Response to Comments Pertaining to the Notice of Intent to List Ethylene Glycol as Causing Reproductive Toxicity under Proposition 65

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
June 2015

On April 11, 2014, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Notice of Intent to List[1] Ethylene Glycol (EG) under Proposition 65[2] as a chemical known to the state to cause reproductive toxicity (developmental endpoint). The action was based on Proposition 65 statutory requirements[3] and on the authoritative bodies provision of the Proposition 65 implementing regulations, Title 27, Cal. Code of Regulations, section 25306[4]. OEHHA found that EG meets the criteria for listing via this mechanism based on conclusions by the National Toxicology Program in a final report by the NTP Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) that EG causes reproductive toxicity, and on the scientific evidence relied on by NTP[5]. NTP (solely as to final reports of the NTP-CERHR) is designated as an authoritative body for purposes of listing chemicals as causing reproductive toxicity. This document responds to comments on the Notice of Intent to List.

Under Section 25306, a chemical is identified as causing reproductive toxicity, including developmental toxicity, if it has been "formally identified" by an authoritative body as causing reproductive toxicity. A chemical has been "formally identified" pursuant to section 25306 if it has been included in a list of chemicals causing reproductive toxicity published by the authoritative body; is the subject of a report which is published by the authoritative body and which concludes that the chemical causes reproductive toxicity; or has been "otherwise identified" as causing reproductive toxicity by the authoritative body in a document that indicates that the identification is a final action, and if the list, report, or document meets specified criteria in section 25306(d)(2).

---

[1] Notice of Intent to List: Ethylene Glycol. Available at http://www.oehha.ca.gov/prop65/CRNR_notices/admin_listing/intent_to_list/041114NOILethyleneglycol.html

[2] The Safe Drinking Water and Toxic Enforcement Act of 1986 (codified at Health and Safety Code section 25249.5 *et seq*.) hereinafter referred to as Proposition 65 or the Act.

[3] Health and Safety Code section 25249.8(b)

[4] Title 27, Cal. Code of Regulations, section 25306; all further references are to sections of Title 27 of the California Code of Regulations, unless otherwise indicated.

[5] National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) (2004). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Ethylene Glycol. Research Triangle Park, NC, National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction: NIH Publication No. 04 – 4481. Available online at http://ntp.niehs.nih.gov/?objectid=4980AA81-E919-4E85-60B789CA36E59FA5

OEHHA has reviewed the conclusions and statements in the 2004 NTP-CERHR report titled, "NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Ethylene Glycol" and determined that these conclusions and statements satisfy the Section 25306(d)(1) requirement that EG is the subject of a report published by the authoritative body that concludes that EG causes reproductive toxicity, and that the document meets the section 25306(d)(2) criteria, thus satisfying the formal identification criteria in the Proposition 65 regulations. In the 2004 report, NTP concludes there is clear evidence of adverse effects for reproductive toxicity (developmental endpoint) in laboratory animals at high oral doses:

> "[T]he panel concluded that EG produces developmental toxicity in rodents after oral exposure to high doses. The critical developmental rodent studies showed that oral exposure of pregnant females to high doses of EG (≥500 mg/kg bw/day in mice and ≥1,000 mg/kg bw/day in rats) caused increased fetal deaths, skeletal malformations and external malformations, as well as reduced body weights in offspring." (NTP-CERHR, 2004: NTP Brief, page 2)

> "There were sufficient data to conclude that oral gavage exposure to high doses of ethylene glycol (CD-1 mice, ≥ 500 mg/kg bw/day on gd 6–15; Sprague-Dawley rats, ≥1,000 mg/kg bw/day on gd 6–15) causes developmental toxicity in mice and rats, including axial skeletal malformations, reduced body weights, external malformations, and increased post-implantation loss." (NTP-CERHR, 2004: Summary and Conclusions of Reproductive and Developmental Hazards, page II-116)

The studies cited by NTP-CERHR in support of these conclusions were reviewed by OEHHA with regard to the sufficiency of evidence criteria in Section 25306(g)(2). OEHHA has concluded that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between the adverse reproductive effects in humans and the toxic agent in question is biologically plausible. This meets the sufficiency of evidence criteria in Section 25306.

