Regulatory Toxicology and Pharmacology 65 (2013) 229–241

Contents lists available at SciVerse ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Regular Article

Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect

William M. Snellings [a,*,1], Richard A. Corley [b], Kenneth E. McMartin [c], Christopher R. Kirman [d], Sol M. Bobst [e,2]

[a] Snellings Toxicology Consulting, LLC, 23 Trails End Lane, Ridgefield, CT 06877, USA
[b] Battelle Pacific Northwest Division, Richland, WA 99352, USA
[c] Louisiana State University Health Sciences Center, Shreveport, LA 71130, USA
[d] Summit Toxicology, L.L.P., 29449 Pike Drive, Orange Village, OH 44022, USA
[e] Shell Oil Company (Formerly), Houston, TX 77002, USA

ARTICLE INFO

Article history:
Received 23 August 2012
Available online 22 December 2012

Keywords:
Ethylene glycol
EG
Human-based oral RfD
Calcium oxalate crystal
Renal tubule degeneration
Concordance mode of action
Toxicokinetics
Toxicodynamics
HED

ABSTRACT

Several risk assessments have been conducted for ethylene glycol (EG). These assessments identified the kidney as the primary target organ for chronic effects. None of these assessments have incorporated the robust database of species-specific toxicokinetic and toxicodynamic studies with EG and its metabolites in defining uncertainty factors used in reference value derivation. Pertinent *in vitro* and *in vivo* studies related to one of these metabolites, calcium oxalate, and its role in crystal-induced nephropathy are summarized, and the weight of evidence to establish the mode of action for renal toxicity is reviewed. Previous risk assessments were based on chronic rat studies using a strain of rat that was later determined to be less sensitive to the toxic effects of EG. A recently published 12-month rat study using the more sensitive strain (Wistar) was selected to determine the point of departure for a new risk assessment. This approach incorporated toxicokinetic and toxicodynamic data and used Benchmark Dose methods to calculate a Human Equivalent Dose. Uncertainty factors were chosen, depending on the quality of the studies available, the extent of the database, and scientific judgment. The Reference Dose for long-term repeat oral exposure to EG was determined to be 15 mg/kg bw/d.

1. Introduction

Ethylene glycol (EG; CAS No. 107-21-1) is the smallest member of the chemical family named glycols, which are characterized by two hydroxyl groups attached to separate carbons in an aliphatic chain. EG is a clear, colorless, relatively non-volatile, liquid, which has a sweet odor and a sweet/acrid taste.

EG is used as antifreeze or a heat transfer fluid in cooling and heating systems; in hydraulic brake fluids; as an industrial humectant; as an ingredient of electrolytic condensers; as a solvent in the paint and plastics industries; in the formulations of printers' inks, stamp pad inks, and inks for ballpoint pens; and in the synthesis of safety explosives, plasticizers, synthetic fibers, and synthetic waxes (O'Neil et al., 2001). EG is also used to de-ice airport runways and aircraft (Forkner et al., 2004) and as a component in pesticide products used as an antifreeze or deactivator for pesticides applied before the crop emerges from soil and as a component in herbicides before or after crop emerges (USEPA, 2010).

USEPA (2000), Technology Transfer Network cites the following on potential exposures: Dermal or inhalation exposure to workers may occur during the manufacture or use of the chemical (HSDB, 1993). EG may be discharged into wastewaters from its production and use. It may also enter the environment from its uses in deicing airplanes and runways and from spills and improper disposal of used antifreeze, coolant, and solvents containing EG (ATSDR, 2010). The National Research Council (2001) has reported examples of waterborne outbreaks in the past associated with EG contamination of drinking water distribution systems as a result of inappropriate cross connections with an air conditioning or heating system (Blackburn et al., 2004).

Several government agencies have performed risk assessments for the oral exposure route. Assessments cited below have used the No Observable Adverse Effect Level (NOAEL) from certain rat studies divided by a factor of 100, the default value to account for inter- and intra-species variation. In this publication, we review what is known about the mode of action for EG and summarize the data that contribute to a reduction in the uncertainty in developing a Reference Dose (RfD) for the oral route of exposure.

* Corresponding author. Fax: +1 203 431 6667.
E-mail address: toxdoc143@aol.com (W.M. Snellings).
[1] Retired.
[2] Present address: Nexeo Solutions LLC, The Woodlands, TX 77380, USA.

0273-2300/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yrtph.2012.12.005

2. Summary of selected regulatory dose–response assessments

The two significant endpoints that have been identified for short- and long-term exposure to EG are developmental and renal toxicity. The NTP (2004) concurred with the Ethylene Glycol/Propylene Glycol Expert Panel under the Center for the Evaluation of Risks to Human Reproduction (CERHR) that there is negligible concern of adverse developmental toxicity from EG at exposures below 125 mg/kg bw. This calculation was based on the differences between rat and human for the saturation points (*in vitro*) of the metabolism of glycolic acid, the proximate developmental toxicant. The NTP–CERHR monograph further states that the known exposure scenarios for EG suggest that expected human exposures are at least 100- to 1000-fold lower than those expected to result in metabolic saturation. In addition, as stated in Environment Canada and Health Canada Final Report (2010) further work (Corley et al., 2005a) with physiologically based pharmacokinetic models (PBPK) predicted humans would only achieve the threshold for developmental effects at an even higher dose of EG (>350 mg/kg). This along with the point that saturation is expected to require much higher doses for slower dose-rate (non-bolus) exposures supports that renal toxicity is the critical effect of concern from oral exposures to EG.

Risks of adverse renal effects (crystal nephrotoxicity) from long-term oral EG exposures have been evaluated by several organizations resulting in a number of reference values (Table 1). These values are generally considered an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects over a lifetime. Comparisons of these evaluations demonstrate the variations in recommended risk values as new data emerged:

- USEPA's Office of Drinking Water (USEPA Health Advisory, 1987) and Office Research and Development (USEPA Health Effects Assessment, 1987) conducted two separate risk assessments for EG in 1987. Several risk factors were presented, including calculation of a Reference Dose (RfD). Their two RfD values (1 and 2 mg/kg/bw/d, respectively) were derived by dividing the NOAEL values (100 mg/kg bw in chronic rat by Blood (1965) or 200 mg/kg bw in the chronic rat study by DePass et al. (1986a), respectively), by an uncertainty factor of 100 (10 for interspecies variation, 10 for intraspecies variation).
- USEPA IRIS published their Reference Dose (RfD) for EG in 1989 (USEPA IRIS, 1989). This RfD, 2 mg/kg bw/d, was derived by dividing the NOAEL (200 mg/kg bw; DePass et al. 1986a) by an uncertainty factor of 100 (10 for interspecies variation, 10 for intraspecies variation). USEPA also noted that this value is protective of teratogenic and reproductive effects, which have higher NOAELs in comparison to the renal effects (USEPA IRIS, 1989).
- Environment Canada and Health Canada published their Tolerable Intake (TI) for EG in a report entitled Final Report (April, 2010), Priority Substance List Assessment Report, Follow-Up to the State of Science Report, 2000 on Ethylene Glycol. The TI for EG (1.2 mg/kg bw/d) was derived by dividing the benchmark response level of 5% extra risk (BMD_{05}), 120 mg/kg bw/d (calculated from ACC, 2005) by an uncertainty factor of 100 (10 for interspecies variation, 10 for intraspecies variation).
- ATSDR published their Minimal Risk Levels (MRL) in a Toxicological Profile for Ethylene Glycol in 2010. The chronic-duration MRL (1.5 mg/kg bw/d) was derived by dividing the NOAEL (150 mg/kg bw/d; Corley et al. 2008) by an uncertainty factor of 100 (10 for interspecies variation, 10 for intraspecies variation). However, ATSDR noted that this chronic MRL value (1.5 mg/kg bw/d) is higher than the acute-duration oral MRL (0.8 mg/kg bw/d), which was also determined in this report. Since it is against ATSDR policy to derive a chronic-duration MRL that is higher than the acute-duration MRL, ATSDR published the chronic-duration oral MRL at the same value as the acute MRL (0.8 mg/kg bw/d).

3. Hazard identification

EG has been causally linked to a number of adverse health effects. ATSDR (2010) reports the information on the health effects of oral exposure in humans is largely limited to case reports of acute accidental or intentional ingestion of EG. These case reports have identified three stages of acute oral EG toxicity. The first stage involves central nervous system depression and gastrointestinal upset. During the second stage, metabolic acidosis and associated cardio-pulmonary symptoms become evident. During stage three, renal involvement becomes evident. This third stage is characterized by flank pain and oliguria/anuria. Histopathological findings show renal tubular necrosis and deposition of calcium oxalate crystals. In rats, long-term oral exposures to EG in the diet (Blood, 1965; DePass et al., 1986a; Corley et al., 2008) have resulted in metabolic acidosis, oliguria/anuria, and comparable renal tissue effects. ATSDR (2010) concludes even though there are no human epidemiological studies, that available information suggests EG is likely to cause the effects in humans similar to those found in animals. At high doses in the diet or fast dose rates by gavage (Carney, 1994), EG can cause developmental toxicity in rats and mice. Similar effects have not been reported for human exposure.

Animal studies indicate that oral exposure to EG can cause effects in different organ systems. The developing fetus is sensitive to acute exposure to EG, and this is discussed in detail by Carney

Table 1
Selected published risk values for chronic oral exposure.

Year	Agency	Risk designation	Study based on	Uncertainty factor	Risk value (mg/kg bw/d)
1987, March	USEPA, Office of Drinking Water, EG Health Advisory	Reference Dose	Blood (1965), Sprague–Dawley rat, kidney effects	100	1
1987, July	USEPA, Office Research and Development, Health Effects Assessment for EG	Reference Dose	DePass et al. (1986a), Fischer 344 rat, kidney effects	100	2
1989	USEPA IRIS	Reference Dose	DePass et al. (1986a), Fischer 344 rat, kidney effects	100	2
2006	USEPA, Office of Drinking Water, EG Health Advisory	Reference Dose	DePass et al. (1986a), Fischer 344 rat, kidney effects	100	2
2010	Environment Canada and Health Canada	Tolerable Intake	ACC (2005), Wistar rat, kidney effects	100	1.2[a]
2010	ATSDR	Minimal Risk Level	Corley et al. (2008), Wistar rat, kidney effects	100	1.5[b]

[a] Environment Canada and Health Canada used raw data from ACC (2005) and calculated BMD_{05} of 120 mg/kg bw/d based on incidence. Corley et al. (2008) used raw data from same study and calculated BMD_{05} of 170 mg/kg bw/d using model based on incidence x severity.

[b] ATSDR indicated the scientific derivation of the risk value of 1.5 mg/kg bw/d. ATSDR also derived an acute MRL at 0.8 mg/kg bw/d. Since it is against their policy to derive chronic MRL that is higher than the acute MRL (0.8 mg/kg bw/d), the reported MRL is the same value (0.8 mg/kg bw/d) as the acute MRL.