Propylene glycol used as an excipient

Report published in support of the 'Questions and answers on propylene glycol used as an excipient in medicinal products for human use' (EMA/CHMP/704195/2013).

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Introduction

This document and the related questions and answers [86] have been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the label and package leaflet of medicinal products for human use' [5, 34].

Propylene glycol is commonly used as an excipient in a variety of drugs and it is also authorised in food products and cosmetics. In addition it has a wide range of other practical applications e.g. used as antifreeze, dicing solution, and as an additive to latex paints and coatings to improve freeze-thaw capability. Propylene glycol is also used in the generation of artificial mists and fogs used in fire safety training and theatrical and stage productions.

According to Lessmann et al. [62], propylene glycol production capacity has been reported to have been about 600,000 tons in the US (1998) and about 325,000 tons in Western Europe (1989). Of this volume, 40–45% is estimated to be used as intermediate in the synthesis of other chemicals, especially unsaturated polyester resins.

The remainder of the production volume is used in a multitude of industrial products, for example:

    (1) as solvent in lacquers and varnishes (about 4% of the production)

    (2) for certain resins and also as plasticiser, for example, in vinyl resins (about 4–10%)

    (3) as component in antifreeze products, lubricants, cutting-fluids, inks (about 10–13%)

And in many products for private use:

    (1) as component in many cosmetics and pharmaceutical preparations and as food additive (for example, as solvent for food colours or flavours) due to its low toxicity (about 12–17%)

    (2) in household cleansers, liquid laundry or detergents (about 9–15%)

    (3) in pet foods (about 5%) or

    (4) as humectant in tobacco (about 4%).

This widespread use of propylene glycol stems from its assumed low level of toxicity. It is included in the list of food additives generally regarded as safe (GRAS) by the US Food and Drug Agency and is considered to raise negligible concern for adverse effects on development and reproduction in the NTP-CERHR Monograph (National Toxicology Program, Centre for the Evaluation of Risks to Human Reproduction, 2004). Propylene glycol is also accepted for use as a food additive (E 1520) in Europe [28].

The WHO has set a maximum permissible daily intake of propylene glycol as a food additive at 25 mg/kg bodyweight [47], which was maintained at the fifty-seventh meeting FAO/WHO [48]. These estimates of human exposure are for food products and do not include exposure from pharmaceutical products or exposure through inhalation.

In the EU, the Guideline on Excipients in the Label and Package Leaflet of Medicinal Products for Human Use [27] requires that the warning: "May cause alcohol-like symptoms" is included in the package leaflet of parenteral and oral drugs containing propylene glycol doses in excess of 400 mg/kg if used in adults and 200 mg/kg if used in children. These thresholds were also advised by the Dutch Medicines Evaluation Board as maximum tolerable daily dosages of propylene glycol in cough medicines [108].

While propylene glycol is generally considered safe as a food additive, concerns have been raised repeatedly with regard to potential toxicity of propylene glycol and its acidic metabolites in patients following pharmacologic exposure.

The following adverse events have been linked to propylene glycol exposure in patients, when administered as an excipient with various medicinal products [77, 115, 124]:

- Hyperosmolality, lactic acidosis;

- Renal dysfunction (acute tubular necrosis), acute renal failure;

- Cardiotoxicity (arrhythmia, hypotension);

- Central nervous system (depression, coma, seizures);

- Respiratory depression, dyspnoea;

- Liver dysfunction;

- Haemolytic reaction (intravascular hemolysis) and haemoglobinuria;

- Multisystem organ dysfunction.

Because of the high public exposure to propylene glycol several literature reviews have been undertaken by governmental or non-governmental organisations such as FAO-WHO [47], FDA (Food and drug administration, 1997 and 2008), Agency for Toxic Substances and Disease Registry [1], NTP-CERHR [77], and OECD [80] based upon extensive literature publications.

The current review will use as basis the NTP-CERHR Monograph [77] based upon the NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol (May 2003). The format for Expert Panel Reports includes synopses of studies reviewed and an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation.

The conclusions of the NTP review, completed and/or challenged by any additional relevant literature data published so far (only the latter data are tabulated and discussed in detail), will be used to provide guidance on the safe incorporation of propylene glycol in pharmaceutical preparations.

Scientific discussion

1. Quality

1.1. Physico-chemical properties
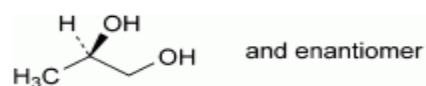
Propylene glycol or 1,2-dihydroxypropane or 1,2-propanediol, is a clear, colourless, viscous, practically odourless liquid with a density of 1.038 g/cm³ at 20°C and a molecular weight of 76.095. It is miscible with water, acetone and chloroform. It is miscible in water.

Propylene glycol monographs are included in the PhEur, the USP and the JP.

1.2. Use in medicinal products

It is a well-known pharmaceutical excipient that is used for several purposes in a wide range of pharmaceutical dosage forms e.g. as a humectant in topicals (15%), as a preservative in solutions

(15–30%) or as a co-solvent in aerosols (10–25%), parenterals (10–60%), oral solutions (10–25%) and topicals (5–80%). It is also used as plasticiser in aqueous film-coating formulations.

2. Pharmacokinetics

2.1. Absorption

2.1.1. Oral and IV pharmacokinetics

Animal data

Available animal data including structure-activity relationships point toward very rapid and complete absorption after oral administration. This is plausible for a highly water-soluble small molecule which will cross membranes with bulk flow of water across aqueous pores.

Morshed et al. [72] found that propylene glycol blood concentration (41.04 mM or 312.3 mg/dL) reached its maximum level 1 hour after administration to 4 New Zealand White (NZW) rabbits of 38.66 mM/kg BW (2.942 g/kg BW) as a 28.4% aqueous solution by gavage. Morshed et al. [73] orally administered an aqueous solution of propylene glycol at 4.83–77.28 mmol/kg BW (0.4–5.9 g/kg bw) to 6 male Wistar rats/group and found that time to peak absorption was related to dose and ranged from roughly 10 minutes at the low dose to 2 hours at the high dose. Peak plasma concentration of 29.21 mM/L (222 mg/dL) was measured at the highest dose of 5.9 g/kg BW. Calculation based on the analysis of data by Michaelis-Menten kinetics yielded the propylene glycol metabolising rate as 0.63 g/kg/h. Propylene glycol metabolism saturation seems to occur at 2.9 g/kg. Of note, no CNS side effects were detected in rats given 2.9 g/kg for up to 45 consecutive days.

Adults

These data are consistent with those of Yu et al. [122] following repeated oral doses in adult patients of either 20.7 g three times daily or 41.4 g two times daily, for a minimum of 3 days (administered in conjunction with phenytoin); rapid absorption (T_{max} within 1 hour of dosing), distribution into total body water (volume of distribution ~ 0.5 L/kg), relatively short half-life (2.4–5.2 hours), and rapid total body clearance (0.1 L/kg/h). Even if oral bioavailability was considered to be close to 100%, accurate bioavailability was not determined because of concomitant exposure to ethanol. Nevertheless the half-life estimates are generally consistent with the results of Speth et al. [100] following IV administration.

In these Speth's studies propylene glycol was used as a solvent in the formulation of mitoquidone, a new potential cytostatic agent. Propylene glycol pharmacokinetics was studied following IV (4h infusion) administration of 3 and 4.5 g/m2 (about 85 and 130 mg/kg assuming a 60 kg BW) for 5 consecutive days, or 7.5 and 15 g/m2 (about 225 and 350 mg/kg assuming a 60 kg BW) on day 1 of 3-week cycles. All patients had normal renal and hepatic functions. Pharmacokinetics was nonlinear, based upon a saturable clearance (clearance decreased as the dose increased). There is an average terminal half-life of 2.3 + 0.7 h, varying from 1.4 h (at the lower doses) to 4.4 h (at the higher doses). No accumulation was observed following the repeated daily administration, and during the 4h infusions the exposure slowly increased, the steady state being not achieved at the end of the infusion. Pharmacokinetic parameters reported for patient receiving 21 g of propylene glycol IV were in the same range than those reported following oral administration of 20.7 g [122] indicative of a close to complete bioavailability following oral administration. And as in the study of Yu et al., wide interpatient plasma concentration differences were observed. Cmax ranged from 48 to 131 µg/mL (4.8–

13.1 mg/dL) following daily administration of about 85 and 130 mg/kg and from 168 to 425 μg/mL (16.8 to 42.4 mg/dL) following once every three weeks administration of about 225 and 350 mg/kg, AUC ranged from 261 to 762 μg.h/mL following daily administration and from 938 to 3719 μg.h/mL following once every three weeks administration. There was no evidence of lactic acidosis, hemolysis or increase in osmolarity.

Several more recent studies evaluated propylene glycol accumulation following continuous lorazepam infusion to critically ill adult patients. Propylene glycol concentration measured following at least 48 h infusion generally correlate with the infusion rate and the previous cumulative (at least over 24 h) dose [7, 10, 42, 79].

Horinek et al. [42] followed critically ill patients who were given lorazepam at doses titrated according to the sedation agitation score. The patient were distributed into two groups, those showing propylene glycol serum concentration below 25 mg/dL and those showing concentrations higher than 25 mg/dL. The first were given a mean load of propylene glycol of 23.1 g/day, the others (42% of the patients) a load of 65.7 g/day. Propylene glycol serum concentrations reached 9.1 versus 130.8 mg/dL respectively. Serum propylene glycol concentrations were correlated with lorazepam infusion rate and previous 24 h cumulative dose.

Interestingly, in studies where patients with renal dysfunction were enrolled, propylene glycol concentration did not differ between patients with normal or impaired renal function [42, 10].

Mixed findings were reported as concerns the correlation between osmolar gap and propylene glycol concentration. Nevertheless osmolar gap increases with increase in propylene glycol concentration and in general the correlation is strongest as lorazepam infusion dose escalates, to the extent that osmolar gap should be considered a predictive signal of propylene glycol accumulation and of potential toxicity. Depending on the publication accumulation was defined as a serum concentration of 25 mg/dL or higher based upon recommendation from the World Health Organisation [42] or when osmolar gap is greater than 10.

Increased anion gap (poor indicator of propylene glycol accumulation) and metabolic acidosis were detected following the highest infusion rates of about 0.16 mg lorazepam/kg/h, equivalent to 1.6 g propylene glycol/kg/day [7]. Corresponding osmolar gaps were 24.3–67.1. A strong correlation between osmolar gap and propylene glycol was determined ($R^2 = 0.804$, $p = 0.001$). Predicted propylene glycol concentration may be calculated as follows from the osmolar gap: propylene glycol concentration = (-82.12 + [osmol gap X 6.5]). In these studies doses of propylene glycol ranged roughly from 0.3 to 1.6 g/kg/day and serum concentrations ranged from 5 to 350 mg/dL.

Interestingly, no new-onset of renal impairment was attributed to propylene glycol in any of the studies. This may be due to the too short duration of the infusions. In the report by Yaucher et al. [120] the median time to serum creatinine increases was 9 days (3 to 60 days). In the case report from Parker [83], acute renal failure was observed following 24-days lorazepam infusion in a critically ill patient presenting with an osmolar gap of 97 and serum propylene glycol concentration of 520 mg/dL.

Children

Accumulation during (continuous) propylene glycol exposure has also been repeatedly reported in various paediatric cases, cohorts or populations, including preterm and term neonates and children.

Datasets on intravenous exposure are available in infants and newborns. In 11 paediatric intensive care patients (1–15 months) given 0.96 to 3.17 g/kg/day [15] slight propylene glycol accumulation

was described from 51.9 mg/dL 48 h after the start of the infusion up to 76.3 mg/dL at the end of the infusion (mean values) during continuous intravenous lorazepam exposure for 3 to 14 days, but PK estimates were not calculated and no biochemical markers of toxicity were observed. While a significant correlation was demonstrated between cumulative dose of propylene glycol (143 mg on average) and the propylene glycol serum concentration at the end of the infusion which ranged between 10 to 220 mg/dL, this was not demonstrated for serum lactate or osmolar gap.

Pharmacokinetic estimates are mainly limited to intravenous formulations in neonates and young infants. In a cohort of preterm neonates (<1.5 kg), the elimination half-life was estimated by Glasgow et al. [32] to be 10.8–30.5 h (compared to adults = 2–5 h).

More recently [18], pharmacokinetic estimates have been described based on 372 propylene glycol plasma concentrations from 62 (pre)term neonates (birth weight 630–3980 g, postnatal age 1–30 days). Birth weight and postnatal age were hereby identified as most important covariates for clearance of propylene glycol in neonates. Large differences in clearance values are seen between neonates of 1 kg (0.013 L/h) and neonates of 4 kg (0.13 L/h) at day of birth. This 10-fold difference in clearance is still seen one month after birth. The largest increase in clearance was observed during the first two weeks of life. The distribution volume was estimated to be 0.96 L/kg [18]. The final model shows that for commonly used dosing regimens, the population mean propylene glycol peak and trough concentrations are highly variable and range between 3.3–14.4 and 2.8–21.8 mg/dL (peak) and 1.9–10.9 and 0.6–11.2 mg/dL (trough) for paracetamol and phenobarbital formulations, respectively, depending on birth weight and age of the neonates.

These PK data have been quantified following overall low propylene glycol exposure (median 40 mg/kg/day) and do not necessary apply (zero versus first order kinetics may apply) when much higher propylene glycol exposure exists.

Data on non-intravenous routes of administration are much more limited. Similar to adults, it is reasonable to assume that oral absorption is high (~100%).

2.1.2. Rectal

The study of Kollöffel et al. [52] demonstrated that absorption of propylene glycol through the rectum was rapid with peak concentrations obtained at 1±0.6 h (average ±SD) in children (5–12 years old) and 1.5±0.3 hours in adults. Peak plasma concentrations were measured at 17.1 mg/dL (2.2 mM) in 4 children dosed with 173 mg/kg BW propylene glycol and 11.9 mg/dL (1.6 mM) in 10 adults dosed with 8.64 g propylene glycol (123 mg/kg BW assuming a 70 kg BW). The serum half-life was determined to be 2.8±0.7 hours in adults and 2.6±0.3 hours in children. These values are in agreement with alcohol dehydrogenase (the limiting step for metabolism) reaching adult levels by the age of 5 years [85]. The apparent volume of distribution was 0.79±0.30 L/kg in adults and 0.77±0.17 L/kg in children [52].

2.1.3. Dermal

The dermal absorption of propylene glycol, a highly water-soluble substance, through the intact skin is expected to be very limited. In a study of human skin biopsy specimens from adult 19–50 years of age, MacKee [66] found no penetration of radioactive tracer materials after up to 1 hour permeation time using propylene glycol alone as a vehicle. A rat dermal penetration in vitro study [103] also showing no uptake, and given the difficulty water soluble molecules generally have penetrating the stratum corneum, it may be concluded that the dermal absorption rate across intact skin is likely to be low.

Nevertheless, studies [29, 32, 57] indicate that once the stratum corneum is impaired (removed such as in burns, toxic epidermolysis, or irritated such as in diaper rash), dermal absorption may become a significant source of exposure. Transcutaneous absorption and accumulation has been described in a limited number of pediatric cases in a setting of extensive cutaneous wounds (burns,) [11, 29]. Peleg et al. [84] reported the case of a premature baby with dermal application of gauze dressing of nitrofurazone on burned skin. On day 4 of life the baby became lethargic and apneic, requiring re-initiation of mechanical ventilation. Metabolic acidosis preceded coma which resolved within hours of cessation of topical treatment. High peak of propylene glycol was measured in urines. More recently, Willis et al. [113] described the case of a 3-year-old male receiving topical application of silver sulfadiazine on burns covering approximately 60% TBSA. Increased osmolality and urine output, osmolar gap of 56 mOsm/kg, cyclic increases in serum lactate and doubling of serum creatinine concentration were observed. Propylene glycol toxicity was evidenced from day 47 of treatment, possibly related to decrease in renal function (increased creatinine concentration). Within 24h of the cessation of the sulfadiazine treatment the lactic acidosis and osmolar gap resolved.

In addition it was demonstrated that enhancers such as surfactant [66] or oleic acid [102] may increase propylene glycol absorption.

2.1.4. Inhalation

Conclusion from the NTP was that it is reasonable to predict that propylene glycol would be absorbed by the lungs if incorporated in a carrier medium (absorption of vapor is expected to be low given the low vapor pressure of 0.07 mmHg). This is confirmed by the studies of Werley et al. [111] where C_{max} and AUC reached values up to 1376 and 91 µg/mL and 450 159 and 52 037 µg/mL min in rats and dogs given 216 or 60 mg/kg (estimated lung deposit) respectively by inhalation for 28 consecutive days.

2.2. Distribution

Speth et al. [100] reported apparent volume of distribution ranging from ~0.55 to 0.94 L/kg in adult patients. In other studies with oral or rectal exposure, apparent volumes of distribution ranged from ~0.52 to 0.79 L/kg. In neonates, the median apparent volume of distribution is 0.96 L/kg. This indicates that propylene glycol is uniformly distributed in total body water without a significant distribution to specific tissues. The higher volume of distribution in infancy reflects the higher body water content and consequently, it is reasonable to assume a lower distribution volume in geriatric patients. It can also be predicted with certainty that propylene glycol will distribute into the water compartment of the placenta and fetus as has been demonstrated for ethanol.

Kelner and Bailey [51] found a significant correlation of lactate concentrations in the serum and CSF to the corresponding propylene glycol concentrations in these fluids in patients receiving medication containing propylene glycol as a vehicle. PG concentrations ranged from undetectable to 71.1 mg/dL in serum and from 1.1 to 56.6 mg/dL in the CSF. The CSF/serum propylene glycol concentration ratios ranged from 0.74 to 0.85. Fifty-five minutes after propylene glycol IV administration the CSF contained propylene glycol at a concentration of 1.1 mg/mL, indicating that propylene glycol penetrates the spinal fluid in less than one hour. The authors stated that all patients had normal hepatic and renal function based upon laboratory tests.

The Du et al. study [24] using magnetic resonance spectroscopy confirmed that significant propylene glycol concentrations may be achieved in the brain (up to ~0.456 mg/g tissue) following a single IV dose of 1 mg/kg BW.

As hemodialysis can readily counteract propylene glycol toxicity in patients, a low protein binding may be assumed [83]. In this study dialysis was used to reduce exposure (serum concentration) to propylene glycol in a critically ill patient with acute renal failure, given lorazepam. Propylene glycol is an alcohol of small molecular weight (76.1 daltons). This type of agent bears several properties which favor excellent clearance by hemodialysis, including small size, non-ionic state, high water solubility and lack of significant protein binding.

2.3. Metabolism

In what is considered to be the main pathway of propylene glycol metabolism in mammals [1, 72], propylene glycol is oxidised by alcohol dehydrogenase to lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is further metabolised to pyruvate, carbon dioxide, and water. Lactate also contributes to glucose formation through gluconeogenic pathways [16]. Lactate, via phosphoenol pyruvate, can be detoxified into glucose and stored as glycogen, as has been demonstrated by Wittman et al. [116] for propylene glycol in rats.

As previously described, Morshed et al. [74] orally administered single doses of an aqueous solution of propylene glycol at 0.4–5.9 g/kg BW to rats and measured blood lactate concentrations thereafter. Lactate was rapidly formed (Tmax: 15–30 minutes) and a 1.83 to 4.01 fold increase in plasma concentrations were recorded. Metabolism of propylene glycol into lactate seemed saturated from the dose of 2.9 g/kg BW. In summary, Morshed et al demonstrated that administration of propylene glycol up to 5.9 g/kg to rats induces hyperlactemia. Lactate concentrations up to 2.68 mEq/L were recorded during this study. Of note, lactic acidosis in human is quoted at lactate concentrations of at least 5 mEq/L.

In human, excess production of lactic acid resulting from very large exposures to propylene glycol was demonstrated to produce a metabolic anion gap [anion gap = (Na+) - (Cl- + total CO_2)] and metabolic acidosis [1].

2.3.1. Propylene Glycol Stereospecific Metabolism in Mammals

Synthesis of propylene glycol results in a 1:1 ratio of D and L stereoisomer forms. There is some, although incomplete, information in the literature about stereospecificity of the enzymes in the propylene glycol metabolic pathways.
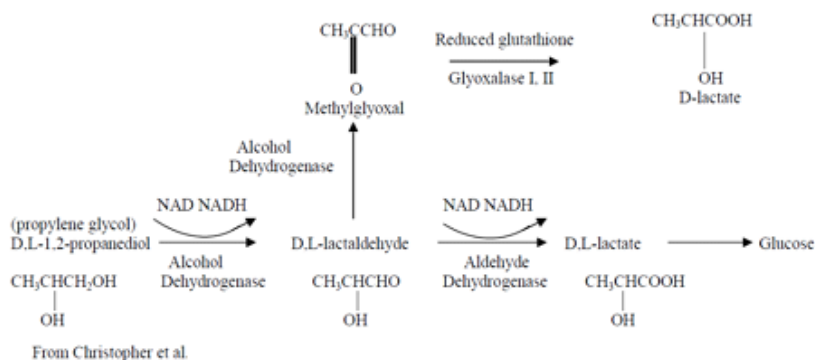
L-lactate is indistinguishable from endogenous lactate, which in human is a good substrate for gluconeogenesis. D-lactate is less readily converted to glucose than L-lactate, which prolongs its half-life leading, under conditions of prolonged exposure (e.g. IV infusion), to D-lactic acidosis. It is difficult

to cause L-lactic acidosis even with very high doses of propylene glycol because of its efficient detoxification via gluconeogenesis. D-lactate is metabolised to pyruvate and CO_2 [55].