The April 11 notice initiated a 30-day public comment period that was scheduled to close on May 12, 2014. OEHHA extended the public comment period to June 11, 2014 after receiving a request for an extension from the Ethylene Glycols Panel of the American Chemistry Council. OEHHA subsequently received a request from the PET Resin Association seeking an additional extension, and OEHHA extended the public comment period until June 25, 2014.

Seven sets of comments were submitted by the following organizations:

- American Chemistry Council Ethylene Glycols Panel (ACC-EGP), submitted by William P. Gulledge
- Unifi Manufacturing, Inc. (Unifi), submitted by Jane Johnson
- Writing Instrument Manufacturers Association (WIMA) and The Art & Creative Materials Institute (ACMI), submitted by Ann Grimaldi
- Consumer Specialty Products Association (CSPA), submitted by Steven Bennett and Kristin Power
- Information Technology Industry Council (ITIC), submitted by Chris Cleet
- PET Resin Association (PETRA), submitted by Ralph Vasami
- Old World Industries, LLC (OWI), submitted by Daniel M. Leep

OEHHA reviewed all of the comments and accompanying materials submitted in the context of the regulatory criteria for listing chemicals under the authoritative bodies mechanism in Section 25306.

Comments relevant to the Notice of Intent to List (NOIL) from the individuals and groups listed above are grouped and numbered by topic, and responses follow below.

1. **Comments that data considered by NTP did not meet the requirements of Title 27, Cal. Code of Regs., section 25306(g)(2).**

<u>Comment:</u>
OWI noted the lack of reproductive toxicity data in humans, and cited the negligible level of concern expressed by NTP for developmental and reproductive effects in humans.

<u>Response:</u>
Section 25306(g) provides that the criteria for “as causing reproductive toxicity” can be met either because studies in humans indicate that there is a causal relationship between the chemical and reproductive toxicity, or studies in experimental animals indicate that there are sufficient data indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible. As discussed in detail above, the latter criterion has been met for ethylene glycol. The level of concern expressed by NTP was explicitly “based on the limited exposure data, estimated occupational exposure scenarios, metabolism studies, and laboratory animal toxicity studies”. Although NTP stated that current exposures to ethylene glycol were “probably not” high enough to cause concern, this does not conflict with the authoritative body’s conclusion that ethylene glycol causes developmental toxicity in animals at high oral doses, nor does it indicate that developmental toxicity in humans is not biologically plausible.

2. Comments that data not considered by NTP show that the requirements of Title 27, Cal. Code of Regs., section 25306(g)(2) are not met

2.1 Comment:

ACC-EGP presented their findings and discussions from 11 peer-reviewed reports published in 2004 and later that were not included in the 2004 NTP-CERHR monograph, and concluded that the sufficiency of evidence criteria are not met because adverse developmental effects in humans are not biologically plausible at non-lethal doses of EG (pp. 2-10). Citing the same references as those relied upon by ACC-EGP, WIMA and ACMI present a similar argument as that by ACC-EGP. CSPA and PETRA support the comments by ACC-EGP.

Response:

Section 25306(h) provides that the chemical will not be listed if scientifically valid data which were not considered by the authoritative body clearly establish that the sufficiency of evidence criteria contained in Section 25306(g) are not met. Under those sufficiency of evidence criteria, a chemical causes reproductive toxicity when either "(1) studies in humans indicate that there is a causal relationship between the chemical and reproductive toxicity, or (2) studies in experimental animals indicate that there are sufficient data…indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible."

