival in 4 kittens of the 10% propylene glycol-fed group and in 4 control kittens. Kittens with Heinz body formation induced by 10% propylene glycol had significantly decreased erythrocyte survival compared to controls, with half-life of 8.3 days for kittens of the propylene glycol group, and of 12.6 days for kittens of the control group.	
Cats (adult) / n=7/dose (7 males and 14 females)	13 weeks; feed	0%, 6% (1260 mg/kg bw/day) and 12% in the diet	-	1260 mg/kg bw/day	Dose-related increase in Heinz bodies within two weeks and the increase persisted through the study; punctuate reticulocytes were significantly increased in the high dose group; decreased RBC survival (30% (low dose) and 55% (high dose)); however, the animals did not develop clinical anaemia	Bauer *et al.*, 1992[97]
Cats (kittens) / n=7/dose	13 weeks; feed	0%, 6% and 12% in the diet (0, 2750 and 5290 mg/kg bw/day)	-	2750 mg/kg bw/day	Significant decreases in haemoglobin concentrations in the 2750 mg/kg bw/day group but not in the 5290 mg/kg bw/day group; significant decreases in the numbers of erythrocytes in both the 2750 and 5290 mg/kg bw/day group; increased Heinz bodies in both the 2750 and 5290 mg/kg bw/day group within two weeks and persisted throughout the study (mechanism of injury involves changes in the tertiary structure of haemoglobin (due to a propylene glycol metabolite) ultimately leading to the collapse of the molecule and aggregation into discrete masses termed Heinz bodies); significantly increase in punctuate reticulocytes indicating accelerated erythropoiesis; significant increases in numbers of aggregate reticulocytes in the 5290 mg/kg bw/day group; decreased RBC survival (44% (2750 mg/kg bw/day) and 63% (5290 mg/kg bw/day)). The increase in reticulocyte count and reduction in RBC lifespan was greater than observed in adult cats. The greater effect in kittens may be due to greater food (propylene glycol) intake and to an innate susceptibility of kitten RBC to propylene glycol; however, the animals did not develop clinical anaemia	Bauer *et al.*, 1992[96]

Cats (male) (n=2/dose group)	Up to three months	0, 80, 443, 675, 1763 and 4239 mg/kg/day	80 mg/kg/day	443 mg/kg/day	An increase in the incidence of red blood cell Heinz body formation at the higher dose levels; also a slight increase in the amount of iron pigment in the reticuloendothelial cells of the liver and spleen. There were no treatment-related changes detected in cats ingesting 80 mg/kg/day of propylene glycol, the lowest dose level evaluated.	Quast *et al.*, 1980[94]
Dogs	8 weeks	3 g/kg bw/day	3 g/kg bw/day	-	A careful examination of the blood taken from the animals revealed no clear indications of abnormalities	Burger, 1977 cited in BIBRA, 1996[117]
Dogs	14 weeks	14.5 g/kg bw/day	-	14.5 g/kg bw/day	Marked blood effects. These effect were not present 9 weeks after the end of the treatment	CIVO-TNO, 1977 cited in BIBRA, 1996[117]
Chicken	3 weeks	5% and 10% propylene glycol in the diet (6 and 12 g/kg bw/day)	-	6 g/kg bw/day	5%: weight gains were lowered 10%: increased mortality	Persons *et al.*, 1968 cited in BIBRA, 1996[117]
Chicken	unspecified	2.5%-10% propylene glycol in the diet	-	2.5% propylene glycol in the diet	Toe deformities	Bowen and Waldroup, 1968 cited in BIBRA, 1996[117]
Dermal						
Mice (hairless; hr/h Oslo strain) / n=12 males	Propylene glycol was injected subcutaneously 3 times a week for 3 months	0.2 ml propylene glycol	-	0.2 ml propylene glycol	Urinary bladder epithelium: the proportion of diploid cells in the S-phase fluctuated but was not significantly altered; the proportion of tetraploid cells in the S-phase was significantly reduced, and at 3 months, there was no DNA synthesis in these cells; overall, the proportion of diploid cells increased, the number of tetraploids was slightly reduced, and almost all of the octoploid cells disappeared; the alterations reported were described as disturbed regenerative reactions resulting from propylene glycol-induced acute cellular toxicity; some of the bladder epithelial cells were killed and the mechanism of repeated DNA synthesis was disturbed. Propylene glycol caused a disturbance in the proliferative response of rat urinary bladder epithelial cells.	Farsund, 1974 cited in Anonymous, 1994[50], Farsund, 1978[161] and Farsund, 1981[162]
Rabbits	30 days	0.16 and 5.0 ml/kg bw (0.17 and 5.2 g/kg bw) to 100 cm^2	5.2 g/kg bw	-	No renal toxicity	Hanzlik *et al.*, 1947 cited in Lakind *et al.*, 1999[15]

