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ScienceDirect
Toxicology and Applied Pharmacology 228 (2008) 165–178
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicity ☆

R.A. Corley [a,*], D.M. Wilson [b], G.C. Hard [c], K.E. Stebbins [b], M.J. Bartels [b], J.J. Soelberg [a], M.D. Dryzga [b], R. Gingell [d], K.E. McMartin [e], W.M. Snellings [b]

[a] *Battelle Pacific Northwest Division, Richland, WA 99352, USA*
[b] *The Dow Chemical Company, Midland, MI 48674, USA*
[c] *Tairua, 2853, New Zealand*
[d] *Shell Oil Company, Houston, TX 77002, USA*
[e] *Louisiana State University Health Sciences Center, Shreveport, LA 71130, USA*

Received 5 October 2007; revised 26 November 2007; accepted 26 November 2007
Available online 4 December 2007

Abstract

Male Wistar rats have been shown to be the most sensitive sex, strain and species to ethylene glycol-induced nephrotoxicity in subchronic studies. A chronic toxicity and dosimetry study was therefore conducted in male Wistar rats administered ethylene glycol via the diet at 0, 50, 150, 300, or 400 mg/kg/day for up to twelve months. Subgroups of animals were included for metabolite analysis and renal clearance studies to provide a quantitative basis for extrapolating dose–response relationships from this sensitive animal model in human health risk assessments. Mortality occurred in 5 of 20 rats at 300 mg/kg/day (days 111–221) and 4 of 20 rats at 400 mg/kg/day (days 43–193), with remaining rats at this dose euthanized early (day 203) due to excessive weight loss. Increased water consumption and urine volume with decreased specific gravity occurred at 300 mg/kg/day presumably due to osmotic diuresis. Calculi (calcium oxalate crystals) occurred in the bladder or renal pelvis at ≥300 mg/kg/day. Rats dying early at ≥300 mg/kg/day had transitional cell hyperplasia with inflammation and hemorrhage of the bladder wall. Crystal nephropathy (basophilic foci, tubule or pelvic dilatation, birefringent crystals in the pelvic fornix, or transitional cell hyperplasia) affected most rats at 300 mg/kg/day, all at 400 mg/kg/day, but none at ≤150 mg/kg/day. No significant differences in kidney oxalate levels, the metabolite responsible for renal toxicity, were observed among control, 50 and 150 mg/kg/day groups. At 300 and 400 mg/kg/day, oxalate levels increased proportionally with the nephrotoxicity score supporting the oxalate crystal-induced nephrotoxicity mode of action. No treatment-related effects on the renal clearance of intravenously infused ^{3}H-inulin, a marker for glomerular filtration, and ^{14}C-oxalic acid were observed in rats surviving 12 months of exposure to ethylene glycol up to 300 mg/kg/day. In studies with naïve male Wistar and F344 rats (a less sensitive strain), a significant difference was observed in oxalate clearances between young rats (i.e. Wistar clearance < F344) but not in age-matched old rats. Regardless, the ratios of oxalate:inulin clearances in these two strains of rats, including those exposed to ethylene glycol, were all < 1, suggesting that a fraction of the filtered oxalate is reabsorbed. Other species, including humans, typically have clearance ratios >1 and are more effective at clearing oxalic acid by both glomerular filtration and active secretion. Thus, the lower renal clearance and kidney accumulation of oxalates in male Wistar rats enhances their sensitivity, which will be a factor in human risk assessments. The benchmark dose values (BMD05, BMDL05) were 170 mg/kg/day and 150 mg/kg/day for nephropathy, and 170 mg/kg/day and 160 mg/kg/day for birefringent crystals, using incidence times severity data in each case. The NOAEL of 150 mg/kg/day is the same as that reported after 16-week exposure and appears to be a threshold dose below which no renal toxicity occurs, regardless of exposure duration.

Keywords: Ethylene glycol; Oxalic acid; Renal clearance; Chronic toxicity; Nephrotoxicity

☆ The study was sponsored by the Ethylene Glycol Panel, American Chemistry Council. Six co-authors (DMW, KES, MJB, MDD, RG, and WMS) are employees of ACC member companies.

* Corresponding author. Biological Monitoring and Modeling, 902 Battelle Blvd., P.O. Box 999, MSIN P7-59, Richland, WA 99352, USA. Fax: +1 509 376 9064.
E-mail address: rick.corley@pnl.gov (R.A. Corley).

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doi:10.1016/j.taap.2007.11.024

Introduction

Ethylene glycol is one of the most well-studied high production volume industrial chemicals due to its decades of use and variety of applications. Although considerable variability has been observed in sensitivity across species, strains and sexes, renal toxicity has long been recognized as a potential outcome following repeated exposures to several hundred mg/kg/day. Ethylene glycol also induces developmental toxicity at higher doses (1000 mg/kg/day) and dose rates (gavage) in rats and mice but not in rabbits. Several recent reviews have summarized the current state of knowledge of the modes of action for these two endpoints (e.g. Carney, 1994; Carney et al., 1999; CERHR, 2003; Corley et al., 2005b). What became clear from these analyses is the critical importance of metabolism and pharmacokinetics in potential susceptibilities and the importance of quantitating these relationships in animal-to-human extrapolations.

For example, developmental effects observed in animals are associated with a dose-rate dependent buildup of the intermediate metabolite, glycolic acid, due to saturation of enzymes responsible for further metabolism (Carney, 1994, Carney et al., 1999; Corley et al., 2005b). The initial steps, metabolism of ethylene glycol, accumulation and distribution of glycolic acid, and the metabolic and renal clearance of glycolic acid were therefore incorporated into a physiologically based pharmacokinetic model to improve risk assessments for glycolic acid-induced developmental toxicity (Corley et al., 2005a; Corley and McMartin, 2005). Based upon the now extensive pharmacokinetic and mode of action database for developmental toxicity, there is negligible concern that developmental toxicity will occur in humans under normal use conditions (CERHR, 2003; NTP, 2004).

Kidney toxicity, however, occurs in all species studied at dose levels lower than those causing developmental toxicity in rats and mice and thus remains the primary organ of concern for current human health risk assessments. Although notable quantitative differences have been observed, the mode of action for kidney toxicity is essentially the same in animals and humans and involves three key events: (1) the metabolism of ethylene glycol to oxalic acid via glycolic acid; (2) precipitation of calcium oxalate crystals in the kidneys; and (3) degeneration of renal tubule epithelium due to physical trauma or localized oxidative stress (Corley et al., 2005b).

Chronic studies have previously been conducted in F344 rats and B6C3F1 mice with rats found to be more sensitive to nephrotoxicity than mice and males more sensitive than females (DePass et al., 1986; NTP, 1993). No treatment-related tumors were observed in either species or sex. For rats, all high dose (1000 mg/kg/day) males died by 15months due to nephrotoxicity with the next lowest dose (200 mg/kg/day) representing a no-observed adverse effect level (NOAEL). Such a dramatic dose–response relationship precludes effective benchmark dose analysis favored by several regulatory agencies.

Risk assessments for chronic exposures have been further complicated by an apparent strain difference in the sensitivity of male rats to ethylene glycol-induced nephrotoxicity suggesting that Wistar rats may be more susceptible. In an unpublished 16-week dietary study, a NOAEL of 71 mg/kg/day, nearly three-fold lower than the chronic NOAEL in F344 rats, was reported in male Wistar rats (Gaunt et al., 1974). However, comparisons of NOAEL's from these two studies are problematic given that the doses used in the chronic study were adjusted periodically to maintain targeted mg/kg/day doses based upon group mean body weights and feed consumption while the subchronic study utilized a constant concentration of ethylene glycol in the diets. Thus, in the Gaunt et al. study, significantly higher dose levels were achieved during the first week of the study than at week 16. As a result, risk assessors are faced with the dilemma of using either the chronic NOAEL of 200 mg/kg/day from the male F344 rat or from the subchronic NOAEL of 71 mg/kg/day from the Wistar rat with an additional uncertainty factor (possibly 10-fold) for lack of a chronic study in this strain, potentially producing a 30-fold difference in reference dose.

We have therefore undertaken a series of studies aimed at refining human health risk assessments for lifetime exposures based upon nephrotoxicity as an endpoint and developed a framework for making quantitative comparisons across strains and species. In our subchronic toxicity study, Cruzan et al. (2004) verified that the male Wistar rat is more sensitive than male F344 rats following identical exposures and criteria for pathological evaluation. While the no-observed adverse effect level (NOAEL) for renal toxicity in both male Wistar and F344 rats was 150 mg/kg/day following 16 weeks of exposure when dietary concentrations were adjusted weekly to achieve targeted dose levels, the severity of nephrotoxicity was significantly greater in Wistar rats at 500 and 1000 mg/kg/day, leading to lower benchmark doses in the Wistar rat when incidence and severity data are taken together. Cruzan et al. also included the analysis of blood, urine and kidneys for ethylene glycol and its metabolites, glycolic acid and oxalic acid, following one and sixteen weeks of exposure and demonstrated that the greater nephrotoxicity seen in male Wistar rats vs. the F344 rat was associated with greater kidney oxalate levels, consistent with the mode of action.

The purpose of this current study was, therefore, to extend the work of Cruzan et al. (2004) and establish the NOAEL and benchmark dose levels for nephrotoxicity following 12 months of chronic exposure in the most sensitive animal model (male Wistar rat). To assist in dose, route and cross-species extrapolations for human health risk assessments, the concentrations of ethylene glycol, glycolic acid and total oxalates were determined in blood, urine and kidney. In addition, the ability of male Wistar rat kidneys to excrete oxalic acid in the urine was evaluated as a function of age and prior ethylene glycol exposure. Oxalate clearances were also evaluated in age-matched naïve male F344 rats. Quantitative relationships were thus developed between male Wistar and F344 rats, and published data in other species including humans, to facilitate comparisons.