The overall conclusion from all data is that acute exposure to D, L-propylene glycol can cause L-lactic acidosis (if the dose is very high) due to the more rapid biotransformation (alcohol dehydrogenase being the rate-determining step) of L-propylene glycol to L-lactate, whereas subchronic/chronic exposure leads to D-lactic acidosis due to accumulation of D-lactate derived from the glyoxylase/ GSH pathway and from being a poor substrate for gluconeogenesis.

Methylglyoxal synthetase can convert the substrate, dihydroxyacetone phosphate, to methylglyoxal. However, in conditions where ketone levels are high, such as diabetes or starvation, methylglyoxal synthetase activity is increased, producing more methylglyoxal and D-lactate. Excessive production of D-lactate may result in its accumulation, especially in the brain, which has a low level of catabolizing enzymes [16]. Therefore, in cases of ketosis, excess levels of D-lactate may be exacerbated by propylene glycol [86].

2.3.2. Phosphorylated propylene glycol metabolism in mammals

In a third possible metabolic pathway, propylene glycol can be phosphorylated, converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate, and lactic acid. Metabolism of D and L forms of propylene glycol in this pathway is species-specific. The rabbit converts the L-form of phosphorylated propylene glycol to lactic acid, whereas the rat and mouse can convert both forms [43, 68].

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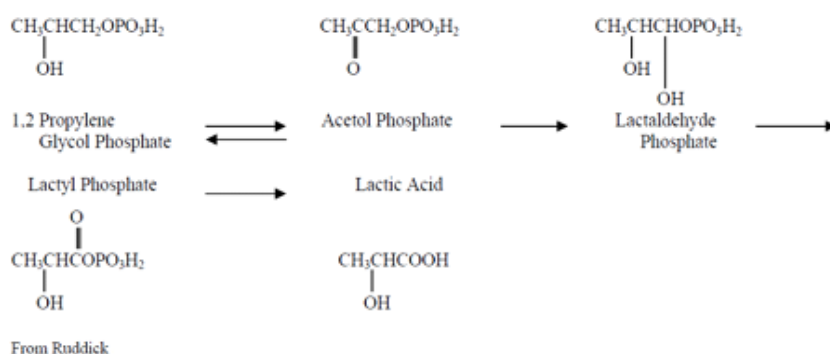
2.3.3. Saturation of metabolic clearance

Total body clearance occurs by metabolic clearance and by renal excretion.

Morshed et al. [73] provide evidence in the rat that the rate-determining step in the metabolic clearance of propyleneglycol is NAD-dependent alcohol dehydrogenase.

From the data of Speth et al. [100] it may be conclude that humans clear propylene glycol similarly to rats and rabbits, but saturation of metabolic clearance occurs at lower doses in humans than in rats and rabbits. Saturation of metabolism appears to occur in rats and rabbits at a dose of about 1.6 to 2 g/kg BW, whereas in humans this seems to happen at a dose of about 0.2 g/kg BW.

From the data of Speth et al. [100] and Yu et al. [122] it was determined that metabolic clearance follows a first-order process (up to doses of approximately 12 g/day) with a constant half-life of 1.6 ± 0.2 h (± SD). Beyond this dose, the serum half-life becomes dose dependent (zero order process) with a serum half-life above 3 hours.

Activities of enzymes such as ADH (alcohol dehydrogenase) and ALDH (aldehyde dehydrogenase) can affect how fast propylene glycol is cleared from the body, thus affecting potential toxicity.

2.3.4. Placental metabolic capacity

Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolise propylene glycol. In rats, placenta was found to have no ADH activity and ALDH activity in placenta was found to be 4–7% of liver activity [96].

2.3.5. Developmental aspects of metabolic capacity

There are consistent data in both animals and humans showing that alcohol dehydrogenase is much lower prenatally and during pediatric life. In humans, adult levels were reached by the age of 5 years and in rats on day 47 after parturition.

Sjoblom et al. [96] found that in Wistar rats ADH activity in liver was low before birth, being 5 and 16% of adult activity on gestation day (gd) 15 and 20, respectively. There was a rapid increase at birth: 53% of adult levels on postnatal day (pnd) 1 with a continued gradual increase with age to 82% of adult activity on post-natal day 47. Similar developmental patterns were noted for ALDH in rat liver.

Similar findings were demonstrated by Raïhä et al. [87] who found that rat liver ADH activity was about 25% of adult activity at birth and reach adult activity AT post-natal day 18. Injection of ethanol to the mother or the offspring did not influence ADH activity in the offspring.

Pikkarainen and Raiha [85] measured in vitro ADH activity in the livers of human fetuses, children, and adults (n=1–3/age group) using ethanol as a substrate. The ADH activity in 2-month-old fetal livers was about 3–4% that of adults. In 4–5-month-old fetuses, ADH activity was roughly 10% that of adults, and in infancy, activity was about 20% that of adults. ADH activity increased in children with age, and at 5 years of age, activity reached a level within ranges noted for adults. Great variation was noted in adult ADH activity. Tran et al [105] also found that mean alcohol dehydrogenase content in liver from perinatal infants is approximately 10-fold lower than in adults.

The lower metabolism capability in newborns and infants, however, may partially protect them from metabolic acidosis after ingestion of propylene glycol but will result in more pronounced accumulation of the parent compound in the case of limited renal clearance and to a higher exposure to propylene glycol itself which has known alcohol-like CNS side effects.

2.4. Excretion

In most mammals, propylene glycol is eliminated via either metabolic or renal clearance. Part of the propylene glycol dose is eliminated unchanged by the kidney and part is metabolised by the liver to lactic acid and further metabolised to pyruvic acid; the remainder is conjugated with glucuronic acid and eliminated in the urine.

The amount of propylene glycol eliminated by the kidneys has been estimated for humans (adults) at 45% [6], for dogs at 55–88% [90], and for rabbits at 24–14.2% [123]. Morshed et al. [73] provided evidence in the rat that increasing doses of propylene glycol increased elimination by the kidneys. Dosages of 19, 38, and 77 mmol/kg BW resulted in 2.3, 7, and 17% renal excretion of propylene glycol.

In the rabbit [123] there was evidence of a saturation of propylene glycol metabolism at the 2.0 g/kg BW acute dose, as evidenced by the decreased metabolic clearance. The rate of renal elimination was dependent upon urine flow.

The infant studies suggest prolonged half-lives of propylene glycol [29, 32] in the range of 10.8–30.5 hours in infants (BW < 1.5 kg) receiving a dose of about 3 g propylene glycol that may be attributed to either renal immaturity (< 1 year of age) and/or lower metabolic clearance (< 5 years of age).

As mentioned earlier, propylene glycol clearance in early infancy depends on weight and postnatal age [18]. More recently, the datasets in urine and plasma [18, 58] were combined to quantified hepatic metabolic and renal primary elimination.

It seems that postmenstrual age is one of the covariates of propylene glycol renal elimination [58], but somewhat different to what we initially anticipated. A lower PMA resulted in a proportionally higher renal elimination of propylene glycol. This might reflect either a difference in ontogeny between alcohol + dehydrogenase and primary renal elimination in favor of primary renal elimination, or might reflect differences in renal tubular transport.

Although these data are at present only submitted [19], it was documented that in – absolute values – both hepatic and renal elimination are much lower in neonates than in adults.

De Cock et al. [19] studied the contribution of renal elimination to clearance of propylene glycol in preterm and term neonates. They demonstrated that renal elimination of propylene glycol in (pre)term neonates is low (15% of total clearance), particularly compared to the reported percentage of 45% in adults, but may increase with time to 25% and 30% respectively 24 h and 48 h after the first dose of propylene glycol.

2.5. Pharmacokinetic drug/drug interactions

Despite the rapid increase over time of renal clearance after dose [19], renal elimination of propylene glycol in neonates still remains substantially lower compared to adults, for which renal clearance of propylene glycol was reported to be 45%. The consequence of this finding is that maturational changes in the ratio between renal and metabolic clearance may influence the magnitude of drug-drug interactions. As in neonates hepatic clearance of propylene glycol proves the most important elimination route, drug-drug interactions for the alcohol dehydrogenase enzyme will become more important in neonates compared to adults. It was also demonstrated that the largest increase in renal clearance is observed during the first 2 weeks of life. In this perspective, the advice of the FDA to avoid Kaletra®, a propylene glycol and ethanol containing solution, in premature babies until 14 days after their due birth date or in full-term neonates younger than 14 days of postnatal age is of relevance since intoxications were observed only in pre-term and term neonates (with the exception of one 1-month baby who was overdosed). Kaletra® is a solution, which contains a combination of lopinavir and ritonavir solved in ethanol (356.3 mg ethanol/mL Kaletra®) and propylene glycol (152.7 mg propylene glycol/mL Kaletra®).

Dean et al. [20] have also showed that propylene glycol is susceptible to significantly increase microsomal metabolism of aniline and p-nitroanisole, and decrease aminopryridine demethylation with no significant change in p-nitrobenzoic acid metabolism, and to prolonged sleeping time induced by hexobarbital, potentially indicative of inhibition of metabolism, following 3 days i.p. administration of 4.152 g/kg propylene glycol BID.

The potential for pharmacokinetic drug/drug interactions as also been demonstrated by Hughes et al. [44], Snawder et al. [97], and Kelava et al. [50] who showed in vitro and in vivo that in mice the

toxicity of paracetamol may be prevented presumably by inhibition of its metabolism by CYP 2E1 into the toxic metabolite N-acetyl–p-benzoquinone imine, at doses of propylene glycol ranging from 0.62 to 4.2 g/kg BW (i.p.).

Propylene glycol has also been shown to block the increased permeability of the blood brain barrier which occurs following ischemic stroke/reperfusion [99], when administered at 7.8 g/kg of BW (high dose!) in the rat.

As described before, proportionally, the contribution of metabolic clearance to primary renal clearance is much higher in neonates when compared to the 55/45 elimination described in human adults [90]. Although speculative, this also suggests that interactions during co-administration of other compounds that also undergo ADH metabolisation (e.g. ethanol) are more likely in neonates, while associated renal dysfunction likely will be less relevant in neonates.

2.6. Pharmacokinetics summary

The absorption, distribution, metabolism and excretion of propylene glycol have been studied in human and in the animal species used in the toxicity studies (rat, mouse, dog and monkey). No major differences were noted between pharmacokinetics in these species supporting the clinical relevance of their use in the assessment of the toxicology profile of propylene glycol.

Absorption by oral route is rapid and nearly complete. Pharmacokinetic parameters are generally considered similar whenever propylene glycol is administered by oral, intravenous or intraperitoneal route. Absorption by the rectal route or by inhalation is also important. Propylene glycol is mainly distributed to the aqueous compartment including in the brain (CSF) and the fetus. Absorption through intact skin is negligible but may be increased in case of skin abrasion.

The major metabolic pathway in mammals is considered to be propylene glycol oxidation by alcohol dehydrogenase to lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is further metabolised to pyruvate, carbon dioxide, and water. Lactate also contributes to glucose formation through gluconeogenic pathways.

Exaggerated formation of lactate may induce lactic acidosis through accumulation of D- or L-lactate.

Significant correlation has been demonstrated between lactate concentrations in the serum and CSF to the corresponding propylene glycol concentrations in these fluids in patients receiving medication containing propylene glycol as a vehicle.

This metabolic clearance is saturable in all species but occurs at lower doses in humans than in rats and rabbits. Saturation of metabolism appears to occur in rats and rabbits at a dose of about 1.6 to 2 g/kg BW, whereas in humans this seems to happen at a dose of about 0.1 to 0.2 g/kg BW.

Activities of enzymes such as ADH (alcohol dehydrogenase) and ALDH (aldehyde dehydrogenase) can affect the rate of propylene glycol clearance from the body which may partially explain why exposure in patients is highly variable, and thus affecting potential toxicity.

Propylene glycol may be excreted unchanged or conjugated with glucuronic acid and eliminated into urine. This excretion has been shown to be flow dependent in the rabbit, or to vary with increasing dose.

In case of metabolic clearance saturation or impaired renal function, propylene glycol accumulates in the serum inducing hyperosmolarity. Osmolar gap increases with increase in propylene glycol concentration and in general the correlation is strongest as lorazepam infusion dose escalates, to the

extent that osmolar gap should be considered a predictive signal of propylene glycol accumulation and of potential toxicity.

Finally propylene glycol is an inducer/inhibitor of specific P450 metabolic pathways and as such may affect the pharmacokinetic parameters of co-administered drugs. Nevertheless this occurred when propylene glycol was administered to rats or mice at relatively high dose levels above no adverse effect levels and the clinical relevance should be established on a case by case basis.

2.7. Special populations

Liver and/or renal impairment

In patients with liver or renal impairment propylene glycol is expected to accumulate. Nevertheless it has been shown in studies where patients with renal dysfunction were enrolled, that propylene glycol serum concentration did not differ between patients with normal or impaired renal function [42, 10].

Children

Absorption has been documented in infants and children following oral, rectal and even cutaneous (when barrier is defected). The distribution volume reflects body water content. Consequently, it is somewhat higher in infants. Metabolic and renal clearance are lower, and in neonates depends more on the metabolic compared to primary renal elimination. As a consequence, accumulation is more likely in neonates and toddlers, when compared to adults and may occur faster in the setting of liver failure or metabolic competitive inhibition (e.g. ethanol) particularly in neonates and very young infants with immature renal function.

Elderly

No data were found on pharmacokinetic and metabolism of propylene glycol in the elderly population.

3. Non-Clinical safety assessment

3.1. Pharmacodynamics

3.1.1. Primary pharmacodynamics

Propylene glycol is used as an excipient in drug products and no primary pharmacodynamic properties are expected.

3.1.2. Secondary pharmacodynamics

Some in vitro mechanistic studies on the effects of propylene glycol on calcium homeostasis have been published. Chu and Brazeau [17] state that propylene glycol influences calcium release in rabbit skeletal muscle sarcoplasmic reticulum. Another publication [38] reported that intracellular calcium concentration in rat pheochromocytome cells was raised via an influence on voltage-dependent Ca^{2+} channels. The same group also found an effect of propylene glycol on neuromuscular transmission in the mouse [37] via its stimulatory effect on Ca^{2+} efflux from the nerve terminals. A similar finding, i.e. propylene glycol - increased Ca^{2+} concentration in rat cerebrocortical synaptosomes, was reported by Satoh et al. [92].

3.1.3. Safety pharmacology

A number of publications describe cardiovascular effects of propylene glycol, usually administered intravenously, to rats and dogs. For example, a pronounced antiarrhythmic activity after repeated IV injection of propylene glycol in rats and dogs has been reported by Eichbaum and Yasaka [25] and Yasaka [119]. Bost and Ruckebusch [13] describe a hypotensive effect of propylene glycol in dogs. In anesthetised dogs, starting at 400 mg/kg, dose-related but transient decreases in heart rate and arterial pressure were seen [2].

The action of propylene glycol on the central nervous system in dogs was reported to be similar to that of ethanol, although larger doses are necessary to bring about the effects. The narcotic action of propylene glycol is about one-third that of ethanol [61].

Furthermore, Morshed et al. [72, 74] noticed increased blood lactate concentrations in rats after oral treatment with ≥ 368 mg/kg. At doses ≥ 2.9 g/kg, blood glucose was also increased. In another experiment in rats, they found an effect on brush border membrane enzymes and intestinal uptake of nutrients.

3.1.4. Pharmacodynamic drug interactions

Lau et al. [60] reported that phenobarbital produced significantly more apoptosis when used in combination with propylene glycol; intraperitoneal administration of 5 mg/kg phenobarbital prepared in a subtoxic dose of 68% (v/v) propylene glycol produced apoptosis in juvenile mice, while for phenobarbital dissolved in saline, apoptosis was only detected from 40 mg/kg. It is unknown whether this is the result of a pharmacokinetic or pharmacodynamic interaction.

3.2. Toxicology

Very high doses of propylene glycol cause CNS, hematologic/hyperosmotic, and perhaps cardiovascular effects, as well as lactic acidosis. Animals lethally intoxicated undergo CNS depression, narcosis, and eventual respiratory arrest.

3.2.1. Cytotoxicity

Cytotoxic properties of propylene glycol were reported. Using primary cultured human proximal tubule cells, Morshed et al. [75, 76] have demonstrated toxic effects after single (≥ 131 mM) and repeated exposure (≥ 25 mM) to clinically relevant concentrations of propylene glycol. More recently, the acute toxic effect has been confirmed in an MTT assay in respiratory epithelial cells [93]. However, compared to other solvents like ethanol (LC50=771 mM), the LC50 value for propylene glycol was remarkably higher (LC50=3350 mM). No cellular toxicity was detected in mouse fibroblast-like cells but this can probably be explained by the relatively low concentrations that were tested (up to 100 μM) [98].

3.2.2. Single dose toxicity

Propylene glycol has a very low order of acute toxicity. The following oral LD50 values were found [77].

Species	LD50 (g/kg)
Rat	8–46
Mouse	25–32

Rabbit	18–20
Dog	19
Guinea pig	18–20

Subcutaneous LD50 values are reported to be 25–28 g/kg BW in the rat and 19 g/kg BW in the mouse. Intravenous LD50 values of 5–8 g/kg BW and 4–6 g/kg BW were reported in the mouse and rabbit respectively.

The general symptom of acute intoxication was CNS depression. In female rats, also transient haematological changes including decreased hemoglobin and red cell counts occurred after single oral doses [91].

3.2.3. Repeat-dose toxicity

In the NTP-CERHR Monograph (2004), chronic oral toxicity studies provide evidence of slight liver damage as a high dose effect in the rat (effects starting at doses around 2 g/kg), and of enhanced erythrocyte destruction with signs of increased erythropoiesis in the dog. For the effects in dogs, a NOAEL of 2 g/kg was determined [110]. The hemolysis potential of high doses of propylene glycol is firmly established in dogs and reasonably well substantiated in other species including man. Saini et al. [91] reported reversible hematologic effects of propylene glycol in rats administered single doses of 0.7 or 3 g/kg BW by gavage. This confirms that the hematopoietic system is also a target of propylene glycol in rats. The absence of such findings in the chronic study with rats by Gaunt et al. [31] (see also section 2 d) – carcinogenicity) may be related to adaptation following repeated administration or lower peak plasma levels of both propylene glycol and lactic acid given the different mode of administration (diet admixture instead of gavage). The NOEL in this study was the highest dose tested (1700/2100 mg/kg BW per day in males/females respectively). Furthermore, Morshed et al. [73] report that no CNS abnormalities were observed in adult rats ingesting 2.9 g/kg once daily for 45 days. With respect to inhalation toxicity, the NTP Monograph (2004) states that studies performed in the rabbit, rat, and monkey seem to indicate that aside from local effects such as enlarged goblet cells and some nasal hemorrhages, exposure by inhalation to propylene glycol does not pose a significant toxicology problem. Primates (rhesus monkeys) safely inhaled about 1 g of propylene glycol per day [89].

More recently, the following data were published:

- Thackaberry et al. [104] conducted a study (sponsored by Merck) to assess the safety and tolerability of some formulation vehicles, including propylene glycol, in general toxicology studies. Propylene glycol (1000 mg/kg) in purified water has been administered by oral gavage to mice (100 mg/mL), rats (200 mg/mL), dogs (200 mg/mL), and cynomolgus (200 mg/mL) monkeys for approximately 90 days and the effects of this formulation on clinical observations, body weight and food consumption parameters, clinical pathology and histopathology were evaluated across all species. According to the study results, the suitability of the formulation containing up to 1000 mg/kg propylene glycol for use in preclinical safety studies was confirmed by a lack of effects on all the parameters examined.

- Werley et al. [111] evaluated aerosolised propylene glycol toxicity in a battery of non-clinical studies intended to assess its potential inhalation and systemic toxicity in rats and dogs. These included safety pharmacology, pharmacokinetic (PK) studies, single dose toxicity studies, and repeated dose toxicity studies. In the rat, 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, which is commonly observed in inhalation studies in the rat. In the female Beagle dog, treatment-related decreases in haemoglobin, red blood cells and haematocrit 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. These effects were not clinically significant and the changes were still within normal historical ranges for dogs of this age, strain and sex. Furthermore, histopathological evaluations did not reveal any tissue related findings. Under the conditions of these studies, the NOEL for the rat was determined to be 20 mg/kg/day for the 28-day study. In the Beagle dog, the NOEL was approximately 6.05 mg/kg/day for the 28-day study.

- Finally, Montharu et al. [70] assessed in a 4-day toxicity study the pulmonary tolerance of propylene glycol in a rat model of intratracheal administration. Biochemical analysis on bronchoalveolar lavage (BAL) fluid and histological examinations showed that 30% propylene glycol was tolerated in a qualitatively similar way as deionised water.

3.2.4. Genotoxicity

Several studies described in the NTP Monograph (2004) point out the low genotoxic potential of propylene glycol. These conclusions were confirmed by the results of in vitro and in vivo assays from FDA and from Hayashi et al. [39].

On the other hand, the results of recent studies conducted by Aye et al. [9] demonstrated that propylene glycol could produce in vitro DNA-damage, in the presence and absence of S9 mix, leading to chromosomal mutations in CHO cells. Nevertheless, it should be pointed out that high cytotoxic concentrations of propylene glycol were tested (50–150 mg/mL and IC50 for cytotoxicity = 24.1 mg/mL), in order to evaluate its potential effects when used as a cryoprotectant in oocyte vitrification.

3.2.5. Carcinogenicity

The NTP Monograph (2004) identified two useful long-term toxicity studies for evaluating the carcinogenicity potential of propylene glycol.

In a 2-year study in rats from Gaunt et al. [31] the mean daily intakes of propylene glycol were approximately 0, 0.2, 0.4, 0.9, and 1.7 g/kg in males and 0, 0.3, 0.5, 1.0, and 2.1 g/kg in females for the 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol dose groups, respectively (diet admixture). No abnormalities were observed among groups in deaths, behavior, or food consumption. The authors reported no significant differences between the control and treated groups with respect to blood chemistry or renal concentration tests, organ weights (including gonads) and organ weights relative to terminal body weight. Incidence of neoplasms was similar between control and treated groups. This study establishes a highly credible NOEL for propylene glycol in terms of chronic toxicity in both male (1.7 g/kg) and female (2.1 g/kg) rats.