Rabbits / n=5 females	Daily treatment for 100 days with a mixture of equal parts of propylene glycol and diethylene glycol	a mixture of equal parts of propylene glycol and diethylene glycol (0.5 ml)	0.5 ml of a mixture of propylene glycol and diethylene glycol (1:1)	-	One dermal long-term toxicity study was available. Five female rabbits were exposed daily to a 0.5 ml mixture of equal parts of propylene glycol and diethylene glycol for 100 days (exposure area 100 cm^2; no specification of exposure time per day; not specified whether the substance was removed after each day). There were no macroscopic changes. Microscopic examination after 20-30 days showed a slightly thickened stratum granulosum and signs of proliferation in the stratum basale. Superficial portions of the dermis showed some infiltration with cells of the lymphatic series and histiocytes. The collagen fibers were slightly fragmented and scattered. The findings remained unaltered in later stages. From the study description, no data are available on systemic effects.	Rantuccio *et al.*, 1979[79]
Other routes						
Rabbits (New Zealand) / n=5 males	One intramuscular injection of propylene glycol (1 ml) for three times (every two weeks)	40% (v/v) propylene glycol	-	40% (v/v) propylene glycol	Increased corrected area under the serum kinase activity versus time.	Brazeau and Fung, 1989 cited in Anonymous, 1994[50]
Rats (i.v.) (male) (n=10)	30 days	80 and 240 mg/kg bw/day	80 mg/kg bw/day	240 mg/kg bw/day	Haemoglobin in the urine (presumably arising from red blood cell haemolysis)	Horspool and Joseph, 1991 cited in BIBRA, 1996[117]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E-5 Long-term animal toxicity studies of propylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Dose or concentration tested	NOAEL[a]	LOAEL[b]	Critical effect	Reference
Inhalation						
Rats / n=39 The number of rats in each group was increased by birth of young. Breeding was controlled to produce about equal populations in the two groups.	18 months	170-350 mg/m^3 (supersaturation)	350 mg/m^3	-	Comparative observations on the growth rates, blood counts, urine examination, kidney function tests, fertility and general condition of the test and control group, exhibited no essential differences between them with the exception that the rats in the glycol atmospheres exhibited consistently higher weight gains (at 12 months the weights of the exposed animals were about 50% increased compared to the control group). Microscopy of tissues from the major organs revealed no effects.	Robertson *et al.*, 1947[99]
Oral						
Rats (albino SD) / n=6	14 months	Propylene glycol was painted onto the hard palate 3 times a week	-	-	No intraoral changes were found, all organs remained uninfluenced.	Wallenius and Lekholm, 1973 cited in Nordic-chemicals-group; Mortensen B, 1993[2] and in IUCLID dataset, 2000[1]
Rats (inbred albino) / n=10/dose group (4 females and 6 males)	2 years	2.45% and 4.9% in the diet (1225 and 2450 mg/kg bw/day)	2450 mg/kg bw/day	-	Renal NOAEL; mild liver injury = minor chronic liver damages: diffuse centrilobular atrophy, bile-duct proliferation and/or fatty degeneration.	Morris, 1942 cited in Lakind *et al.*, 1999[15], in BIBRA, 1996[117] and in Suber *et al.*, 1989[90]
Rats (Charles River CD) / n=30/sex/dose	104 weeks (2 years)	0, 6250, 12500 and 50000 ppm propylene glycol in the diet (0, 0.3125, 0.625 and 2.5 g/kg bw/day)	2.5 g/kg bw/day	-	No effects on the blood, kidney function, weights of the major organs or the macroscopic and microscopic appearance of tissues from a wide range of organs.	Gaunt *et al.*, 1972[98]
Dogs	8 weeks-2 years	4.3-5.1 g/kg bw/day	-	4.3-5.1 g/kg bw/day	Diuresis; changes in the blood profile, including increases in the level of methaemoglobin and Heinz bodies, but no tissue damage was observed.	Burger, 1977, Weil *et al.*, 1971[100] and CIVO-TNO, 1977 cited in BIBRA, 1996[117]