As noted above, OEHHA has determined that studies of EG in experimental animals (rodents and rabbits) indicate that there are sufficient data indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible. That determination is consistent with the conclusion by NTP-CERHR that "EG may adversely affect human development if oral exposures are sufficiently high", and the statement by NTP-CERHR that the "toxicokinetic, absorption, distribution, metabolism, and excretion data from rats, mice, and humans indicate that the observed adverse effects in rodents are likely to be relevant to humans."[6]

OEHHA reviewed the 11 publications[7,8,9,10,11,12,13,14,15,16,17] cited by ACC-EGP as not having been considered by the authoritative body, NTP. Of the 11 publications, four are

---

[6] National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) (2004). NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Ethylene Glycol. Research Triangle Park, NC, National Toxicology Program, Center for the Evaluation of Risks to Human Reproduction: NIH Publication No. 04 – 4481. Available online at http://ntp.niehs.nih.gov/?objectid=4980AA81-E919-4E85-60B789CA36E59FA5 (page 2).

[7] Booth E.D., Dofferhoff O, Boogaard PJ, Watson WP (2004). Comparison of the metabolism of ethylene glycol and glycolic acid in vitro by precision-cut tissue slices from female rat, rabbit and human liver. *Xenoiotica* 34:31-48.

[8] Carney, E.W., Scialli, A.R., Watson, R.E. and DeSesso, J.M. (2004). Mechanisms regulating toxicant disposition to the embryo during early pregnancy: An interspecies comparison. *Birth Defects Res., Part C, Embryo Rev.* 72:345-360.

[9] Carney, E. W., Tornesi, B, Markham, D.A., Rasoulpour, R.J., 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):573–581.

study reports focusing largely on characteristics of EG metabolism in rats and rabbits and the role of EG metabolism in producing developmental toxicity in rats or rabbits. Two publications focus on physiologically-based pharmacokinetic modeling and one reported an in vitro study using human skin to determine the dermal penetration rate of EG. The other references are literature reviews, three published in journals and one book chapter.

All of the original laboratory studies are well-designed and provide scientifically valid data for consideration. The three literature reviews and one book chapter provide comprehensive discussions on the characteristics of the developmental toxicity and possible modes of actions (MOA).

The data from these eleven publications related to the developmental toxicity and possible MOA of EG are discussed briefly below, together with the findings and conclusions in the 2004 NTP-CERHR report.

- NTP (NTP-CERHR, 2004) concluded that gestational exposure to high oral doses of EG produces developmental toxicity in rodents. Oral administration of EG (≥500 mg/kg bw/day in mice or ≥1,000 mg/kg bw/day rats) caused increased fetal deaths, skeletal malformations and external malformations, as well as reduced body weights in offspring. None of the comments disagree with this conclusion. The new study by Carney et al. (2011)[18] re-confirmed the developmental effects of EG in rats at a dose of 1000 mg/kg-day.

---

[10] 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., Corley, R.A. (2011). The Impact of Dose Rate on Ethylene Glycol Developmental Toxicity and Pharmacokinetics in Pregnant CD Rats. *Toxicol Sci.* 119(1), 178–188.
[11] Carney, E.W. (2011). Ethylene Glycol. In Reproductive and Developmental Toxicology (Ramesh C. Gupta, ed.). Elsevier Inc. pp. 607-615.
[12] Corley, R. A., Bartels, M. J., Carney, E. W., Weitz, K. K., Soelberg, J. J., Gies, R. A., and Thrall, K. D. (2005a). Development of a Physiologically Based Pharmacokinetic Model for Ethylene Glycol and Its Metabolite, Glycolic Acid, in Rats and Humans. *Toxicol Sci.* 85:476–490.
[13] Corley, R.A., Meek, M.E. and 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.
[14] 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.
[15] Ellis-Hutchings, R.G., Moore, N.P., Marshall, V.A., Rasoulpour, R.J., Carney, E.W. (2014). Disposition of glycolic acid into rat and rabbit embryos in vitro. *Reprod Toxicol.* 46C:46-55.
[16] Saghir S.A., Bartels MJ, Snellings WM (2010). Dermal penetration of ethylene glycol through human skin in vitro. *Int J Toxico* 29: 268-76.
[17] Slikker W. Jr, Andersen ME, Bogdanffy MS, Bus JS, Cohen SO, Conolly RB, David RM, Doerrer NG, Dorman DC, Gaylor DW, Hattis D, Rogers JM, Setzer RW, Swenberg JA, Wallace K (2004). Dose-dependent transitions in mechanisms of toxicity: case studies. *Toxicol Appl Pharmacol*. 201: 226-94.
[18] Carney, E.W., B. Tornesi, A.B. Liberacki, D.A. Markham, K.K. Weitz, T.M.Luders, K.G. Studniski, J.C. Blessing, R.A. Gies and R.A. Corley (2011). The Impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats. *Toxicol Sci.* 119(1): 178-188.

