38941 Minnesota Department of Health Notice of Hearing (Initial Comment Period)

Closed Mar 08, 2023 · Discussion · 5 Participants · 1 Topics · 6 Answers · 0 Replies · 1 Votes

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William Reeves · Citizen · (Postal Code: unknown) · Mar 08, 2023 2:37 pm

0 Votes

Please find attached Bayer Crop Science's comments on the health risk level proposal.

Bill Gulledge · Citizen · (Postal Code: unknown) · Mar 08, 2023 4:34 pm

0 Votes

Please see attached comments from the ACC Ethylene Glycols Panel.

Via Email: nancy.rice@state.mn.us

March 8, 2023

Ms. Nancy Rice, MPH
Minnesota Department of Health
625 Robert Street North
P.O. Box 64975
Saint Paul, MN 55164-0975

Re: Ethylene Glycol (EG)- Proposed Health Risk Limit Rules

Dear Ms. Rice:

The Ethylene Glycols Panel (EGs Panel) of the American Chemistry Council (ACC) appreciates the opportunity to discuss our progress on developing research to address ethylene glycol (EG) risk assessments. We understand that MDH used what CERHR, 2004 stated was apparently the most sensitive species for developmental effects. However, we also understand that the exposure for a toxicity assessment must be by a route that, as you stated in your January 20, 2023 response to me, should "**represent a similar exposure as a person consuming ethylene glycol in their drinking water daily over a period of time.**"

The EGs Panel published the following studies addressing dose rate to determine the toxic response for EG.

> Pottenger, L. H., Carney, E. W. and Bartels, M. J. Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites in pregnant (GD 10) and nonpregnant Sprague-Dawley rats following oral administration of ethylene glycol. Toxicol Sci 2001; 62: 10-9.
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> E.W. Carney, B. Tornesi, A.B. Liberacki, D.A. Markham, K.K. Weitz, T.M. Luders, K.G. Studniski, J.C. Blessing, R.A. Gies, R.A. Corley, The impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats, Toxicol. Sci., 119 (2011), pp. 178-188.

The Panel maintains that using the Neeper-Bradley gavage study (fast dose rate) is not the appropriate study to determine point of departure (POD) in risk determination for a drinking-water daily exposure over a period of time, particularly for EG.

Please note that before the death of the major ethylene glycol researcher (Dr. Ed Carney) in the field of developmental toxicity, he published his final set of experiments. In this publication, Dr. Carney provides in detail, the importance of dose rate on EG developmental toxicity and pharmacokinetics. He concludes that for an EG risk determination, gavage administration (fast dose rate) would not be appropriate for determining risk for drinking water contaminated with EG.

Following is a key part of the Carney et al., 2011, paper including references to the Pottenger et al., 2001 that he coauthored (highlights have been added):

> "**In both nonpregnant and pregnant rats (GD 10) given EG via gavage, the dose-dependent shift to nonlinear GA kinetics was evident at dose levels ≥ 500 mg/kg** (Pottenger et al., 2001). The point at which GA kinetics become nonlinear just slightly precedes the apparent threshold for developmental toxicity in rats based on a no-observed effect level (NOEL) of 500 mg/kg/day and a lowest-observed effect level (LOEL) of 1000 mg/kg/day, suggesting that this dose-dependent transition is required for developmental toxicity… **A toxicokinetic study in pregnant rats revealed peak maternal blood GA values at the NOEL (500 mg/kg/day) and LOEL (1000 mg/kg/day) for developmental toxicity of 1.7 and 4.8mM, respectively** (Pottenger et al., 2001). These in vivo data correspond closely with in vitro rat whole-embryo culture data, which indicate a no-effect concentration of 3mM (Klug et al., 2001). Collectively, the available data led to the hypothesis that developmental toxicity in rats requires peak GA levels > 2mM in maternal blood and > 3mM in embryo (Corley et al., 2005b). Such high levels of GA are **plausible only after high-dose bolus exposure and**, **conversely, would seem unlikely to occur for low dose rate and/or low-dose exposures to EG.** Understanding the effect of dose rate is important to human risk assessment because human exposures to EG typically occur via the dermal or inhalation routes where the slow rate of exposure and/or absorption make saturation of GA kinetics highly unlikely (Frantz et al., 1996b; Sun et al., 1995)."