Dogs / n=4 females	9 months, two times a day for a period of 1 h	5% in drinking water;	5% in drinking water;	-	Study was focussed on kidney and liver function: no functional deficits were reported and no pathological tissue changes were found in these organs.	Van Winkle and Newman, 1941 cited in Lakind *et al.*, 1999[15] and in Anonymous, 1994[50]
Dogs / n=4 males	5-6 months; daily	600 ml of 10%;	600 ml of 10%;	-		
Dogs (Beagle) / n=5/sex/dose	2 years; feed	0, 2000 and 5000 mg/kg bw/day	2000 mg/kg bw/day	5000 mg/kg bw/day	Decreased erythrocytes (increased erythrocyte haemolysis), haemoglobin and haematocrit with increases in anisocytosis, poikilocytes and reticulocytes, assumed to indicate erythrocyte destruction and compensation by bone marrow; slight increase in serum total bilirubin concentration. 5000 mg/kg/d was insufficient to produce any irreversible changes and the authors reported no evidence of damage to bone marrow or spleen; increased urinary output and decreased water intake; however, blood was not examined for Heinz bodies.	Weil *et al.*, 1971[100]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E-6 Genotoxicity of propylene glycol *in vitro.*

Test system	Dose or concentration	End point	Result	Reference
Ames test with Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538	Propylene glycol diluted to final concentrations of 1-10000 µg/plate in 0.1 ml DMSO	Gene mutations	- (both without and with metabolic activation)	Clark *et al.*, 1979[78]
Ames test with Salmonella typhimurium strains TA92, TA94, TA98, TA100, TA1535 and TA1537	10 mg/plate (solvent: DMSO)	Gene mutations	- (with metabolic activation)	Ishidate *et al.*, 1984[101]
(modified) Ames test with Salmonella typhimurium	Concentrations between 5-300 µmol/plate	Gene mutations	- (without metabolic activation)	Pfeiffer and Dunkelberg, 1980[102]
Ames test with Salmonella typhimurium strains G46 and TA1530 (plate test)	Not reported	Gene mutations	- (without metabolic activation)	Green, 1977[103]
Ames test with Salmonella typhimurium strains TA98 and TA100	Not reported	Gene mutations	- (not specified whether metabolic activation is used)	Kawachi *et al.*, 1980[104]
Silk worms	Not reported	Gene mutations	- (not specified whether metabolic activation is used)	Kawachi *et al.*, 1980[104]
Escherichia coli microsuspension assay with E. coli strains WP2, WP2uvrA, WP67, CM611, WP100, W3110polA$^+$ and p3478pola	Not reported	Chemically-induced preferential kill	- (both without and with metabolic activation) ($1*10^4$ µg/well was the minimal inhibitory concentration for the E. coli repair-deficient strains)	McCarroll *et al.*, 1981[105]
Rec assay with Bacillus subtilis	Not reported		- (without metabolic activation)	Kawachi *et al.*, 1980[104]

Host-mediated assay with Salmonella typhimurium G46 and TA1530 (resident in the peritoneal cavity of mice treated by gavage with doses up to 5 g/kg given singly or daily for five days)	up to 5 g/kg	Gene mutation	-	Litton Biokinetics Inc., 1974 cited in BIBRA, 1996[117]
Host-mediated assay with Saccharomyces cerevisiae D3 (resident in the peritoneal cavity of mice treated by gavage with doses up to 5 g/kg given singly or daily for five days); In addition, treatment in culture was performed.	up to 5 g/kg	Gene mutation	Weak mutagenic activity (in the absence of mutagenic activity)	Litton Biokinetics Inc., 1974 cited in BIBRA, 1996[117]
Host-mediated assay: Salmonella typhimurium resident in the blood of mice	Mice received an i.v. injection of 2.7 g/kg propylene glycol	Gene mutation	-	Solt and Neale, 1980[106]
Chromosome aberration test; Chinese hamster fibroblast cells	32 mg/ml propylene glycol (420 mM) (solvent: physiological saline): without metabolic activation	Chromosome aberrations	+ (without metabolic activation)	Ishidate *et al.*, 1984[101] and Ishidate *et al.*, 1988[108]
	64 mg/ml propylene glycol (840 mM): with metabolic activation		- (with metabolic activation)	
Chromosome aberration test with Human embryonic lung cells (WI 38)	Not reported	Chromosome aberrations	- (without metabolic activation)	Green, 1977[103]
Chromosome aberration test with Human embryonic lung cells (WI 38)	0.001, 0.01 and 0.1 µg/ml	Chromosome aberrations	- (not specified whether metabolic activation is used)	Litton Biokinetics Inc., 1974 cited in BIBRA, 1996[117] and in IUCLID dataset, 2000[1])
Chromosome aberration test with Human embryo fibroblasts	Not reported	Chromosome aberrations	- (not specified whether metabolic activation is used)	Kawachi *et al.*, 1980[104]
Cytogenic assay (OECD Guideline 473)	476, 1910 and 3810 µg/l	Chromosome aberrations	- (both without and with metabolic activation)	EC Erdolchemie GmbH cited in IUCLID dataset, 2000[1]
Mitotic recombination with Saccharomyces cerevisiae strain D3 (plate test)	Not reported	Mitotic recombination	- (without metabolic activation)	Green, 1977[103]
Sister chromatid exchange assay; HE 2144 human fibroblast cell line	Concentrations of 3.805, 7.61 and 22.83 mg/ml propylene glycol in HBSS	Chromosome aberrations	-	Sasaki *et al.*, 1980[107]
Sister chromatid exchange assay; Don-6 Chinese hamster cell line	Concentrations of 3.8, 7.6 and 22.8 mg/ml propylene glycol in HBSS	Chromosome aberrations	weak dose-dependent increase in the frequency of sister chromatid exchanges: 6.6, 6.8, 9.6 and 10.0 chromatid exchanges/cell at propylene glycol concentrations of 0; 3.8; 7.6; and 22.8 mg/ml;	Sasaki *et al.*, 1980[107]
Alkaline elution assay with Chinese hamster lung fibroblast V79 cells	up to 10 mM propylene glycol	Chromosome damage	- (both without and with metabolic activation)	Swenberg *et al.*, 1976[109]

Transformation assay with embryo cells from Syrian hamsters	4 ml propylene glycol at concentrations ranging from 0.125 to 8.0% propylene glycol (actual concentrations not stated)	- (not specified whether metabolic activation is used)	Pienta, 1980[110]

Table E-7 Genotoxicity of propylene glycol *in vivo*.

Test system	Dose or concentration	End point	Result	Reference
Micronucleus test; Swiss female mice / n=5	Single (unspecified) oral dose, five (unspecified) oral doses and a (unspecified) single i.p. injection)	Chromosome aberrations	-	Vargova *et al.*, 1980[111]
Micronucleus test (i.p.); mice (ddY mice) / n=5/dose	Single i.p. injections of 0, 2.5, 5, 10 and 15 g/kg bw in saline (the latter injection killing 3/6 animals) or daily injections of 0, 2.5 and 5 g/kg bw for 5 days	Chromosome aberrations	-	Hayashi *et al.*, 1988[112]
Dominant lethal test; mice (Charles River) / n=12 males	Single i.p. dose of 10 mg/kg	Gene mutations	-	Kennedy *et al.*, 1975[113]
Mice	Single i.p. administration of 0.1 ml	Chromosome aberrations	Remark: Evaluation not possible because the original study is not available + (chromosome aberrations in spermatocytes)	Razvi *et al.*, 1979 cited in HSDB, 2004[14]

Table E-8 Animal carcinogenicity studies of propylene glycol.

Species/ Strain/No. per Sex per Group	Exposure duration	Dose or concentration tested	NOAEL[a]	LOAEL[b]	(Critical) effects	Reference
Inhalation						
Rats / n=20	18 months	170-350 mg/m3 (supersaturation)	350 mg/m^3	-	No indication of carcinogenic action in the kidney, lung, liver, spleen or bladder (limited study).	Robertson *et al.*, 1947[99]
Monkey (Macacus Rhesus) / n=29	13 months	100-220 mg/m3 (about 60% saturation) 230-350 mg/m3 (supersaturation)	350 mg/m^3	-	No indication of carcinogenic action in the kidney, lung, liver, spleen or bladder (limited study).	Robertson *et al.*, 1947[99]
Oral						
Rats (inbred albino) / n=10/dose group (4 females and 6 males)	2 years	2.45% and 4.9% propylene glycol in the diet (1225 and 2450 mg/kg bw/day)	2450 mg/kg bw/day	-	No convincing evidence of carcinogenicity; only tissues from the major organs of a random sample were (probably half) of the treated rats were microscopically examined.	Morris, 1942 cited in Lakind *et al.* 1999[15], in BIBRA, 1996[117] and in Suber *et al.*, 1989[90]
Dogs (Beagle) / n=5/sex/dose	2 years; feed	0, 2000 and 5000 mg/kg bw/day	2000 mg/kg bw/day	5000 mg/kg bw/day	No tumours were observed after 2 years.	Weil *et al.*, 1971[100]

Rats (Charles River CD) / n=30/sex/dose	104 weeks (2 years)	0, 6250, 12500 and 50000 ppm propylene glycol in the diet (2.5 g/kg bw/day)	2.5 g/kg bw/day	-	A wide range of tissues were examined in detail, no convincing evidence of carcinogenicity was observed.	Gaunt *et al.*, 1972[98]
Dermal						
Mice (Balb/c) / n=17 females	one single subcutaneous injection	0.2 ml (8400 mg/kg) propylene glycol	0.2 ml (8400 mg/kg) propylene glycol	-	No tumours were observed after 2 years	Dehwurst *et al.*, 1972 cited in Nordic-chemicals-group; Mortensen B, 1993[2]
Mice (Swiss) / n=50 females	110 weeks (skin-painting study)	10, 50 and 100% propylene glycol; uncovered skin was in twice weekly contact with 0.02 ml of the same concentration; the highest exposure was equivalent to 800 mg/kg bw per application	800 mg/kg bw	-	No treatment-related increases in skin or systemic tumours; the extent of the systemic microscopic examination was not clearly defined but would have probably included tissues from all the major organs.	Stenbäch and Shubik, 1974 cited in BIBRA, 1996[117] and in Anonymous, 1994[50])
Rabbits / n=5/dose	220 weeks	0.02 ml of a 10% or 50% solution in acetone or 0.02 ml neat propylene glycol to the uncovered ears; top dose group: 8 mg/kg bw per application	8 mg/kg bw	-	No increase in local or systemic tumours were reported; the extent of the microscopic examination is unclear, but it is likely to have included tissues from all the major organs.	Stenbäch, 1977 cited in BIBRA, 1996[117]
Other routes						
Rats (SD) / n=60 females	up to 22 weeks	Unspecified volume of neat propylene glycol to the palate	-	-	No evidence of local carcinogenicity; only the palate was examined in detail	Svensson and Heyden, 1982[163]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level

Table E-9 Animal reproduction toxicity studies of propylene glycol.

Species/Strain/No. per Sex per Group	Exposure duration	Dose or concentration tested	NOAEL[a]	LOAEL[b]	Critical effect	Reference
Fertility						
Inhalation						
Rats / initially groups of 10 rats animals per sex	18 months	170-350 mg/m^3 (supersaturation)	350 mg/m^3	-	The animals reproduced normally; no foetal malformations were reported	Robertson *et al.*, 1947[99]

Mice (Swiss CD-1) / n=20/sex/dose; At the time this study was conducted, the protocol called for no necropsy of F0 animals in the absence of a fertility effect, so the F0 mice were killed and discarded without necropsy. Since there was no effect on fertility, a Task 3 crossover study was not performed.	14-week continuous breeding study according to RACB protocol; dose-range finding study (task 1) and main study (task 2) at 0.0, 1.0, 2.5, 5.0% propylene glycol in drinking water; calculated consumption estimates nearly equal to 1819, 4796, and 10118 mg/kg bw/day	0, 1, 2.5 and 5% propylene glycol in drinking water (0, 1819, 4796 and 10118 mg/kg bw/day)	10118 mg/kg bw/day	-	F0 generation: higher water consumption (6-15%), not statistically different from controls; no effect on body weights during the continuous cohabitation period; all groups had ≥ 4,6 litters/pair with ≥ 11,9 pups/litter; no treatment related effect on pup weight adjusted for litter size (control value: 1,55 g); viability and growth of the final litter was unaffected by propylene glycol consumption. F1 generation: no effect on body and organ weights in males and females; no change in sperm endpoints; no change in estrous cycle parameters; total calcium levels in serum unchanged. For F2, only the control and 5% propylene glycol groups were evaluated: no treatment related effect on mating, fertility, or on the number, weight, or viability of the F2 offspring. Conclusion: no effect on fertility and reproduction in either generation up to 10 g/kg/day.	NTP, 1985[114], Anonymous, 1997[164] and Morrissey *et al.*, 1989[165]
Mice (female QS)	The feeding started 3 days before introducing males and continued until mating or for 10 days	0.1 ml of 50% propylene glycol in water (1900 mg/kg/day)	-	1900 mg/kg/day	Reduced mating frequency to 30% of normal and the litters to 15% of normal (reduced fertility).	Emmens, 1971[166]

