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Mr. William Gulledge
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Via Electronic Mail: Bill_Gulledge@americanchemistry.com

Re: New Information on Ethylene Glycol (EG) For Determining More Accurate Ethylene Glycol Health Risk Limits in Groundwater

Dear Mr. Gulledge:

Thank you for submitting comments from the Ethylene Glycols Panel (EGs Panel) of the American Chemistry Council (ACC) on the proposed amendment to the Health Risk Limits Rule (HRL) for ethylene glycol dated March 8th, 2021.

In your letter you mentioned 14 peer-reviewed publications that were not explicitly called out in the reference section of our summary sheet for ethylene glycol, 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. The list of sources at the end of our summary sheet includes the references that were impactful for water guidance development. It does not include the entire list of all references consulted as part of the review. The more specific comments in your letter regarding how this information might influence our assessment will now be addressed.

Minnesota Department of Health (MDH) risk assessors selected the Neeper-Bradley (1995) developmental mouse study as the critical study for the short-term duration guidance. Ethylene glycol is a known developmental toxicant for two different species of mammals (rodents). Although developmental toxicity has not been observed in accidental or occupational exposures, these do not represent a similar exposure as a person consuming ethylene glycol in their drinking water daily over a period of time. Our methods state that "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." The occupational and accidental exposures do not overcome the evidence in rodents (Statement of Need and Reasonableness, SONAR, 2009, page 27).

The lowest point of departure (POD) from Neeper-Bradley (1995) shows that a sensitive lifestage (i.e., developing fetuses) is affected. 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. The selected POD from the critical study was the lower confidence limit of a benchmark dose corresponding to a 10% ($BMDL_{10\%}$) increase in skeletal malformations over control animals. The selection of this study and use of benchmark dose modeling to derive a POD is in accord with the guidance of our published methods laid out in our 2009 SONAR. Modeled results are often considered to be superior as modeling takes into account the entire dose response curve rather than a few discrete data points as does the LOAEL/NOAEL approach.

MDH risk assessors did not find the available toxicological and toxicokinetic information on ethylene glycol to be sufficient to develop chemical specific adjustment factors to extrapolate the dose from mice to humans. It appears that saturation of glycolic acid kinetics is likely needed to see the developmental effects captured in the various rodent studies, and in mice this threshold may indeed be near 150 mg/kg-d as you assert based on the Neeper-Bradley (1995) NOAEL and LOAELs. However, there is no *in vivo* human data that identifies the dose needed for glycolic acid metabolic saturation; therefore potential developmental effects could occur at a similar or even lower dose. Additionally, both the NTP and MDH identified the developmental NOAEL in the Neeper-Bradley (1995) study at 150 mg/kg-d, suggesting that kinetic saturation had not yet been reached at the lower POD, $BMDL_{10\%}$ of 76.5 mg/kg-d. Therefore, a body weight scaling based dosimetric adjustment factor (DAF) was used.

MDH follows the hierarchy that the EPA laid out in 2002 for applying HEDs. The preferred option is to use a chemical-specific physiologically based pharmacokinetic (PBPK) model. A PBPK model estimates the dose to a target tissue or organ by taking into account the rate of absorption into the body, distribution among target organs and tissues, metabolism, and excretion. Constructing a PBPK model is an information intensive process that requires a significant quantity of chemical-specific data, including route-specific data. 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).

MDH reviewed your comments in Table 1 of your letter on our guidance value derivation for the short-term duration. A benchmark response level (BMR) of 10%, which as stated by ATSDR in their 2010 assessment, is the lowest BMR that was supported by the data. Usually MDH would usually apply a BMR of 5% for a developmental critical effect because fetuses are more sensitive to chemicals, but it was not supported by the data. You also commented on MDH's choice of an appropriate relative source contribution factor (RSC). It is based on volatility as you noted, and MDH classified ethylene glycol as nonvolatile contaminant using the Henry's Law Constant. However, the critical endpoint occurred after *in utero* exposure, so a pregnant woman's intake rate was used because it represents the exposure of

concern, and therefore the default RSC for all other non-infant life stages (RSC= 0.2) for nonvolatile chemicals was used rather than the infant-based RSC of 0.5 (see SONAR, 2008, pg. 51). This is explained in the Toxicological Summary Sheet's footnote as well.

> The RfD is based on malformations that occur in utero, therefore, the intake rate for a pregnant woman is utilized rather than the default infant intake rate as described in the MDH 2008 SONAR (page 46). Effects relevant to post-natal development occurred at higher dose levels. As the short-term duration intake is based on pregnant women, not infants, a Relative Source Contribution of 0.2 is utilized. (MDH, 2020).