> "Research linking the developmental toxicity, mode of action, and pharmacokinetics of EG has been underway for a number of years with an aim toward refining human health risk assessments of this high production volume chemical. This integrated research program had already established that EG developmental toxicity required administration of very high doses and that saturation of GA oxidation was an essential step in the mode of action (Corley et al., 2005b; National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR), 2004; Slikker et al., 2004). **Based on the linkage between developmental NOELs/ LOELs in vivo and pharmacokinetic data in pregnant rats, it was hypothesized that developmental toxicity in rats required GA levels > 2mM in maternal blood and > 3mM in the embryo (Corley et al., 2005b). These putative threshold values have been widely accepted (e.g., NTP-CERHR, 2004), and it is readily appreciated that very high doses, particularly by the gavage route, are necessary to exceed these threshold values, whereas this is highly unlikely for nongavage exposures.** Nonetheless, the gavage route of

exposure has become entrenched in regulatory assessments for developmental toxicity, and there often is reluctance to base risk assessments and classification/labeling decisions on nongavage studies, even when these studies are available and human exposures occur by nongavage routes. To further our understanding of the impact of gavage versus other modes of human exposure, **the present study examined the variable of dose rate and its impact on pharmacokinetics and developmental toxicity.** To model a high dose, but low dose-rate scenario, a novel implantable and refillable spring-loaded infusion pump system was utilized which allowed for the maintenance of maternal blood GA levels at ~1mM, i.e., below the putative threshold, continuously from GD 6–15. Developmental outcomes following this slow dose-rate regimen were compared with those of rats given equivalent doses of EG in the form of daily sc bolus injections for the same period of time. Based on prior knowledge of EG kinetics, it was expected that the blood levels of the sc bolus group dams would each day temporarily exceed 2mM of the GA metabolite, which would then rapidly clear to undetectable levels by 18–24 h postdose (Carney et al., 1999).

Pharmacokinetic analyses in the present study verified that the sc bolus injections achieved GA concentrations greater than 2mM, whereas the infusion groups maintained GA concentrations below 2mM (Fig. 2B). In support of the hypothesis, developmental toxicity was observed in fetuses from dams given EG as a bolus, whereas no developmental effects were observed when the same doses of EG were given by infusion. The effects in the sc bolus groups were consistent with those seen in previous studies in which similar doses were given by gavage (Carney, 1994). These results also provided further support that a high Cmax, rather than AUC, is the key driver for developmental toxicity. As seen in Table 3, AUC values for embryonic GA were nearly identical in the gavage 1000 mg/kg group (52.1mMh) and 2000 mg/kg infusion groups (50.5mMh), yet only gavage exposure resulted in developmental toxicity. In contrast, Cmax values for embryonic GA were quite different in the 1000 mg/kg gavage group (6.3mM) versus the 2000 mg/kg infusion group (2.4mM), and these differences were correlated with developmental toxicity. Finally, a remarkable degree of correspondence was evident between the Cmax value (6.3mM) for the 1000 mg/kg gavage group (the in vivo LOEL for developmental toxicity) and the LOEC (6.0mM) identified in rat whole-embryo culture (Carney et al., 1996; Klug et al., 2001**). This consistency across in vivo developmental toxicity, in vivo toxicokinetics, and in vitro whole-embryo culture data provides a significant degree of confidence in these conclusions.**

The second part of this study examined the impact of dose rate on pharmacokinetics. Although numerous kinetic studies have been

conducted on EG given to male, female, and pregnant rats, this was the first comprehensive study to include specific analysis of EG, GA, and oxalic acid in maternal target organs (kidney), as well as the developing embryo, and the exocoelomic fluid contained within the visceral yolk sac placenta. A time-course assessment was conducted during GD 11–12, a time period known to show high susceptibility to EG induced teratogenic effects (Khera, 1991). Dose rate only had a moderate effect on the kinetics of unmetabolized EG and essentially no impact on oxalate. The fact that oxalate remained relatively constant across a large dose range further supports its lack of a causative role in EG developmental toxicity. The lack of change in oxalate despite large differences in levels of the upstream metabolite, GA (Fig. 4), is explained by differences in conversion and respiratory elimination of CO2 as shown by others (Frantz et al., 1996a). Based on previous rat developmental toxicity and pharmacokinetic data (Corley et al., 2005a,b; Neeper-Bradley et al., 1995), oxalate-induced kidney toxicity and secondary effects on pharmacokinetics were unlikely to have been present given the doses and relatively short durations of exposure evaluated in this study.

