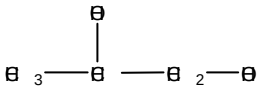
PROPYLENE GLYCOL

SYNONYMS

(+-)-1,2-Propanediol, (+-)-Propylene glycol, (RS)-1,2-Propanediol, 1,2-Dihydroxypropane, 1,2-Propylene glycol, 1,2-Propylenglykol, 1,2-Propylenglykol [German], 2,3-Propanediol, 2-Hydroxypropanol, 3-01-00-02142 (Beilstein Handbook Reference), AI3-01898, All purpose lubricant, BRN 1340498, CCRIS 5929, Caswell No. 713, DL-1,2-Propanediol, Dowfrost, EPA Pesticide Chemical Code 068603, General lube, Isopropylene glycol, Methylethyl glycol Methylethylene glycol, Monopropylene glycol, NSC 69860, PG 12, Propane-1,2-diol, Propylene Glycol USP, Sentry Propylene Glycol, Sirlene, Solar Winter BAN, Solargard P, Trimethyl glycol, UNII-6DC9Q167V3, Ucar 35, alpha-Propyleneglycol, dl-Propylene glycol

CHEMICAL STRUCTURE



CHEMICAL FORMULA

$C_3H_8O_2$

IDENTIFIER DETAILS

CAS Number	:	57-55-6
CoE Number	:	2065
FEMA	:	2940
EINECS Number	:	200-338-0
E Number	:	1520

SPECIFICATIONS

Melting Point: -59°C

Boiling point: 189°C

PURPOSE

Humectant

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
0-25	JECFA	1973	Retained in 2002

FDA Status: [CFR21]

Section Number	Comments
184.1666	Propylene Glycol (PG) (GRAS)

HUMAN EXPOSURE

Natural Occurrence: Found in several varieties of mushrooms, roasted sesame seed, Parmesan cheese, cocoa, pecans and truffle [Fenaroli, 2005].

Reported Uses: PG is reportedly used (max. levels) in baked goods at 2.44 ppm, other grains at 0.01 ppm, fats and oils at 0.32 ppm, milk products at 0.38 ppm, cheese at 0.62ppm, frozen dairy at 2.13ppm, fruit juice at 0.82ppm, meat products at 0.53ppm, poultry at 0.1 ppm, processed vegetables at 5.0 ppm, soft candy at 1.44 ppm, confection frosting at 50.86 ppm, sweet sauce at 4.21ppm, gelatins & puddings at 0.69ppm, snack foods at 0.92 ppm, non-alcoholic beverages at 1.24 ppm, alcoholic beverages at 5.88 ppm, reconstituted vegetables at 0.06ppm, gravies at 0.98 ppm, hard candy at 1.35 ppm, and chewing gum at 3.0ppm [Fenaroli, 2005].

PG is widely used in the cosmetics, pharmaceutical, food and tobacco industries as a solvent, moistening agent, viscosity-decreasing agent and vehicle [Nikitakis, 1988]. It is also used in some intravenously administered pharmaceuticals [NTP, 2003].

The Cosmetic Ingredient Review [CIR] state that contact with PG in some form is likely to occur on a daily basis [CIR, 1994].

PG has been used as a source of energy in animal diets, as a cryoprotective agent in kidney & blood preservations, and as a therapy for ketosis in cattle [Emmanuel, 1976; Pegg *et al*., 1987; Ruddick, 1972].

TOXICITY DATA

This ingredient has been registered under REACH. Under REACH, registrants have an obligation to provide information on substances they manufacture or import. This information includes data on hazardous properties (covering various toxicological endpoints), guidance on safe use and classification and labelling. The European Chemicals Agency (ECHA) makes this information publicly available on its website: http://echa.europa.eu/.

Carmines (2002), Rustemeier *et al*., (2002), Roemer *et al*., (2002) and Vanscheeuwijck *et al.,* (2002) reported on a testing program designed to evaluate the potential effects of 333 ingredients added to typical commercial blended test cigarettes on selected biological and chemical endpoints. The studies performed included a bacterial mutagenicity screen [Ames assay] a mammalian cell cytotoxicity assay [neutral red uptake], determination of

smoke chemical constituents and a 90-day rat inhalation study. Based on the findings of these studies, the authors concluded that the addition of the combined ingredients, including PG at levels up to 47,225 ppm, "did not increase the overall toxicity of cigarette smoke" [Carmines, 2002].

Gaworski *et al*., (2010) reported on a testing program designed to evaluate the potential effects of experimental cigarettes containing no added propylene glycol against otherwise similar cigarettes with three different target amounts of propylene glycol (40,000ppm, 70,000ppm, 100,000ppm) added to the tobacco. The main toxicological comparison was a sub-chronic inhalation study with mainstream smoke in Sprague-Dawley rats (exposures of 150mg/m^3 of total particulate matter, 6 hour exposure per day, for 90 consecutive days). Additional studies with mainstreams smoke were bacterial mutagenicity (Five *S. Typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA102 ±S9), cytotoxicity of both particulate and gas/vapour phases (using neutral red uptake assay), and determination of selected smoke constituents. Broadly similar responses were seen across the four cigarette types, and the responses were similar to those previously described in the scientific literature. Most of the changes produced in the 90-days of exposure were resolved in a 42 day post-inhalation period [Gaworski *et al*., 2010].

In Vivo Toxicity Status

Species	Test Type	Route	Reported Dosage
Mouse	LD_{50}	Oral	>20g/kg
Rat	LD_{50}	Oral	>20g/kg
Rabbit	LD_{50}	Oral	>19g/kg
Guinea Pig	LD_{50}	Oral	>18g/kg
Mouse	LD_{50}	I.P	9.73g/kg

[BIBRA, 1996]

The toxic effects observed at doses in the LD_{50} range involved the central nervous system [depression] and damage was also seen in the intestine, kidney and liver. In rats, oral doses of 3.1-6.2 g/kg bw produced diuresis. In dogs [three], oral doses of 8.3-20.8 g/kg bw resulted in CNS effects [loss of muscular control, depression, sleeplessness] but no effect on liver or kidney. Again, CNS depression [and loss of muscular control] has been demonstrated in horses that drank between 2.25-4.5 litres of PG. They recovered within 3 days. Consumption of 9 litres was fatal to one horse and autopsy revealed gastro-intestinal tract, kidney, brain and liver damage [BIBRA, 1996].

One of three dogs dosed with neat PG at a level of 0.78-3.1 g/kg bw/day for up to 3 days, exhibited slight gastro-intestinal tract irritation [BIBRA, 1996].

Liver and kidney damage as well as haemolysis have been demonstrated in cows, rabbits, chickens, rats, and sheep receiving 10-80% PG in water, saline or ethanol via intravenous injection. Smaller concentrations [2-3%] were ineffective in rats and dogs although slight alterations in blood flow were seen in the heart and kidneys of dogs [BIBRA, 1996].

Rapid I.V injections in cats of doses equivalent to 0.2-1 g/kg bw produced disturbances in heart rhythm and rapid transient decreases in blo od pressure. This is in contrast to slower infusions [1 ml/min or less] of 3.6 g/kg bw which had minimal effect [BIBRA, 1996].

Life-threatening abnormal heart rhythms occurred in a 15 month year old patient who received 700-770 mg/kg bw/day of PG for 8 days in a vitamin C preparation [oral] [no information is given to indicate what the patient was initially suffering from]. An 11 year old receiving 4.1-4.8 g PG/day in a vitamin D preparation for 13 months, developed epileptic seizures. No further symptoms were seen in either of these cases when PG exposure was stopped [BIBRA, 1996].

Sixteen patients received a dose of 21g PG every 8 hours for at least 3 days. The other six patients received 42g every 12 hours. No clear dose dependent response could be established, probably because of the large individual differences in metabolic breakdown or tissue susceptibility. CNS effects were seen when 18 patients were given around 62g PG [and 22ml ethanol]/day for up to 20 days. The effects were described as moderate and included dizziness, fatigue and hot flushes [BIBRA, 1996].

No effect was seen on gross liver function when 10 volunteers received 5.1g PG orally every 5 hours for 48 hours. Liver function was determined by antipyrine clearance [BIBRA, 1996].

In other human studies, PG produced hyperosmolarity in the blood of patients suffering from extensive skin burns. PG was applied to the skin in an antibiotic cream (no other information provided). BIBRA highlight one patient who received an estimated dose of about 9 g/kg bw/24 hours for 70 hours, who had a peak serum PG level of 1060 mg/dl. In four patients with skin disease, neither hyperosmolarity nor metabolic acidosis developed when daily doses of 1.5-6.1 g/kg bw were applied to their skin for a period of 5 days [BIBRA, 1996].

Intravenous injection of PG [1g/ day, increased by 1g/ day up to a maximum of 10g/day] in four men failed to produce any overt toxic effects [BIBRA, 1996].

Accumulation of PG has been reported to differ markedly between indivi duals, demonstrated by two studies where patients were treated orally, or by intravenous injection. These differences were largely due to the variability in clearance, with premature infants treated intravenously having the slowest clearance rate compared with adults [BIBRA, 1996].

Rabbits demonstrated a complex and unpredictable metabolic and kidney clearance of PG infused intravenously [no details concerning dose are given]. The authors suggested from this that long-term treatment with PG may result in toxicity [BIBRA, 1996].

Following an unspecified single high dose [oral] of PG in one individual, lactic acidosis developed. Blood PG levels were measured at 70 mg/dl. The individual was suspected to have poor kidney function before this suicide attempt [BIBRA, 1996].

Intramuscular injection of 2.1 g PG in humans resulted in burning sensations at the site of injection, lasting for 5-10 minutes. This effect was seen in 3 out of 6 patients tested. In another individual receiving 480g PG by intravenous injection, lactic acidosis and hyperosmolarity was evident [BIBRA, 1996].

Lactic acidosis has been reported in 5 patients receiving intravenous injections of 60-770mg/kg bw/day (time period not stated) [BIBRA, 1996].

I.P injections of 6.2-8.3 g/kg bw/day PG for 3 days, administered to rats, produced changes in a number of liver enzyme activities [no further details given]. BIBRA (1996), give mention to an abstract that reports the presence of haemoglobin in the urine of rats following an intravenous inje ction of 240 mg/kg bw/day of PG, for 30 days. Neither this nor any other effect was seen when 10 males [presumably rats] received a smaller dose (80 mg/kg bw/day for 30 days) via the same route of administration [BIBRA, 1996].

These effects were not seen in four patients receiving four intramuscluar injections of 0.5, 0.5, 1 and 2g PG on four successive days. The only obvious effect was a slight burning sensation at the site of administration which lasted for 1-2 hours [BIBRA, 1996].

PG administered to mice at a level of 10 g/kg bw/day by stomach tube produced signs of "mild overt toxicity" [no further details given]. BIBRA also refer to an abstract where subtle changes in several tissues are noted including the spleen, lymph nodes and blood in mice. T he authors state that this may have a modulating effect on the immune system f ollowing the administration of 4 g/kg bw/day for 21 days. However, no convincing evidence of immune system effects were seen when groups of 30 female mice were given 1.25, 2.5 or 5 g/kg bw/day for 5 days [BIBRA, 1996].

In groups of male & female rats [15 of each sex], no effects on the blood, kidney function, weights of major organs or the microscopic appearance of tissues was seen when they were administered with 5% PG in the d iet for 15 weeks. This result is replicated in groups of 30 male and 30 female rats receiving the diet for 2 years. In a similar 20 week study (groups of 5 male & 5 female rats), again no indication of toxicity was apparent following microscopic examination of several tissues. However, a 2 year study with groups of 10 rats receiving smaller doses [2.45 % or 4.9% in the diet] resulted in mild liver injury [BIBRA, 1996].

In other oral studies, rabbits receiving up to 3.2 g/kg bw/day by stomach tube for 50 days did not suffer any obvious toxic effects. When the dose was raised to 4.2 g/kg bw/day they demonstrated a lose of appetite [no detail is given as to what tissues were examined]. There was no change in body weight in rabbits given 8 g/kg bw/day PG as a 5% solution in drinking water. The rate of mortality has been shown to increase in chickens given 10% PG in the diet for 3 weeks, and weight gain was reduced at 5% PG. In chicks, similar doses [2.5-10 %] produced a dose-dependent increase in toe defor mities [this effect has also been seen in chicks dosed with lactose] [BIBRA, 1996].

Rats receiving 2.1 g/kg bw/day or more, orally, for between 5 and 12 weeks failed to demonstrate any increases in liver weight but exhibited changes in their blood cellular profile. In a separate study, oral doses upwards of 2.9 g/kg bw/day gave altered lipid profiles of the blood, heart and liver. At oral doses of 5 g/kg bw/day kidney injury, brain injury and changes in liver enzymes occurred with diuresis and increases in kidney and caecum weights. A further oral rat study using doses of 5 and 10 g/kg bw/day identified transient "subtle" effects on red blood cell membranes, but without changes to Heinz bodies or methaemoglobin levels. Liver injury is seen at administration levels of 12 g/kg bw/day but not at 4-7.7 g/kg bw/day for 100-120 days [in five rats]. Dietary levels of 15-18 g/kg bw/day resulted in depressed growth and death within one month [rats] [BIBRA, 1996].

Detailed examination of blood and major organ tissues in groups of two male cats receiving an oral dose of 80 or 443 mg/kg bw/day for 94 days via the diet, failed to reveal any toxic effects. At a higher dose, upwards of 675 mg/kg bw/day for 21-101 day, effects on the blood were evident in both male and female cats. These included a marked increase in the number of Heinz bodies. In addition, very mild effects were produced on the liver and spleen [no details given]. In another separate study on cats, diuresis occurred following higher oral doses of 2.4 and 4.8 g/kg bw/day and BIBRA report that there was some indication of increased liver weight. Further signs of toxicity are seen with the increase of dose, 8 g/kg bw/day for 21 days resulted in "excessive thirst" and "slight to moderate" ataxia. The cats receiving 4.8 g/kg bw/day recovered from any alterations in the blood 40-50 days after PG administration ceased [BIBRA, 1996].

BIBRA (1996), report that following microscopic examination of a wide range of tissues and detailed examination of the blood, there was no evidence of toxicity when five male and five female dogs were dosed 2 g/kg bw/day PG in the diet for 2 years. Similarly, dogs dosed with 3 g/kg bw/day for 8 weeks had no abnormalities of the blood. However, at doses of 4.3-5.1 g/kg bw/day for 8 wk-2 yr., diuresis and changes in blood profile [increases in the level of methaemoglobin and Heinz bodies] were apparent. No other tissues examined were damaged. A similar experiment demonstrated that the marked blood effects were temporary when the blood returned to normal 9 weeks after 14 weeks exposure to 14.5 g/kg bw/day PG [BIBRA, 1996].

Carcinogenicity and Mutagenicity

A mouse skin painting study investigated the carcinogenicity of condensate prepared from cigarettes containing a number of additives in combination, including PG at 14,740 ppm. The authors concluded that the study "did not indicate any substantive effect of these ingredients on the tumorigenicity of cigarette smoke condensate" [Gaworski *et al.*, 1999]. [It should be noted that the cigarettes contained a typical American blend humectant and sugar component (*i.e.* glycerine 20,000 ppm, PG at 24,000 ppm, and brown invert sugar at 24,000 ppm)].

Similarly, rats dosed by stomach tube 5 g/kg bw/day for 5 days, had no effect on chromosomal damage [BIBRA, 1996].

In a dominant lethal assay, rats were exposed to a single PG dose of 5 g/kg by stomach tube, or daily doses of 5 g/kg bw by the same route for 5 days. The ten rats dosed in each group gave no convincing indications of genetic damage in sperm cells. An increased incidence of early foetal deaths would have been indicative of a genotoxic effect when untreated females were mated with treated males [BIBRA, 1996].

Mice [groups of 6] receiving either single intraperitoneal injections of 2.5, 5, 10 or 15 g/kg bw PG or daily injections of up to 5 g/kg bw (for 5 days) did not exhibit any increase in chromosomal damage (micronuclei) in the bone marrow. The highest single intraperitoneal injection killed 3 of the 6 animals in that group, [BIBRA, 1996].

Subcutaneous injections of 8 g/kg bw of PG in mice, 3 times a week for 3 months, resulted in a decrease in the extent of bladder cell polyploidy. BIBRA question the study by stating that "The significance of this finding is uncertain." [BIBRA, 1996].

Neat application of PG to the palette of 60 rats, 3 times per week for 22 weeks failed to produce any evidence of local carcinogenicity following observation for 80 weeks [no details regarding quantity of dose are given and only the palate was examined closely] [BIBRA, 1996].

An 18 month inhalation study, wher e 20 rats were exposed to 0.17-3.5 g/m^3 PG, gave no indication of carcinogenic potential in the lung, liver, kidney or spleen [BIBRA, 1996].

BIBRA comment on the carcinogenicity studies they reference stating that none of the protocols used would be considered adequate by current (1996) regulatory standards. In essence, BIBRA infer that the size of the test groups were too small, needing to be around 50 per sex, and that the leng th of exposure should be 2 years for rats and 18 months for mice, with a 'comprehensive' range of tissues being microscopically examined [BIBRA, 1996].

BIBRA (1996) report that there is no convincing evidence of carcinogenicity in rats or mice. Groups of 30 male & 30 female rats given PG in the diet [0.62%, 1.25%, 2.5% or 5%] for 2 years failed to produce any signs of carcinogenicity in a wide range of tissues examined in detail.

In a mouse skin-painting study, there were no treatment related increases in skin or systemic tumours, when groups of 50 females received 0.02 ml applications, twice weekly, to uncovered skin, of 10%, 50% [in acetone] or neat PG for up to 110 weeks [BIBRA, 1996].

Similarly, no increase in local or systemic tumours were reported in rabbits when groups of 5 received 0.02 ml applications, twice weekly, to uncovered ears, of 10%, 50% (in acetone) or neat PG for up to 220 weeks [BIBRA, 1996].

Dermal Toxicity

A 15% PG solution [presumably aqueous] failed to produce any irritation to the penile mucosa of rabbits [BIBRA, 1996].

A number of eye irritation studies [more than 20 laboratories] have demonstrated that neat PG is 'at worst minimally irritating' at a level of 0.1 ml applied to the rabbit eye [draize test]. A study based on OECD guidelines [Jacobs, 1992] concludes that PG is not irritating to the eyes in accordance with EC criteria. In contrast, one study notes that a drop of neat PG applied to the human eye caused immediate stinging, blinking and tear formation lasting a few seconds , though there was no residual discomfort or injury [BIBRA, 1996].

PG applied directly to human skin sores and burns produced strong local inflammation, which left scars once healed [BIBRA do not comment as to whether the scars were a result of the PG effect or a direct effect of the initial sore/burn]. When PG [presumably neat] was applied directly to the human tongue, it produced a temporary burning sensation. Intramuscular injections of neat PG in humans resulted in pain / burning sensations at the site of application, which continued for up to 10 minutes [BIBRA, 1996].

One study, where 50 healthy volunteers were exposed to PG via a 48 hour patch test, reports the highest non-irritant concentration as being 20% [BIBRA, 1996].

PG, both neat and in 10-50 % solution, has been shown to induce skin irritation in normal & sensitive individuals [including those suffering from dermatitis]. Additionally, a small proportion of individuals (23 out of 817 patients tested) demonstrated irritancy to 10 % PG solution and 31 out of the 817 to a 5% solution. However, a number of studies demonstrate that the neat material is a non-irritant. Individuals exposed to neat PG for 24 hours, or daily for 21 days [covered patch], or daily for 36 days [uncovered] showed that PG was virtually a non-irritant [BIBRA, 1996].

Two studies investigating oral sensitisation of PG have produced conflicting results. In one study 17 out of 43 patients who exhibited a local skin reaction to dermally applied PG, produced a skin rash after ingesting up to 15.7 g PG. Twenty volunteers who were not sensitive to dermal application were also insensitive to oral administration of PG. BIBRA report on 2 patients s uffering from PG intolerance; an intermittent burning sensation of the mouth was eliminated by the removal of PG [and sorbic acid in one case] from the diet. Similarly, certain patients exhibiting skin sensitisation towards PG suffered recurrence of the c omplaint following ingestion of PG through food [level unspecified] [BIBRA, 1996].

Following an initial exposure of 48- or 72-hr closed patches of 12% PG in a cream, for 10 applications over a 3 week period; 204 volunteers were challenged with the same cream in a 72-hr closed patch test 2 weeks after the end of the induction period. None of the 204 subjects suffered skin sensitization reactions. Another similar experiment involving 203 men

exposed to neat PG through a 48/72-hr closed patch gave some indication of sensitizing potential & 13 of the 203 exhibited a "significant response" following a 48/72 hour challenge patch 2 weeks later. However, their response was considered low as they did not react to neat PG applied daily for 7 days [BIBRA, 1996].

In a number of larger surveys involving 100-5202 patients with skin complaints, sensitization to PG ranged from 0.2% to 3.9% of the patients. This was indicated by response to 24/48 hour closed patch tests using 2-10% PG. However, a small number of patients gave inconsistent responses, failing to respond to challenge 2 days post induction, but responding once again 2 days later to challenge. BIBRA warn that the low concentration range used makes interpretation of these results difficult [BIBRA, 1996].

Applied to uncovered rabbit ears twice per week for up to 220 weeks, 0.02 ml PG solution failed to produce any signs of toxicity at concentrations of 10, 50 or 100%. The same result was produced when groups of 50 mice received the same doses on their uncovered skin for up to 110 weeks [BIBRA, 1996].

A review reports that PG is a very weak contact sensitizer but exposure to particular PG-containing products may be associated with increased risk of sensitization. Patch test data of 45,138 patients tested with 20% PG in water were reviewed and it was concluded that PG exhibited a very low sensitization potential, [Lessmann *et al,* 2005].

Reproductive and Developmental toxicity

A vitamin preparation containing 30% PG was administered intravenously over a 19 month period to premature infants. The doses given were around 3g/day for a least two weeks and resulted in an increased incidence of nervous system seizure [exact details of toxic effect and number of participants is not given] [BIBRA, 1996].

An NTP report (2003), stated that no human or reproductive data is available for PG. Although the serum half-life was greater for infants than adults, post natal developmental toxicity in infants and children below the age of 5 was diminished by low levels of alcohol dehydrogenase. Further published data have reported the continuous therapeutic infusion in paediatric intensive care patients in which paediatric intensive care patients 15 months and below were without any acute toxicity effects. In the light of the knowledge that PG saturates at 8-10x lower dose in humans than rats or rabbits it is reported to provide confidence that PG is of negligible concern for reproductive or developmental toxicity in humans [NTP, 2003].

NOAEL 10 g/kg bw/d mice (highest dose tested).
NOAEL 1.6 g/kg bw/d rats (highest dose tested).
NOAEL 1.55 g/kg bw/d hamsters (highest dose tested).
NOAEL 1.23 g/kg bw/d rabbits (highest dose tested) [NTP, 2003].

Similar intravenous doses of PG in another vitamin preparation resulted in

hyperosmolarity in premature infants receiving periodic doses for 5 days. This effect was also seen in a child receiving high doses of PG via the same root (estimated blood levels of 10g/l). Again, hyperosmolarity was the result of 340g/day "almost neat" PG administered intravenously for 8 days to a patient suffering from poor kidney function. Lactic acidosis and CNS depression were also evident in this instance. For a short period during the second day of treatment, PG and a blood trans fusion were administered through the same intravenous line, resulting in haemolysis of the red blood cells [BIBRA, 1996].

In a reproductive study, rats received PG as 30% of their diet. Their overall capacity to breed and then to feed their offspring suf fered adverse effects [Exact details not given]. Additionally, the second generation failed to produce any offspring. The authors suggested that these effects were a direct result of malnutrition. Therefore further investigative studies were carried out using groups of 3 male and 6 female rats, feeding them between 1.9 & 22.1% PG in the diet whilst receiving mineral, vitamin and protein supplements. For those animals receiving up to 14.7% PG in the diet, birth rates were comparable with control animals. Half of the animals receiving 22.1 % failed to give birth. Further studies mating unproductive females with untreated males suggested female sterility, although no abnormalities were identified in the reproductive organs following microscopic examination [BIBRA, 1996].

Oral administration [stomach tube] of 1.65 g/kg bw/day of PG on days 6-15 of pregnancy, failed to induce any adverse reproductive effects in groups of 20-40 rats. Again, no adverse reproductive effects were seen in a group of 8 rats receiving 6.2 g/kg bw/day on days 10,11,12 and 14 of pregnancy. Similarly, in another study, where groups of 20-23 mice were dosed 1.55 g/kg bw/day on days 6-10 and no adverse effects on reproduction were observed. This same result was produced when group of 11-14 rabbits received 1.23 g/kg bw/day PG on days 6-18 of pregnancy [administration was presumably by stomach tube] [BIBRA, 1996].

The growth and viability of offspring of 30 mice administered PG orally [10 g/kg bw/day] on days 8-12 of pregnancy was una ffected. However, this dose did produce "slight maternal toxicity" [no other details given] [BIBRA, 1996].

Drinking water containing 5% PG had no overt adverse effects on the reproduction of 20 male & 20 female mice housed together for 14 weeks [BIBRA, 1996].

No reproductive effects were see when rats were exposed to 0.17-0.35 g/m ^{3} PG, for up to 18 months [groups of 10 male & 10 females were treated]. No foetal malformations were observed [BIBRA, 1996].

PG administered to 21 pregnant mice by subcutaneo us injection at a dose of 10.4 g/kg bw/day, on days 9, 10 or 11 of pregnancy, did not result in any significant increase in foetal malformation [BIBRA, 1996].

The National Toxicology Program (NTP) (2003) Centre for the Evaluation of Risks to Human Reprodu ction (CERHR) conducted an evaluation of the

potential for PG (PG) to cause adverse effects on reproduction and development in humans. PG was selected for evaluation because of the potential for widespread human exposure through its use in food, tobacco, pharmaceutical products, cosmetics, various paints and coatings and as an antifreeze and de-icing solution. PG is a small, hydroxy-substituted hydrocarbon used as a chemical intermediate in the production of unsaturated polyester resins and in the production of plasticizers. The NTP concluded that there is negligible concern for adverse developmental and reproductive effects in humans at current, proposed, or estimated exposure levels. There is no direct evidence that exposure of people to PG adversely affects reproduction or development. Studies in pregnant laboratory animals at oral doses of PG greater than 1,200 mg/kg body weight/ day and up to 10,400 mg/kg body weight/day in mice, did not produce developmental toxicity in offspring. In a continuous breeding study, no effects on fertility were observed in male or female mice at doses up to 10,100 mg/kg body weight/day in drinking water. The pharmacokinetics of PG indicates that the lack of adverse effects observed in laboratory animals is relevant to humans. The rate-limiting step in PG metabolism is conversion to the more toxic lactaldehyde product by alcohol dehydrogenase. It is estimated that the average daily intake of PG from food products in the US is 34 mg/kg body weight/day for a 70 kg person, which is over 300 -fold lower than the highest dose tested in laboratory animals.

Inhalation Toxicity

Species	**Dosage and/or Duration**	**Results**
Rat	321ppm (90dy) 0.17-0.35 mg/L (18month)	Enlarged goblet cells LOAEL 112 ppm [50 % bw]
Rabbit	10 – 20%	Increased degeneration of goblet cells
Monkey	32-112ppm 13 month	LOAEL 112ppm

Summary of inhalation toxicity data (as obtained from NTP report, 2003))

No overt toxic effects or haemolysis were found when dogs were exposed to 10-20% PG aerosols for 15 minutes [BIBRA, 1996].

Children exposed to PG at atmospheric concentrations of 94 mg/m^3, there was no effect on the mucous membranes of the respiratory passageways [no details given concerning length of exposure or number of subjects] [BIBRA, 1996].

Wieslander *et al*., (2001) reported on a study in which 27 volunteers (22 males and 5 females, average age 44 11) were exposed to PG mist over a period of 1 minute at an average concentration of 3 60 mg/m^3 (range 176-851 mg/m^3). None of the volunteers were asthmatic or had previous occupational exposure to PG. Examinations were carried out within 15-min prior to and following

exposure. Results revealed an increase in ocular and throat symptoms, a reduction in forced expiratory volume in 1 sec (FEV_1)/forced vital capacity with an increase in self rated dyspnoea. There were no changes in nasal patency, vital capacity, forced vital capacity or nasal symptoms. Four subjects reported an irritative cough during exposure, with FEV_1 reduced by 5 %. However, FEV_1 remained unchanged among those that did not develop coughs. The authors concluded that 'short exposure to PG mist from artificial smoke generators may cause acute ocular and upper airway irritation, [Wieslander *et al*., 2001].

Heck *et al*., (2002) reported a study in which the effects of humectants were evaluated in a 13-week inhalation study in Fischer 344 rats. In this study animals were exposed to smoke generated from American-style tobacco blends containing Glycerin and PG (PG). PG was included in these blends at a concentration of (5660, 11,800 and 22,480 ppm). Rats were also exposed to test cigarettes containing only PG at a level of 21,760 ppm. The result of this study indicated that the addition of glycerin or PG to cigarette tobacco did not substantially alter the incidence, distribution, or severity of biological effects normally seen in the respiratory-tract of rodents after 13-wk of cigarette exposure, [Heck *et al*., 2002].

In a 90 day nose-only inhalation study, where rats were exposed to 160 mg/m^3 of PG aerosol for 6 hours per day, 5 days/wk, local effects occurred which included nasal haemorrhage and ocular discharge. There were also a number of inconsistent changes in, for example, haemato logical and clinical parameters that did not show clear relationships with exposure concentrations. The authors of the study suggested that it confirmed the NOEL at 1 mg/litre, and that PG would not cause adverse health effects when exposures are based on the NOEL [Suber *et al.,* 1989; BIBRA, 1996].

A recent study investigated the effect of cigarettes, containing various additives in three combinations, in a 90-day nose-only smoke inhalation study in rats. These ingredients included PG at 47,225 ppm, a l evel described as a multiple of its typical use in a US cigarette. The data from this study along with that from a number of other biological and chemical studies indicate that the addition of the combined ingredients "did not increase the inhalation toxicity of the smoke, even at the exaggerated levels used " [Vanscheeuwijck *et al.*, 2002].

The addition of propylene glycol at 83,300 ppm to reference cigarettes, used in a 90 day-sub-chronic inhalation exposure in rats, led to a series of pathological changes to smoke exposure that were indistinguishable from those changes caused by the control cigarettes. This indicated that addition of propylene glycol to a reference cigarette had no discernable effect upon the type or severity of the treatment related pathological changes associated with tobacco smoke exposure [Baker *et al.,* 2004].

In a "limited" inhalation study, 39 rats were exposed to 0.17-0.35 g/m^3 for up to 18 months. No overt toxic effects were found. In the same study, 29 monkeys were also exposed to PG at 0.23-0.35 g/m^3 for up 18 months. Again no overt toxicity was identified. In both cases tissues from major organs were examined microscopically [BIBRA, 1996].

Werley *et al*., (2011) exposed (nose-only) Wistar rats (31/sex/group) to aerosolized propylene glycol at a target concentration of 30 mg/L for 4, 12, 40 or 120 minutes per day, 7 days/week for 28 days. Based on a deposition fraction of 10% the authors calculated that the average doses were 0, 7.2 , 21.6, 72.0 or 216 mg/kg-day. The author states that the only biologically relevant findings included clinical signs of ocular and nasal irritation indicated by minor bleeding around the eyes and nose, and minimal laryngeal squamous metaplasia. The author has determined the NOEL for the study to be 20 mg/kg/day.

Werley *et al*., (2011) exposed Beagle dogs via the oropharyngeal route using a closed face mask (4/sex/group) to aerosolized propylene glycol at a target concentration of 5 mg/L for 6, 12, 36 or 120 minutes per day, 7 days/week for 28 days. Based on a deposition fraction of 20% the authors calculated that the average doses were 0, 3, 6, 18 or 60 mg/kg-day. The author states that in the female Beagle dog, treatment-related decreases in haemoglobin, red blood cells and hematocrit were observed in the two highest exposure groups, equivalent to approximately 18 and 60 mg/kg/day. In male dogs from the high dose group, similar small decreases, albeit, non-statistically significant decreases were observed in these haematological markers as well. The author has determined the NOEL to be 6.05 mg/kg/day for the study.

Werley *et al*., (2011) also conducted pharmacokinetic evaluations of the propylene glycol aerosol in Sprague Dawley rats and Beagle dogs. These studies showed that the absorption of PG following pulmonary inhalation exposure occurs rapidly and equilibrium between lung tissue and plasma is achieved quickly. Inhalation exposure to PG aerosols via the capillary aerosol generator achieved PG concentrations in the systemic circulation that were similar to those attained via the oral route.

Other Relevant Studies

Organic water-miscible solvents such as PG (PG) used for drug delivery were assessed for their cardiovascular effects when injected intra-arterially in a sheep model after infusion rapidly at three different volumes (0.1. 0.5 and 1 ml). PG was classed as a solvent with marked cardiovascular toxicity in the sheep model employed characterised by an increase in arterial pressure and concomitant decrease in venous pressure (Laurant *et al.,* 2007).

Mild irritation of the gastro-intestinal tract was exhibited in rats dosed with neat PG [3 g/kg bw/day] by stomach tube daily for 3 days. Contrary to this, rats receiving the same dose as a 75% solution did not exhibit the same irritation [BIBRA, 1996].

The i.v administration of PG (to six cancer patients who were sufficiently healthy to care for themselves with accompanied nor mal liver and kidney function) were reported to show reduced clearance with increasing dose over a dose range of 3-15 mg/m^2. The average terminal half-life was reported to be 2.3 0.7 with an apparent volume of distribution reported to be ~0.55-0.94

L/Kg. Other studies with oral or rectal exposure reported apparent volumes of distribution within the range of ~0.52-0.79 L/kg, [Stu dy as cited from NTP, 2003].

PG did not modulate the cell-mediated or humoral immune responses *in vivo* up to concentrations of 5000 mg/kg/day [Gaworski *et al.,* 1994].

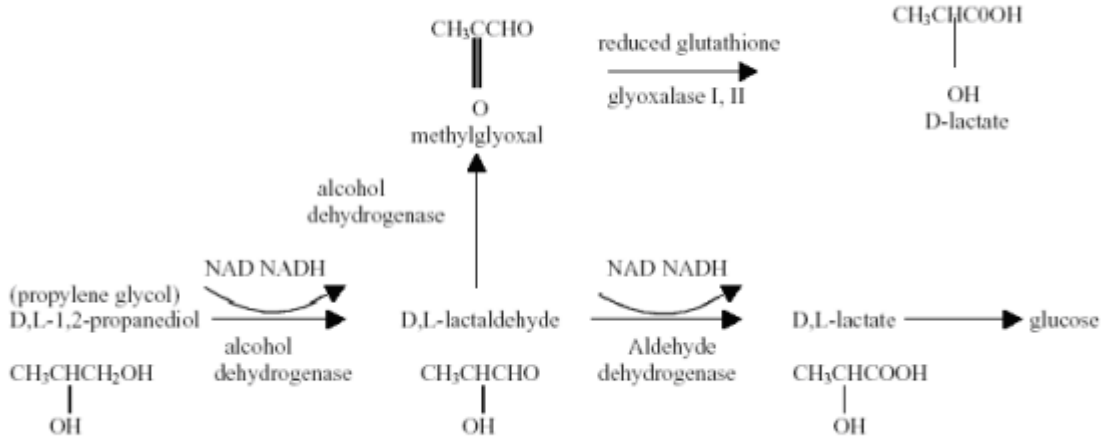
The major route of PG metabolism in mammals is conversion to lactaldehyde and then to l actate via hepatic alcohol and aldehyde dehydrogenases. In an alternative pathway, lactaldehyde is metabolised to methylglyoxal before lactate is formed [Christopher *et al*., 1990a].

Propylene glycol (PG) (in mammals) is reported to be mainly metabolised by oxidation (alcohol dehydrogenase) to produce lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is then reported to be further metabolised to pyruvate, carbon dioxide and water. Lactate is also reported to contribute to glucose format ion through gluconeogenic pathways. An excess production of lactic acid (on exposure to large amounts of PG) is reported to produce a metabolic anion gap and metabolic acidosis. Serum levels of >180 mg/L [2.37 mM] are reported to result in toxicity, [NTP, 2003].

In mammals most of the absorbed PG is reported to be eliminated unchanged via the kidney (with a remaining portion excreted by the kidneys as the glucuronic acid conjugate). In humans it has been estimated that approximately 45 % of PG is excreted via the kidney. [NTP, 2003].

The figure below outlines the pathways identified for PG metabolism in mammals (NTP, 2003).

Figure 2-1. Propylene Glycol Metabolism in Mammals.



From Christopher et al., (1990a)

In humans the absorption of PG after oral exposure is reported to reach maximum plasma concentrations within 1-hour of dosing wit h an average serum half-life between 1-4 hours. Rectal absorption studies revealed half-life values of 2.8 0.7 hrs (adults). 2.6 0.3 hrs (children 5-12). The average half life in infants is approximately 10x greater in adults (this is observed as alcohol dehydrogenase activity is up to 10x lower in infants) [NTP, 2003].

The NTP, (2003) reported that PG clearance in humans is similar to that seen in rabbits and rats. However, saturation of this pathway occurs at lower doses in humans. The panel also reported that the metabolic clearance of PG follows a first order process (up to doses of 12g/day) with a constant half-life of 1.6 0.2 hr. If this dose is exceeded the serum half-life is dose dependant (zero order process) with a serum half life of three h ours. As already stated PG is converted to lactic acid by ADH then further to pyruvate, which subsequently provides energy through the Krebs cycle; lactate is then detoxified into glucose and stored as glycogen, proving a source of energy, [NTP, 2003].

Log K_{ow} was reported to be –0.912, [NTP, 2003].

No TLV-TWA has been established for PG however, the American Industrial Hygiene Association (AIHA) Workplace Environmental Exposure Level (WEEL) guide of 50 ppm (total exposure) and inhalation aerosol exposure of 10 mg/m3 has been reported, [NTP, 2003].

A 46-year-old patient diagnosed with respiratory failure required pressure control ventilation and sedation by continuous infusion of lorazepam also developed Stenotrophomonas maltophilia pneumonia, which was t reated by intravenous trimethoprim-sulfamethoxazole administration. Both drugs contain several hundred mg/ml of PG. Acute renal failure consistent with acute tubular necrosis developed three days after treatment. Further investigations revealed that PG to xicity was responsible (Hayman *et al.,* 2003). Eight patients also developed PG induced renal toxicity after lorazepam infusion. All patients experienced elevated levels of serum creatinine concentrations after receiving continuous infusion of lorazepam (2 -28mg/hr), concluded by the author to be likely associated with PG exposure (Yaucher *et al.,* 2003).

The toxicological profiles of monopropylene glycol (MPG), dipropylene glycol (DPG), tripropylene glycol (TPG) and polypropylene glycols (PPG; including tetra-rich oligomers) were collectively reviewed by the authors, and assessed considering regulatory toxicology endpoints. The review confirms a rich data set for these compounds, covering all of the major toxicological endpoints of interest. The metabolism of these compounds share common pathways, and a consistent profile of toxicity is observed. The common metabolism provides scientific justification for adopting a read-across approach to describing expected hazard potential from data gaps that may exist for specific oligomers. None of the glycols reviewed by the authors presented evidence of carcinogenic, mutagenic or reproductive/developmental toxicity potential to humans. The pathologies reported in some animal studies either occurred at doses that exceeded experimental guidelines, or involved mechanisms that are likely irrelevant to human physiology and therefore are not pertinent to the exposures experienced by consumers or workers. At very high chronic doses, MPG causes a transient, slight decrease in hemoglobin in dogs and at somewhat lower doses causes Heinz bodies to form in cats in the absence of any clinical signs of anemia. Some evidence for rare, idiosyncratic skin reactions exists for MPG. However, the larger data set indicates that these compounds have low sensitization potential in animal studies, and therefore

are unlikely to represent human allergens. The existing safety evaluations of the FDA, USEPA, NTP and ATSDR for these compounds are consistent and point to the conclusion that the propylene glycols present a very low risk to human health. (Fowles *et al* 2013)

Behavioural Data

Da Silva *et al*., (2001) report that experimental drugs and/or plant extracts are often dissolved in solvents, including propylene glycol. The authors present the results of a hole-board test. Rats that were given 10% propylene glycol demonbstrated no modification in head-dipping behaviou. However, 30% propylene glycol induced an increase in the number of head-dips (46.92 +/- 2.37 compared to 33.83 +/- 4.39, P<0.05, A NOVA/Student-Newman-Keuls), an effect comparable to that obtained with 0.5 mg/kg diazepam (from 33.83 +/- 4.39 to 54 +/- 3.8, P<0.01, ANOVA/Student-Newman-Keuls). The authors suggest that these results demonstrate that 30% propylene glycol has significant anxiolytic effects in this model and therefore cannot be used as an innocuous solvent [Da Silva *et al*., 2001]

In Vitro Toxicity Status

Carcinogenicity and Mutagenicity

Roemer *et al.,* (2002) reported on a study in which cigarettes containing various additives in three different combinations were produced. Smoke condensates prepared from these cigarettes were then tested in two different *in vitro* assays. The mutagenicity of the smoke condensate was assayed in the *Salmonella* plate incorporation [Ames] assay with tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of an S9 metabolic activation system. The cytotoxicity of the gas/vapour phase and the particulate phase was determined in the neutral red uptake assay with mouse embryo BALB/c 3T3 cells. The authors concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients which included PG at levels up to 47,225 ppm [a multiple of its typical use in a US cigarette] [Roemer *et al.,* 2002].

Baker *et al.,* [2004]; examined the effects of the addition of 482 tobacco ingredients upon the biological activity and chemistry of mainstream smoke. The ingredients, essentially different groups of flavourings and casings, were added in different combinations to reference cigarettes. The addition of 1,2-PG at 83,300 ppm was determined not to have affected t he mutagenicity of the total particulate matter (TPM) of the smoke in either the Ames, *in vitro* micronucleus assay or the neutral red assay when compared with that of the control cigarettes [Baker *et al.,* 2004].

The mutagenicity of the smoke condensate w as assayed in the *Salmonella* plate incorporation [Ames] assay with the tester strain TA98 in the presence of an S9 metabolic activation system. The cytotoxicity of the cigarette condensate was determined in the neutral red uptake assay and the (3-(4,5-

dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt assay (MTS assay) with the human hepatocellular liver carcinoma cell line, HEP-G2. It was concluded that the *in vitro* mutagenicity and cytotoxicity of the cigarette smoke was not increased by the addition of the ingredients, which included propylene glycol at levels up to 50000 ppm.

PG did not induce sister chromatid exchange or overt chromosomal damage in human cells in culture [no details are given regarding test conditions] [BIBRA, 1996].

A very weak dose-dependent effect on sister chromatid exchange has been demonstrated in hamster cells exposed to PG in the absence of S9 [no other details given] [BIBRA, 1996].

PG has also increased chromosomal damage in the absence of S9, but not in its presence in hamster cells in culture [again, no further information given] [BIBRA, 1996].

BIBRA report that an assay looking for DNA damage in hamster cells gave negative results with PG, as did a transformation assay [no further details given] [BIBRA, 1996].

In the Ames mutagenicity assay, no mutagenic effect was induced by PG in the presence or absence of a liver metabolic activation system, when tested using *S. typhimurium* [no details of strain or conditions are given]. Similar negative results have also been reported in a host-mediated assay where *S. typhimurium* bacteria were resident in the peritoneal cavity of mice receiving 5 g/kg bw/day PG by stomach tube for 5 days. In *Saccharomyces cerevisiae*, weak mutagenic activity has been seen when the yeast cells were resident in the peritoneal cavity of mice receiving the same dose as previously described above, or when treated in culture [BIBRA, 1996].

Another host-mediated assay monitoring mutagenicity using *E. coli* produced negative results. Mice received an intravenous injection of 2.7 g/kg bw of PG when the bacteria were present in the blood. No evidence of DNA damage in *E. coli* was induced by PG in the presence or absence of a liver metabolic activation system, or in *B. subtilis* in the absence of a metabolic fraction [BIBRA, 1996].

PG has been found to be cytotoxic to blood cells drawn from a healthy volunteer. The tissue culture was exposed to 0.5-1.0% PG [no other details regarding test conditions are given] [BIBRA, 1996].

Additional information concerning the *in vitro* mutagenicity of this material may be found in "An Interim report on data originating from Imperial Tobacco Limited's Genotoxicity testing programme September 2003" or "An updated report on data originating from Imperial Tobacco Limited's external Genotoxicity testing programme – Round 2 August 2007".

PYROLYSIS AND TRANSFER STUDIES

Information relating to the pyrolysis and/or transfer of PG is detailed in the Report on Thermochemical Properties of Ingredi ents document. In the aforementioned document, the term 'pyrolysis' means the heating of an ingredient in isolation under controlled conditions in an analytical device to examine its degradation potential. The expression 'transfer data' on the other hand is used to describe the fate of an ingredient in qualitative and quantitative terms following the smoking of a tobacco product to which it has been applied.

The thermal decompn. mechanisms found theor. for propylene glycol and triacetin were validated by a qual. exptl. investigation using gas-phase chromatog.-mass spectroscopy, with excellent agreement. The results provide a validation of the novel simulation framework and shed light on the potential hazard to the health that propylene glycol and triacetin may have when exposed to high temps. [Laino *et al.,* 2012]

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