MDH acknowledges your comments on the subchronic and chronic duration guidance, namely that they should not be based on a gavage developmental study and that rodent studies evaluating renal effects as seen in the Corley et al. (2008) and Cruzan et al. (2004) studies are more appropriate. However, as per our methods, "the longer-duration HRLs must be protective of short exposures that may occur within the longer duration", therefore a developmental study would still be appropriate for a subchronic and chronic guidance as the short-term period of 2-30 days is also contained in those longer durations (SONAR 2009, p.23). This rulemaking does not address methods; the parameter specific comments laid out in Tables 2 and 3 will not be addressed further.

MDH reviewed the EGs Panel additional statements on 1) applicability of gavage route of administration in rodent developmental studies, 2) appropriateness of gavage route of administration for estimated risk from EG contaminated drinking water and dose-rate phenomenon, and 3) mouse and rat model versus the rabbit in developmental/reproductive risk assessment.

In choosing the critical study and POD for an assessment, MDH takes into consideration all available data from toxicology studies using oral routes of administration including gavage studies. It is true that many of the available animal toxicology studies utilize gavage (bolus) dosing and form the basis of assessments meant to estimate risk from lower, continuous exposures to the contaminant via water ingestion; this is not a new concern. Although our methods do not explicitly discuss bolus dosing versus continuous dosing, we carefully consider the limitations of all gavage studies we use in guidance development, and frequently choose to use them if they are the best available study in a sensitive species.

Your comments concerning the dose-rate phenomenon in rats for ethylene glycol and the inclusion of extensive comparison of effect levels taken from bolus vs non-bolus rat studies was helpful to MDH in examining this issue. We again reviewed the evidence and did not find the bolus dosing to be problematic. Additionally, our methods describe PODs as the lowest dose level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group; or the closest lower dose tested (the highest dose level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects) (SONAR, 2009). In addition to the NOAEL/LOAEL method for identifying a POD explained above, our methods also recommend Benchmark Dose (BMD) modeling as well. Although

we consider modeling to provide a higher quality POD, in some cases the data is not suitable to modeling and the other approach is used.

Neeper-Bradley (1995) was selected as the critical study because it provided the lowest POD in the most sensitive species identified, the mouse. The developmental and reproductive mouse studies that administered ethylene glycol via drinking water were not selected as the critical study because they used higher doses (Gulati et al., 1986; Morrissey et al., 1989, and Lamb et al., 1985 as discussed in NTP, 2004).

MDH also recognizes that there is evidence of species, strain, and sex differences in the metabolism and clearance of ethylene glycol. As the EG 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.

It is also important to note that the time course for fetal development varies greatly between mammalian species. Processes that are observed at day 4 in mouse development likely do not appear in human fetuses on day 4. Additionally, the placenta is complex and dynamic during a pregnancy. Transporters that allow for the passage of nutrients and some chemicals across the placenta may be expressed differently at different times during a pregnancy.

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.

It is MDH's mission to protect the health of all Minnesotans, including sensitive populations and the most vulnerable. The EGs Panel suggests using a higher DAF and RSC in the short-term guidance

derivation, and moreover that the route of administration and model species in the critical study were not appropriate. Applying these changes would result in a higher water guidance value that would not be protective of early life stages. Disregarding the developmental effects seen in fetal mice and reported in the Neeper-Bradley et al. (1995) study because of the route of administration and species sensitivity without more conclusive information would contradict MDH's mission to protect, maintain, and improve the health of all Minnesotans. Therefore, to be protective for all populations, MDH will retain the short-term proposed critical study, as well as the DAF and RSC without modification.

Sincerely,

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**Works Cited**

Corley RA, DM Wilson, GC Hard, KE Stebbins, MJ Bartels, JJ Soelberg, MD Dryzga, R Gingell, KE McMartin, WM Snellings. 2008. Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicityl. Tox Appl Pharm 228:165-178.

Cruzan G. RA Corley, GC Hard, JJWM Mertens, KE McMartin, WM Snellings, R Gingell, JA Deyo. 2004. Subchronic Toxicity of Ethylene Glycol in Wistar and F-344 Rats Related to Metabolism and Clearance of Metabolites. Tox Sci 81:502-511.

Neeper-Bradley, TL, RW Tyle, LC Fisher, MF Kubena, MA Vrbanic, PE Losco. 1995. Determination of a No-Observed-Effect Level for Developmental Toxicity of Ethylene Glycol Administered by Gavage to CD Rats and CD-1 Mice. Fund Appl Tox 27: 121-130.

NTP-CERHR 2004. NTP-CERHR Expert Panel report on the reproductive and developmental toxicity of ethylene glycol. Reproductive Toxicology 18:457-532.