In contrast, the effect of dose rate on the kinetics of its metabolite and proximate teratogen, GA, was dramatic. Peak GA levels in maternal and conceptus tissues/fluids were 49– 100 times higher when 1000 mg/kg of EG was given by oral gavage relative to an equivalent daily dose given by infusion. AUCs also were higher in the gavage group relative to infusion, but the differences were not as great (16- to 38-fold). The profound impact on GA kinetics, especially Cmax values, is consistent with the saturation of GA's metabolism to glyoxylic acid (Fig. 1), which is the key rate-limiting step in EG's metabolic pathway. Another noteworthy finding was that the concentrations of GA in exocoelomic fluid and embryos were consistently higher (1.4- to 3-fold) than maternal blood levels (Figs. 3C and 3D), particularly for the first 6–12 h postdose. These ratios are very similar to those reported in an initial developmental kinetics study (Carney, unpublished data) where exocoelomic fluid levels of GA ranged from 1.3- to 1.8-fold higher than corresponding maternal blood levels in the first 3 h after dosing with either 500 or 2500 mg/kg EG by oral gavage. Most likely the higher levels of GA in rat exocoelomic fluid and embryo were due to pH-dependent ion trapping, driven by the more alkaline pH of rat exocoelomic fluid relative to maternal blood (Carney et al., 2004; Nau and Scott, 1986; Scott and Nau, 1987; Srivastava et al., 1991). **The results of this study strongly support the hypothesis that dose rate is a critical determinant of EG developmental toxicity, a phenomenon which is related to the saturation of the metabolism of GA, the proximate teratogen.**

Quantifying the effect of dose rate is important to human risk assessment because the most common routes of human exposure to EG are dermal,

characterized by very slow rates of absorption (Saghir et al., 2010; Sun et al., 1995), or secondarily, inhalation, for which exposures tend to be spread out over time (NTP-CERHR, 2004). Although the data support a value of 2mM peak maternal blood GA as the critical internal dose metric differentiating safe from potentially teratogenic EG exposures, the preferential disposition of GA in the rat embryo indicates that the critical dose metric at the level of the embryo is even higher. As mentioned previously, rat whole-embryo culture studies identified no-observable and LOEL concentrations of 3 and 6mM, respectively, following 48 h of continuous exposure to these test concentrations (Klug et al., 2001).

Comparative data suggest that it may be even more difficult to achieve these GA concentrations in the human embryo based on the fact that the celomic fluid of the first trimester human conceptus is ~0.2 pH units more acidic than the maternal blood (Jauniaux et al., 1994). Based on GA's pKa (3.83), the size and direction of the maternal blood-conceptus fluid pH gradient in humans and application of the Henderson-Hasselbach acid-base distribution equation, **one would predict GA concentrations in the human conceptus to be approximately half those of maternal blood GA. Interestingly, the yolk sac cavity fluids surrounding the rabbit embryo also are acidic with respect to maternal blood (Tornesi and Carney, 2003). Embryonic GA levels in the rabbit are correspondingly less than those of the maternal blood (Carney et al., 2008), and EG is not developmentally toxic in the rabbit, even following gavage exposure to doses as high as 2000 mg/kg/day (Tyl et al., 1993).**

