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

March 8, 2021

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

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

Dear Ms. Rice:

The Ethylene Glycols Panel (EGs Panel) of the American Chemistry Council (ACC) presents significant new information that should be considered in developing a regulatory risk assessment. The ACC EGs Panel represents the manufacturers of ethylene glycols in North America. ACC represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier, and safer.

The Panel's comments identify an additional 14 peer-reviewed publications, not listed in the Minnesota Department of Health Toxicological Summary for Ethylene Glycol. Applying this new information would modify the derivation approaches of the health based value used by MDH and provide state of the science alternatives. These studies include new work on:

- Kinetics and Modeling
    - absorption,
    - distribution,
    - biotransformation
    - elimination
- Toxicodynamics,
- Mode and Mechanism of action

The EGs Panel believes this information will assist in developing a more up-to-date risk assessment. We would be glad to provide any of the references discussed in the comments below.

Background

The Minnesota Department of Health (MDH) requested (December 18, 2020) comments on amendments to the rules governing Health Risk Limits (HRLs) for Ethylene Glycol (EG) in groundwater. The proposed amendment for EG is to replace outdated HRL values in the existing Health Risk Limits Tables found in Minnesota Rules, parts 4717.7500 and 4717.7860. The EGs Panel understands it will have the opportunity to resubmit further comments after the rules are formally proposed.

The MDH August 2020 Toxicological Summary for Ethylene Glycol states that the Short-term Non-Cancer Health-based Value ($nHBV_{Short\text{-}term}$) is based on the results from the Neeper-Bradley et al., 1995 gavage study.

MDH's stated derivation is as follows:

Short-term Non-Cancer Health Risk Limits ($nHBV_{Short\text{-}term}$) = 2,000 µg/L

$$\frac{\text{(Reference Dose, mg/kg-d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Short-term Intake Rate, L/kg-d)}}$$

$$= \frac{\text{(0.33 mg/kg-d) x (0.2) x (1000 µg/mg)}}{\text{(0.038 L/kg-d)}}$$

= 1,736 rounded to 2,000 µg/L

Comments on MDH's $nHBV_{Short\text{-}term}$

Based on the considerable amount of research, the EGs Panel proposes that the science supports the following for Derivations of Short-term Non-Cancer Health-based Value ($nHBV_{Short\text{-}term}$) and differs from the one derived by MDH.

The mode of action (MOA) for EG-induced developmental toxicity has been described by an independent panel of developmental toxicity experts for NTP (CERHR, 2004. *U.S. Department of Health and Human Services, National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction (2004). CERHR Monograph on the potential human reproductive and developmental effects of ethylene glycol. Retrieved from ntp.niehs.nih.gov/ntp/ohat/egpg/ethylene/eg_monograph.pdf.)* and involves the formation of the metabolite, GA. Within this MOA, a key event that precedes and is required for the manifestation of developmental toxicity is the saturation of GA metabolism. The dose at which GA metabolism becomes saturated has been well characterized in rats (~500 mg/kg-d, non-bolus exposures) and humans (~150 mg/kg-d, non-bolus exposures) based on pharmacokinetic data and modeling (CERHR, 2004; Corley et al., 2011. *Corley, R.A., Saghir, S.A., Bartels, M.J. et al., 2011. 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, 229–244.).*

For bolus exposures, the saturating doses of EG are approximately 3-fold lower (e.g., ~150 mg/kg-d and ~50 mg/kg-d for rats and humans, respectively (Corley et al., 2011). Although a

PBPK model has not been developed for EG in mice, the pharmacokinetic data of Frantz et al. (*Frantz SW, Beskitt JL, Grosse CM, Tallant MJ, Dietz FK, Ballantyne B (1996a) Pharmacokinetics of ethylene glycol. I. Plasma disposition after single intravenous, peroral, or percutaneous doses in female Sprague-Dawley rats and CD-1 mice. Drug Metabolism and Distribution, 24:911-921; Frantz SW, Beskitt JL, Grosse CM, Tallant MJ, Dietz FK, Ballantyne B (1996b) Pharmacokinetics of ethylene glycol. II. Tissue distribution, dose-dependent elimination, and identification of urinary metabolites following single intravenous, peroral or percutaneous doses in female Sprague-Dawley rats and CD-1 mice. Xenobiotica, 26:1195–1220; Frantz SW, Beskitt JL, Tallant MJ, Zourelias LA, Ballantyne B (1996c) Pharmacokinetics of ethylene glycol. III. Plasma disposition and metabolic fate after single increasing intravenous, peroral, or percutaneous doses in the male Sprague-Dawley rat. Xenobiotica, 26:515–539)* clearly show that mice, like other species, also exhibit saturation of GA metabolism, and that this saturation occurs in mice at doses that are approximately 3-fold lower than observed in rats (see **Figure 1**).