- As also discussed in NTP-CERHR (2004), oral dosing with EG in drinking water caused no developmental toxicity in rabbits at a dose of 2,000 mg/kg-day, which resulted in maternal deaths and whole litter loss. "The Expert Panel concluded that data are sufficient to demonstrate a lack of developmental toxicity in rabbits following gavage of does with up to 2,000 mg/kg bw/day ethylene glycol on [gestation days] GD 6–19."

- One of the studies submitted by the commenters is an in vitro study using rabbit embryo cultures by Carney et al. (2008)[19]. This study showed no developmental toxicity to rabbit embryos following in vitro exposure to high concentrations of glycolic acid (GA), the active metabolite of EG in vivo. The authors proposed that this lack of effect may result from significantly reduced exposure of rabbit embryo to GA, due to the structural characteristics of rabbit placenta and other factors. A second study from the same laboratory, Ellis-Hutchings et al. (2014)[20], provided additional information on comparative exposures of rat and rabbit embryos to GA using a whole embryo culture paradigm, with exposure of rabbit fetuses being substantially lower. This set of data appeared to be a follow-up of preliminary data included in the NTP-CERHR monograph (2004). NTP-CERHR stated that "these data by Carney et al. (1998)[21] are very interesting, but due to their preliminary nature, they are not very useful for the CERHR evaluative process. They do present a plausible explanation for differences in developmental toxicity between rats and rabbits. These results should be followed up by a more thorough experimentation."

- With regard to the MOA underlying the developmental toxicity of EG in rodents, NTP-CERHR concluded that unmetabolized EG is not likely to be the proximate teratogen in rodents. Glycolic acid (GA), possibly in combination with resulting metabolic acidosis, is the most likely cause of developmental toxicity following exposure of rodents to EG. The findings from new studies submitted to OEHHA support this conclusion by NTP-CERHR. It should be emphasized that GA-induced metabolic acidosis is a major exacerbating factor for EG-induced developmental toxicity in rodents but does not appear to be the mechanism by which developmental toxicity is induced, as reported and concluded by Carney et al. (1999)[22]. Detailed findings from the Carney et al. (1999) study indicate that metabolic acidosis is not an absolute requirement for EG-induced developmental toxicity. The data from the 1999 Carney et al. study showed that some of the developmental effects of EG were induced by the glycolate anion in the absence

[19] Carney, E. W., B. Tornesi, D. A. Markham, R. J. Rasoulpour and N. Moore (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): 573-581.
[20] Ellis-Hutchings, R.G., Moore, N.P., Marshall, V.A., Rasoulpour, R.J., Carney, E.W. (2014). Disposition of glycolic acid into rat and rabbit embryos in vitro. *Reprod Toxicol.* 46C:46-55
[21] Carney, E. W., Pottenger, L. H., Bartels, M. J. and Quast, J. F. (1998). Ethylene glycol: comparative pharmacokinetics and metabolism probe in pregnant rabbits and rats. Midland (MI): The Dow Chemical Company, Health and Environmental Research Laboratories.
[22] Carney, E. W., N. L. Freshour, D. A. Dittenber and M. D. