



May 23, 2016

Via E-Mail

Ms. Monet Vela
Office of Environmental Health Hazard Assessment
P.O. Box 4010, MS-23B
Sacramento, California 95812-4010

p65public.comments@oehha.ca.gov

Re: Ethylene Glycol MADL

Dear Ms. Vela:

The Ethylene Glycols Panel¹ (Panel) of the American Chemistry Council appreciates the opportunity to comment on the California Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA) proposed Maximum Allowable Dose Level (MADL) for ethylene glycol (EG). Panel members include manufacturers of EG, which is used in many commercial and industrial applications including antifreeze and coolant. EG also is used as a raw material in the production of a wide range of products including polyester fibers for clothes, upholstery, carpet and pillows; fiberglass used in products such as jet skis, bathtubs, and bowling balls; and polyethylene terephthalate resin used in packaging film and bottles.

In its notice dated April 9, 2016, OEHHA proposes an oral ingestion MADL of 8,700 micrograms per day. This proposed MADL was developed using the most sensitive study of "sufficient quality"- the Neeper-Bradley et. al. (1995) developmental toxicity study in which pregnant mice were oral gavaged with EG on gestation days 6 through 15.² The No Observed Effect Level (NOEL) was determined to be 150 mg/kg bw/day. Applying the default assumptions and principles delineated in Article 8 of the Proposition 65 regulations, the MADL was calculated to be 8,700 micrograms per day.

The Panel believes that the proposed MADL is conservative for several reasons. First, the method of exposure (gavage) does not closely represent human exposure. Human exposure to

¹ The EGs Panel is comprised of the following companies: Celanese, The Dow Chemical Company, Eastman Chemical Company, Huntsman Corporation, LyondellBasell Industries N.V., and Shell Chemical LP

² Neeper-Bradley, T. L., Tyl, R. W., Fisher, L. C., Kubena, M. F., Vrbanic, M. A. and Losco, P. E. (1995) *Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice*. *Fundam Appl Toxicol* 27: 121-130.

EG more closely resembles *dietary* exposure rather than exposure by gavage, which amounts to a large daily bolus dose. The use of an oral developmental toxicity study using the dietary route of exposure would result in a higher MADL.

Second, the proposed MADL does not adjust for the differences in pharmacokinetics in humans compared to rodents. The proximate developmental toxicant is glycolic acid, a metabolite of EG. Developmental toxicity occurs in rodents only at doses that saturate the metabolism of glycolic acid, resulting in a sharp increase in peak levels of glycolic acid (Carney, 2008, 2011).³ In comparison to the high dose testing in rodents, saturation of metabolism is unlikely to occur in humans exposed to EG in consumer products or through workplace exposure.

Finally, the NOEL used for this proposed MADL comes from a developmental toxicity study in mice, yet the mouse may not be the most relevant species for purposes of estimating a MADL for humans. Carney et al. (2008) concluded that the rabbit may be the most scientifically appropriate model for estimating the potential risk of developmental toxicity to humans based on the negligible role of the rabbit visceral yolk sac in placental transfer (humans lack a visceral yolk sac) and similar rates of EG metabolism and extraembryonic fluid turnover. Using the mouse study as the basis for the proposed MADL, even though the rabbit study may be more scientifically appropriate, is conservative because the NOEL in the mouse study (150 mg/kg bw/day) was more than 13 times lower than the NOEL in the rabbit study (>2000 mg/kg bw/day).

As discussed in its comments dated June 25, 2014, the Panel again urges OEHHA to consider eleven peer-reviewed, mechanistic reports published since 2004 that were not available to NTP-CERHR, the authoritative body OEHHA relied upon to list EG as a reproductive toxicant. These studies provide important insights into EG kinetics of uptake and metabolism, formation and elimination of the proximate developmental toxicant, glycolic acid (GA), and new information on species differences for mode of action (MOA). This new information clearly establishes that the sufficiency-of-evidence criteria were not met because adverse developmental effects in humans are not biologically plausible at non-lethal doses of EG.

The key mechanistic studies are presented in the following table.

³ Carney, E.W., Tornesi, B., Markham, D.A., Rasoulopour, R.J., and Moore, N. (2008) *Species-specificity of ethylene glycol-induced developmental toxicity: toxicokinetic and whole embryo culture studies in the rabbit*. Birth Defects Res B Dev Reprod Toxicol 83(6):573-81.

Carney, E.W., Tornesi, B., Liberacki, A.B., Markham, D.A., Weitz, K.K., Luders, T.M., Studniski, K.G., Blessing, J.C., Gies, R.A., and Corley, R.A. (2011) *The impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats*. Toxicol Sci 119(1):178-88.

<u>Year</u>	<u>Title</u>	<u>First Author</u>	<u>Major Findings</u>
2004	Comparison of the metabolism of ethylene glycol and glycolic acid <i>in vitro</i> by precision-cut tissue slices from female rat, rabbit, and human liver.	Booth	“The results...indicate that levels of glycolic acid, if formed <i>in vivo</i> , following exposures to similar concentrations of ethylene glycol, would be lower in humans than in rabbits and rats.”
2004	Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison.	Carney	This article presents reasons why EG is a developmental toxicant in rats but not in rabbits. “The dose of toxicant reaching the embryo is a critical determinant of developmental toxicity...” “The rabbit...shares several characteristics in common with humans, such as large embryonic and extraembryonic fluid volumes, a yolk sac that is free floating in the yolk sac cavity..., and a relatively large mass of chorionic tissue present from very early in pregnancy.”
2004	Dose-dependent transitions in mechanisms of toxicity: case studies	Slikker	“In animal studies using high doses of EG,...All of these factors converge to bring about very high levels of parent EG, which is rapidly converted to GA. The resultant high levels of GA saturate the enzymatic conversion of GA to glyoxylic acid, leading to a disproportionate rise in GA levels....For workplace, consumer, or other typical human exposures, the large capacity of metabolizing systems available to completely metabolize EG without ever approaching saturation of GA oxidation are thought to effectively preclude the possibility of EG induced developmental effects in humans.”
2005a	Development of a Physiologically Based Pharmacokinetic Model for Ethylene Glycol and Its Metabolite, Glycolic Acid, in Rats and Humans	Corley	“...a physiologically based pharmacokinetic (PBPK) model was developed to integrate the extensive mode of action and pharmacokinetic data on EG and GA for use in developmental risk assessments....When internal dose surrogates were compared in rats and humans over a broad range of

			<p>exposures, it was concluded that humans are unlikely to achieve blood levels of GA that have been associated with developmental toxicity in rats following occupational or environmental exposures.”</p> <p>“After inhalation exposures, however, where species differences in respiratory rates and cardiac output become more important (as compared with bolus oral dosing), the Cmax levels for GA are significantly higher in rats than in humans (Fig. 10b). For occupational exposures, this route of exposure is more important than bolus oral dosing.”</p> <p>“Given the low volatility of EG (0.06 mm Hg at 20°C), the theoretical maximum vapor concentration for ethylene glycol is only ~79 ppm (~200 mg/m³). This low volatility, coupled with the potential irritancy of EG aerosols, effectively limits the possibility for developmental toxicity in humans after inhalation exposures to EG.”</p>
2005b	Mode of Action: Oxalate Crystal-Induced Renal Tubule Degeneration and Glycolic Acid-Induced Dymorphogenesis—Renal and Developmental Effects of Ethylene Glycol	Corley	<p>“The initial steps in the postulated MOA for developmental toxicity involve metabolism of EG to GA, saturation of GA oxidation leading to its accumulation, distribution of GA to the conceptus, and induction of developmental toxicity, the hallmark of which is malformation of the axial skeleton and craniofacial structures.”</p>
2005c	Incorporation of Therapeutic Interventions in Physiologically Based Pharmacokinetic Modeling of Human Clinical Case Reports of Accidental or Intentional Overdosing with Ethylene Glycol	Corley	<p>“...the modifications to the human PBPK described in this article to include various treatment regimens used clinically to treat accidental or intentional ingestions of EG enabled Corley et al. (2005a) to compare internal dose surrogates of the intermediate metabolite, glycolic acid, over a broad dose range in rats and humans with a greater degree of confidence” in the PBPK model.</p>
2008	Species-specificity of	Carney	<p>“High-dose gavage exposure to ethylene</p>

	ethylene glycol-induced developmental toxicity: toxicokinetic and whole embryo culture studies in the rabbit.		glycol (EG) is teratogenic in rats, but not rabbits. To investigate the reason for this species difference, toxicokinetic and whole embryo culture (WEC) studies were conducted....The toxicokinetic profile suggested that the lower GA levels in rabbits were due to a slower rate of maternal metabolism of EG to GA, slow uptake of GA into the yolk sac cavity fluid which surrounds the embryo, and negligible transfer via the visceral yolk sac (VYS) placenta....Integration of these findings with published human data suggest that the rabbit is the more relevant model for human EG exposure,..."
2010 (online) 2011a (pub)	The Impact of Dose-rate on Ethylene Glycol Developmental Toxicity and Pharmacokinetics in Pregnant CD Rats	Carney	"Toward the goal of developing EG risk assessments based on internal dose metrics this study examined the differences between fast (bolus) and slow (continuous infusion) dose-rate exposures to EG on developmental outcome and pharmacokinetics.....In the sc bolus groups, increases in... malformations...and variations...were seen.... In contrast, equivalent daily doses of EG given slowly via infusion did not cause any developmental effects....In the case of EG, we can see clearly that high-dose gavage studies cause a shift from linear to nonlinear GA kinetics, which appears to be a prerequisite for EG-induced developmental toxicity. However, most human exposures involve much lower doses occurring via the dermal or inhalation routes, which are non-bolus. Given our understanding of GA kinetics, it is clear that gavage studies greatly overestimate the risk...."
2010	Dermal Penetration of Ethylene Glycol Through Human Skin <i>In Vitro</i>	Saghir	"...exposure of EG... to intact skin, either through dermal routes that may occur during normal working hours or by accidental exposure of the whole body for a short duration, will not elevate the systemic levels of toxic metabolites....Likely these kinetic explanations account for the lack of

			<p>any developmental toxicity in mice after dermal exposure, even though total penetration of the dermally applied dose is much higher in rodents than humans. Overall, these findings demonstrate that EG dermal penetration in humans is expected to be very low and to be slow, indicating very limited systemic or internal dose of EG due to dermal exposure.”</p>
2011b	Book chapter, Ethylene Glycol	Carney	<p>“By using an integrated approach which combined animal developmental toxicity studies, whole embryo culture experiments and an extensive array of toxicokinetic data, a strong body of evidence was compiled to implicate GA as the proximate toxicant. Furthermore, studies in rats supported a proposed threshold of at least 2mM GA in maternal blood (4 mM in conceptus) which must be exceeded in order to induce developmental effects. Achievement of such high internal concentrations of GA is favored by some specific conditions, namely: high dose, route characterized by rapid absorption (e.g., oral) and/or high dose-rate (Figure 45.5). These three variables converge to bring about very high blood levels of EG, which is readily metabolized to GA. The resulting high GA levels saturate the metabolism of GA to glyoxylic acid, such that GA production exceeds the rate of its further metabolism, resulting in a disproportionate or non-linear increase in blood GA concentrations. In species such as the rat and mouse, which concentrate weak acids within the conceptus, exposure of the embryo to GA is further increased, whereas in species such as the rabbit, GA is poorly distributed to the conceptus and embryonic GA levels are less than those of maternal blood. Given similarities in conceptus fluid volumes and pH gradients, GA disposition in the human is more likely to mirror the rabbit.”</p>
2014	Disposition of glycolic acid	Ellis-	<p>“This research explored the mechanisms of</p>

	into rat and rabbit embryos <i>in vitro</i>	Hutchings	GA disposition into rat and rabbit conceptuses using whole embryo culture (WEC)...Results for this research study suggest GA disposition into rat and rabbit embryos is energy- and pH-dependent, and carrier-mediated...These support and further refine an existing body of data indicating that the pregnant rat model is not relevant to humans due to fundamental differences in maternal metabolism coupled with qualitative differences in the direction of pH-dependent transport.”
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In addition, Environment Canada, under regulation CEPA 1999 in a 2014-updated report, states that there are no accounts of adverse human developmental effects and gives supporting evidence that EG exposures are of negligible concern for human developmental toxicity.

Thank you for the opportunity to comment on the proposed MADL for EG. If you have any questions, please contact me at (202) 249-6714 or at bill_gulledge@americanchemistry.com.

Sincerely yours,

William Gulledge

William P. Gulledge
Senior Director
Chemical Products & Technology Division