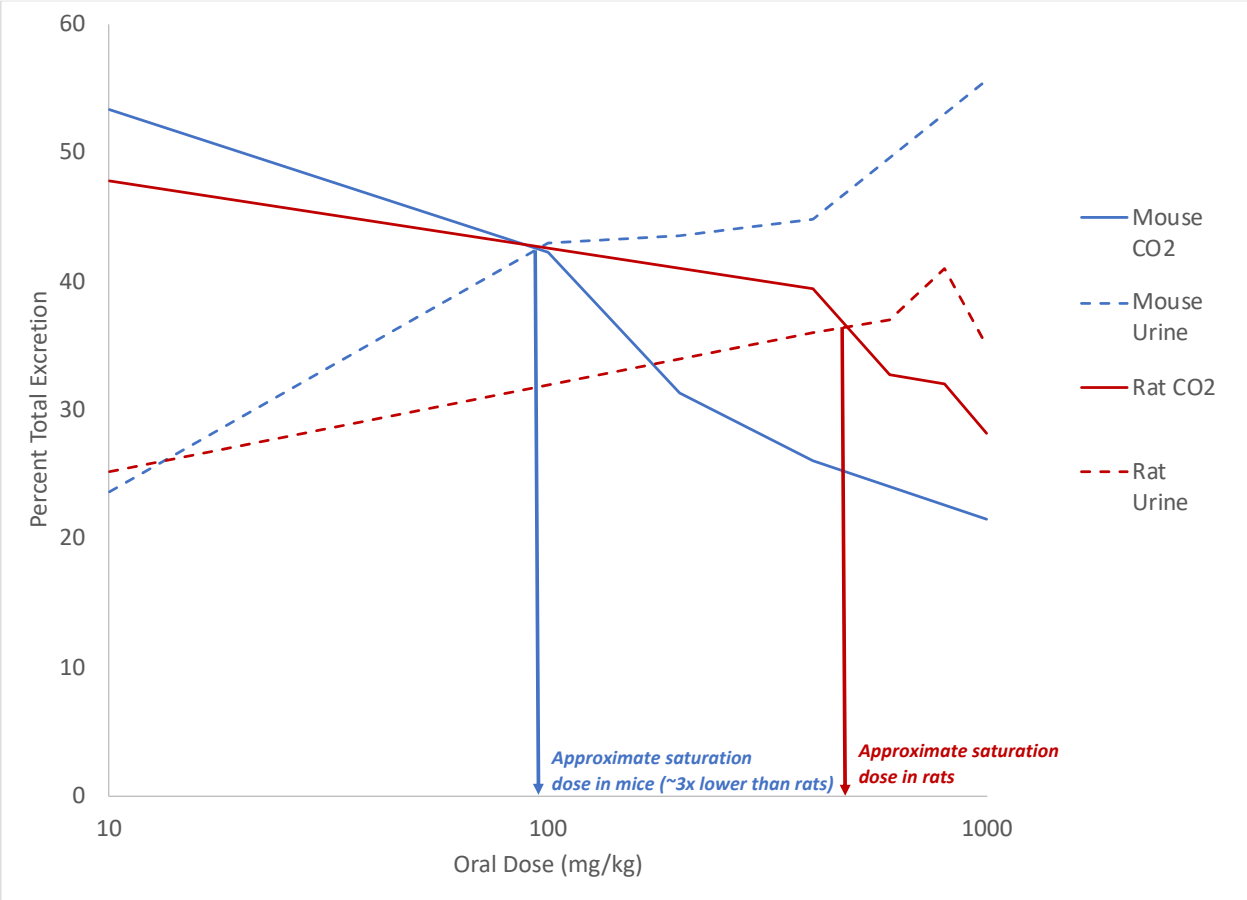

Figure 1. Dose-dependent excretion of radiolabel in rats and mice exposed to EG via gavage (Frantz et al., 1996c). Under linear toxicokinetics, excretion would not be dose-dependent (i.e., data would exhibit flat lines with zero slope). The dose-dependent changes in pathway contributions for EG is consistent with nonlinear toxicokinetics associated with saturation of metabolism. Using the dose at which the pathway contributions cross (urinary vs exhalation) as a crude indicator of the saturation dose, the dose at which metabolism becomes saturated appears lower in mice than in rats by a factor of approximately 3-fold.

It should be noted that allometric scaling practices, as used by MDH for interspecies extrapolation, do not perform well when doses fall within the nonlinear range associated with metabolic saturation (*Kirman CR, Sweeney LM, Meek ME, Gargas ML. Assessing the dose-dependency of allometric scaling performance using physiologically based pharmacokinetic modeling. Regul Toxicol Pharmacol. 2003 Dec;38(3):345-67*). Accordingly, the estimated dose of saturation in mice (~150 mg/kg-d) appears to be very similar to that estimated for humans and is not approximately 7.7-fold higher as would be predicted by default allometric scaling practices (i.e., use of a DAF of 0.13). The estimated saturation dose for mice (~150 mg/kg-d) is consistent with the mouse developmental toxicity data, falling intermediate of the NOAEL (50 mg/kg-d) and LOAEL (500 mg/kg-d) defined by Neeper-Bradley et al., 1995 (*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*).
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Presented in **Table 1** are the EGs Panel suggested modified values used to determine the Short-term Non-cancer Health-based Value based on the Neeper-Bradley et al. (1995) study.

Table 1. Comparison of MDH 2020 Derivation to EGs Panel Derivation for Determining the Short-term Non-cancer Health-based Value ($nHBV_{Short\text{-}term}$) Using Neeper-Bradley et al., 1995 for the POD

	MDH Derivation 2020 Using the Neeper-Bradley et al. (1995)	**EGs Panel Derivation Using the Neeper-Bradley et al. (1995)**	**EGs Panel's Comments and Justification**
Point of Departure (POD)	75.6 mg/kg-d (BMDL10 based on ATSDR's BMD modeling of Neeper-Bradley et al., 1995)	75.6 mg/kg-d	The POD value is considered reasonable. A POD value that is approximately 2-fold lower could be supported based on more recent policy decisions regarding the use of a 5% response rate (i.e., BMDL05) for developmental effects. On the other hand, a POD value that is approximately 2-fold higher could be supported based on the mode of action (MOA) for EG developmental toxicity that involves saturation of GA metabolism. A human dose of 150 mg/kg-d has been estimated for this precursor key event (saturation of GA metabolism) based on human pharmacokinetic data and modeling (CERHR, 2004; Corley et al., 2011)
Dose Adjustment Factor (DAF)	0.13 (Allometric scaling	**1**	MDH (2017) identifies several situations in which allometric scaling may not be appropriate, one of which being "*when there is sufficient chemical-specific information*". In addition to the reasons

	from mice to humans)		identified, allometric scaling may not be appropriate when extrapolating doses at or above metabolic saturation (Kirman et al., 2003), as is evident for EG. Chemical-specific for EG indicate that the dose resulting in saturation of GA metabolism is approximately 500 mg/kg-d in rats and 150 mg/kg-d in humans (CERHR, 2004; Corley et al., 2011). In mice, saturation of metabolism is reached at doses that are approximately 3-fold lower than rats (Frantz et al., 1996a,b,c), which is similar to that estimated for humans (i.e., 150 mg/kg-d). Based upon the ratio of saturation doses in mice and humans (150 mg/kg-d: 150 mg/kg-d), chemical-specific information for EG support a DAF of approximately 1.
Human Equivalent Dose Adjustment (HED)	POD x DAF = 9.83 mg/kg-d	**POD x DAF = 75.6 mg/kg**-d	EGs Panel's proposal would result in modified HED of 75.6 mg/kg-d.
Uncertainty Factor (UF)	30	30	A factor of 10 is considered appropriate for a value based on human data and is comprised of a default factor of 10 for intraspecies variation (UFh). A modifying factor of 3 can be used to account for uncertainty in the exposure timing and intensity of drinking water events, since PBPK modeling predicts that bolus exposure reaches metabolic saturation at doses that are approximately 3-fold lower than corresponding non-bolus exposures to EG.
Reference Dose	9.83 / 30 = 0.33 mg/kg-d	**75.6 / 30 = 2.5 mg/kg-d**	
Relative Source Contribution	0.2	**0.5**	Per MDH administrative rules (4717.7820), the value for RSC is dependent upon chemical volatility. Based on a Henry's Law constant value of 6E-08 atm-m3/mol (PubChem. 2021; https://pubchem.ncbi.nlm.nih.gov/compound/1_2-Ethanediol#section=LogP), EG is considered to fall within in the nonvolatile range. For this reason, an RSC value 0.5 is supported for EG.
Short-term Intake Rate	0.038 L/kg-d	0.038 L/kg-d	

Subchronic Non-Cancer Health Based Value ($nHBV_{Subchronic}$)	1,736 rounded to 2,000 µg/L	33,158 rounded to **30,000 µg/L**	

The MDH August 2020 Toxicological Summary for Ethylene Glycol states that the Subchronic Non-Cancer Health-based Value ($nHBV_{Subchronic}$) is based on the results from the Neeper-Bradley (1995) gavage study.

The MDH August 2020 states "The calculated Subchronic RfD (0.57 mg/kg-d) is higher than the Short-term RfD (0.33 mg/kg-d), which is based on developmental effects. The Subchronic RfD must be protective of all types of adverse effects that could occur as a result of subchronic exposure, including short-term effects (MDH, 2008). Therefore, the Short-term RfD is used in place of the calculated subchronic RfD and the water intake rate for a pregnant woman is used."

EGs Panel believes that MDH should revert to their 2011 stated POD (from Cruzan et al., 2004) for determining Subchronic Non-cancer Health-based Value and not use the default derivation of the Short-term RfD from a gavage study.

In 2011, MDH document stated the derivation is as follows:

Subchronic Non-Cancer Health Risk Limits ($nHRL_{Subchronic}$) = 2,000 µg/L

$$\frac{\text{(Reference Dose, mg/kg/d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Subchronic intake rate, L/kg/d)}}$$

$$= \frac{\text{(0.715 mg/kg/d) x (0.2) x (1000 µg/mg)}}{\text{(0.077 L/kg-d)}}$$

= 1,857 rounded to 2,000 µg/L

Presented in **Table 2** are the EGs Panel suggested modified values used to determine the Subchronic Non-cancer Health-based Value based on the Cruzan et al. (2004) study.

Table 2. Comparison of MDH 2011 Derivation to EGs Panel Derivation for Determining the Subchronic Non-cancer Health-based Value ($nHBV_{Subchronic}$) Using Cruzan et al., 2004 (*Cruzan, G., Corley, R.A., Hard, G.C. et al., 2004. Subchronic toxicity of ethylene glycol in Wistar and F 344 rats related to metabolism and clearance of metabolites. Toxicol. Sci. 81 (2), 502–511*) for the POD

	MDH Derivation 2011	**EGs Panel Derivation Using Cruzan et al. (2004)**	**EGs Panel's Comments and Justification**

	<u>Using the Cruzan et al. (2004)</u>		
Point of Departure (POD)	71.5 mg/kg-d (BMDL10 based on nephropathy by Cruzan, et al 2004. NOAEL/LOAEL were 150/500 mg/kg-d)	71.5 mg/kg-d	
Dose Adjustment Factor (DAF)	1	**0.6** (~1/1.6, rounded to 1 significant figure)	Chemical-specific information is available for EG based on PBPK modeling predictions. Based on PBPK modeling to estimate the internal dose associated with toxicity (cmax for total oxalate in rat and human kidney following dietary exposure), humans are expected to experience internal doses that are ~1.6-fold higher than rats (Snellings et al., 2013; **Figure 3. (***Snellings, W.M., Corley, R.A., McMartin, K.E., Kirman, C.R., Bobst, S.M., 2013. Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect. Regul. Toxicol. Pharmacol. 65 (2), 229–241.)*
Human Equivalent Dose Adjustment (HED)	Insufficient data for adjustment	POD x DAF = 71.5 x 0.6 = **42.9 mg/kg-d**	As noted above, chemical-specific information is available from PBPK modeling for EG, which can be used to account for species differences between rats and humans in estimating the an HED.
Uncertainty Factor (UF)	100	**10** [1 for interspecies extrapolation and 10 for intraspecies variability]	By using PBPK modeling to estimate an HED, UFa should be reduced to 1, because humans are less sensitive than rats A default value for UFh (10) is considered appropriate for EG **<u>REDUCTION in UF</u>** For detailed justification of the reductions in uncertainty factors, refer to Table 7 in Snellings, et al., 2013 (*Snellings, W.M.,*

			Corley, R.A., McMartin, K.E., Kirman, C.R., Bobst, S.M., 2013. Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect. Regul. Toxicol. Pharmacol. 65 (2), 229–241.) The justification for UF of 1 for interspecies extrapolation is as follows: There is a robust database to support this reduction because of the following toxicokinetics studies in rats and humans. <u>PHARMAKOKINETICS</u> a. *Corley, R.A., McMartin, K.E., 2005. 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 (1), 491–501*; b. *Corley, R.A., Bartels, M.J., Carney, E.W., et al., 2005a. Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic acid, in rats and humans. Toxicol. Sci. 85 (1), 476–490*; c. *Corley, R.A., Meek, M.E., Carney, E.W., 2005b. 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, 691–702*; d. *Corley, R.A., Saghir, S.A., Bartels, M.J., et al., 2011. 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, 229–244.*

			There is a robust database to support this reduction because of the following toxicodynamics studies. <u>TOXICODYNAMICS</u> *a) Guo, C., McMartin, K.E., 2005. The cytotoxicity oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation. Toxicology 208, 347–355.* *b) Guo, C., McMartin, K.E., 2007. Aluminum citrate inhibits cytotoxicity and aggregation of oxalate crystals. Toxicology 230, 117–125.* *c) Guo, C., Cenac, T.A., Li, Y., et al., 2007. Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol. Toxicol. Lett. 173, 8–16.* *d) Li, Y., McMartin, K.E., 2009. Strain differences in urinary factors that promote calcium oxalate crystal formation in the kidneys of ethylene glycol-treated rats. Am. J. Physiol. Renal Physiol. 296, F1080–F1087.* *e) Li, Y., McLaren, M.C., McMartin, K.E., 2010. Involvement of urinary proteins in the rat strain difference in sensitivity to ethylene glycol-induced renal toxicity. Am. J. Physiol. Renal Physiol. 299, F605–F615.* *f) McMartin, K.E., Wallace, K.B., 2005. Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function. Toxicol. Sci. 84, 195–200.* A default value for UFh (10) is considered appropriate for EG (Snelling et al., 2013)
Reference Dose	71.5/100 = 0.715 mg/kg-d	42.9 / 10 = **4.29 mg/kg-d**	RfD is recalculated using the values supported above.
Relative Source Contribution	0.2	0.2	
Subchronic Intake Rate	0.077 L/kg-d	0.077 L/kg-d	

Subchronic Non-Cancer Health-based Value ($nHBV_{Subchronic}$)	1,857 rounded to 2,000 µg/L	11,143 rounded to **11,000 µg/L**	

Figure 3.

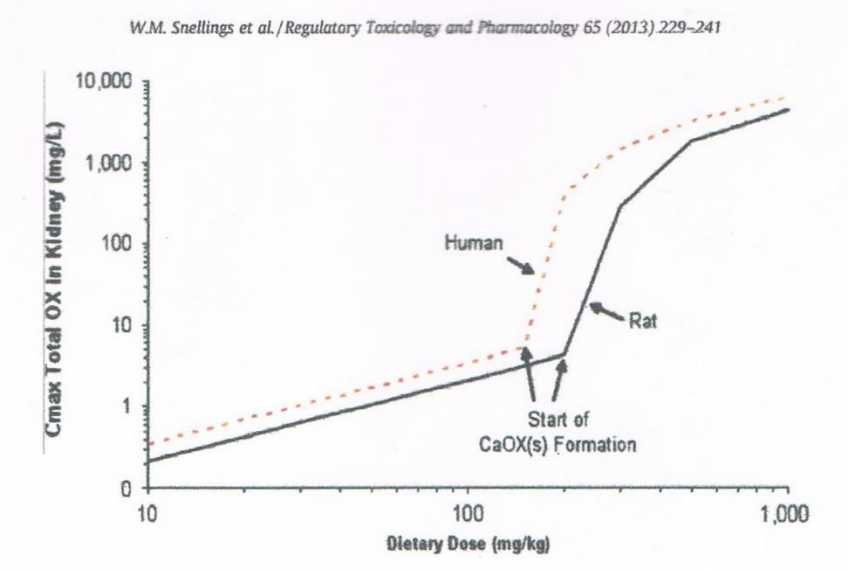

Figure. 3. PBPK predicted Cmax for total oxalate in kidney. Dose-response of internal dose surrogate Cmax for total oxalates in kidney following single day of dietary administration of EG in male Wistar rats and humans.

The MDH August 2020 Toxicological Summary for Ethylene Glycol states that the Chronic Non-Cancer Health-based Value ($nHBV_{Chronic}$) is based on the results from the Neeper-Bradley (1995) gavage study.

The MDH August 2020 document states "The calculated Chronic RfD (0.44 mg/kg-d) is higher than the Short-term RfD (0.33 mg/kg-d), which is based on developmental effects. The Chronic RfD must be protective of all types of adverse effects that could occur as a result of chronic exposure, including short-term effects (MDH, 2008). Therefore, the Short-term RfD is used in place of the calculated Chronic RfD and the water intake rate for a pregnant woman is used. (Intake rate: MDH 2008, Section IV.E.1. and US EPA 2019, Exposure Factors Handbook, Tables 3-1, 3-3, and 3-5). The calculated Chronic nHBV, before consideration of the Short-term RfD and HBV, resulted in the same water guidance value after rounding to one significant digit. Therefore, the chronic duration additivity endpoints of Male Reproductive system and Renal (kidney) system are added to Developmental. Additivity endpoints: Developmental, Male Reproductive system, Renal (kidney) system."

The EGs Panel believes that MDH should revert to their 2011 stated POD from Corley et al., 2008 (*Corley, R.A., Wilson, D.M., Hard, G.C., et al., 2008. Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicity. Toxicol. Appl. Pharmacol. 228,*

***165–178*) for determining Chronic Non-cancer Health-based Value and not use the default derivation of the Short-term RfD from a gavage study.**

In 2011, MDH stated the derivation is as follows:

$$\frac{\text{(Reference Dose, mg/kg/d) x (Relative Source Contribution) x (Conversion Factor)}}{\text{(Chronic intake rate, L/kg/d)}}$$

$$= \frac{\text{(0.5 mg/kg/d) x (0.2) x (1000 µg/mg)}}{\text{(0.043 L/kg-d)}}$$

= 2326 rounded to 2,000 µg/L

Presented in **Table 3** are the EGs Panel suggested modified values used to determine the Chronic Non-cancer Health-based Value based on the Corley et al., 2008 study.

Table 3. Comparison of MDH 2011 Derivation to EGs Panel Derivation for Determining the Chronic Non-cancer Health-based Value ($nHBV_{Chronic}$) Using Corley et al. (2008) for the POD

	MDH Derivation 2011 Using Corley, et al. (2008)	**EGs Panel Derivation using Corley, et al. (2008)**	**EGs Panel's Comments and Justification**
Critical effect	Critical effect(s): Decreased adult body weight; increased water intake resulting in lower urine specific gravities and higher urine volumes; increased kidney weight; gross and histological changes in kidney and bladder.	Rat kidney effects (Corley et al., 2008)	
Point of Departure (POD)	150 mg/kg-d (NOAEL based on kidney	**BMDL05 = 16 mg Eq OX/L**	Chemical-specific information (PBPK model for EG) supports characterizing the POD for nephrotoxicity in rats (Corley et al., 2008)

	changes reported by Corley et al., 2008. LOAEL was 300 mg/kg-d)	(PBPK-derived internal dose)	using internal dose as assessed in Snellings et al. (2013)
Dose Adjustment Factor (DAF)	NA	NA	Chemical-specific information (PBPK modeling) was used to estimate the human equivalent dose of EG instead of relying upon allometric scaling or dose equivalency assumptions
Human Equivalent Dose Adjustment (HED)	Insufficient data for adjustment	**150 mg/kg-d**	Chemical-specific information PBPK model was used to estimate HED
Uncertainty Factor (UF)	300 [10 for interspecies extrapolation, 10 for intraspecies variability, 3 for subchronic-to-chronic UF (comparison of the 16 week (Cruzan et al., 2004) and 12 month study (Corley, et al., 2008) suggests increased severity with increased duration, however, since the study is 12 months in length a factor of 3 rather than 10 was used]	**10** [1 for interspecies extrapolation, 10 for intraspecies variability, and no need for UF in duration]	**<u>CORLEY 12-MONTH STUDY DURATION</u>** The EGs Panel disagrees with the conclusion that the Corley 12-month study showed an increase in severity in comparison to the 16-weeks study. Increasing exposure length did not change the toxic findings or effect levels for EG when increasing testing duration from 16 weeks to 52 weeks. As Corley et al., 2008 (*Corley, R.A., Wilson, D.M., Hard, G.C., et al., 2008. Dosimetry considerations in the enhanced sensitivity of male Wistar rats to chronic ethylene glycol-induced nephrotoxicity. Toxicol. Appl. Pharmacol. 228, 165–178*) states, "the 12-month study recapitulated the results from the 16- week study…Comparison of these two studies also confirms that there is **no progressive or cumulative effect of ethylene glycol with increased duration of exposure** at dose levels that were non-toxic in short-term studies as was observed at higher dose levels causing toxicity….Identical NOAEL's of 150 mg/kg/d for the subchronic and chronic studies indicate that there is a threshold dose for renal toxicity below which

			exposure of any duration will not be expected to result in adverse renal effects." In addition, the Corley 12-month study is more than sufficient to satisfy the requirements of a well-established chronic study and is appropriate for human chronic oral toxicity risk assessment based on the following: 2. Extensive background of target organ knowledge from repetitive dosing studies in rodents a. *DePass, L.R., Garman, R.H., Woodside, M.D., et al., 1986a. Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. Fundam. Appl. Toxicol. 7 (4), 547–565.* b. *DePass, L.R., Woodside, M.D., Maronpot, R.R., et al., 1986b. Three-generation reproduction and dominant lethal mutagenesis studies of ethylene glycol in the rat. Fundam. Appl. Toxicol. 7 (4), 566–572.* 3. Use of a more sensitive test strain and gender (male Wistar), which was confirmed in a comprehensive subchronic study. a. *Cruzan, G., Corley, R.A., Hard, G.C., et al., 2004. Subchronic toxicity of ethylene glycol in Wistar and F 344 rats related to metabolism and clearance of metabolites. Toxicol. Sci. 81 (2), 502–511.* 4. Use of a large number of animals/group (15) 5. Use of four dose groups plus a control over a narrow dose range (50-400 mg/kg-d) 6. Moreover, the use of a chronic exposure duration (12 months, 7 days/week) is acceptable length for different regulatory agencies including FDA* and Health Canada for chronic oral toxicity testing.

			REDUCTION in UF For detailed justification of the reductions in uncertainty factors, refer to Table 7 in Snellings et al., 2013 (*Snellings, W.M., Corley, R.A., McMartin, K.E., Kirman, C.R., Bobst, S.M., 2013. Oral Reference Dose for ethylene glycol based on oxalate crystal-induced renal tubule degeneration as the critical effect. Regul. Toxicol. Pharmacol. 65 (2), 229–241*.) The justification for UF of 1 for interspecies extrapolation is as follows: There is a robust database to support this reduction because of the following toxicokinetics studies in rats and humans. PHARMAKOKINETICS a. *Corley, R.A., McMartin, K.E., 2005. 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 (1), 491–501*; b. *Corley, R.A., Bartels, M.J., Carney, E.W., et al., 2005a. Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic acid, in rats and humans. Toxicol. Sci. 85 (1), 476–490*; c. *Corley, R.A., Meek, M.E., Carney, E.W., 2005b. 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, 691–702*; d. *Corley, R.A., Saghir, S.A., Bartels, M.J., et al., 2011. 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, 229–244. There is a robust database to support this reduction because of the following toxicodynamics studies. <u>TOXICODYNAMICS</u> *g) Guo, C., McMartin, K.E., 2005. The cytotoxicity oxalate, metabolite of ethylene glycol, is due to calcium oxalate monohydrate formation. Toxicology 208, 347–355.* *h) Guo, C., McMartin, K.E., 2007. Aluminum citrate inhibits cytotoxicity and aggregation of oxalate crystals. Toxicology 230, 117–125.* *i) Guo, C., Cenac, T.A., Li, Y., et al., 2007. Calcium oxalate, and not other metabolites, is responsible for the renal toxicity of ethylene glycol. Toxicol. Lett. 173, 8–16.* *j) Li, Y., McMartin, K.E., 2009. Strain differences in urinary factors that promote calcium oxalate crystal formation in the kidneys of ethylene glycol-treated rats. Am. J. Physiol. Renal Physiol. 296, F1080–F1087.* *k) Li, Y., McLaren, M.C., McMartin, K.E., 2010. Involvement of urinary proteins in the rat strain difference in sensitivity to ethylene glycol-induced renal toxicity. Am. J. Physiol. Renal Physiol. 299, F605–F615.* *l) McMartin, K.E., Wallace, K.B., 2005. Calcium oxalate monohydrate, a metabolite of ethylene glycol, is toxic for rat renal mitochondrial function. Toxicol. Sci. 84, 195–200.* A default value for UFh (10) is considered appropriate for EG (Snelling et al., 2013) SUMMARY. By using PBPK modeling to estimate an HED, UFa should be reduced to 1, because humans are less sensitive than rats (as described in Snellings et al. (2013).

Reference Dose	Reference Dose / Concentration: 0.5 mg/kg-d (laboratory animal)	**15 mg/kg-d**	As assessed in Snellings et al. (2013). This value represents the more conservative of 2 RfD values derive (the other value being 47 mg/kg-d)
Relative Source Contribution	0.2	0.2	
Chronic Intake Rate	0.043 L/kg-d	0.043 L/kg-d	
Secondary effects	a. Decreased fetal/pup body weight; decreased embryo/fetal viability; b. Increased pre-implantation loss; c. Decreased adult body weight; d. Proteinurea; decreased testis weight and sperm count; increased incidence of renal lesions; and increased mortality		
Chronic Non-Cancer Health-based Value ($nHBV_{Chronic}$)	2326 rounded to 2,000 µg/L	69,767 rounded to 70,000 µg/L, but reduced to **11,000 µg/L**	The calculated Chronic RfD (15 mg/kg-d) is higher than the Subchronic RfD (4.29 mg/kg-d), which is also based on renal effects. Therefore, the nHBVsubchronic value of 11,000 µg/L calculated above is adopted here for the nHBVchronic value.

* July 2007, Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Chapter IV.C.5.a. Chronic Toxicity Studies with Rodents. IV. Experimental Design, A. Duration of Testing: The test animal should be exposed to the test substance 7 days per week for at least 12 months (one year). (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/redbook-2000-ivc5a-chronic-toxicity-studies-rodents)

<u>It should be noted that the EGs Panel is currently supporting the following:</u>

1. **Rodent developmental toxicity by gavage route of administration is probably not the best way for determining the point of departure in EG risk assessment.**
   - CERHR (2004) states there is negligible concern for adverse <u>human</u> developmental toxicity below 125 mg/kg
   - PBPK has been developed and predicts that human would only achieve the threshold for developmental effects at >350 mg/kg bd wt
   - Developmental toxicity in mice by gavage 500 mg/kg bd wt results in one skeletal variation (extra 14 rib; minor variation not considered significant by some researchers) and not until 750 mg/kg b dwt was there decrease in body weight and axial skeleton malformations.
   - Considerable research has been conducted on does-rate effects from EG treatments. Calculating the RfD using NOEL for gavage route of administration is not the best way to determine risks for drinking water.
   - Saturation (needed to increase glycolic acid above threshold) is expected to require much higher doses for slower dose-rate (non-bolus) exposures supports the renal toxicity is the critical effect of concern from oral exposure to EG
   - The NOEL for developmental toxicity is 150 mg/kg/b dwt. Chronic renal toxicity tests show that at 300 mg/kg bd wt there is death and the NOEL for any adverse effects on the kidney is 150 mg/kg. Both have the same NOEL, but the slope for renal toxicity is dramatically much greater.
   - Recent investigation demonstrated that GA uptake into the rat embryo occurs predominantly by a specific, pH-dependent, active uptake transporter protein, consistent with the proton-linked monocarboxylate transporters (MCT). Two isoforms of the MCT exist in the placenta, a high-affinity isoform (MCT1) and a low affinity isoform (MCT4). The published results indicate that polarity of these isoforms in the mouse and rat placenta syncytiotrophoblast is opposite to that in the rabbit and human placenta. In the rodent, MCT1 lies on the side of the maternal blood, while MCT4 lies on the side of the embryonic blood; in rabbits and humans MCT1 lies on the side of the embryonic blood while MCT4 lies on the side of the maternal blood (*Nigel P. Moore, Catherine A. Picut, Jeffrey H. Charlap, "Localisation of Lactate Transporters in Rat and Rabbit Placentae", International Journal of Cell Biology, vol. 2016, Article ID 2084252, 6 pages, 2016. https://doi.org/10.1155/2016/2084252*).
   - It is proposed that the rabbit, and not rat/mouse, is the appropriate species for the assessment of human relevance findings of the EG-induced developmental toxicity.

2. **Gavage is not the appropriate route of administration to determine human oral risks from ingesting EG contaminated drinking water**

   Dose-rate phenomenon must be considered.

   - Considerable toxicokinetic research has been conducted showing that EG is one of the best examples of the importance of dose-rate effects in determining the toxic response for certain chemicals. It is important to note, that dose rate (fast as in a gavage treatment) is paramount in understanding the mechanism of action for EG's

developmental toxicity. If EG is given as a non-bolus dosage (slow diet consumption), it is not a developmental toxicant, as it is when given at the same dose by gavage (fast bolus treatment).

- Supporting research
  - LOEL developmental toxicity (decreased body weights, axial skeleton malformations and variations) reported in rats given EG by gavage was 1000 mg/kg-d. [*Neeper-Bradley TL. 1990. Developmental toxicity evaluation of ethylene glycol administered by gavage to CD (Sprague-Dawley) rats: Determination of a "no observed effect level" (NOEL). Bushy Run Research Center. CMA Project Report 52-656.*]
  - NOEL developmental toxicity reported in rats given EG in the diet was >1000 mg/kg-d. [*Maronpot RR, Zelenak JP, Weaver EV, et al. 1983. Teratogenicity study of ethylene glycol in rats. Drug Chem Toxicol 6(6):579-594.*]
  - Recent peer-reviewed publications show that saturation (needed to increase glycolic acid (GA), the proximate developmental toxicant, above threshold) is expected to require much higher doses for slower dose-rate (non-bolus) exposures as in drinking water ingestion.
  - Carney et al. in 2011 (*Toxicol Sci. 119:178-88*) published a pivotal study on EG. The title explains the importance of this study. "**The Impact of Dose Rate on Ethylene Glycol Developmental Toxicity and Pharmacokinetics in Pregnant CD Rats.**" Corley states that "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, supported by information on internal dose, so as to increase relevance of the data to humans….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, which are nonbolus. Given our understanding of GA kinetics from this publication, it is clear that gavage studies greatly overestimate the risk of typical environmental exposures that are characterized by low doses and/or low dose rates as in drinking water contamination.
  - For an excellent review of the studies linking developmental toxicity and kinetics, refer to Carney, 2011 (*Book chapter, Ethylene Glycol (Reproductive and Developmental Toxicology, Gupta editor, ISBN: 978-0-12-382032-7, 607-615.)* Briefly stated, several studies have been performed to show the relevance of dose-rate effects. Pharmacokinetics studies included Pottenger et al., 2001 (*Dose-dependent nonlinear pharmacokinetics of ethylene glycol metabolites*

in pregnant and nonpregnant Sprague-Dawley rats following oral administration of ethylene glycol. Toxicol. Sci. 62, 10–19) and Klug et al., 2001. (*Effects of ethylene glycol and metabolites on in vitro development of rat embryos during organogenesis. Toxicology in Vitro, Volume 15, Issue 6, Pages 635-642*), and a truly relevant discussion on dose rate is a study by Carney et al., 2011, (*The Impact of Dose-rate on Ethylene Glycol Developmental Toxicity and Pharmacokinetics in Pregnant CD Rats, Toxicol Sci. 119:178-88*). Carney (2011) in his book chapter states "…threshold values of 2mM GA in maternal blood and 4 mM GA in embryo were proposed….To test the validity of these proposed threshold values, a study was done to compare equivalent doses of EG given as a bolus (fast dose-rate) vs. as a slow continuous infusion (slow dose-rate) for their impact on kinetics and developmental outcome (Carney, 2011)….the fast dose-rate groups had peak maternal blood GA levels in excess of the putative 2 mM threshold and the fetuses from these dams showed significant increases in skeletal malformations and variations. In the slow dose-rate groups, GA levels remained below the putative threshold and there was no increase in the incidence of skeletal defects."

3. <u>Mouse and rat are not the appropriate species for developmental/reproductive risk assessment.</u>

Recent research is supporting that the rabbit is a better species to select for determining human oral risks.

- Supporting research
  - Carney et al., 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: 573-81*) report "High-dose gavage exposure to ethylene 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,…"
  - Ellis-Hutchings et al., 2014, (*Disposition of glycolic acid into rat and rabbit embryos in vitro (Reprod Toxicol 46:46-55*) report that "This research explored the mechanisms of 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."

- Moore et al., 2016 (*Nigel P. Moore, Catherine A. Picut, Jeffrey H. Charlap, "Localisation of Lactate Transporters in Rat and Rabbit Placentae", International Journal of Cell Biology, vol. 2016, Article ID 2084252, 6 pages, 2016. https://doi.org/10.1155/2016/2084252*) report that the rabbit, and not rat/mouse, is the appropriate species for the assessment of human relevance findings of the EG-induced developmental toxicity. The mechanisms underlying molecular mechanisms and species differences in the developmental toxicity was discussed.

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

Sincerely,

Bill Gulledge

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