Stenback and Shubik [101] conducted a skin-painting experiment with, among other chemicals, propylene glycol. The dose was 0.02 mL pure propylene glycol or 50 and 10% solutions in acetone twice a week, during the entire life-time of the animals. There were no skin tumors in treated mice, although this strain of mice (Swiss females) is exquisitely sensitive to the induction of skin tumors. The highest dose tested translates to approximately 0.8 g/kg bw twice a week.

3.2.6. Reproductive and developmental toxicity

Prenatal and perinatal toxicity studies

Data from the Driscoll et al. [23] GLP compliant prenatal developmental study indicate that oral exposure to propylene glycol from gestation day 6 to 15 is not a developmental toxicant at doses of up to 10 g/kg BW/day in CD-1 mice. The maternal NOAEL was also 10 g/kg BW/day, considering that the only effect in dams at 5.0 and 10.0 g/kg BW/day was an increased water consumption most probably attributable to the physiological response to the high dose of propylene glycol administered. Aside from increased water consumption no treatment effects were seen in the dams.

Besides, recently, a series of studies were conducted by Enright et al. [26] (sponsored by Merck) to assess some alternative formulation vehicles, including propylene glycol, in developmental and reproductive toxicology (DART) studies. In a rat fertility study, 1,000 mg/kg propylene glycol was administered orally before/during mating, and on gestation Day (GD) 0–7, followed by an assessment of embryonic development on GD 14. In the rat and rabbit teratology studies, the same dose of propylene glycol was administered. In these teratology studies, pregnant females were dosed during the period of organogenesis, followed by an assessment of fetal external, visceral, and skeletal development. In the rat fertility and rat teratology studies, propylene glycol did not exhibit toxicity. Similarly, in the rabbit teratology study, there was no propylene glycol related toxicity observed.

The only data available to evaluate postnatal effects of propylene glycol are those from the continuous breeding study in mice conducted for an assessment of possible fertility effects [71]. When mice were exposed throughout gestation and lactation and to 34 weeks of age with estimated doses as high as 10 g/kg BW/day, no adverse effects were observed on fertility indices. These data suggest that pre- and postnatal development was not significantly impaired.

Multigenerational reproductive toxicity study

NTP tested propylene glycol for reproductive/developmental toxicity (GLP study). Using the reproductive assessment by continuous breeding (RACB) protocol, they investigated the reproductive function of male and female mice exposed to propylene glycol in drinking water (the results of this study are summarised in Morrissey et al. [71]. Animals were exposed to propylene glycol (> 99% purity) in drinking water for a total of 18 weeks. Chemical consumption estimates in this study were 0, 1.82, 4.80, and 10.1 g/kg BW/day for each of the respective dose groups. Live litters born were weighed, examined and then sacrificed. Approximate delivery time and number of dead and cannibalised pups were noted. Offspring from the last litter (5th litter) of the control and high-dose groups were allowed to mature (F1) and reproductive performance was evaluated.

Propylene glycol had no significant effect on any of the following reproductive parameters in F0 animals: number of litters per pair, number of live pups per litter, sex ratio, pup weights, number of days to litter, and dam weights at delivery. F0 parents were not necropsied.

F1 animals received exposure to propylene glycol from their dosed dam during gestation and lactation up to weaning and then continuous exposure from drinking water (estimated daily dose of propylene glycol, 14.4 g/kg bw/day). Propylene glycol had no effect on F1 pup survival or body weight gain. Also, mating and fertility index were not different from the control group. No effects were seen on pups from F2 either. At necropsy of F1 animals, there was no difference between high dose and control groups with respect to body weight, organ weights (kidney, liver and gonads), sperm parameters and estral cyclicity.

Juvenile toxicity study

In the continuous breeding study by NTP, as described above, newborn mice were exposed to propylene glycol via lactation, followed by an exposure to high doses (estimated to 14.4 g/kg/day) via drinking water up to adulthood. Although limited endpoints were investigated in this study, no adverse effects were detected.

However, in a recent study in newborn and juvenile C57BL/6 mice of several ages (PND4 to PND30) exposed to a single i.p dose of 100% v/v propylene glycol the authors have observed a dose-dependent induction of widespread apoptotic neurodegeneration in the brain with doses from 2 mL/kg (or 2.076 g/kg). Damage was observed in animals aged PND 4, 7, 14 and 17 and was greatest in Postnatal Day 7 animals. No damage was detected in animals aged PND 24 and 30. At 10 mL/kg, animal death occurred. At this dose level death rate was about 8% across all times, ages, pilot work and cause of death appeared to be cardio/respiratory failure (personal communication from Prof. N. Farber, Feb. 2013). At 1 mL/kg no apoptotic effects were observed [60].

It is not clear why these results look different from the results of the two-generation studies in mice mentioned above. No behavioural examination or CNS histopathology is reported in the NTP study, while Lau study focuses on brain histopathological examination. The mode of propylene glycol administration is different. The i.p. rather than oral in drinking water administration of propylene glycol suggests that there might be a difference in pharmacokinetic and distribution, with higher serum and brain propylene glycol in the mice from Lau study which is more susceptible to induce ethanol like effects as demonstrated in the acute toxicity studies.

Similarly to the results described above, in a series of recent in vivo studies in the same mouse strain (C57BL/6), it has been shown that ethanol, when administered to immature rodents during the period of synaptogenesis, triggers widespread apoptotic neurodegeneration throughout the developing brain [81, 82]. The deletion of large numbers of neurons from many different regions of the developing brain by ethanol is suggested to provide an explanation for the reduced brain mass and lifelong neurobehavioral disturbances associated with the human foetal alcohol syndrome (FAS). The period of synaptogenesis, also known as the brain growth spurt period, occurs in different species at different times relative to birth.

In rodents the critical period of synaptogenesis occurs during the first three postnatal weeks of life, peaking during week 2, while in humans, the proliferation of synapses begins around 20 weeks of gestation, density increases rapidly after birth, particularly within the early postnatal months, to reach a level approximately 50% higher than that seen in adults by 2 years of age [94]. The timing of this synapse proliferation is region-dependent; for example, synaptic density peaks in the primary visual cortex as early as 8–12 months of age, compared to 2–4 years of age in the prefrontal cortex.

3.2.7. Local tolerance

Propylene glycol was tested on the clipped skin of NZW rabbits according to three protocols (the cosmetic protocol, the "Association française de normalisation" protocol, and the OECD protocol); in all three tests, propylene glycol was classified as a non-irritant (NTP Monograph, 2004).

Results from guideline studies (OECD 405) in the rabbit demonstrate that undiluted propylene glycol is minimally irritating to the eye [46], producing no more than slight transient conjunctivitis which resolves by 24–48 hours.

3.2.8. Allergy/hypersensitivity

Non-clinical studies including several guinea pig maximisation tests, a modified mouse ear swelling test in BALB/c mice and a local lymph node assay with 100% propylene glycol, failed to detect any sensitizing properties of propylene glycol [62].

Nevertheless, sensitisation potential of propylene glycol, although minimal, has been established in man.

3.3. Summary of non-clinical safety assessment

There is an adequate database to assess the toxicity of propylene glycol.

- Propylene glycol is not acutely harmful after ingestion or skin contact.

- Several repeat-dose toxicity studies in adult animals are available.

3.3.1. Oral administration

Weil. et al [110] concluded that the NOAEL for chronic propylene glycol toxicity in dogs was 2 g/kg/d because at the higher dose tested, i.e. 5 g/kg, some changes in haematological parameters were observed. Most of these were not statistically significant compared to concurrent controls and all remained within normal biological ranges. Therefore, in our opinion no real adverse propylene glycol - related effects appear to occur up to the highest dose in this study. Interestingly, the authors determined plasma concentrations of a single 5 g/kg dose in an additional experiment. Cmax was 560 mg/dL.

The oral subchronic study by Thackaberry et al. [104] provides supporting evidence that the NOAEL in mice, rats, dogs, and cynomolgus monkeys is higher than 1 g/kg, the highest dose tested.

In a chronic dietary study with rats [31] the NOEL was the highest dose tested (1700/2100 mg/kg/day in males/females respectively).

In a mouse prenatal developmental toxicity study [23], no relevant treatment-related effects were seen in the dams at the only dose tested, leading to a maternal NOAEL ≥ 10 g/kg /day.

In rat fertility and rat teratology study, as well as a rabbit teratology study [26], propylene glycol did not exhibit toxicity after oral dosing of 1 g/kg/day.

3.3.2. Inhalation

In rats given propylene glycol by inhalation, Werley et al. [111] reported a NOEL of 20 mg/kg/day (deposited dose in the lung) based on minimal laryngeal squamous metaplasia at the higher doses. However, this finding is commonly observed as a local effect in inhalation studies in the rat and hence not considered relevant with respect to the determination of a NOAEL for systemic propylene glycol toxicity. In fact, after 28 days of inhalation exposure, no systemic effects were seen up to 200 mg/kg (highest dose tested, corresponding to a plasma Cmax of 137.6 mg/dL). Moreover, in the 7-day inhalation study in rats, no effects were observed up to the highest dose tested, i.e. 41.0 mg/L and corresponding to plasma Cmax of 350.8 mg/dL.

In the same publication, a NOEL of 6 mg/kg was determined for Beagle dogs due to statistically significant decreases in haemoglobin, red blood cells and haematocrit in female dogs of the two highest exposure groups. However, these changes were not clinically relevant and were within the historical control ranges for dogs of this age and strain and were not accompanied by any other clinical signs or

tissue-related changes. It may therefore be concluded that propylene glycol did not cause adverse effects up to the highest dose tested, i.e. 60 mg/kg/d corresponding to plasma Cmax of 9.2 mg/dL.

3.3.3. Conclusion

In conclusion, after repeated exposure, propylene glycol has a rather low systemic toxicity in experimental adult animals. No treatment-related adverse effects were observed up to the highest doses tested, although in dogs at 5 g/kg/day orally or 0.06 g/kg/day by inhalation changes in haematological parameters start to occur and in one study in rats at 2 g/kg/d slight liver changes were reported. Based on the results of safety pharmacology studies, high doses of propylene glycol may also cause CNS, hematologic/hyperosmotic, and perhaps cardiovascular effects, as well as lactic acidosis.

In mouse, rat and rabbit teratology studies and in a rat fertility study, propylene glycol did not exhibit toxicity. From the continuous breeding study conducted by NTP, it can be concluded that propylene glycol administered in the drinking water at up to doses corresponding to 10 mg/kg bw/day had "no effect on the fertility and reproduction in adult or second generation CD-1 mice. Furthermore, there was no apparent effect with respect to body and organ weights (both absolute and adjusted), sperm motility, sperm counts per g caudal tissue, incidence of abnormal sperm, estrual cyclicity, and calcium levels in blood-serum of second generation mice."

Overall, NOAELs seem to be in the same range for rats, dogs and mice, i.e. 2 g/kg/d, 5 g/kg/d and 10 g/kg/d, respectively. Since there is only one recent study reporting toxicity data in monkeys in which no effects were observed, the data in monkeys are considered insufficient for deriving a meaningful threshold.

Dose causing no <u>adverse</u> systemic effects	Rat	Dog	Mouse	Monkey
Gaunt et al., 1972 [31] 2yr diet	NOAEL ≥1.7/2.1 g/kg/day			
NTP [77]	LOEL 2 g/kg/day Slight liver effects			
Saini et al. 1996 [91] Oral gavage single dose	LOAEL 0.7 g/kg/day Reversible hematological effects			
Weil et al., 1971 [110] 2yr diet		NOEL 2 g/kg/d NOAEL 5 g/kg/day Some hematological effects Cmax = 560 mg/dL		
Thackaberry et al., 2010 [104]	NOAEL	NOAEL	NOAEL	NOAEL

90d oral gavage	≥1 g/kg/day	≥1 g/kg/day	≥1 g/kg/day	≥1 g/kg/day
Werley et al., 2011 [111] 28d inhalation	NOAEL ≥0.2 g/kg/day (28-day) Cmax = 137.6 mg/dL NOAEL ≥1.1 g/kg/day (7-day) Cmax = 350.8 mg/dL	NOAEL ≥0.06 g/kg/day Cmax = 9.2 mg/dL		
Robertson et al. 1947 [89] 1y inhalation				NOAEL ≥1 g/kg/day
Driscoll et al., 1993 [23] Oral prenatal development study			NOAEL ≥10 g/kg/day	
Enright 2010 [26] Oral fertility & teratology study	NOAEL ≥1 g/kg/day			
NTP Continuous breeding study, 1989 [77] Propylene glycol in drinking water			NOAEL ≥10.1 g/kg/dV	
Stenback and Shubik, 1974 [101] Life-time skin painting			NOAEL ≥0.8 g/kg twice a week	
Overall NOAEL	**2 g/kg/day**	**5 g/kg/day**	**10 g/kg/day**	Insufficient data

With respect to propylene glycol toxicity in juvenile animals, in the continuous breeding study by NTP, newborn mice were exposed to propylene glycol via lactation, followed by an exposure to high doses (estimated to 14.4 g/kg/day) via drinking water up to adulthood. Although limited endpoints were investigated in this study, no adverse effects were found.

However, a juvenile mouse study [60] shows that propylene glycol produces ethanol-like apoptotic neurodegeneration in the developing mouse CNS starting at doses of 2 mL/kg. At 1mL/kg no apoptotic effects were observed. It is unknown whether this apoptosis could result in long-term cognitive and behavioural abnormalities. This issue for propylene glycol has not been addressed in either humans or other animals. Moreover, when propylene glycol was administered with phenobarbital the apoptotic

effect of the anticonvulsant was potentiated. It is unknown whether this is the result of a pharmacokinetic or pharmacodynamic interaction.

The apoptotic effects seen in the juvenile mouse study [60] are considered relevant for the risk assessment of propylene glycol particularly following acute administration in children less than 5 years of age and the NOAEL of 1 g/kg/day is taken forward for the PDE calculation.

Dose causing no <u>adverse</u> systemic effects	Juvenile mice
NTP Continuous breeding study, 1989 [77] Propylene glycol in drinking water	NOAEL ≥10.1 g/kg/day
Lau et al. 2012 [60] Single i.p. dose	NOAEL 1 g/kg/day LOAEL 2 g/kg/day
Overall NOAEL	1 g/kg/day

In line with the ICH guideline on impurities: Residual Solvents [45], permitted daily exposures (PDE) of propylene glycol were calculated based upon the NOAELs derived from the most relevant animal studies as follows:

PDE = NOAEL x Weight Adjustment / F1 x F2 x F3 x F4 x F5

F1 = A factor to account for extrapolation between species (5 for extrapolation from rats to humans, 12 for extrapolation from mice to humans, 2 for extrapolation from dogs to humans)

F2 = A factor of 10 to account for variability between individuals

F3 = A variable factor to account for toxicity studies of short-term exposure (1 for studies that last at least one half lifetime e.g. 1 year for rodents, 2 for a 6-month study in rodents, 5 for a 3-month study in rodents or a 2-year study in non-rodents, 10 for studies of a shorter duration)

F4 = A variable factor that may be applied in cases of severe toxicity

F5 = A variable factor that may be applied if the no-effect level was not established

Permitted daily exposures (PDE):

Species	Rat	Dog	Mouse	Monkey	Juvenile mice
NOAEL for PDE calculation	2000 mg/kg/day	5000 mg/kg/day	10000 mg/kg/day	Insufficient data	1000 mg/kg/day
F1 (extrapolation between species)	5	2	12		12
F2 (variability between individuals)	10	10	10		10
F3 (exposure duration)	1	5	1		10

F4 (severe toxicity) and F5 (no-effect level not established) = 1					
PDE (mg/kg/day)	40	50	83		1

PDEs of 50 mg/kg and 1 mg/kg were derived for adults and children below five years of age, respectively. These PDEs represent conservative safety limits based upon non-clinical data. The use of propylene glycol as pharmaceutical excipient in lower doses than these PDEs is considered acceptable without further justification.

The cut-off age of 4 years is based upon the following arguments:

- The no adverse effect level determined in juvenile mice [60] is applicable to mice younger than 3 weeks of age (equivalent to 2 years of age in children, based upon reproductive and central nervous systems post-natal maturation). The cerebral lesions observed in mice (apoptotic neurodegeneration) when treated up to 17 days of age (but not observed in older mice) are attributed to an effect on synaptogenesis, similar to the effect induced by phenobarbital and ethanol in the same model. The period of synaptogenesis, also known as the brain growth spurt period, occurs in different species at different times relative to birth. In rodents the critical period of synaptogenesis occurs during the first three postnatal weeks of life, peaking during week 2, while in humans, the proliferation of synapses begins around 20 weeks of gestation, density increases rapidly after birth, particularly within the early postnatal months, to reach a level approximately 50% higher than that seen in adults by 2 years of age [94]. The timing of this synapse proliferation is region-dependent; for example, synaptic density peaks in the primary visual cortex as early as 8-12 months of age, compared to 2-4 years of age in the prefrontal cortex.

- While it is known that the ADH activity, the rate limiting step of metabolic clearance, reaches adult value around 5 years of age in humans, it already reaches about 30 to 50% of the adult activity at 2 years of age.

- Renal clearance, the other elimination pathway, is mature at about 1-year of age.

With respect to genotoxicity the results of recent in vitro studies show that propylene glycol could produce DNA damage. These effects occurred however at high doses, resulting in strong cytotoxicity. Overall, the weight of evidence suggests that propylene glycol is not genotoxic.

There is no evidence to suggest that propylene glycol has any carcinogenic potential.
Studies demonstrate that it is not irritating to skin or eye, nor does it cause sensitisation by skin contact.

4. Clinical Safety Assessment

4.1. Adults

Despite an apparent low toxic potential, adverse events have been linked to propylene glycol exposure in patients when administered as an excipient with various medicinal products. A detailed analysis of the published clinical case studies and safety studies (retrospective or prospective) was performed in order to determine the doses and exposure levels at which these effects were observed. In addition, proposed monitoring tools and rescue therapies were taken into consideration.

These studies are summarised in Appendix 3.

4.1.1. Adult case studies

The potential for systemic toxicity secondary to propylene glycol has traditionally been considered to be low in adult patients; however several case reports associating propylene glycol with hyperosmolality, elevated anion gap metabolic acidosis, hemolysis, neurotoxicity, and acute renal insufficiency have challenged this assumption.

Case studies provide an overview of the different toxicities which were observed in clinical practice together with an evaluation of the dosing regimen responsible for the side effects. Nevertheless, several limitations have to be taken into account when interpreting the data:

- Most of the time the drugs products containing propylene glycol as an excipient, are administered to critically ill patients with co-medications. This hampers the identification of effects specifically due to propylene glycol.

- Neurologic assessment is often not possible because the drug products containing propylene glycol is intended to induce sedation (e.g. lorazepam).

- Most cases deal with IV continuous infusions, often of lorazepam. Propylene glycol side effects have been described over a wide range of cumulative lorazepam doses, serum propylene glycol concentrations, and infusion duration.

- Missing information may hamper the accuracy of the evaluation, such as:

  - Body weights (BW): by default males were considered to weigh 75 kg, females 60 kg, unless otherwise stated.

  - Complete data sets and timing of analyses (e.g. of serum propylene glycol concentration).

- Most of the reports provide the total propylene glycol load. The daily doses expressed in g/kg/day are mean values calculated over the treatment duration. As often treatments were titrated to reach an effect (e.g. sedation), higher doses may have been administered occasionally.

Summary of adult case studies stratified taking into account the severity of the observations.

References + Treatment	PG load	Exposure duration	PG dose (g/kg/day)	PG serum concentration (mg/dL)	Reversibility
No changes attributed to propylene glycol:					
Krakoff et al., 2001 [54] Etomidate	7-36 g/day	5.5 months	0.48	-	Improvement of renal function during treatment, no worsening of metabolic acidosis
Hyperosmolarity / Metabolic acidosis:					
Arbour and Esparis, 2000 [6] Lorazepam	540 g	5 days	1.44	78	Resolved within 3 days weaning off lorazepam
Cawley, 2001[14] Lorazepam	284 g	3 days	1.6	12	Resolved following weaning off lorazepam

Parker et al., 2002 [83] Lorazepam	3000 g	24 days	2	520	There is a threefold reduction in PG levels post-dialysis The osmol gap drops proportionally to PG concentration
Wilson et al., 2005 [115] Diazepam	970 g	5 days	2.6	108	Metabolic disorders resumed within 24h weaning off diazepam
Neale et al., 2005 [78] Lorazepam	5167 g	18 days	4.8	1100	Lactic acidosis tend to resume following weaning off lorazepam (day 18) The renal function remained normal as measured by BUN, serum creatinine, and urine output throughout hospitalisation until death on day 26
Tsao et al., 2008 [106] Lorazepam	398.4g	4 days	1.3	382	Resolved following weaning off lorazepam and hemodyalisis
Yan et al., 2010 [118] Lorazepam	498 g	5 days	1.7	250.4	Osmol gap and PG concentration decreased following weaning off lorazepam and hemodyalisis, but metabolic acidosis persisted
Hyperosmolarity / Metabolic acidosis / Renal impairment (increase in serum creatinine level):					
Reynolds and Teiken, 2000 [88] Lorazepam	50-169 g/day	Intermit-tent over 31 days	Up to 2.3	Calculated from osmol gap : 80.4	Resolved following weaning off lorazepam
Wilson et al., 2005 [115] Lorazepam	444 g	7 days	0.85	144	Resolved following weaning off lorazepam

Wilson et al., 2005 [115] Lorazepam	4565 g	25 days	2.4	Not measured	Resolved within 24h weaning off lorazepam
Wilson et al., 2005 [115] Lorazepam	899 g	7 days	1.7	Not measured	Tend to resolved following weaning off lorazepam
Miller and Forni, 2008 [69] Pentobarbital	173.7 g	24h	2.9	170	Metabolic disorders resumed following weaning off pentobarbital
Hyperosmolarity / Metabolic acidosis / Acute renal failure and/or Clinical deterioration:					
Wilson et al., 2000 [114] Diazepam	-	24h	-	1300	Resolved with one dialysis session
Hayman and Seidl, 2003 [40] Lorazepam + Trimethoprin-sulfamethoxazole	219 g + 304 g	6 days + 5 days	0.175* 0.292* *(BW: 208 kg)	30 (24h following weaning off trim/sulfa)	Death from respiratory failure 5 days following weaning off lorazepam
Wilson et al., 2005 [115] Lorazepam	1912 g	9 days	3.4	Not measured	Metabolic disorders tend to return to normal within 24h weaning off lorazepam
Zar et al., 2007 [125] Lorazepam	1699 g	7 days	3.2	810	Osmol gap improved following two hemodialysis sessions, and returned progressively to normal thereafter
Ganesh and Audu, 2008 [30] Etomidate	507.6 g	29h	7.1	580.9	Severe metabolic acidosis with hyperglycemia which resolved following weaning off etomidate
Bledsoe and Kramer, 2008 [12] Pentobarbital + phenobarbital	-	2 weeks	Not provided	55 (measured 24h following weaning off barbiturates)	Changes resume following weaning off barbiturates. Patient died several weeks later from septic shock

Zosel and Egelhoff, 2010 [126] Lorazepam (over dose during 10h)	500 g	10h	16	659	Following hemodialysis serum PG returned to 45 mg/dL. The patient died (family consent to extubation) from renal failure and hypoxic brain injury due to initial cardiac arrest.

From these data it may be concluded that early manifestation of propylene glycol toxicity includes hyperosmolarity and lactic acidosis, which may progress when dosage/exposure increases into hypotension, hyperglycemia, renal dysfunction detected by increase in serum creatinine level and decrease in serum bicarbonate level, acute renal failure consistent with proximal tubular necrosis, and finally severe clinical deterioration. With excessive doses, proximal tubular secretion decreases and renal clearance slows down [12]. Assessment of neurologic effects of propylene glycol is in most of the case not possible because of the underlying pathologies or the co-medications.

The appearance and severity of these manifestations seem to be related to the dose of propylene glycol administered both in term of mean daily dose but also of propylene glycol load, and to the exposure (serum propylene glycol concentration) and the treatment duration. It appears that manifestation of polypropylene toxicity generally occurs following the administration of mean daily doses close to or higher than 1 g/kg/day. Serious clinical deterioration seems to generally correspond to daily dosing higher than 3 g/kg/day. In one case report severe effects were seen in one patient at relatively low dose and exposure levels [40], but this patient was largely obese (208 kg BW) and the general status of health of this individual may have contributed to this exceptionally high sensitivity to propylene glycol.

From these data it is difficult to directly correlate exposure to propylene glycol to the severity of the effects. Nevertheless it seems that severe toxicity generally occurs at serum concentrations well above 500 mg/dL.

Finally, metabolic changes and renal dysfunction seem to respond well to weaning off propylene glycol and in more severe cases to hemodialysis.

4.1.2. Adults retrospective/prospective observational safety studies and reviews

Based upon the evidence that propylene glycol is susceptible to induce toxicity, retrospective or prospective observational studies and reviews were performed. Again most of the studies evaluated propylene glycol side effects following lorazepam continuous infusion. Indeed the American College of Critical Care Medicine (ACCM) published clinical practice guidelines [127] for the sustained use of sedatives and analgesics in the critically ill adults that recommend lorazepam as the drug of choice for both intermittent and long-term sedation (≥72 hours), with the cautionary statement that there is a potential for propylene glycol toxicity with prolonged high-dose lorazepam infusions (>18 mg/h). This explains the large number of publications on the subject.

Nelsen et al. [79] followed 50 critically ill patients given low doses of continuous infusion of lorazepam. These patients received a mean dose of propylene glycol of 0.280 g propylene glycol/kg/day. While hyperosmolality and serum propylene glycol concentrations higher than 25 mg/dL were noted, no

convincing evidence of propylene glycol - related toxicity particularly anion gap and metabolic acidosis or renal dysfunction were detected.

Arroliga et al. [7] and Barnes et al. [10] demonstrated that following short term infusion (< 50h) of propylene glycol at relatively high doses (up to 1.6 g/kg/day for Arroliga, and 1 g/kg/day for Barnes) no signs of renal impairments were detected based upon creatinine clearance or concentration, or increase incidence of renal failure.

Similarly, no signs of renal dysfunction were detected by Yahwak et al. [117] following 3 weeks continuous infusion of low dose propylene glycol (up to 0.078 g/kg/day) while following doses up to 0.62 g/kg/day for about 6 days increases in creatinine concentrations were observed in about one third of the patients. Regression analysis showed that serum propylene glycol concentrations >18 mg/dL would be associated with lorazepam doses >1.4 mg/kg/day (0.581 g propylene glycol/kg/day). The authors concluded that propylene glycol serum concentrations >18 mg/dL are associated with a higher risk of metabolic acidosis and renal dysfunction. This study supports the establishment of a lorazepam safety threshold dose of 1 mg lorazepam/kg/day (~0.415 g propylene glycol/kg/day).

Yaucher et al. [120] reviewed retrospectively the data collected on 128 critically ill patients receiving continuous infusion of lorazepam. Few (eight) patients whose serum creatinine concentrations increased progressively during lorazepam administration were identified. The median time to serum creatinine concentration rise was 9 days (3 to 60 days), following administration of 0.51 to 1.19 g/kg/day (mean cumulative propylene glycol dose per day). As no increase in serum creatinine levels were detected in patients following 24 to 48 h propylene glycol administration (similar daily doses) in the studies from Arroliga et al. [7] and Barnes et al. [10], these data suggest that the probability to observe signs of renal dysfunction increases with the treatment duration. These eight patients [120] also developed hyperosmolality and metabolic acidosis. Propylene glycol serum concentration measured at the peak of creatinine concentration ranged from 18.6 to 345 mg/dL. Serum creatinine concentration was back to normal within 3 days after lorazepam treatment cessation with one exception (unknown reason).

Wilson et al. [115] performed a prospective study on 21 patients given propylene glycol - containing benzodiazepines. They identified 4 cases of propylene glycol metabolic changes. The data suggest that propylene glycol toxicity has a spectrum of severity ranging from common metabolic abnormalities to infrequent clinical deterioration. The threshold level beyond which propylene glycol accumulation is detrimental is unknown.

- In subjects with clinical deterioration due to propylene glycol toxicity, propylene glycol levels ranged from 104 to 144 mg/dL.

- In contrast, in subjects with only metabolic abnormalities due to propylene glycol toxicity, serum propylene glycol levels ranged from 58 to 127 mg/dL.

This suggests that higher concentrations of propylene glycol are more likely to be associated with clinical deterioration, although overlap exists.

Similar results were obtained by Levy et al. [63] who followed seven patients (14 to 68-year-old) with traumatic or ischemic elevation of intracranial pressure given etomidate or pentobarbital continuous infusions. Three received etomidate continuous infusion for 24 to 72 hours, four received Pentobarbital. The patients treated with etomidate were given about 0.7 to 1.2 g of propylene glycol/kg/day, more than 10 times the amount received by the patients treated with pentobarbital. All patients in the Etomidate group showed hyperosmolality, metabolic acidosis, and renal impairment (Low creatinine

clearance ~41 mL/min, and increases in BUN and creatinine). Renal impairment resolved following etomidate discontinuation.

Interestingly, a different toxicity profile was observed when etomidate was administered as bolus injection. Doenicke et al. [21, 22] demonstrated in patients or healthy volunteers that bolus administrations of etomidate (over 30 s) corresponding to the administration of 54.5 mg of propylene glycol per kg of BW are susceptible to induce hemolysis, pain, phlebitis, and histamine release. In comparison, a patient given 1 g of Propylene glycol per kg of BW per day with lorazepam continuous IV infusion would receive 0.347 mg of propylene glycol per kg of BW over a 30 s period. These effects were not seen with etomidate formulated in a lipid emulsion. The authors conclude that etomidate formulated in propylene glycol may induce direct injury to the vascular endothelium attributed to local hyperosmolality and resulting in pain and venous sequelae, whereas etomidate in lipid emulsion does not. There was no relationship between pain or venous sequelae and histamine release.

Zar et al. [125] concluded following a thorough literature review that detection of propylene glycol toxicity could be based upon the onset of metabolic acidosis, hyperosmolarity, and increase in the osmolar gap 48 hours after the start of propylene glycol administration. Treatment would consist in discontinuation of propylene glycol administration, and intermittent hemodyalisis if needed. As others, the authors suggest to limit propylene glycol administration to 1g/kg/day, a dose that would be reached when administering the maximum recommended dose lorazepam (0.1 mg/kg/h). Close monitoring of patients given high doses lorazepam (>10 mg/h) is recommended. This dose of lorazepam would correspond to the administration of 1.7 g of propylene glycol/kg/day for a patient weighing 60 kg of BW or 1.3 g/kg/day for a patient weighing 75 kg.

The review by Kraut et al. [55] brought forward the same conclusions.

In 2011, Kraut et al. [56] discussed the relationship between osmolar gap and anion gap following administration of toxic alcohols. According to these authors, accumulation of the parent unmetabolised toxic alcohol accounts for the increase in serum osmolality, but toxicity of these alcohols is largely due to accumulation of their metabolites. The evolution of changes in serum osmolality with toxic alcohols exposure therefore is dependent primarily on the rate of decrease in concentration of the parent alcohol. If the patient is observed early after exposure to the alcohol, serum osmolality might be elevated, whereas serum anion gap might be unchanged. Thereafter anion gap might increase whilst osmol gap decreases. As increased osmol gap might result from pathophysiological changes (kidney disease, ketoacidosis, etc.), it has been suggested that serum osmol gap > 20 mOsm/kg is indicative of toxic alcohol exposure.

4.2. Potentially sensitive populations

4.2.1. Individuals with compromised liver or kidney function

Theoretically patients with impaired liver or renal function could be more susceptible to propylene glycol accumulation in serum and consequently to toxicity. This is particularly true for neonates and young babies with both low renal and metabolic clearances.

Nevertheless in studies where adult patients with renal dysfunction were enrolled, propylene glycol concentrations did not differ between patients with normal or impaired renal function. This seems to indicate that metabolic clearance may compensate for impaired renal excretion.

4.2.2. Children

Intoxications due to propylene glycol have also been described in children particularly in pre-term and term neonates and in infants. As previously described, decreased size and increased elimination half-life predispose the youngest children to a greater probability of toxic effects from propylene glycol accumulation.

Intoxications have been observed following oral accidental ingestion, oral/iv administration, or dermal absorption following application on burned skin or diaper rashes.

Toxic effects similar to those observed in adults were noted (i.e. hyperosmolality, metabolic acidosis, renal impairment) which resolved following weaning off propylene glycol. In a 9-year-old patient given etomidate by IV infusion [107], these symptoms were recorded with propylene glycol serum concentration of 230 mg/dL measured 4h following infusion interruption.

Additional symptoms were recorded in some cases, indicative that propylene glycol may also affect the gastrointestinal, respiratory, or central nervous systems:

- Frequent watery stools were observed in a 9-month old patient [67] given lorazepam by oral route (about 3 g propylene glycol/kg/day). This effect was attributed to high enteral osmotic load.

- Treatment resistant seizures [8] were induced following long term oral administration of dihydrotachysterol in a solution of alcohol and propylene glycol. Exacerbation of fever induced seizures was detected in a 16-year-old boy [121] given pentobarbital/phenobarbital infusions containing propylene glycol. Acute renal failure was also detected in this patient, and a renal biopsy showed alterations of the proximal tubular epithelial cells (vacuolation, swollen mitochondria, debris in lysosomes attributed to osmotic nephrosis). This child was given up to ~1.5 g propylene glycol/kg/day.

- Lethargy was observed in infants and preterm neonates following oral or dermal ingestion of propylene glycol [33, 36, 84].

- Apnea or superficial respiration was noted in the same lethargic patients.

- An additional case of cardiorespiratory arrest in an 8-month old infant treated with silver sulfadiazine in propylene glycol for burns was described in the NTP-CERHR Monograph [29]. An elevated serum propylene glycol concentration (369 mg/dL) was temporally related to the cardiorespiratory arrest and may have been causally related, through production of cardiac arrhythmia, CNS depression, or synergism with a second CNS depressant (diphenhydramine). A peak serum level of 1,059 mg/dL was measure in this patient.

The evidence that propylene glycol is susceptible to induce toxicity when administered as excipient in different types of medications has triggered the interest particularly of pediatricians in charge of neonatalogy units.

Shehab [95] and Whittaker [112] used retrospective medical record reviews to describe the sources of propylene glycol exposure (oral or IV routes of administration) and to calculate cumulative intravenous propylene glycol exposure. Both studies pointed out daily loads of propylene glycol close to or higher than the WHO recommendation (25 mg/kg/day). In patients who received propylene glycol by continuous infusion, a median cumulative daily dose of 4.6 g/kg/day was found to be approximately 180 times this recommended maximum daily intake. The lack of outcome data limits the possibility to draw conclusions as to the clinical impact of such high propylene glycol exposure. Nevertheless, these data confirm that hospitalised neonates may be given propylene glycol daily doses which exceed the doses above which toxicity has been reported in infants.

Several studies evaluated the correlation between propylene glycol intake, propylene glycol serum concentration, elimination half-life and related toxic effects in neonates administered different multivitamine solutions administered with parenteral nutrition.

Glasgow et al. [32] studied 10 children weighing 1 to 4.5 kg, given 10mL of MVI12 multivitamin solution per day containing 3 g propylene glycol. No clinical problems could be ascribed to propylene glycol, but the study was initiated because of a case of propylene glycol intoxication in 890g preterm neonate (27 week of gestation) who had unexplained acute renal failure following this parenteral nutrition. Serum osmolality and propylene glycol concentration up to 407 mOsm/kg and 930 mg/dL were recorded respectively.

MacDonald et al. [65] compared then the effects of the administration of three different multivitamin solutions MVI12 (3 g propylene glycol/day), MVI (300 mg propylene glycol/day), or a solution free of propylene glycol.

There was no evidence of increased incidence of hepatic or renal toxicity, hematuria, or apnea using either formulation but a higher incidence of patient with seizures was observed with patients given MVI12 compared to those given MVI (33 vs 11%).

Allegaert et al. [3] collected data from 69 neonates given paracetamol formulated in propylene glycol which were compared to data from patients given paracetamol formulated with mannitol (n=149, historical controls). No short term biochemical impact was detected during or following a median propylene glycol exposure of 34 mg/kg/24 h (ranges 14 to 252). Exposure to propylene glycol seemed well tolerated and did not affect normal postnatal maturational changes in renal, metabolic and hepatic functions. De Cock et al. [18] built a pharmacokinetic model that showed that for these commonly used dosing regimens, the population mean propylene glycol peak and trough concentrations range between 3.3–14.4 and 2.8–21.8 mg/dL (peak) and 1.9–10.9 and 0.6–11.2 mg/dL (trough) for paracetamol and phenobarbital formulations, respectively, depending on birth weight and age of the neonates.

More recently Agenerase® (amprenavir) received a contraindication for infants and children below 4 years of age because of its content in propylene glycol exceeding the recommended WHO-limit of 25 mg/kg/day.

FDA issued a warning for lopinavir/ritonavir, (Kaletra®, Abbott Laboratories, IL, United States) oral suspension containing ethanol (42.4%, v/v) and propylene glycol (15.3%, w/v) because of safety issues (cardiac, renal and respiratory) attributed to its content in propylene glycol and ethanol, both substrates of alcohol dehydrogenase. The review of the adverse event reporting system database identified 10 cases of intoxication attributed to Kaletra® administration. All cases occurred in neonates (8 born prematurely). Eight of them had treatment started the day of birth or the day after birth. They received between 76.4 and 117.5 mg/kg/day of propylene glycol. One baby received once 10X the expected dose (451.2 mg/kg) on post-natal day 34 and died. One last case has not been clarified. This review resulted (see section III: pharmacokinetics, point 4: excretion) in the recommendation to avoid this formulation before both the postmenstrual age of 42 weeks and/or postnatal age of 2 weeks has been attained (www.fda.gov, accessed 01.08.11). It is not possible to extrapolate directly to the administration of propylene glycol only, as the amount of ethanol was higher, but the affinity of alcohol dehydrogenase is higher for ethanol compare to propylene glycol. However, it illustrates that a cautionary approach is justified.

4.2.3. Elderly

No data were found associating propylene glycol toxicity in the elderly population to specific exposure levels.

4.3. Local tolerance/irritation/sensitisation

Several publications [4, 59, 41, 53, 62, 109] pointed out the weak irritation and sensitisation potential of propylene glycol.

4.4. Summary of clinical safety assessment

Propylene glycol toxicity profile was established mainly on the basis of the effects induced by IV infusion of lorazepam, benzodiazepine, or etomidate in both critically ill adults and children. In addition propylene glycol safety profile was evaluated following paracetamol IV administration to neonates. These studies showed that hyperosmolality and metabolic acidosis, then renal dysfunction and finally acute renal failure and clinical deterioration are the major effects of increasing doses and serum concentrations of propylene glycol.

In addition several case reports of intoxication pointed out the potential CNS (lethargy, coma, seizures) or local vascular affects (including hemolysis) most probably associated to higher plasma levels of propylene glycol and hyperosmolality following acute toxicity.

4.4.1. In adults

The review of the case reports suggests that manifestation of propylene toxicity generally occurs following the administration of mean daily doses close to or higher than 1 g propylene glycol/kg of BW/day. Serious clinical deterioration seems to generally correspond to daily dosing higher than 3 g/kg/day. From these data it is difficult to directly correlate exposure to propylene glycol to the severity of the effects. Nevertheless it seems that severe toxicity generally occurs at serum concentrations well above 500 mg/dL.

Data collected by Arroliga et al. [7] and Barnes et al. [10] following short term infusion (<50h) of lorazepam confirmed that no signs of renal impairments could be detected at doses of propylene glycol up to 1.6 g/kg/day for Arroliga, and 1 g/kg/day for Barnes).

Nevertheless, Yaucher et al. [120] showed that during longer term infusions the probability to observe signs of renal dysfunction increases with the treatment duration following administration of 0.51 to 1.19 g/kg/day (mean cumulative propylene glycol dose per day). These patients also developed hyperosmolality and metabolic acidosis. Propylene glycol serum concentration measured at the peak of creatinine concentration ranged from 18.6 to 345 mg/dL. Serum creatinine concentration was back to normal within 3 days after lorazepam treatment cessation with one exception (unknown reason).

Similarly, signs of renal dysfunction were detected by Yahwak et al. [117] following 3 weeks continuous infusion of propylene glycol up to 0.62 g/kg/day. Regression analysis showed that serum propylene glycol concentrations >18 mg/dL would be associated with lorazepam doses >1.4 mg/kg/day (0.581 g propylene glycol/kg/day). This study supports the establishment of a lorazepam safety threshold dose of 1 mg lorazepam/kg/day (~0.415 g propylene glycol/kg/day).

Of note, Wilson et al. [115] suggested that propylene glycol toxicity has a spectrum of severity ranging from common metabolic abnormalities to infrequent clinical deterioration. The threshold level beyond which propylene glycol accumulation is detrimental is unknown.

- In subjects with clinical deterioration due to propylene glycol toxicity, propylene glycol levels ranged from 104 to 144 mg/dL.

- In contrast, in subjects with only metabolic abnormalities due to propylene glycol toxicity, serum propylene glycol levels ranged from 58 to 127 mg/dL.

Zar et al. [125] concluded following a thorough literature review that detection of propylene glycol toxicity could be based upon the onset of metabolic acidosis, hyperosmolarity, and increase in the osmolar gap 48 h after the start of propylene glycol administration. Kraut et al. [56] suggested that serum osmolar gap >20 mOsm/kg is indicative of toxic exposure to propylene glycol.

Importantly, metabolic changes and renal dysfunction seem to respond well to weaning off propylene glycol and in more severe cases to hemodialysis.

Finally, Doenicke et al. [21, 22] demonstrated in patients or healthy volunteers that bolus administrations of etomidate (over 30 s) corresponding to the administration of 54.5 mg of propylene glycol per kg of BW are susceptible to induce hemolysis, pain, local phlebitis, and histamine release.

4.4.2. In children

Shehab [95] and Whittaker [112] reviewed data that confirm that hospitalised neonates may be given propylene glycol daily doses which exceed the doses above which toxicity has been reported in infants.

Glasgow et al. [32] and MacDonald et al. [65] demonstrated that parenteral administration of 300 mg/kg of propylene glycol in a multivitamin solution to children weighing 1.5 to 4 kg was safe, while 3 g/kg induced seizures.

Similarly, Allegaert et al. [3] demonstrated that no short term biochemical impact was detected during or following a median propylene glycol exposure of 34 mg/kg/24 h (range 14–252). Exposure to propylene glycol seemed well tolerated and did not affect normal postnatal maturational changes in renal, metabolic and hepatic functions. De Cock et al. [19] built a pharmacokinetic model that showed that for these commonly used dosing regimens, the population means propylene glycol peak concentrations range between 2.8–21.8 mg /dL depending on birth weight and age of the neonates.

Additional symptoms were recorded in some intoxication cases, indicative that propylene glycol may also affect the gastrointestinal, respiratory, or central nervous systems. For example seizures and renal failure were detected in a 16-year-old boy given ~1.5 g polypropylene glycol/kg/day [121], or cardiorespiratory arrest was observed in an 8-month old boy with 369 mg/dL propylene glycol in serum [29].