Rats	Multi-generation study (six generations)	2.5; 5; 7.5; 10; 20 and 30% propylene glycol in diet Feeding studies were continued with diets supplemented with minerals, vitamins, and protein. Small groups (3 males, 6 females) received 1.9 -22.1% propylene glycol in their diet for up to six generations.		10% propylene glycol in the diet (5440 mg/kg bw/day)	Reduced number of litters born, reduced size of the litters, reduced weight of the young at weaning and impaired ability of females to feed their offspring. At dietary 22.1% propylene glycol, half of the animals failed to give birth in the first generation, whereas in the other groups receiving up to 14.7% (8 g/kg bw/day) birth rates were comparable to those of untreated controls. Further studies on mating of unproductive females with untreated males suggested female sterility, but reproductive organs showed no microscopic abnormalities.	Guerrant *et al.*, 1947[167] cited in BIBRA, 1996[117] and in Suber, 1989[90]
Developmental toxicity						
Oral						
Mice (CD-1) / n=20-23/dose	Days 6-15 of pregnancy; gavage	Up to 1.55 g/kg bw/day in water	1.55 g/kg bw/day	-	No adverse reproductive effects (no effects on nidiation, maternal survival, foetal survival and skeletal abnormalities)	Food and Drug Research Laboratories, Inc., 1973 cited in BIBRA, 1996[117] and in Suber *et al.*, 1989[90]
Mice (CD-1) / n=30 females	Days 8 to 12 of gestation via gavage (developmental screening assay)	10 g/kg bw/day	10 g/kg bw/day	-	No significant effects found on maternal death, numerous reproductive endpoints (fertility rate, number of resorptions, average litter size, birth weight) and postnatal pup weight gain. The dose was sufficient to produce slight maternal toxicity	Kavlock *et al.*, 1987[115]
Rats / n=20-40/dose	Days 6-15 of pregnancy; gavage	Up to 1.6 g/kg bw/day in water	1.6 g/kg bw/day	-	No adverse reproductive effects (no effects on nidiation, maternal survival, foetal survival and skeletal abnormalities)	Food and Drug Research Laboratories, Inc., 1973 cited in BIBRA, 1996[117] and in Suber *et al.*, 1989[90]
Rats / n=8	Day 10, 11, 12 and 14 of pregnancy	6.2 g/kg bw	6.2 g/kg bw	-	No adverse reproductive effects	Seidler 1970 cited in BIBRA, 1996[117]

Hamsters (golden)	5 days; gavage	Up to 1.6 g/kg bw/day in water	1.6 g/kg bw/day	-	No adverse reproductive effects (no effects on nidiation, maternal survival, foetal survival and skeletal abnormalities)	Food and Drug Research Laboratories, Inc. 1973 cited in Suber *et al.*, 1989[90]
Rabbits (Dutch-belted)	Days 6-18 of pregnancy; gavage	Up to 1.23 g/kg bw/day in water	1.23 g/kg bw/day	-	No adverse reproductive effects (no effects on nidiation, foetal survival and skeletal abnormalities)	Food and Drug Research Laboratories, Inc. 1973 cited in BIBRA, 1996[117] and in Suber *et al.*, 1989[90]
Dermal						
Mice (ICR/Jcl) / n=21 females	Days 9, 10 and 11 of pregnancy (s.c. injection)	10.4 g/kg bw	10.4 g/kg bw	-	No significant increases in foetal malformations.	Nomura, 1977[168] and BIBRA, 1996[117]
Other routes						
Rabbits (i.v.) (pregnant New Zealand)	Day 8-12 of pregnancy; daily	No details concerning the doses were found	-	-	No effect was found with regard to malformations or the number of visible implantation sites; propylene glycol tended to cause a small increase in the percentage of retardation and resorbed foetuses	Schumacher *et al.*, 1968 cited in Schardein, 1993[169]

[a] NOAEL = No Observed Adverse Effect Level
[b] LOAEL = Lowest Observed Adverse Effect Level