Finally, the findings from this study have broader implications for the practice of developmental toxicity testing, as regulatory guidelines for prenatal developmental toxicity studies in animals require testing at maximally tolerated doses, with gavage as the default route of exposure. **This study exemplifies the tremendous disparities in pharmacokinetics that can occur following high-dose and high dose rate exposures relative to expected kinetic profiles at lower doses and dose rates. Increasingly, the wisdom of high-dose and high dose rate exposures, which run the risk of inducing shifts to nonlinear kinetics, is being questioned for the evaluation of chemicals present at low levels in the environment.** For these types of chemicals, an alternative approach to the maximum-tolerated dose garnering support calls for setting the high-dose level based on the point of transition to nonlinear kinetics [*e.g., kinetically-derived maximum dose or KMD*], supported by information on internal dose, so as to increase relevance of the data to humans (Saghir et al., 2009**).**

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. [*This*

observation is consistent with that made for many chemicals in which bolus gavage exposures achieve nonlinear toxicokinetics at oral doses that are approximately an order of magnitude lower than corresponding continuous oral exposures (Kirman et al., 2003).] However, most human exposures involve much lower doses occurring via the dermal or inhalation routes, which are nonbolus. Given our understanding of GA kinetics, it is clear that gavage studies greatly overestimate the risk of typical environmental and workplace exposures, which occur by the dermal and inhalation routes and are characterized by low doses and/or low dose rates."

To summarize, when large doses of EG are given by a fast dose rate as in gavage, the saturation of the oxidative enzyme systems for EG occurs, and developmental toxicity can occur. This fast rate or gavage would represent a suicide attempt and does not "represent a similar exposure as a person consuming ethylene glycol in their drinking water daily over a period of time." (quote from MDH January 20, 2023 response) **Please also note that this key dose-rate phenomenon has been shown in rats where EG was given at 1000 mg/kg in the diet (slow dose-rate), and no developmental toxicity was observed (Maronpot et al., 1983, Teratogenicity study of ethylene glycol in rats), but when rats were given 1000 mg/kg by gavage (fast dose-rate), developmental toxicity was observed (Neeper-Bradley et al. 1985).**

We now have discovered there are 21 peer-reviewed pertinent additional studies conducted AFTER the CERHR 2004 publication (Appendix I). We incorrectly stated in our March 8th, 2021 submission to MDH that there were only 14 studies. In your January 20, 2023, response it was stated "and while some of these publications were not individually cited, they are **reviewed or summarized** as part of larger reports like the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction's (CERHR) 2004 review." Please note that we have reviewed the CERHR 2004 report in detail and **none of these studies was mentioned.**

We feel that this large, significant, pertinent database is extremely useful in risk assessment determination and must be considered to develop a meaningful risk assessment. We feel that at some point in time, this new research must be recognized.

ACC's reply to statements made by MDH in the January 20, 2023 correspondence to Bill Gulledge

To address some of the MDH statements in the January 20, 2023 response to ACC, we have included your "statements" in quotes and followed with our reply in brackets [ ].

- MDH stated: "MDH identified an administered no observed adverse effect level (NOAEL) of 150 mg/kg-d and an administered lowest observed adverse effect level (LOAEL) of 500 mg/kg-d based on increased **skeletal malformations**."

[We believe this statement is incorrect**. Neeper Bradley, et al., 1985 did not classify the extra 14th rib as a malformation, but as a variation**. And moreover, in the CERHR 2004 report it is stated "The incidences of one individual **skeletal variation (extra lumbar rib)** in litters from the 500 mg/kg bw/day group and 23 individual skeletal variations (i.e., poorly ossified thoracic and lumbar centra, extra lumbar ribs) in litters of the 1,500 mg/kg bw/day group were

significantly increased." Since the OECD Test Guideline definition of a variation is "Variation/Minor Abnormality: Structural change **considered to have little or no detrimental effect on the animal; may be transient and may occur relatively frequently in the control population.**" Please also note in the Neeper-Bradley et al., 1985 publication, this variation was noted in the controls. In addition, in Carney, 1994 (An integrated perspective of the developmental toxicity of ethylene glycol. Reprod. Toxicol. 8 (2), 99–113.), it is discussed that **the mouse and rat are more similar than a quick look at only the NOELs would indicate**. Carney states, "In evaluating species differences in sensitivity, one must consider that the **mouse LOEL of 500 mg/kg/day is based solely on an increase incidence of one skeletal variation** (14^{th} rib). In contrast, **decreased fetal body weights** and **increased incidence of two malformations** and **12 skeletal variations were noted at the rat LOEL of 1000 mg/kg/day.** Thus, rat and mouse embryos/fetuses are probably more similar than a cursory glance at the NOELS would indicate."]

- MDH stated: "Such sophisticated data and models are usually available for only a small subset of chemicals that have extensive databases (SONAR, 2009). While the PBPK database for ethylene glycol may be rich for animal models, it is not complete enough to construct a realistic model for humans. Responses to chemicals are often incongruent between laboratory animals and humans. In the absence of strong evidence showing that the rodent PBPK is similar to humans, MDH defaults to developing an HED using a dosimetric adjustment factor (DAF) using body weight scaling (SONAR 2009)."

[ACC disagrees and believes there is "extensive," "complete enough," and "strong evidence" for the PBPK database for rodents and **humans**. Following are our key publications to support this statement:

E.W. Carney, B. Tornesi, A.B. Liberacki, D.A. Markham, K.K. Weitz, T.M. Luders, K.G. Studniski, J.C. Blessing, R.A. Gies, R.A. Corley, The impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats. Toxicol. Sci., 119 (2011), pp. 178-188

R.A. Corley, K.E. McMartin, Incorporation of therapeutic interventions in physiologically based pharmacokinetic modeling of **human clinical case reports** of accidental or intentional overdosing with ethylene glycol. Toxicol. Sci., 85 (2005), pp. 491-501

R.A. Corley, M.J. Bartels, E.W. Carney, K.K. Weitz, J.J. Soelberg, R.A. Gies, K.D. Thrall, Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic Acid, in rats and **humans.** Toxicol. Sci., 85 (2005), pp. 476-490.

Corley, R. A., Meek, M. E., and Carney, E. W. Mode of action: oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—renal and developmental effects of ethylene glycol. Crit. Rev. Toxicol. 35, (2005b). 691–702.

R.A. Corley, D.M. Wilson, G.C. Hard, K.E. Stebbins, M.J. Bartels, J.J. Soelberg, M.D. Dryzga, R. Gingell, K.E. McMartin, W.M. Snellings, Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicity. Toxicol. Appl. Pharmacol., 228 (2008), pp. 165-178

R.A. Corley, S.A. Saghir, M.J. Bartels, S.C. Hansen, J. Creim, K.E. McMartin, W.M. Snellings, Extension of a PBPK model for ethylene glycol and glycolic acid to include the competitive formation and clearance of metabolites associated with kidney toxicity in rats and **humans.** Toxicol. Appl. Pharmacol., 250 (2011), pp. 229-244]

- MDH stated: Our methods state "It is assumed that humans are at least as sensitive as the most sensitive mammalian species for which there are toxicological data. Substantial evidence that the response seen in laboratory animals is due to a mechanism that does not exist in humans can overcome this assumption."

[ACC does not agree with MDH's statement regarding the sensitivity of human vs. rodents' developmental effects of EG proximate toxicant, glycolic acid (GA). Again, there is a clear species difference in the active disposition facilitated by opposite polarity of rodent MCT transporters vs. that of rabbits and humans. Recent investigations conducted after the 2004 CERHR evaluation demonstrated that GA uptake into the rat embryo occurs predominantly by a specific, pH-dependent, active uptake transporters MCT1 and MCT4.]

- MDH stated: "MDH also recognizes that there is evidence of species, strain, and sex differences in the metabolism and clearance of ethylene glycol. As the EGs Panel has pointed out, rabbits exposed in utero to ethylene glycol do not exhibit the same developmental effects as rodents do. The EGs Panel asserts that the mechanistic and toxicokinetic findings from Carney et al. (2008), Ellis-Hutchings et al. (2014), and Moore et al. (2016) conclude that rodents are inappropriate animal models for testing potential developmental effects following exposure to ethylene glycol and rabbits are more appropriate, however, MDH risk assessors do not agree and consider the findings preliminary."
  Research by Ellis-Hutchings et al. 2014 used whole embryo cultures to explore the rat and rabbit's ability to concentrate ethylene glycol. Their findings suggest that the ability of the rat embryo to concentrate glycolic acid is pH dependent and may involve a protein transporter.
  The expression of these transporters has been investigated in the rabbit and rat placenta by Moore et al., 2016, who concluded that the arrangement of transporters in the placenta of rats had an opposite polarity compared to the rabbit placenta, which they report is similar to the humans. There is no functional consequence reported."

[ACC disagrees that there is no functional consequence reported. A clear argument has been made that the major, active pathway of glycolic acid (GA) disposition into the rat and mouse developing embryo is via the MCT transporters located in the placenta. The orientation of the rabbit and human MCT transporters is opposite to rodents; this polarity would not allow for a significant accumulation of GA into rabbit and human embryo during the critical window of development compared with that of rodents where developmental toxicity is observed. While there is a passive disposition of the proximate toxicant into the developing embryos, this accounts for a minor percentage of GA disposition into accumulation within developing embryos.]

- MDH stated: "While the studies cited above do provide some insight as to why there may be species differences in susceptibility to developmental effects due to differences in placental biology, they do not fully elucidate how these differences functionally change the processing of ethylene glycol. They also do not sufficiently demonstrate that the findings from the critical study in mice are irrelevant to human health risk assessment. As directed by our methods (SONAR 2009, p.27, also cited above) MDH selected a POD based on developmental effects from the most sensitive species, the mouse in this case, to derive the short-term guidance value."

[ACC argues that the active disposition of GA via MCT transporters, accounting for major proportion of GA disposition from maternal blood to the developing embryo, underlies the species differences seen in developmental toxicity. TK effects of rodents vs. that not seen in rabbits at 2000 mg/kg/d are not expected for humans. These species differences functionally result in rabbit and human placenta with similar polarity of MCT1 and MCT 4 which is opposite to that of the rat and mouse MCTs. Hence, GA is preferentially sequestered in the mouse and rat embryo and not the rabbit embryo. By extension, rat and mouse developmental effects are not appropriate model for human hazard characterization and risk assessment for EG and GA.]

- MDH stated: "In your letter you mentioned 14 peer-reviewed publications…while some of these publications were not individually cited, they are reviewed or summarized **as part of larger reports like the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction's (CERHR) 2004 review**."

[We appreciate that MDH appears to have spent significant time on reviewing our submission. MDH is not the first regulatory group that has used the CERHR 2004 report as their main supportive evidence for using the Neeper-Bradley et al., 1985 gavage study for the basis of a risk assessment. ACC is hoping that time would allow for one regulatory group to feel as we do, that the database for EG is now extensive with strong evidence that renal toxicity is the best POD for a risk assessment and that gavage or fast dose rate is not appropriate for human health risk assessment. (W.M. Snellings, R.A. Corley, K.E. McMartin, C.R. Kirman, S.M. Bobst, Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect. Regul. Toxicol. Pharmacol., 65 (2) (2013), pp. 229-241.)

Unfortunately, none of the following new studies could have been reviewed or summarized, as you stated in the CERHR 2004 report, since they were not completed until after the 2004 CERHR review. We now note that there are a total of 21 new important and pertinent peer-reviewed studies (Appendix I), including many studies on metabolism, pharmacokinetics, and pharmacodynamics, that should be considered when performing a risk assessment on EG. We realize that it would be easier to just stick with the Neeper-Bradley 1985 study but would hope we can help in determining why the EG case should be re-opened for risk determination.]

ACC Conclusion

Finally, we feel strongly that MDH should consider what the **National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction's (CERHR) 2004** review states in **support of our position on the kidney being the appropriate endpoint for risk assessment**. They state in their conclusion:

> "5.3 Overall Conclusions **Available data from rat studies suggest that oral doses associated with developmental toxicity (1,000 mg/kg bw) are greater than doses associated with renal toxicity (500 mg/kg bw).** Developmental toxicity, and evidence of some renal toxicity, are observed in rodents at doses that exceed saturation of glycolic acid metabolism, which clearly occurs at 500 mg/kg bw in rats. Limited human in vitro data suggest that saturation of glycolic acid metabolism occurs at ~125 mg/kg bw, but saturation is expected to require much higher doses for slower dose-rate (non-bolus) exposure or for routes characterized by poor absorption (e.g., dermal). **The Panel believes that ethylene glycol exposures resulting in blood levels below the level of saturation should not result in hazard associated with developmental toxicity in humans.** There are no data that are viewed as reliable estimates of human exposure in the general human population. It was noted that Health Canada had estimated a worst-case-scenario for persons living in the immediate vicinity of an ethylene glycol point source in the range of 0.022–0.088 mg/kg bw/day. The Panel also constructed two occupational exposure scenarios based on data presented in Section 1.2.4.2:
>
> • Occupational inhalation exposure to 188 mg/m3 (irritation limit) for 15 minutes resulting in a burden of 0.8 mg/kg bw for 15 minutes (21 L/minute, 70 kg bw).
>
> • Occupational inhalation exposure of 10 mg/m3 (the Expert Panel-estimated median of deicing data) for 480 minutes resulting in a total exposure burden of 1.4 mg/kg bw/8 hours (21 L/minute, 70 kg bw).
>
> A comparison of the exposures associated with these scenarios to the dose where saturation of human metabolism is estimated to occur (125 mg/kg bw) **shows that all of these expected exposures in the human are at least 100- to 1,000-fold lower than those expected to result in**

> **metabolic saturation. Scenarios involving continuous rather than acute exposures would have even a larger margin of safety due to dose rate phenomena**. This comparison does not take into account the potential impact of human interindividual variability. **The Expert Panel judges the likelihood of adverse developmental toxicity in the humans from such levels of exposure to be of negligible concern. The Panel concludes that the lack of reproductive toxicity in experimental animal studies indicates there is negligible concern for reproductive effects in humans."**

In addition, as stated by another regulatory agency, Environment Canada and Health Canada, in their Final Report (Environment Canada and Health Canada Final Report April, 2010, Priority Substance List Assessment Report, Follow-Up to the State of Science Report, 2000 on Ethylene Glycol. http://www.ec.gc.ca/lcpe cepa/default.asp?lang=En&n=4B7409ED-1.)

> "These PBPK models have predicted that it is unlikely to achieve levels of human blood glycolic acid concentrations that could lead to developmental toxicity. Humans would only achieve the threshold for developmental effects determined in rats of 2 mM if they **consumed bolus oral doses greater than 350 mg/kg** (> 20 g ethylene glycol for a 58 kg female) during the critical window of susceptibility based on simulations of peak maximum blood concentrations of glycolic acid."

We conclude that 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.**

We can shortly supply these 21 publications in **Appendix I** and would offer you any time you may need to discuss these findings or to summarize the importance of each so that you can see that this significant new research should be considered for determining risk and that for the reasons given here that kidney should be used to determine the POD in a health risk assessment.

Should you have any questions regarding these comments, please contact me at (202) 249-6714 or bill_gulledge@americanchemistry.com .

Sincerely,

Bill Gulledge

Bill Gulledge

Senior Director, Chemical Products & Technology Division

Appendix I

Booth et al., 2004. Booth, E.D., Dofferhoff, O., Boogaard, P.J., Watson, W.P. "Comparison of the metabolism of ethylene glycol and glycolic acid in vitro by precision-cut tissue slices from female rat, rabbit and human liver" (Article) Xenobiotica, Volume 34, Issue 1, January 2004, Pages 31-48.

Carney et al., 2004. Carney, E. W., Scialli, A. R., Watson, R. E., and DeSesso, J. M. "Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison." Birth Defects Res. C Embryo Today 72, 2004, 345–360.

Carney et al., 2008. E.W. Carney, B. Tornesi, D.A. Markham, R.J. Rasoulpour, N.P. Moore "Species-specificity of ethylene glycol-induced developmental toxicity: toxicokinetic and whole embryo culture studies in the rabbit" Birth Defects Res, 8B 83 (2008), pp. 573-581.

Carney et al., 2011.E.W. Carney, B. Tornesi, A.B. Liberacki, D.A. Markham, K.K. Weitz, T.M. Luders, K.G. Studniski, J.C. Blessing, R.A. Gies, R.A. Corley. "The impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats" Toxicol. Sci., 119 (2011), pp. 178-188.

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Corley, et al., 2005b. Corley, R. A., Meek, M. E., and Carney, E. W. "Mode of action: oxalate crystal-induced renal tubule degeneration and glycolic acid-induced dysmorphogenesis—renal and developmental effects of ethylene glycol." Crit. Rev. Toxicol. 35, (2005b). 691–702.

Corley et al., 2008. R.A. Corley, D.M. Wilson, G.C. Hard, K.E. Stebbins, M.J. Bartels, J.J. Soelberg, M.D. Dryzga, R. Gingell, K.E. McMartin, W.M. Snellings. "Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicity" Toxicol. Appl. Pharmacol., 228 (2008), pp. 165-178.

Corley et al., 2011. R.A. Corley, S.A. Saghir, M.J. Bartels, S.C. Hansen, J. Creim, K.E. McMartin, W.M. Snellings. "Extension of a PBPK model for ethylene glycol and glycolic acid to include the competitive formation and clearance of metabolites associated with kidney toxicity in rats and humans" Toxicol. Appl. Pharmacol., 250 (2011), pp. 229-244

Cruzan et al., 2004. . G. Cruzan, R.A. Corley, G.C. Hard, J.J. Mertens, K.E. McMartin, W.M. Snellings, R. Gingell, J.A. Deyo. "Subchronic toxicity of ethylene glycol in Wistar and F-344 rats related to metabolism and clearance of metabolites" Toxicol. Sci., 81 (2004), pp. 502-511.

Ellis-Hutchings et al., 2014. R.G. Ellis-Hutchings, N.P. Moore, V.A. Marshall, R.J. Rasoulpour, E.W. Carney. "Disposition of glycolic acid into rat and rabbit embryos in vitro" Reprod. Toxicol., 46 (2014), pp. 46-55.

Guo and McMartin, 2005. C. Guo, K.E. McMartin. “The Cytotoxicity of oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation” Toxicology, 208 (2005), pp. 347-355.

Guo et al., 2007. C. Guo, T.A. Cenac, Y. Li, K.E. McMartin. “Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol” Toxicol. Lett., 173 (2007), pp. 8-16.

Hovda et al., 2010. K.E. Hovda, C. Guo, R. Austin, K.E. McMartin. “Renal toxicity of ethylene glycol results from internalization of calcium oxalate crystals by proximal tubule cells” Toxicol. Lett., 192 (2010), pp. 365-372.

Li et al., 2010. Y. Li, M.C. McLaren, K.E. McMartin. “Involvement of urinary proteins in the rat strain difference in sensitivity to ethylene glycol-induced renal toxicity” Am. J. Physiol. Renal Physiol., 299 (2010), pp. F605-615.

Manini et al., 2009. A.F. Manini, R.S. Hoffman, K.E. McMartin, L.S. Nelson. “Relationship between serum glycolate and falsely elevated lactate in severe ethylene glycol poisoning” J. Anal. Toxicol., 33 (2009), pp. 174-176.

McMartin and Wallace, 2005. K.E. McMartin, K.B. Wallace. “Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function” Toxicol. Sci., 84 (2005), pp. 195-200.

Moore et al., 2016. N. Moore, C. Picut, J. Charlap 2016. “Localisation of lactate transporters in rat and rabbit placentae” International J. Cell Biol., 2016 (2016), 10.1155/2016/2084252.

Saghir et al., 2010. S.A. Saghir, M.J. Bartels, W.M. Snellings. “Dermal penetration of ethylene glycol through human skin in vitro” Int. J. Toxicol., 29 (2010), pp. 268-2762.

Snellings et al., 2013. W.M. Snellings, R.A. Corley, K.E. McMartin, C.R. Kirman, S.M. Bobst. “Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect?” Regul. Toxicol. Pharmacol., 65 (2) (2013), pp. 229-241.

Upadhyay et al., 2008. S. Upadhyay, J. Carstens, D. Klein, T.H. Faller, S. Halbach, W. Kirchinger, W. Kessler, G.A. Csanády, J.G. Filser. “Inhalation and epidermal exposure of volunteers to ethylene glycol: kinetics of absorption, urinary excretion, and metabolism to glycolate and oxalate” Toxicol. Lett., 178 (2008), pp. 131-141.