Theoretically liver and/or renal impairment are susceptible to induce accumulation and increased risk of toxicity. But this particularly critical in the case of neonates, and even more importantly in case of competitive inhibition of metabolic clearance (e.g. when another aldehyde dehydrogenase substrate is coadministered) in neonates with very low renal excretion (see Kaletra®) due to renal immaturity.

Finally propylene glycol was considered to have low irritation and sensitizing properties.

Discussion, safety limits

This literature review confirms that propylene glycol may generally be considered as a safe ingredient in pharmaceutical preparations even though side effects are expected to occur in case of over dosing or of sensitive populations such as very young children (neonates) or patients with impaired renal or metabolic clearances. Its pharmacokinetics and toxicity profiles are well defined and are consistent between human beings and the different animal species usually used in drug development (mouse, rat,

monkey, dog) supporting the clinical relevance of their use in the assessment of the safety profile of propylene glycol.

Absorption by oral route is rapid and nearly complete in all species. Pharmacokinetic parameters are generally considered similar whenever propylene glycol is administered by oral, intravenous or intraperitoneal route. Absorption by the rectal route or by inhalation is also important. Absorption through intact skin is negligible but may be increased in case of skin abrasion. The same dose limits are proposed in terms of mg/kg of body weight for all these routes of administration with the exception of inhalation because of potential local irritation side effects. In this particular case not enough data are available to define safety limits.

Propylene glycol is widely distributed to the aqueous compartment including to the brain (CSF) and via the placenta to the fetus.

The major metabolic pathway in mammals is considered to be propylene glycol oxidation by alcohol dehydrogenase to lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is further metabolised to pyruvate, carbon dioxide, and water. Lactate also contributes to glucose formation through gluconeogenic pathways.

This metabolic clearance is saturable in all species but occurs at lower doses in humans than in rats and rabbits. Propylene glycol is also excreted unchanged or conjugated with glucuronic acid and eliminated into urine.

In adult patients metabolic and renal clearances account equally for about 50% of total clearance and each may compensate for the other in case of impairment of one of the elimination pathways. This is not true in very young children (preterm and term neonates) where renal elimination accounts for only about 15% of total clearance.

In animal species as well as in human it can be assumed that propylene glycol toxicity can, in principle, result from different mechanisms. These include its direct effects on the central nervous system as an alcohol particularly in the case of parenteral bolus administration (similar to ethanol, but about 3 times less toxic, [61]), its effects on the kidneys, and the effects of its acidic metabolites on acid-base homeostasis. Hyperosmolality aggravated by accumulation (e.g. following continuous or repeated administration particularly in patients with lower metabolic and/or renal clearances and in preterm/term neonates) is also to be taken into consideration. Propylene glycol may induce local intolerance such as vascular injection site reactions in case of IV bolus or airways irritation when administered by inhalation.

Permitted daily exposures (PDE)

A very comprehensive set of non-clinical data were reviewed and allow the determination of no-adverse-effect-levels (NOAEL) and subsequently of a permitted daily dose.

PDEs of 50 mg/kg and 1 mg/kg were derived for adults and children below five years of age, respectively. These PDEs represent very conservative safety limits based upon non-clinical data. Therefore, when the use of propylene glycol as pharmaceutical excipient is below these PDEs (respectively), there is no need for a specific information in the package leaflet.

Safety limits

However, at the present time the published clinical data demonstrate that higher propylene glycol load may be safely administered to patients particularly to children above 4 years of age and adults. A more cautious approach is still recommended for patients below 5 years of age because of the paucity of clinical data in this population.

Adult patients and children ≥ 5 years of age:

Papers from Yaucher et al. [120] and Yahwak et al. [117] indicate that doses up to 500 mg of propylene glycol/kg/day could be administered safely to adult patients even for long term periods.

Children < 5 years of age

In children below 5 years of age a dose limit of 50 mg/kg for which no effects are expected is being proposed based upon the following data:

- Allegaert et al. [3] demonstrated that no short term biochemical impact was detected during or following a median propylene glycol exposure of 34 mg/kg/24 h (range 14–252). Exposure to propylene glycol seemed well tolerated and did not affect normal postnatal maturational changes in renal, metabolic and hepatic functions.

- The human equivalent dose to the NOAEL of 1000mg/kg in the juvenile mouse was calculated to be 192 mg/kg for a neonate (3.5 kg), 150 mg/kg for a 1-year-old child (9 kg), and 126 mg/kg for a 4 year old child (15 kg)[1]. The proposed dose limit of 50 mg/kg is still 2.5 times lower.

- Model-based simulated concentration-time profiles of propylene glycol in a term neonate (birth weight 3500 g) following the administration of 34 mg propylene glycol/kg/day in paracetamol, did not show accumulation (no increase in propylene serum concentration following repeated administration). Therefore the risk of accumulation may be considered limited above the age of 1 month in children administered 50 mg propylene glycol/kg with non-impaired liver and/or renal functions. This is confirmed by data from Chicella et al. [15].

Nevertheless this is not applicable for **children below 1 month of age**. In (pre)term neonates De Cock et al. [19] have demonstrated that total body clearance is very low compare to the adult clearance [122], but also that the contribution of renal clearance to the total body clearance is very low. The results of this study [18] may indicate that due to maturational changes, some drug/drug metabolic interactions are more relevant for this specific population. This may explain the toxicities observed in neonates given Kaletra® which contains 356.3 mg ethanol/mL and 152.7 mg propylene glycol/mL.

Considering also the data produced by Shehab [95] and Whittaker [112] showing the multiple sources of propylene glycol and ethanol in neonatology units, it is proposed to restrict the safety limit for no effects to 1 mg/kg in preterm neonates below 44 weeks of post menstrual age, or below one month post-natal age for term neonates.

Esters:

No systematic review has been performed for the esters of PG. According to the literature, there is evidence that the propylene glycol esters of fatty acids are hydrolysed to propylene glycol and fatty acids. Toxicological evaluation can be based on the content of propylene glycol, for which an acceptable daily intake has been established [47]. It is therefore considered that the package leaflet information on propylene glycol applies also to its esters.

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[1] Using the Haycock formula BSA (m²) = 0.024265 x Height(cm)$^{0.3964}$ x Weight(kg)$^{0.5378}$

Conclusions

The following limits, expressed in terms of maximum daily dose, are considered to be safe and with no noticeable effects whatever the duration and the route of administration, with the exception of inhalation. Special attention will have to be taken to avoid hyperosmolality, CNS, cardiovascular, and/or respiratory effects during bolus parenteral administration.

Age group	neonates up to 28 days (or 44 weeks post menstrual age for pre-terms)	1 month (29 days) up to 4 years	5 years up to 17 years and adults
Safety limits	1 mg/kg	50 mg/kg	500 mg/kg

As a general remark, it should be kept in mind that higher doses may be administered, when justified on a case by case basis in order to support the safety of the proposed formulation.

The justification should consider:

- Non-clinical investigations on the potential drug/drug interactions between propylene glycol and the active substance tested with special attention to CNS toxicity, reversibility, and toxicokinetics, especially when products are intended to be used in children < 5 years.

- Pivotal clinical studies using the final formulation where signs of hyperosmolality, metabolic acidosis, and/or renal failure need to be monitored. Measurements include but are not limited to:

  – Propylene glycol serum concentration

  – Osmolality

  – Osmolar gap

  – Serum creatinine concentration, creatinine clearance

  – Serum lactate concentration

  – Serum bicarbonate concentration

5. Updated information for the package leaflet

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Propylene glycol and esters of propylene glycol	All routes of administration	1 mg/kg/day	This medicine contains x mg propylene glycol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	
	Oral, parenteral	1 mg/kg/day	If your baby is less than 4 weeks old, talk to your doctor or pharmacist before giving them this medicine, in particular if the baby is given other medicines that contain propylene glycol or alcohol.	Co-administration with any substrate for alcohol dehydrogenase such as ethanol may induce serious adverse effects in neonates.
		50 mg/kg/day	If your child is less than 5 years old, talk to your doctor or pharmacist before giving them this medicine, in particular if they use other medicines that contain propylene glycol or alcohol.	Co-administration with any substrate for alcohol dehydrogenase such as ethanol may induce adverse effects in children less than 5 years old.
			If you are pregnant or breast-feeding, do not take this medicine unless recommended by your doctor. Your doctor may carry out extra checks while you are taking this medicine.	While propylene glycol has not been shown to cause reproductive or developmental toxicity in animals or humans, it may reach the foetus and was found in milk. As a consequence, administration of propylene glycol to pregnant or lactating patients should be considered on a case by case basis.
			If you suffer from a liver or kidney disease, do not take this medicine unless recommended by your doctor. Your doctor	Medical monitoring is required in patients with impaired renal or hepatic functions because various adverse events attributed to propylene

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
			may carry out extra checks while you are taking this medicine.	glycol have been reported such as renal dysfunction (acute tubular necrosis), acute renal failure and liver dysfunction.
		500 mg/kg/day	Propylene glycol in this medicine can have the same effects as drinking alcohol and increase the likelihood of side effects. Do not use this medicine in children less than 5 years old. Use this medicine only if recommended by a doctor. Your doctor may carry out extra checks while you are taking this medicine.	Various adverse events, such as hyperosmolality, lactic acidosis; renal dysfunction (acute tubular necrosis), acute renal failure; cardiotoxicity (arrhythmia, hypotension); central nervous system disorders (depression, coma, seizures); respiratory depression, dyspnoea; liver dysfunction; haemolytic reaction (intravascular haemolysis) and haemoglobinuria; or multisystem organ dysfunction, have been reported with high doses or prolonged use of propylene glycol. Therefore doses higher than 500 mg/kg/day may be administered in children > 5 years old but will have to be considered case by case. Adverse events usually reverse following weaning off of propylene glycol, and in more severe cases following hemodialysis. Medical monitoring is required.
	Cutaneous	50 mg/kg/day	Propylene glycol may cause skin irritation. Do not use this medicine in babies less than 4 weeks old with open wounds or large areas of broken or damaged skin (such as burns)	

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
			without talking to your doctor or pharmacist.	
		500 mg/kg/day	Propylene glycol may cause skin irritation. Because this medicine contains propylene glycol, do not use it on open wounds or large areas of broken or damaged skin (such as burns) without checking with your doctor or pharmacist.	

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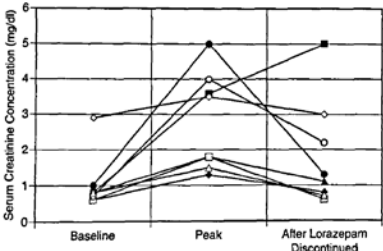
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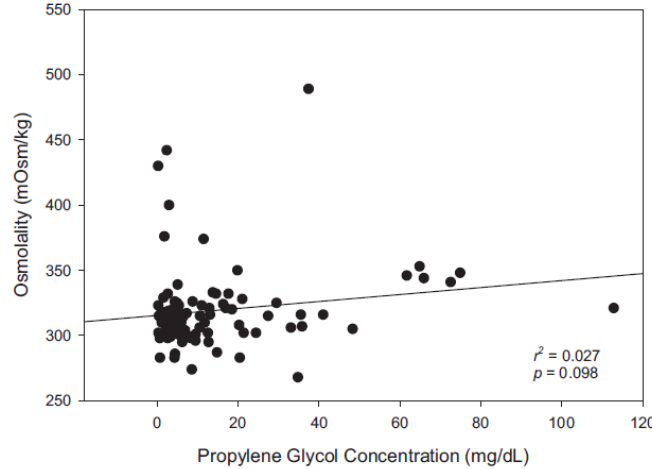
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Appendix 1: Tabulated summaries of clinical pharmacokinetic studies

References	Patients (age, weight, n)	Treatment (drug, dose, duration)	Route	Propylene glycol (PG) dose	Major findings
Clinical studies: adults					
Parker et al, 2002 [83]	34 y female with adult respiratory distress syndrome complicating varicella pneumonia	Lorazepam infusion (up to 24 mg/h) up to dialysis	IV	24-days cumulative PG dose: 2890 mL (3000 g over 24 days, 2 g/kg/day, BW 60 kg)	Dialysis performed because patient had increased anion and osmol gaps, and lactic acidosis Before — 2 h — 12 h Dialysis — post-d — post-d Osmol gap — 97 — 27 — 21 PG (mg/dL) — 520 — 170 — No data There is a threefold reduction in propylene glycol levels post-dialysis The osmol gap drops proportionally to propylene glycol concentration
Yaucher et al, 2003 [120]	Restrospective analysis of 128 patients given continuous infusion of lorazepam: - 8 of these critically ill patients showed increase in	Patients develop serum creatinine increase during lorazepam continuous infusion (solution of 2 or 4 mg/mL) titrated to achieve sedation (range: 2–28 mg/h at time of peak creatinine	IV	Mean cumulative PG dose per day: 36 to 83g/day (0.51 to 1.19 mg/kg/day, BW: 70 kg)	Of 128 patients given lorazepam, 8 showed increase in serum creatinine (up to 5 mg/dL in one patient) following 3 to 60 days lorazepam infusion Figure 1. Changes in serum creatinine concentration in response to lorazepam infusion in the eight patients.
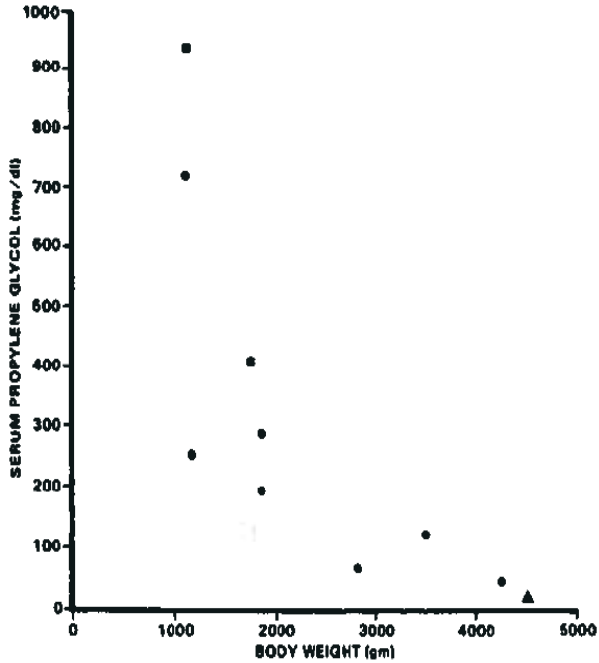
	serum creatinine, - patients with with respiratory failure requiring mechanical ventilation, 29 to 77 y of age, - 2 patients with impaired liver function, one with impaired renal function	concentration) for 7 to 75 days			Serum creatinine concentration back to normal 3 days after lorazepam treatment cessation with one exception (unknown reason) Propylene glycol concentration at peak creatinine level: 18.6 to 345 mg/dL 8/8 patients: hyperosmolarity and metabolic acidosis 7/8 patients: elevated osmol gap (>10, 12 to 64) Weak to moderate correlation between creatinine concentration rise and propylene glycol concentration. Propylene glycol concentration strongly correlated to serum osmolality and osmol gap and had a weak correlation with anion gap. Creatinine increase thought to result from renal tubular injury.
Arroliga et al, 2004 [7]	9 critically ill patients 20–62 years 73–130 kg Mechanical ventilation No exclusion based upon renal or hepatic insufficiency.	Lorazepam, Titration up to: • High dose of ≥10 mg/h for at least 48h (mean of 50h) before blood sampling • Mean rate of infusion during high infusion rate period: 0.16 mg lorazepam/kg/hr	IV continuous infusion	Cumulative PG dose: • Total: 5.1 g/kg/4.9 days • During high dose: 3.3 g /kg/50h (~1.6 g/kg/day)	Propylene glycol plasma concentration following 50h high dose infusion: 94 to 350 mg/dL (mean: 199.6). • A significant correlation was observed between cumulative high dose propylene glycol infusion and propylene glycol serum concentration but not between total cumulative dose and propylene glycol serum concentration. • Osmol gap at 50h: 24.3-67.1 (mean: 48.0) • Osmol gap strongly correlates with propylene glycol concentration (r^2= 0.804, p= 0.001) • Predicted propylene glycol concentration from osmol gap: (- 82.1 + [osmol gap X 6.5]) • In creased anion gap (poor indicator of propylene glycol accumulation) Hyperosmolarity and metabolic acidosis were observed

		• Mean duration of total lorazepam infusion: 4.9 days			• No renal toxicity (no effects on creatinine, creatinine clearance) • Significant risk of propylene glycol toxicity may be associated with continuous infusion of ≥10 mg lorazepam/h
Barnes et al., 2006 [10]	65 intensive care unit adult patients (n = controls: 25, LD:26, HD:14) Patients with mechanical ventilation Exclusion criteria: renal failure requiring dialysis, condition known to be associated with high osmolarity	Lorazepam: Controls: no PG Low dose (LD): 2-5.99 mg/h High dose (HD): ≥6 mg/h For at least 36h before blood sampling (to allow steady state PG exposure)	IV continuous infusion	Cumulative over 36h before blood sampling (g): • Controls: 0 • LD: 53 • HD: 118 (79 g/day, or 1 g/kg/day, BW: 75 kg)	Serum propylene glycol concentrations(mg/dL): • Controls: undetectable • LD: 14 $\pm$ 16 • HD: 39 $\pm$ 20 Propylene glycol concentration predicted by: • Osmol gap (control: 3.4, LD: 4.2, HD: 11.7 respectively) • Amount of propylene glycol administered during the previous 36h • Propylene glycol concentration = 1.40 + (1.182 x osmol gap) + (2.388E-04 x mg of propylene glycol administered in the 36h before serum sampling) • Propylene glycol concentration = 14.221 + (1.937 x osmol gap) Propylene glycol concentration not predicted by: • Anion Gap No relationship was established between propylene glycol concentration and lactate concentration or renal dysfunction (hepatotoxicity was not assessed) based upon creatinine concentration and incidence of renal failure between groups.
Nelsen et al, 2008 [79]	50 critically ill patients (13–88 years) No specific exclusions from	Lorazepam: 2.1 (0.5–18.0) mg/h for 137 h (41–475 h)	IV continuous infusion	Cumulative over the whole period: 128 (17–725) g	Serum propylene glycol concentrations (mg/dL): 5.0 (0.2–212.2) Osmolality: 314 (268 – 489) mOsm/kg Osmol gap: 12.5 (-23 - 194)

	study participation	Blood samplings: • 1st: after 40 to 380 h infusion • Max 5 daily samples		Mean: 871.5 mg PG/h Or 21 g PG/day, (280 mg/kg/day BW: 75kg)	8 patients (16%) had significant propylene glycol accumulation (>25 mg/dL) PG concentration ≥25 mg/dL corresponds to significantly higher infusion rates; [6.4(1.9-11.3) versus 2.0 (0.5-7.4) mg/h (P =.0003)] and had a more elevated % of high anion gap values BUT: osmolality, osmol gap, and lactate did not correlate with serum PG concentrations (measured at different times) No convincing evidence of propylene glycol-related toxicity particularly anion gap and metabolic acidosis or renal dysfunction. No apparent relationship between plasma propylene glycol conc. and changes in renal function (Seven patients (16%) had a significant increase, >25%, in serum creatinine, and 12 patients (27%) had a significant decrease, ≥25%, in serum creatinine from baseline).
Horinek et al,	33 critically ill	Lorazepam:	IV	PG doses:	Serum concentration ≥ 25 mg/dL are expected to be toxic (<literature),

2009 [42]	patients Patients were NOT excluded for renal or hepatic impairment Patients were distributed in two groups: • With accumulation (PG > 25 mg/dL) • Without accumulation (PG < 25 mg/dL)	variable infusion rate according to the Sedation Agitation Score: • No acc.: 48 mg/24h* • Acc: 268.5 mg/24h* Blood samplings: • 1st: after 24-48h infusion • Every 3-5 days after *before blood sampling	continuous infusion	• No acc: 23.1 g/day • Acc: 65.7 g/day	Osmol gap not measured in this study Accumulation if: serum concentration ≥ 25 mg/dL (WHO) 42% of patients showed accumulation: • Propylene glycol serum concentration (130.8 vs 9.1 mg/dL, with acc. vs without acc. respectively) Serum propylene glycol concentrations associated to: • Lorazepam infusion rate • Previous 24h cumulative dose Lack of correlation between propylene glycol concentration and renal or hepatic dysfunction Indeterminate propylene glycol-associated adverse effects (renal, hepatic, respiratory)
Clinical studies: children					
Tran et al, 2007 [105]	Liver from perinatal infants (necropsies)	-	In vitro	-	The mean alcohol dehydrogenase content in liver from perinatal infants is approximately 10-fold lower than in adults (0.11 g/kg versus 1.00 g/kg liver wet weight, respectively)
Bekeris et al., 1979 [11]	14-year and 39-year old patients with severe burns	Topical application of silver sulfadiazine	Dermal application on burned skin		Increase in osmolality and osmol gap attributed to propylene glycol Serum propylene glycol concentrations: 760 and 340 mg/dL for patients 1 and 2 respectively
Peleg et al.,	Premature	Gauze dressing	Dermal	-	On D4 of life, the baby became lethargic and apneic, requiring reinitiation of

1998 [84]	infant : (gestation week 29, BW 1200g)	of nitrofurazone 0.2% dissolved in PG 96.8%	application on burned skin		mechanical ventilation. Metabolic acidosis preceded coma which resolved within hours of cessation of topical treatment. High peak of propylene glycol was measured in urines.
Willis et al., 2013 [113]	3-year-old male with burns covering approximately 60% TBSA.	Topical application of silver sulfadiazine	Dermal application on burned skin	-	In creased osmolality and urine output, osmol gap of 56 mOsm/kg, cyclic increases in serum lactate and doubling of serum creatinine concentration. Propylene glycol toxicity evidenced from day 47 of treatment, possibly related to decrease in renal function (concommitent increased creatinine concentration) Within 24h of the cessation of the sulfadiazine treatment the lactic acidosis and osmolar gap resolved.
Glasgow et al. 1983 [32]	Infants in nursery (n=10, BW: 1 to 4.5 kg)	10mL multivitamin preparation (MVI-12, 30% PG) /day For at least 5 consecutive days immediately before blood sampling	In parenteral nutrition	3g/day for at least 5 days	Direct correlation between propylene glycol serum concentration and osmol gap The highest osmolalities were found in the smallest babies Estimated elimination half-life: 19.3 h (10.8 to 30.5 h)

					Squares and triangle indicate patients receiving additional sources of propylene glycol. No clinical problems could be ascribed to propylene glycol outside of hyperosmolality but the study was initiated because of a case of propylene glycol intoxication in 890 g preterm neonate (27 week of gestation) who had unexplained acute renal failure following this parenteral nutrition. Serum osmolality and propylene glycol concentration up to 407 mOsm/kg and 930 mg/dL were recorded respectively in this patient.
MacDonald et al., 1987 [65]	Retrospective analysis Neonates (≤	Multivitamine solution in parenteral	IV	• MVI: 300 mg/day	• No evidence of increased incidence of hepatic or renal toxicity, hematuria, or apnea during period B.

	1500 g at birth) • 78 given MVI concentrate (period A) • 49 given MVI-12 (period B)	nutrition		• MVI-12: 3 g/day	• Higher incidence of patient with seizures in period B (33 vs 11%). Increased incidence in intraventricular hemorrhage, most probably related to mode of detection changes
MacDonald et al., 1987 [64]	Retrospective analysis Neonates (≤ 1500 g at birth) • 30 given MVI concen-trate (PG) • 30 given MVI-pediatric (Mannitol)	Multivitamine solution in parenteral nutrition	IV	• MVI: 300 mg/day • MVI-Pediatric: No PG	Propylene glycol group: BW < 1000g: • 10/12 neonates with PG >40 mg/dL (41.4-108.9) • Good correlation propylene glycol and hyperosmolality BW > 1000g: • 4/15 neonates with PG >40 mg/dL (40.0-79.4) Mannitol group: • 3/15 neonates with propylene glycol >40 mg/dL (42-61): in children with very low birth weight, < from other treatments.
Chicella et al., 2002 [15]	11 intensive care patients: 1-15 months Patients with renal or	Lorazepam: 0.1 (starting dose) to 0.33 mg/kg/h (dose adapted to condition by the	IV continuous infusion for up to 3-14 days	PG: 40 to 132 mg/kg/h (0.96 to 3.17 g/kg/day)	Serum osmolality considered clinically significant if: ≥ 15 mOsm/kg Base 48h End PG conc 8.5 51.9 76.3 (mg/dL)

	hepatic insufficiency were excluded from the study	clinician) Blood samplings at baseline (some patients had previous lorazepam shots), 48h, and end of treatment			Lactate 1.9 1.7 1.7 (mmol/L) Osmol gap 1.5 7.5 13.3 (mOsm/kg) Propylene glycol accumulated significantly, however no clinically apparent symptoms of propylene glycol toxicity were observed A significant correlation was demonstrated between cumulative dose of propylene glycol (143 mg on average) and the propylene glycol serum concentration at the end of the infusion which ranged between 10 to 220 mg/dL. This was not demonstrated for serum lactate or osmol gap
Shehab et al., 2009 [95]	82 neonates in intensive care unit (3-145 days) Age: 36 w gestation (5.9 days postnatal)	1 to 3 medications per child Amiodarone and lorazepam as primary sources of propylene glycol	Oral or IV	All: 204.9 (17.3 to 9472.7) mg/kg/day <IV infusion: 4554.5 (869.5 to 9472.7) mg/kg/day	In patients who received propylene glycol by continuous infusion, the median cumulative daily dose was approximately 180 times the recommended daily intake of 25 mg/kg (WHO) The lack of outcome data limits the possibility to draw conclusions as to the clinical impact of such high propylene glycol exposure (not the objective of the study)
Whittaker et al., 2009 [112]	38 preterms at or below 30 w gestation	dexamethasone	Oral	Up to ~425 mg/kg/day	Exposure to propylene glycol close or higher than WHO recommended daily dose (25 mg/kg)

Age-corrected mean (SD) weekly exposure to propylene glycol (PG) in infants receiving dexamethasone treatment. The dotted line represents WHO recommended limit for adults (175 mg/kg/week).

Kulo et al., 2012 [58]	Neonates	IV paracetamol loading dose (20 mg/kg, equal to 16 mg/kg PG, PARANEO study)	IV	34 mg/kg/d (14-252)	Based on 69 propylene glycol plasma measurements following 16 mg/kg propylene glycol administration, peak propylene glycol concentrations were about 4 mg/dL, through concentration 6h later were 2-3 mg/dL. Assuming a one-compartment model, a distribution volume of 0.5 L/kg and an elimination half-life of 6-12h can be estimated:
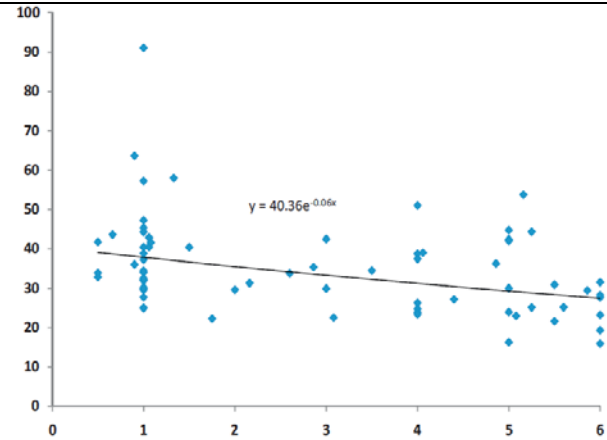
Fig. 1. 69 plasma PG observations collected in neonates following the first administration of iv paracetamol-PG (loading dose, 20 mg/kg iv paracetamol, co-administration of 16 mg/kg PG). X-axis: hours; Y-axis: PG plasma (mg/L).

In cases with a bladder catheter, urine was collected in the first 6 and 24 h time interval following initiation of paracetamol - propylene glycol administration. If we focus on urine collections following the loading dose, the median relative contribution of primary renal elimination to overall propylene glycol exposure was 7 (range 1-37)%. When further extended to the first 24h during repeated IV paracetamol - propylene glycol exposure (median propylene glycol exposure 40 mg/kg), this increased to 15 (range 7.8-78)%:

					Fig. 2. Median PG (retrieved/exposed) in urine collections of 18 neonates is 15 (range 7.8–78) %. There is a higher retrieval (%) in more immature neonates. X-axis = postmenstrual age (PMA, weeks); Y-axis = % PG (retrieved/exposed). It seems that postmenstrual age is one of the covariates of propylene glycol renal elimination, but somewhat different to what we initially anticipated. A lower PMA resulted in a proportionally higher renal elimination of propylene glycol. This might reflect either a difference in ontogeny between alcohol dehydrogenase and primary renal elimination in favor of primary renal elimination, or might reflect differences in renal tubular transport.
De Cock et al. 2013a [19]	Population PK analysis based upon 372 PG plasma concentrations from 62 (pre)term neonates (birth weight:	PG in: • paracetamol: loading dose of 20mg/kg, + 5-10 mg/kg/6h • Phenobarbital: loading dose 20 mg/kg	IV		A pharmacokinetic model with birth weight and postnatal age as covariates for clearance was developed for propylene glycol co-administered with paracetamol or phenobarbital in preterm and term neonates [CL(i) = 0.0849 × {(bBW/2720)(1.69) × (PNA/3)(0.201)}]. As such, large variability in exposure of propylene glycol may be expected in neonates which are dependent on birth weight and postnatal age: • Birth weight was found the most important covariate for clearance while an increase in clearance was seen with postnatal age. • Large differences in clearance values are seen between neonates of 1 kg (0.013 L/h) and neonates of 4 kg (0.13 L/h) at day of birth. This 10-fold difference in

	630-3980 g, postnatal age: 1-30 days)	+5 mg/kg/day			clearance is still seen one month after birth. • During the first two weeks of life the largest increase in clearance is observed. • The large variability in clearance and volume of distribution as a result of birth weight, PNA and current weight is reflected by the large range in expected peak and trough concentrations that can be expected upon commonly used doses of paracetamol and phenobarbital in neonates varying in birth weight between 630g and 3500g and between a PNA of 1–28 days. • A significant difference in volume of distribution was seen between neonates receiving phenobarbital and paracetamol. The volume of distribution was estimated to be 1.77 times higher (95% confidence interval: 1.35–2.19) for neonates receiving Phenobarbital. • The final model shows that for these commonly used dosing regimens, the population mean propylene glycol peak and trough concentrations range between 3.3–14.4 and 2.8–21.8 mg/dL (peak) and 1.9–10.9 and 0.6–11.2 mg/dL (trough) for paracetamol and Phenobarbital formulations, respectively, depending on birth weight and age of the neonates
De Cock et al. 2013b [19]	Plasma and urine PG concentrations from 69 (pre)term neonates (birth weight: 630-3980 g, postnatal age: 1–29 days, except for one postnatal age	PG in: • paracetamol: loading dose of 20 mg/kg, + 5-10 mg/kg/6h • Phenobarbital: loading dose 20 mg/kg + 5 mg/kg/day	IV		Propylene glycol analysis from 372 plasma concentrations of propylene glycol co-administered with paracetamol, Phenobarbital or both, and 79 urine concentrations of propylene glycol co-administered with paracetamol: • Birth weight: most important covariate for hepatic clearance • Current weight: most important covariate for volume of distribution • Postnatal age: significant covariate for hepatic clearance • Renal excretion of propylene glycol represented 15, 20, 23 and 25% of total clearance at 6, 12, 18 and 24 hours after the administration of the first dose respectively. It increased up to 30% at 48 hours after administration of the first dose.

	of 82 days)				• Renal elimination of propylene glycol increases in a similar manner as hepatic clearance with birth weight and postnatal age. The time at which 50% of the maximum renal clearance is reached, was estimated to be 42.2 hours.

Appendix 2: Tabulated summaries of non-clinical safety studies

References	Study type	Test system	Concentrations/ doses	Major findings
Secondary pharmacodynamics				
Chu et al, 1994 [17]	Mechanistic	NZW rabbits skeletal muscle sarcoplastic reticulum	0, 5.3 or 10.5% (v/v) (0, 55 or 109 mg/mL)	≥ 5.3%: stimulation of calcium release from terminal cysternae of sarcoplastic reticulum (SR) via ryanodine-sensitive Ca channels ≥ 10.5%: reduction of ATP dependent Ca^{2+} accumulation in SR.
Hattori et al, 1999 [38]	Mechanistic	Rat Pheochro – mocytome (PC12)	0.5, 2 or 10% (v/v) (5.19, 20.76 or 108.3 mg/mL)	≥ 0.5%: rise of intracellular calcium concentration by induction of influx via L- and N-type Ca2+ channels
Satoh et al, 2004 [92]	Mechanistic	Rat cerebrocorti-cal synap-tosomes	0.5–5% (v/v)	≥ 0.5%: increased calcium concentration in synaptosomes (from mitochondria)
Hattori et al, 1995 [37]	Mechanistic	Mouse phrenic nerve-diaphragm	1% (v/v) (10.38 mg/mL)	Propylene glycol did not significantly alter either Ca uptake or ATPase activity of the Ca pump. Propylene glycol stimulated Ca efflux from terminal cisternae vesicles Facilitation of neuromuscular transmission
Safety pharmacology				
Eichbaum et al, 1976 [25]	Cardiovascular	Dog and rat	IV admin.	0.1–0.3 mL/kg (70-100% solution): no HR or ECG changes ≥ 0.3 mL/kg in dog and 0.4-0.5 mL/kg in rat: apnea 0.2–0.3 mL/kg (70-100% sol) in dog: transient decrease in blood pressure 0.1–0.3 mL/kg (70-100% sol) in rat and dog: antiarrhythmic-antifibrillatory against spontaneous or drug induced arrhythmias (lengthening of effective refractory period)

Yasaka et al, 1979 [119]	Cardiovascular	Rabbit atria	0.3, 0.55, 1.0, 1.8, 3.0, 5.5 mM (0.023, 0.042, 0.076, 0.14, 0.23, 0.42 mg/mL)	Antiarrhythmic by lengthening of effective refractory period: ED50%: 0.84 mM (0.064 mg/mL)
Al-Khudhairi et al, 1986 [2]	Cardiovascular	Dog	IV admin. 160, 400, or 800 mg/kg	≥ 400 mg/kg: transient decrease in heart rate and blood pressure, reduction of efferent activity in sympathetic nerves followed by increase in heart rate, blood pressure and sympathic activity (back to normal within 1 minute).
Morshed et al, 1989 [74]	Metabolism	Wistar rats	Oral 0, 4.83, 9.66, 19.33, 38.66, or 77.32 mM/kg (0, 367.6, 735.1, 1471, 2942, 5884 mg/kg)	≥ 4.83 mM: Lactatemia: 1.83 to 4.01 fold increase of the control fasting blood lactate concentration of 0.825mM/L • Highest peak level observed at 38.66 mM/kg (metabolic saturation): about 3.3 mM/L. • Lactate levels associated with lactic acidosis in human: ≥5 mM/L No effect on blood pyruvate ≥ 38.66 mM/kg: increased blood glucose
Morshed et al, 1991 [72]	Metabolism	Wistar rats	Oral 0 or 2942 mg/kg for 10, 20 or 30 consecutive days	Body weight: initially reduced, than higher than control values Intestinal enzymes: increase in sucrose, lactase, γ-glutamine transpeptidase. Absorption: increased D-glucose, glycine, L-aspartic acid, L-lysine, and calcium absorption No ultrastructural changes
Cytotoxicity				
Morshed et al, 1994 [75]	Cytotoxicity	Human proximal tubule cells	0, 66, 131, 263 mM (0, 5.016, 9.96, 20.0	! 120 mM reported as toxic in human > 131mM: increased lactate release (enantiomer specific) and inhibition of Na^+ -

			mg/mL) Racemic and enantiomers	independent D-glucose uptake ≥ 263 mM: -increased lactate release (enantiomer specific)and inhibition of Na^+ - independent D-glucose uptake - increased LDH release
Morshed et al, 1998 [76]	Cytotoxicity	Human proximal tubule cells	0, 10, 25, 50 mM (0.76, 1.90, 3.80 mg/mL) Up to 5daily applications of racemic and enantiomers	Following 3 daily applications: > 25 mM: decreased cell proliferation (thymidine incorporation), cell viability (neutral red assay), and mitochondrial metabolic activity (MTT assay) No stereospecificty
Scherließ 2011 [93]	cytotoxicity	Human respiratory epithelial cells (Calu-3 cell line)	Tested concentrations: covering 100% to 0% viability	MTT assay: LC_{50}: 3350 mM (254.6 mg/mL)
Song et al, 2012 [98]	Cytotoxicity	Mouse fibroblast-like cells, L-929	6.25, 12.5, 25, 50, 100 μM (0.475, 0.95, 1.9, 3.8 or 7.6 μg/mL)	- IC_{50} (MTT assay): 394.7 μM - Up to 100 μM: no membrane damage, altered cellular metabolic activity, decreased mitochondrial activity, or altered protein synthesis rate

References	Patients (age, weight, n)	Treatment (drug, dose, duration)	Route	Propylene glycol (PG) dose	Major findings
Repeat-dose toxicity studies:					
Thackaberry et al, 2010 [104]	CD1 Mice 6 weeks 10/sex/group	92-93 days toxicity study	Oral gavage	0, 1000 mg/kg/day	Minimally elevated transaminase levels (ALT/AST) in 2/5 male, no histopathology correlate NOAEL : 1000 mg/kg

GLP compliant	SD Rats 6 weeks 10/sex/group				NOAEL : ≥ 1000mg/kg
	beagle dog 7–17 months 4/sex/group	95–97 days toxicity study			NOAEL : ≥ 1000mg/kg
	Cynomolgus monkeys M:2.8–4.0 kg F:2.0–3.6 kg 4/sex/group				NOAEL : ≥ 1000mg/kg
Montharu et al, 2010 [70]	SD Rats 8 weeks 11 F /group	4 days toxicity study	Intra-tracheal microspray	150 µL of 0 or 30% PG in water/rat	Qualitatively similar to water, minimal inflammation
Werley et al, 2011 [111] GLP compliant	SD Rats 8 weeks 15/sex/group	Acute toxicity study	Inhalation (nose-only)	14.4 / 30.5 / 44.9 mg/L inhaled during 4h	day 1-3 : 5 to 10% decreases in body weight day 7: localised "bleeding" around nose and eyes
	SD Rats 8 weeks 5/sex/group	7 days toxicity study		20.8 / 41.0 mg/L inhaled during 4h/day	No remarkable findings NOEL : ≥ 41.0 mg/L

Fisher 344 Rats 7–8 weeks 31/sex/group	28 days toxicity study		30 mg/L for up to 120min: ->lung deposits: ~0 / 7.2 / 21.6 / 72.0 / 216.0 mg/kg/day (10% nose deposit)	≥ 72.0 mg/kg: laryngeal squamous metaplasia No remarkable systemic findings up to 216 mg/kg NOEL: ~20 mg/kg, lung deposit based on squamous metaplasia Exposure D28: Dose Cmax AUC∞ mg/kg µg/mL µg/mL.min 20 139 22344 216 1376 450159 Lung/plasma ratios : ~1 (Cmax, AUC, $T_{1/2}$) $T_{1/2}$ increases with dose due to clearance saturation (60 to 180 min)
Beagle dogs 2/sex/group 8–9 months	MTD	Inhalation (throat delivery)	- Ascending phase: 1,5 to 30 mg/L for 8-60 min - 7 days: 5 mg/L for 60 min (~30 mg/kg)	Concentration > 5 mg/L: dogs restless and signs of intolerance No remarkable findings, MTD: 5 mg/L for 60 min
Beagle dogs 4/sex/group 6.25 months	28 days toxicity study		Target lung deposits:0 / 3 / 6 / 18 / 60 mg/kg/day	Lungs: background findings in inhalation studies. Not dose-related ≥ 18 mg/kg: decrease in Hb, hematocrite and RBC counts in females NOEL: 6.05 mg/kg, lung deposit NOAEL : 60 mg/kg, lung deposit Exposure D28:

					Dose	Cmax	AUC∞
					mg/kg	µg/mL	µg/mL.min
					6.05	28.1	5157
					60.00	91.9	17810
					Lung/plasma ratios : ~1 (Cmax, AUC, $T_{1/2}$)		
	Beagle dogs 4 dogs 12-24 months	CV	IV (slow IV infusion over 2 min)	Latin square 4X4 cross over design: 0, 0.3, 2.9, 14.3 mg/kg	No effects detected on BP, HR ECG (including QT and QTc).		
Reproductive toxicity studies:							
Enright et al 2010 [26]	SD rats 22 F/group 9-11 weeks	Fertility: dosing before/ during mating (2-3 w) and up to GD 7 in F or 3w after mating in M. Euthanasia: GD 14	Oral gavage	0, 1000 mg/kg/day	No male or female toxicity, effects on fertility, or effects on early embryonic development in rats. No maternal toxicity or effects on embryo-fetal development in rats or rabbits. NOEL ≥ 1000 mg/kg		
	SD rats 22 F/group 11 weeks	Teratology: GD: 6-17 Euthanasia: GD21	Oral gavage	0, 1000 mg/kg/day			
	NZW rabbits 20 F/group 5-6 months	Teratology: GD: 7-19 Euthanasia: GD 29	Oral gavage	0, 1000 mg/kg/day			

Juvenile toxicity studies:					
Lau et al, 2012 [60]	C57BL/6 mice 12 mice PND 7	Single dose CNS toxicity	Intra- peritoneal	0 or 10.38 g/kg	At > 2 mL/kg: dose related increase of apoptotic neurodegeneration (max 12h postdose)
	C57BL/6 mice 36 mice PND 7			0, 1.038, 2.076, 3.114, or 5.19 g/kg	Greatest apoptotic effects when exposure at PND7 No effects seen if PND >24 At > 10mL/kg: death
	C57BL/6 mice 72 mice, PND 4,7,14,17, 24 or 30			0, or 10.38 g/kg	Drowsiness at highest doses
	C57BL/6 mice 26 mice PND7	Single dose CNS toxicity	Intra- peritoneal	5 mg/kg PB + 0, or 0.71g/kg PG	5mg/kg PB; no apoptotic effect idem + propylene glycol: increased apoptosis
	C57BL/6 mice 26 mice PND7			20, or 40 mg/kg PB	At $\geq$ 40mg/kg: increased apoptosis
Genotoxicity:					

Aye et al, 2010 [9]	Commet assay	Chinese ovary cells	0, 50, 75, 100 or 150 mg/mL	Cytotoxicity (24h incubation): IC55: 24.1 mg/mL
				
				Increased DNA strand-breaks:
				
				    from 24.2 mg/mL without S9
				
				    from 31.8 mg/mL with S9	

	Micronucleus			Cytotoxicity (24h incubation): IC55: 24.1 mg/mL increased micronucleated cells:	

				from 15.7 mg/mL without S9
				from 36.4 mg/mL with S9

Appendix 3: Tabulated summaries of clinical safety studies

References	Patients (age, weight, n)	Treatment (drug, dose, duration)	Route	Propylene glycol (PG) dose	Major findings
Adults: case studies					
Arbour and Esparis, 2000 [6]	60 y patient (respiratory failure with mechanical ventilation)	Lorazepam 5 days of escalating infusion rate	IV	PG load over 5 days: 540 g (1.4 g/kg/day BW:75 kg)	Propylene glycol serum concentration: 78 mg/dL (day 5) • metabolic acidosis, and increased osmol gap which resolved within 3 days after discontinuation of lorazepam
Reynolds et al., 2000 [88]	36 y patient (alcohol withdrawal, followed by mechanical ventilation because of respiratory failure)	Intermittent Lorazepam infusion increasing up to 17 mg/h	IV	PG daily load: 50 to 169 g (2.3 g/kg/day, BW: 75 kg)	Propylene glycol serum concentration (estimated from osmol gap, cf Arroliga): 80.4 mg/dL • Osmolarity up to 320 mOsm/kg • Osmol gap up to 25 • Lactate up to 4.7 mmol/L • Possible renal dysfunction, which would explain bicarbonate retention at the level of proximal tubules during propylene glycol infusion. These values went back to normal following discontinuation of lorazepam infusion. Bicarbonate level increased immediately following lorazepam discontinuation, compensatory to hypercapnia (proximal tubular function). The portion of serum osmolality contributed by propylene glycol can be calculated by dividing the serum concentration of propylene glycol (in mg/dL)

					by 7.6. If this component explains an osmolar gap >10, then no other osmotically active particles are contributing to the gap. This would mean that propylene glycol serum concentrations reached ~ 190 mg/dL.
Wilson et al., 2000 [114]	46 y patient (alcohol withdrawal)	Diazepam 3000 mg over 24 hours	IV	-	Propylene glycol serum concentration: 1300 mg/dL • Severe hypotension,tachypnea • metabolic acidosis • creatinine level: 2.2 mg/dL (as compared with 0.6, 15h earlier) • bicarbonate level: 7mmol/L (as compared with 23mmol/L) • anion gap, 31 (as compared with 12) • serum osmolality, 600 mOsm per kilogram. Resolved with one dialysis session
Cawley et al., 2001 [14]	34 y patient with pre-existing renal failure (cocaine)	Lorazepam 3 days	IV	284 g over 72 hours (1.6 g/kg/day BW: 60 kg)	Propylene glycol serum concentration: 12 mg/dL (day 3) • metabolic acidosis • increased osmol gap: up to 24 • hyperlactatemia: 3.3 mmol/L • hyperosmolality: 345 mmol/kg Values returned toward normal following weaning off lorazepam
Krakoff et al., 2001 [54]	39 y patient (ectopic cushing's syndrome)	Etomidate 5.5 months Titration (40-80 mg over 5 h) based upon serum cortisol	IV	7 g/day during acute renal failure Up to 36 g/day when renal function improved (0.48 g/kg/ day,	No worsening of renal function or worsening of metabolic acidosis, on the contrary improved during etomidate treatment: renal failure resolved despite regular use of etomidate.

		levels		BW 75 kg)	
Parker et al., 2002 [83]	34 y patient with adult respiratory distress syndrome complicating varicella pneumonia	Lorazepam infusion (up to 24 mg/h) up to dialysis	IV	24-days cumulative PG dose: 3000 g over 24 days, (2 g/kg/day, BW 60 kg)	Dialysis performed because patient had increased anion and osmol gaps, and lactic acidosis <u>Before</u> <u>2h</u> <u>12h</u> <u>Dialysis</u> <u>post-d</u> <u>post-d</u> Osmol gap 97 27 21 PG (mg/dL) 520 170 ND* *No data There is a threefold reduction in propylene glycol levels post-dialysis The osmol gap drops proportionally to propylene glycol concentration
Hayman et al, 2003 [40]	46 y obese patient (208 kg) (respiratory failure/ infection, normal renal and hepatic functions)	Lorazepam from day 6: bolus of 6 mg followed by infusion: 4 mg/h up to day 19 Trimethoprin-sulfamethoxaz ole: from day 14, boluses every 6 h up to day 18	IV	Hospital days : • 8 to 13 ~36.5 g/day • 14to18 ~60.8 g/day (0.292 g/kg/day, BW 208kg)	From hospital day (HD)14: increase in creatinine up to 3.1 mg/dL on HD17 HD19: serum propylene glycol: 30 mg/dL (24h following weaning off trim/sulfa) • Osmol gap: 15 • Osmolality: 346 mOsm/kg • Creatinine: 5 mg/dL • Glucose: 284 mg/dL Death from respiratory failure 5 days following weaning off lorazepam Renal biopsy: changes in brush borders of proximal tubules (consistent with renal reperfusion following acute tubular nephrosis)
Jorens et al., 2004 [49]	72 y patient Bronchopneu-	Accidental ingestion of content of a	Oral	-	Patient presented with unusual D-Lactic acidosis and became comatose.

	monia (with cardiac decomp. and respiratory distress)	Cold/hot pack			
Wilson et al., 2005 [115]	48 y patient with mechanical ventilation	Lorazepam infusion > 7 days	IV	444 g PG over 7 first days (0.85 g/kg/day, BW 75kg)	Propylene glycol serum concentration: 144 mg/dL was measured once • anion gap up to 22 • osmolality up to 405 mOsm/kg • creatinine up to 2.2 mg/dL • bicarbonate down to: 10 mmol/L Metabolic disorders resumed within 24h discontinuation
	61 y patient (alcohol withdrawal syndrome)	Diazepam infusion > 5 days		970 g PG over 5 first days (2.6 g/kg/day, BW 75 kg)	Propylene glycol serum concentration: 108 mg/dL was measured once • anion gap up to 21 • osmolality up to 386 mOsm/kg • creatinine: no change • bicarbonate down to: 15 mmol/L Metabolic disorders resumed within 24h discontinuation
	41 y patient with HIV (mechanical ventilation)	Lorezepam infusion > 25 days		4565 g PG over 25 first days (2.4 g/kg/day, BW 75 kg)	Propylene glycol not measured • anion gap up to 24 • osmolality up to 380 mOsm/kg • creatinine up to 1.9 mg/dL • bicarbonate down to: 21 mmol/L • hypotension

				Metabolic disorders resumed within 24h discontinuation
	30 y patient (mechanical ventilation)	Lorazepam infusion >9 days		639 g PG over 3 first days, than 1273 g during the 6 following days (3.4 g/kg/day, BW 60 kg)	• Propylene glycol not measured • anion gap up to 27 • osmolality up to 384 mOsm/kg • creatinine up to 5.6 mg/dL: progressive oliguric renal failure • lactate level up to 3.8 mEq/dL Metabolic disorders tend to return to normal within 24h discontinuation
	53 y patient (respiratory failure, mechanical ventilation)	Lorazepam infusion 7 days		899 g PG over 7 days (1 g/kg/day, BW 75 kg)	Propylene glycol not measured • anion gap: ND • osmolality up to 408 mOsm/kg • creatinine up to 3.2 mg/dL Metabolic disorders tend to return to normal within 72h discontinuation
Wilson et al., 2005 (cont'd) [115]	In addition a prospective study on 21 patients given Propylene glycol - containing benzodiazepines describes 4 cases of propylene glycol metabolic changes:				

In addition a prospective study on 21 patients given Propylene glycol - containing benzodiazepines describes 4 cases of propylene glycol metabolic changes:

Cases	Serum Bicarbonate, mmol/L			Anion Gap			Osmolar Gap, mOsm/kg	Lactic Acid, mEq/dL	Propylene Glycol, mg/dL
	Before BZD	Trough	After BZD	Before BZD	Peak	After BZD			
1	26	15	23	14	16	10	57	175(serum),
2	27	21†	26	14	11	9	41†	2.3	58 (serum)
3	22	20	24	7	13†	9			
4	25	21†	25	8	12†	9	35†		

*BZD = benzodiazepine.
†Meets study criteria for propylene glycol toxicity.

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Together the data suggest that propylene glycol has a spectrum of severity ranging from common metabolic abnormalities to infrequent clinical deterioration. The threshold level beyond which propylene glycol accumulation is detrimental is unknown.

	• In subjects with clinical deterioration due to propylene glycol toxicity, we found propylene glycol levels ranging from 104 to 144 mg/dL. • In contrast, in subjects with only metabolic abnormalities due to propylene glycol toxicity, we found serum propylene glycol levels ranging from 58 to 127 mg/dL. This suggests that higher concentrations of propylene glycol are more likely to be associated with clinical deterioration, although overlap exists.			
Neale et al., 2005 [78]	24 y patient (acute respiratory distress syndrome, mechanical ventilation)	Lorazepam continuous infusion 18 days Up to 50 mg/h	IV	cumul. dose of 5167 g (4.8 g/kg/day, 60 kg BW)	Propylene glycol serum concentration: 1100 mg/dL on day 18 • Lactic acid level up to: 12.7 mEq/L • Creatinine: 0.5 mg/dL • Serum osmolality up to: 500 mOsm/kg, • Osmol gap: up to 186 Lactic acidosis tend to resume at discontinuation (day 18) The patient died on day 26 with fever, tachycardia, asystoles, acute decrease in blood pressure and urine output. The renal function remained normal as measured by BUN, serum creatinine, and urine output throughout her hospitalisation until day 26.
Zar et al., 2007 [125]	54 y patient (alcohol withdrawal)	Lorazepam infusion Up to 10–20 mg/h	IV	1699 g PG over 7 days (3.2 g/kg/day, BW 75 kg)	On day 8 of hospitalisation the patient condition deteriorated dramatically with high osmol gap, metabolic acidosis and acute renal impairment (high creatinine) Propylene glycol serum concentration: 810 mg/dL • Osmol gap up to 145 mmol/kg • Anion gap up to 14 mmol/L • Osmolality up to 395 mOsm/kg • Creatinine up to 1.9 mg/dL Osmol gap improved following two hemodialysis sessions, and returned progressively to

					normal thereafter.
Bledsoe et al., 2008 [12]	19 y patient (refractory status epilepticus, mechanical ventilation)	Pentobarbital infusion for 2 weeks + phenobarbital for 24h	IV	Not provided	Propylene glycol serum concentration: 55 mg/dL (24h after weaning off propylene glycol) • hypotension • Osmol gap up to 39 • Osmolality up to 362 mOsm/kg • Increase in creatinine up to 1.5 mg/dL, acute renal failure • Lactate: 5mmol/L Changes resume following weaning off propylene glycol. Patient died several weeks later from septic shock.
Miller et al., 2008 [69]	59 y patient (refractory status epilepticus, mechanical ventilation)	Pentobarbital Titrated up to 10 mg/kg/hr for 12h then down titration	IV	173.7 g over 24h (2.9 g/kg/day, 60 kg BW)	Propylene glycol serum concentration: 170 mg/dL • hypotension • Osmol gap up to 43 • Osmolality up to 344 mOsm/kg • Creatinine up to 1.1 mg/dL (from 0.7 mg/dL) • Lactate: 13.2 mEq/L • Bicarbonate down to 9 mEq/L Changes resume following discontinuation.
Ganesh et al., 2008 [30]	33 y patient (massive subarachnoid hemorrhage, mechanical	Etomidate 2 800 mg over 29 h (containing 362.6 mg PG/mL)	IV infusion preceded by a bolus	507.6 g over 29 h (7.1 g/kg/day, BW 60 kg)	Propylene glycol serum concentration (estimated from osmol gap, cf Arroliga): 580.9 mg/dL • Osmol gap up to 102 • Osmolality up to 422 mOsm/kg

	ventilation)				• creatinine: not provided • lactate: up to 77 mEq/L • Bicarbonate down to 7 mEq/L • Glucose: up to 572 mg/dL Severe metabolic acidosis with hyperglycemia which resolved following weaning off etomidate
Tsao et al., 2008 [106]	62 y patient (pneumonia, mechanical ventilation, normal renal and liver functions)	Lorazepam 10 mg/h for 4 days	IV	398.4 g over 4 days (1.3 g/KG/day, 75 kg BW)	Propylene glycol serum concentration: up to 382 mg/dL • Osmol gap up to 81.4 mOsm/kg • D-lactate: 11.8 mmol/L (metabolic rate of L-lactate by lactate dehydrogenase is 5 times more than that of D-lactate) Resolved following weaning off propylene glycol and hemodyalisis.
Yan, et al., 2010 [118]	67 y patient (shortness of breath, temper-ature, normal liver function, acute renal failure: creatinine 530.4 µmol/L, BUN: 21.4 mmol/L, mechanical ventilation), normal liver	Lorazepam uptitrated to 10 mg/h over 5 days	IV	498 g PG over 5 days (1.7 g/kg/day, BW 60 kg)	Propylene glycol serum concentration: up to 32.9 mmol/L (250.4 mg/dL) • Osmol gap up to 89.4 • L-lactate: 1.9 mmol/L (metabolic rate of L-lactate by lactate dehydrogenase is 5 times more than that of D-lactate) Osmol gap and propylene glycol concentration decreased following hemodyalisis and weaning off lorazepam, but metabolic acidosis persisted. The patient died from pneumonia after two-month hospital stay.

	function				

Zosel et al., 2010 [126]	50 y patient (generalised tonico-clonic seizures presumed to be due to anoxic brain injury following cardiac arrest)	Lorazepam 2 mg/min instead of 2 mg/h (over dose for about 10 h)	IV	About 498 g PG over 10h infusion (16 g/kg/day, 75 kg BW)	Metabolic acidosis and severe hypotension are detected. Peak propylene glycol: 659 mg/dL (3h after infusion discontinuation). At this time: • Serum creatinine: 0.8 mg/dL • Serum lactate: 14.6 mmol/L Serum creatinine increased 42h after hospitalisation and tubulonecrosis and renal failure were diagnosed. 70 h after hospitalisation and following hemodialysis serum propylene glycol returned to 45 mg/dL. The patient died (family consent to extubation) from renal failure and hypoxic brain injury due to initial cardiac arrest.

Adults : retrospective/prospective safety studies

Levy et al., 1995 [63]	7 patients 14 to 68 y old with traumatic or ischemic elevation of intracranial pressure	• 3 received Etomidate continuous infusion for 24 to 72 h • 4 received Pentobarbital	IV infusion	52.6 to 90.0 g PG/day (0.701 to 1.2 g/kg /day, BW: 75 kg)	Propylene glycol serum concentration: not measured All patients in the Etomidate group showed: Renal impairment: • Low creatinine clearance (~ 41 mL/min) • Increase in BUN and creatinine Metabolic acidosis (anion gap > 16 mEq/L) Hyperosmolality: 362±49 mosm/kg Renal impairment resolved following etomidate discontinuation.
Doenicke et al, 1997	49 patients (otolaryngologic	Etomidate single administration:	IV single injection	54.5 mg PG/kg BW	Hemolysis:

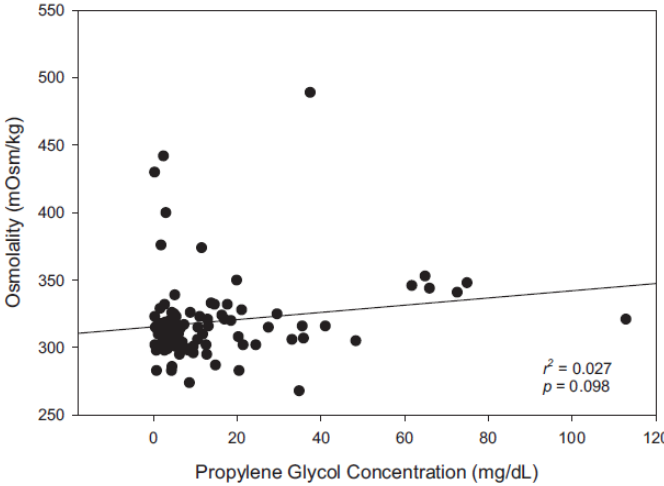| [21]	al surgery)	• N=24: PG formulation • N=25: PG free lipid emulsion		(3.8 g / patient, BW 70 kg)	• Free Hb: from 42.2 to 259 mg/L in the propylene glycol group only • Decreased serum haptoglobin
Doenicke et al, 1999 [22]	20 healthy volunteers (21-27 y)	Etomidate single administration: • N=10: PG formulation • N=10: PG free lipid emulsion	IV single bolus injection, (30s)	54.5 mg PG/kg BW (3.8 g / patient, BW 70 kg)	Pain in 9 out of 10 with propylene glycol Significant increase in plasma histamine concentration in two volunteers + phlebitis in one case(attributed to high osmolality) No haemodynamic changes.
Yaucher et al, 2003 [120]	Restrospective analysis of 128 patients given continuous infusion of lorazepam: - 8 of these critically ill patients showed increase in serum creatinine, - patients with respiratory	Patients develop serum creatinine increase during lorazepam continuous infusion (solution of 2 or 4 mg/mL) titrated to achieve sedation (range: 2–28 mg/h at time of peak creatinine concentration) for 7 to 75 days	IV	Mean cumulative PG dose per day: 36 to 83 g/day (0.51 to 1.19 g/kg/day, BW: M70 kg, F60 kg)	Of 128 patients given lorazepam, 8 showed increase in serum creatinine (up to 5 mg/dL in one patient) following 3 to 60 days lorazepam infusion Figure 1. Changes in serum creatinine concentration in response to lorazepam infusion in the eight patients. Serum creatinine concentration back to normal 3 days after lorazepam treatment cessation with one exception (unknown reason)

	failure requiring mechanical ventilation, 29 to 77 y - 2 patients with impaired liver function, one with impaired renal function				Propylene glycol concentration at peak creatinine level: 18.6 to 345 mg/dL 8/8 patients: hyperosmolarity and metabolic acidosis 7/8 patients: elevated osmol gap (> 10, 12 to 64) Weak to moderate correlation between creatinine concentration rise and propylene glycol concentration Propylene glycol concentration strongly correlated to serum osmolality and osmol gap and had a weak correlation with anion gap. Creatinine increase thought to result from renal tubular injury
Arroliga et al, 2004 [7]	9 critically ill patients 20-62 years 73-130 kg Mechanical ventilation No exclusion based upon renal or hepatic insufficiency.	Lorazepam, Titration up to: • High dose of ≥10 mg/h for at least 48h (mean of 50h) before blood sampling • Mean rate of infusion during high infusion rate period: 0.16 mg lorazepam/kg/h • Mean duration of total lorazepam	IV continu-ous infusion	Cumulative PG dose: • Total: 5.1 g/kg/4.9 days • During high dose: 3.3 g/kg/50h (~1.6 g/kg/day)	Propylene glycol plasma concentration following 50hrs high dose infusion: 94 to 350 mg/dL (mean: 199.6). • A significant correlation was observed between cumulative high dose propylene glycol infusion and propylene glycol serum concentration but not between total cumulative dose and propylene glycol serum concentration. • Osmol gap at 50h: 24.3-67.1 (mean:48.0) • Osmol gap strongly correlates with propylene glycol concentration (r^2= 0.804, p= 0.001) • Predicted propylene glycol concentration from osmol gap: (-82.1 + [osmol gap X 6.5]) • In creased anion gap (poor indicator of propylene glycol accumulation) Hyperosmolarity and metabolic acidosis were observed • No renal toxicity (no effects on creatinine, creatinine clearance) • Significant risk of propylene glycol toxicity may be associated with continuous infusion of ≥ 10 mg lorazepam/h

		infusion: 4.9 days			
Barnes et al., 2006 [10]	65 intensive care unit adult patients (n = controls: 25, LD: 26, HD: 14) Patients with mechanical ventilation Exclusion criteria: renal failure requiring dialysis, condition known to be associated with high osmolarity	Lorazepam: Controls: no PG Low dose (LD): 2–5.99 mg/h High dose (HD): ≥6 mg/h For at least 36h before blood sampling (to allow steady state PG exposure)	IV continu-ous infusion	Cumulative over 36h before blood sampling (g): Controls: 0 LD: 53 HD: 118 (79 g/day or 1 g/kg/day, BW: 75 kg)	Serum propylene glycol concentrations(mg/dL): • Controls: undetectable • LD: 14 ± 16 • HD: 39 ± 20 Propylene glycol concentration predicted by: • Osmol gap (control: 3.4, LD: 4.2, HD: 11.7 respectively) • Amount of propylene glycol administered during the previous 36h • Propylene glycol concentration = 1.40 + (1.182 x osmol gap) + (2.388E-04 x mg of PG administered in the 36h before serum sampling) • Propylene glycol concentration = 14.221 + (1.937 x osmol gap) Propylene glycol concentration not predicted by: • Anion Gap No relationship was established between propylene glycol concentration and lactate concentration or renal dysfunction (hepatotoxicity was not assessed) based upon creatinine concentration and incidence of renal failure between groups.
Zar et al., 2007 [125]	Review: prevention, treatment, prevention of PG toxicity following lorazepam				Detection: • Metabolic acidosis • Hyperosmolarity • Osmol gap at 48 h Treatment:

	infusion				• Shift to midazolam • Intermittent hemodyalisis Prevention: • Max recommended dose of lorazepam: 0.1 mg/kg/h. This suggests a safe dose of propylene glycol of 2.9 g/h or 69 g/day or 1g/kg/day for a patient weighing 70kg BW. • Close monitoring of patients given high doses lorazepam (> 10 mg/h)
Yahwak et al, 2008 [117]	35 critically ill patients (31 with mechanical ventilation, mean age: 69 y) • Mean BW: 80.5 kg • Baseline creatinin: 1 mg/dL	Lorazepam at any dose (0 to 15 mg/day) for 3 weeks	IV	0 to 6.2 g PG/day (0 to 0.078 g/kg/day)	10 patients had osmol gap > 10 (up to 20) on median day 9: With the exception of one patient: • None had serum creatinine > 1.3 mg/dL • These patients had the highest lorazepam dose for the previous 24h and cumulative from day1. • Propylene glycol concentration: ≤ 18 mg/dL One patient had: • Propylene glycol: 28 mg/dL • Osmol gap: 13 • Creatinine : 1.3 mg/dL • Highest dose of lorazepam 7 patients had serum creatinine > 1.3 mg/dL, but no elevated osmol gap These abnormalities resolved with cessation of lorazepam infusion
	14 critically ill patients (mean age:	Lorazepam at doses ≥ 1 mg/kg/day for a		49.4 g PG/day (0.62	Once osmol gap exceeded 10: Propylene glycol concentration and marker of toxicity were assessed: • 9 patients had Propylene glycol > 18 mg/mL

	49 y) • Mean BW: 80.2 kg • Patients with renal dysfunction were excluded from study (creatinine > 1.8 mg/dL or with renal replacement therapy)	mean of 5.5 days Mean daily dose: 123 mg lorazepam (1.5 mg/kg/day)		g/kg/day)	• 7 patients had osmol gap > 10 • 5 patients had creatinine > 2X baseline (one had the highest osmol gap of 34 and propylene glycol of 110 mg/dL) • 3 patients had metabolic acidosis It is not certain that propylene glycol caused all these adverse effects but these abnormalities resolved with cessation of lorazepam infusion.
Yahwak et al, 2008 [117]	*Conclusions :* For adults given ≥ 1mg lorazepam/kg/day: • The osmol gap correlates with serum propylene concentrations • Osmol gap > 10: indicative of propylene glycol > 18 mg/dL • Osmol gap > 12: indicative of possible clinical toxicity Propylene glycol serum concentrations > 18 mg/dL are associated with a higher risk of metabolic acidosis and renal dysfunction. This study supports the establishment of a lorazepam safety threshold dose of 1 mg lorazepam/kg/day (~0.415 g P propylene glycol/kg/day).				
Kraut et al, 2008 [55]	Review on alcohol related intoxications (methanol, ethylene	-	Mostly IV	-	*Pathophysiology :* • Metabolic acidosis • Renal failure in some patients,

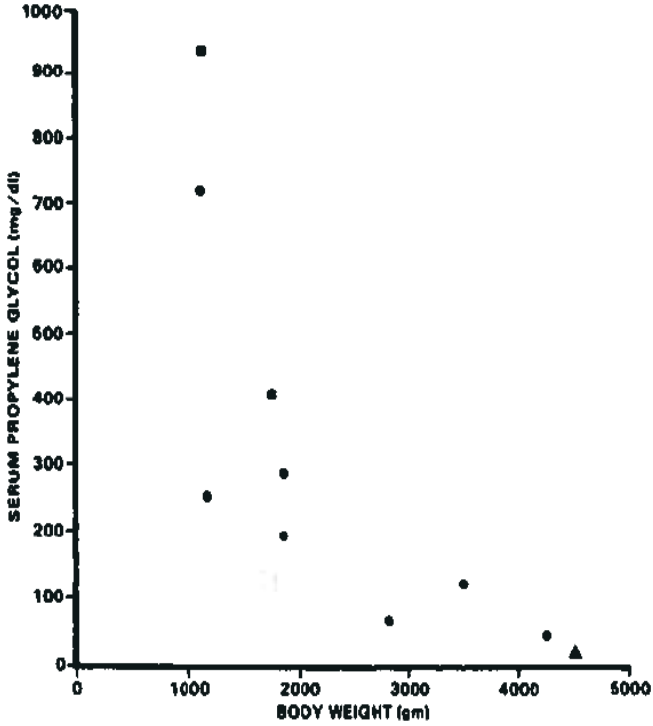|				
	glycol, diethylene glycol and PG)				• Proximal tubular cell injury in cultured renal cells • Pathogenic mechanisms of renal toxicity unclear *Clinical Findings :* in addition • central nervous system depression (!effect of the medication itself in case of benzodiazepines) *Laboratory Findings :* • Increased osmol gap • Serum osmolality normal or high • Mild l-lactic acidosis with serum lactate concentration in the range of 2 to 6 mEq/L • Rare pathologic evidence of tubular necrosis • Serum creatinine may be normal at toxic doses *Treatment :* • Prevention: IV propylene glycol dose < 2.9 g/h (<69 mg/day, or ~ 1 g/kg/day) (eg <166mg lorazepam/day, Zar et al.) • Medication discontinuation • Hemodyalisis (if very high levels of propylene glycol, severe acidosis, renal failure, …) • Fomepizole administration (very high affinity ADH substrate blocking propylene glycol metabolism)
Nelsen et al, 2008 [79]	50 critically ill patients (13-88 years) No specific exclusions	Lorazepam: 2.1 (0.5–18.0) mg/h for 137 h (41–475 h)	IV contin-uous infusion	Cumulative over the whole period: 128 (17–725) g	Serum propylene glycol concentrations(mg/dL): 5.0 (0.2–212.2) Osmolality: 314 (268–489) mOsm/kg Osmol gap: 12.5 (-23–194)

	from study participation	Blood samplings: • 1st: after 40 to 380 h infusion • Max 5 daily samples		Mean: 871.5 mg PG/h Or 21 g PG/day, (280 mg/kg/day BW: 75 kg)	8 patients (16%) had significant propylene glycol accumulation (>25 mg/dL) Propylene glycol concentration ≥ 25 mg/dL corresponds to significantly higher infusion rates; [6.4(1.9-11.3) versus 2.0 (0.5-7.4) mg/h (P =.0003)] and had a more elevated % of high anion gap values BUT: osmolality, osmol gap, and lactate did not correlate with serum propylene glycol concentrations (measured at different times) No convincing evidence of propylene glycol-related toxicity particularly anion gap and metabolic acidosis or renal dysfunction. No apparent relationship between plasma propylene glycol conc. and changes in renal function (Seven patients (16%) had a significant increase, > 25%, in serum creatinine, and 12 patients (27%) had a significant decrease, ≥ 25%, in serum creatinine from baseline).
Kraut et al., 2011 [56]	osmol gap and anion gap	-	-	-	Accumulation of the parent unmetabolised toxic alcohol accounts for the increase in serum osmolality, but toxicity of these alcohols is largely due to accumulation of their

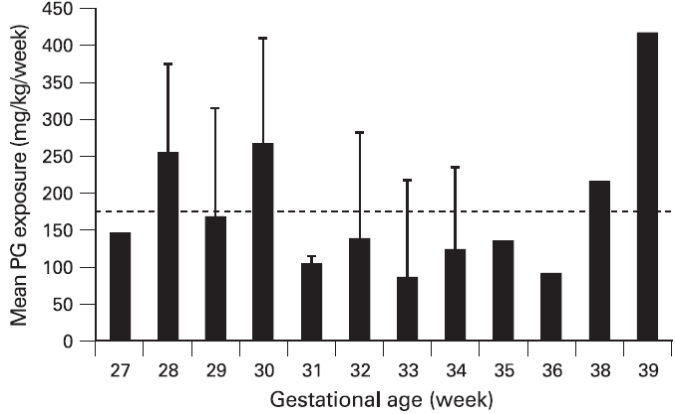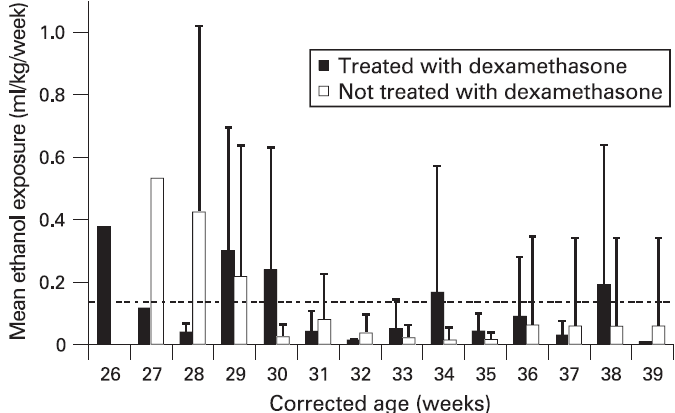|					metabolites.
					The evolution of changes in serum osmolality with toxic alcohols exposure therefore is dependent primarily on the rate of decrease in concentration of the parent alcohol.
changes following administration of toxic alcohols					If the patient is observed early after exposure to the alcohol, serum osmolality might be elevated, whereas serum anion gap might be unchanged. Thereafter anion gap might increase whilst osmol gap decreases.
					As increased osmol gap might results from pathophysiological changes (kidney disease, ketoacidosis...) it has been suggested that serum osmol gap $\geq$ 20 mOsm/kg is indicative of toxic alcohol exposure.
Children: case studies					
Arulanantham et al., 1978 [8]	11-year-old patient	Dihydrotachysterol (1 mg/mL, 2-4 mL bid) dissolved in absolute alcohol with propylene glycol.	Oral	-	Treatment resistant seizures appeared 13 months after the start of this formulation administration: • Directly due to propylene glycol exposure • Due to lower than normal threshold for seizures. Resumed following weaning off propylene glycol
Bekeris et al., 1979 [11]	14-year and 39-year old patients with severe burns	Topical application of silver sulfadiazine (cream)	Dermal application on burned skin	-	Increase in osmolality and osmol gap attributed to propylene glycol. Serum propylene glycol concentrations: 760 and 304 mg/dL for patients 1 and 2 respectively. Both patients died following sepsis, pulmonary infection, and embolism, and in one case renal failure. At necropsy, no evidence of renal damage was detected by light or electron microscopy in either case.
Marshall et al., 1995 [67]	9 month-old patient (11 kg) Mechanical	Enteral lorazepam 30 mg /4h (180 mg/24h) in solution (40%	Oral	PG: 34 g/24h PEG: 50 g/24h	Frequent watery stools. Diarrhea resolved when lorazepam formulation was replaced by crushed tablets. Diarrhea started again with a diazepam solution (50% PG w/v): osmotic diarrhea.

	ventilation	/50% PG w/v)			
Van de Wiele et al., 1995 [107]	9 y patient (34 kg) submitted to surgery for left hemispheric AVM (arteriovenous malformation resection)	Etomidate (brain protection titrated to burst suppression) solution (35%)	IV	~0.4 g/kg/h for 12h.	During etomidate infusion intermittent hemoglobinuria, hemolysis, progressive metabolic acidosis, and increase in serum osmolality (upto 337 mOsm/kg) and osmol gap (up to 52) were noted. 4 h following infusion was stopped serum values were: • PG: 230 mg/dL • Osm: 326 mOsm/kg • Osmol gap: 31 Resolution of metabolic acidosis after etomidate infusion was discontinued. Hemolysis was attributed to the administration of packed erythrocytes and undiluted amidate via the same intravenous line. In vitro studies have shown hemolysis when propylene glycol (> 30%) is added to blood samples. Hemolysis due to propylene glycol has also been demonstrated in patients receiving nitroglycerin (Demey et al., 1988).
Glover, 1996 [33]	2y child (10 kg BW)	Accidental ingestion of hair gel	oral	~ 200 mg/kg of BW	CNS depression and marked metabolic acidosis (that resolved following bicarbonate IV administration): • High anion gap: max 24 mmol/L • Low osmol gap: max 6 mOsm/kg • High lactate and pyruvate levels The low osmol gap may be reflective of delay from time to exposure or high metabolic clearance rate.
Yorgin et al. 1997 [121]	16 y patient (onset of seizures following fever,	IV, High increasing doses pentobarbital and	IV	Hospital days : • 2 to 6	Exacerbation of seizures, reversible acute renal failure propylene glycol serum concentration on day 13: 21 mg/dL Progressive increase in:

	headache)	phenobarbital titrated to control seizures, then progressively decreased from Day 13		~26.2 g/day • 7 to 12 ~63.2 g/day (0.94 g/kg/day) • Max: 90.3 g/day on hosp day 8	• Serum creatinine uo to: 2.1 mg/dL (day 11) • Osmol gap: up to 27.4 mOsm/kg (day 12) Renal biopsy (day 14): • Proximal tubular epithelial cells: vacuolation, swollen mitochondria, debris in lysosomes (osmotic nephrosis) Progressive recovery of serum values following treatment interruption
Peleg et al., 1998 [84]	Premature infant : (gestation week 29, BW 1200 g)	Gauze dressing of nitrofurazone 0.2% dissolved in PG 96.8%	Dermal application on burned skin	-	On D4 of life, the baby became lethargic and apneic, requiring re-initiation of mechanical ventilation. Metabolic acidosis preceded coma which resolved within hours of cessation of topical treatment. Plasma osmolality and propylene glycol were not assessed. High peak of propylene glycol was measured in urines (~3000 mg/dL).
Guillot et al., 2002 [36]	2 y child	Chewing of cleansing towels containing PG	-	-	Lethargy, superficial respiration, metabolic acidosis Serum propylene glycol: 5 mg/dL
Willis et al., 2013 [113]	3-year-old male with burns covering approximately 60% TBSA	Topical application of silver sulfadiazine	Dermal application on burned skin	-	In creased osmolality and urine output, osmol gap of 56 mOsm/kg, cyclic increases in serum lactate and doubling of serum creatinine concentration. Propylene glycol toxicity evidenced from day 47 of treatment, possibly related to decrease in renal function (concomitant increased creatinine concentration) Within 24h of the cessation of the sulfadiazine treatment the lactic acidosis and osmolar gap resolved.
Children: retrospective/prospective safety studies					
Glasgow et al, 1983	Infants in nursery	10 mL multivitamin	In parenter	3 g/day for at least 5	• Direct correlation between propylene glycol serum concentration and osmol gap

| [32] | (n=10, BW: 1 to 4.5 kg) | preparation (MVI-12, 30% PG) /day

For at least 5 consecutive days before blood sampling | al nutrition | days | • The highest osmolalities were found in the smallest babies

• Estimated elimination half-life: 19.3h (10.8 to 30.5h) |

Squares and triangle indicate patients receiving additional sources of propylene glycol.

No clinical problems could be ascribed to propylene glycol, but the study was initiated because of a case of propylene glycol intoxication in an 890g preterm neonate (27 week of gestation) who had unexplained acute renal failure following this parenteral nutrition. Serum osmolality and propylene glycol concentration up to 407 mOsm/kg and 930 mg/dL

					were recorded respectively.
MACDONALD et al., 1987 [65]	Retrospective analysis Neonates (≤ 1500 g at birth) • 78 given MVI concentrate (period A) • 49 given MVI-12 (period B)	Multivitamine solution in parenteral nutrition	IV	• MVI: 300 mg/day (period A) • MVI-12: 3 g/day (Period B)	• No evidence of increased incidence of hepatic or renal toxicity, hematuria, or apnea during period B. • Higher incidence of patient with seizures in period B (33 vs 11%). • Increased incidence in intraventricular hemorrhage, most probably related to mode of detection changes
Macdonald and Fletcher et al, 1987 [64]	Retrospective analysis Neonates (≤ 1500 g at birth) • 30 given MVI concentrate (PG) • 30 given MVI-pediatric (Mannitol)	Multivitamine solution in parenteral nutrition	IV	• MVI: 300 mg/day • MVI-Pediatric: No PG	Propylene glycol group: BW < 1000g: • 10/12 neonates with propylene glycol > 40 mg/dL (41.4-108.9) • Good correlation propylene glycol and hyperosmolality BW > 1000 g: • 4/15 neonates with PG > 40 mg/dL (40.0–79.4) Mannitol group: • 3/15 neonates with propylene glycol > 40 mg/dL (42-61): in children with very low birth weight, < from other treatments.
Chicella et al., 2002	11 intensive care patients:	Lorazepam: 0.1 (starting dose) to	IV continuous	PG: 40 to	Serum osmolality considered clinically significant if: ≥ 15 mOsm/kg

[15]	1–15 months Patients with renal or hepatic insufficiency were excluded from the study	0.33 mg/kg/h (dose adapted to condition by the clinician) Blood samplings at baseline (some patients had previous lorazepam shots), 48 h, and end of treatment	infusion for up to 3-14 days	132 mg/kg/h (0.96 to 3.17 g/kg/day)	Base 48 h End PG conc 8.5 51.9 76.3 (16.5 to 225.8) (mg/dL) Lactate 1.9 1.7 1.7 (mmol/L) Osmol gap 1.5 7.5 13.3 (mOsm/kg) Propylene glycol accumulated significantly, however the propylene glycol accumulation was not associated with significant laboratory abnormalities. Neither serum lactate concentrations nor osmolar gap were significantly elevated over baseline A significant correlation was demonstrated between cumulative dose of propylene glycol (143 mg on average) and the propylene glycol serum concentration at the end of the infusion This was not demonstrated for serum lactate or osmol gap
Shehab et al., 2009 [95]	82 neonates in intensive care unit (3–145 days) Age: 36 w gestation (5.9 days postnatal) Retrospective observational study	1 to 3 medications per child Amiodarone and lorazepam as primary sources of PG	Oral or IV	All: 204.9 (17.3–9472.7) mg/kg/day <IV infusion: 4554.5 (869.5–9472.7) mg/kg/day	Median (range) cumulative dose was 204.9 mg/kg/day (17.3–9472.7 mg/kg/day) for propylene glycol in all kind of medications In patients who received propylene glycol by continuous infusion, the median cumulative daily dose (4.6 g/kg/day) was approximately 180 times the recommended daily intake of 25 mg/kg (WHO) This paper does not assess the clinical outcome of these high exposures to propylene glycol.
Whittaker et al., 2009 [112]	38 premature neonates born ≤ 30 weeks	Weekly exposure (inclusion criteria: exposure for a minimum of 4	Oral dexame-thasone	Only infants given dexa-methasone were exposed	• Weekly exposure to propylene glycol in infants receiving dexamethasone treatment:

	gestation and < 1500 g BW (University Hospitals Leicester NHS Trust)	weeks) to the excipients included into: iron, vitamin drops, domperidone, caffeine, dexamethasone, spironolactone, bendrofluazide and furosemide.		to PG (up to ~60 mg/kg/d)

The dotted line represents WHO recommended limit for adults (175 mg/kg/week)

- Weekly exposure to 96% ethanol for neonates with chronic lung disease either treated or not treated with dexamethasone:

The dotted line (=0.14 ml/kg/week) represents an intake of 1 unit of alcohol per week for an average man weighing 70 kg.

Allegaert et al., 2010 [3]	69 cases including: • 31 preterm (<37 gest w) • 18 low birth weight (<1500 g) Age: PND 2, Range (1–82)	PG in: Paracetamol: n=35 Phenobarb.: n=28 Both (PG+P): n=5 Digoxin: n= 1	IV	34 mg/kg/day (14–252)	The following indicators of toxicity were evaluated in a time interval of 48h before the first administration up to 48 h after the last propylene glycol exposure were collected: • Indicators of renal disturbance (creatinaemia, plasma sodium, diuresis) • Indicator of metabolic disturbance (base excess, AG, lactate, bicarbonate) • Indicator of hepatic disturbance (aspartate aminotransferase (AST), alanine transaminase (ALT), direct bilirubinaemia) Data from patients given paracetamol formulated in propylene glycol were compared to data from patients given paracetamol formulated with mannitol (n=149, historical controls). Data collected from patients given ≤ 40 mg propylene glycol/kg/d were also compared to data from patients given > 40 mg propylene glycol/kg/d. No short-term biochemical impact was detected during or following a median propylene glycol exposure of 34 mg/kg/24h (range 14–252). Exposure to propylene glycol seemed well tolerated and did not affect normal postnatal maturational changes in renal, metabolic and hepatic functions.
Adults/children: local tolerance/irritation/sensitisation					
Andersen and Storrs, 1982 [4]	248 allergic patients were patch-tested (Gentofte Hospital, Denmark)	-	-	-	Five positive reactions (two cases following oral provocation) Skin reactions to propylene glycol are rare
Lamb et al., 2003 [59]	30 y woman	-	-	-	Allergy to topical gels and brassiere gel inserts. Propylene glycol may be a mild contact irritant as well as a contact sensitiser. Therefore low concentration of 10 to 20% are advocated for patch-testing

| Heine et al., 2004 [41] | Review: 285 children (6–12 y of age) and 2175 adolescents (13–18 y of age) patch-tested (20% PG in petrolatum) for suspicion of contact allergy German Information Network of Departments of Dermatology (IVDK – Part of DKG, 1995–2002) | - | - | - | Control groups: 7904 patients aged 60 to 66 y, and all patients: 48180 4% of children, 2.3% of adolescents and 2.5% of patients aged 60–66 y show sensitisation to propylene glycol. |
| Lessmann et al, 2005 [62] | Review: 45 138 patients suspected of allergic contact eczema were submitted to patch-testing (20% PG in water) with | - | - | - | Propylene glycol preparations in concentrations above 20% in water are irritant upon occluded patch testing, exhibiting a clear concentration gradient. This entails the possibility of false-positive patch-test reactions. Positive results to be verified by retesting with 10% and 4% P propylene glycol in water 1044 patients (2.3%) tested positively (probably some false positive):
 • Unremarkable proportion of occupation-related cases
 • Topical therapeutics more frequent in the propylene glycol-sensitive group.
 • Lower leg (stasis) /venous ulcers more frequent in the propylene glycol-sensitive group (predisposing risk factor): may be linked to more frequent use of topical medicines containing propylene glycol but also to an immunostimulant effect of the |

	standard series of the German Contact Dermatitis Research Group (DKG). (1992–2002)				chronic, partly inflammatory disease. Of note several predictive animal tests failed to detect any sensitizing properties of propylene glycol. Taking into account the negative experimental findings in animals and the high level of exposure to propylene glycol in the population, the low frequency of positive reactions to non-irritant propylene glycol concentrations in patch-tested patients indicates a very low sensitisation potential in humans.
Warshaw et al., 2009 [109]	Review: 23 359 patients North American Contact Dermititis Group, (NACDG, 1996–2006)	-	-	-	810 patients (3.5%) had patch-test reactions to 30% propylene glycol. Of which: • 12.8% + reaction to personal products (creams, lotions, cosmetics) • 4.2% of occupational related reactions (very rare)
Kosarek al. 2011 [53]	8 cases of phlebitis following induction of anesthesia with etomidate	Etomidate prepared in PG	IV	-	• Pain during injection • Phlebitis near injection site Attributed to high osmolality of the propylene glycol preparations.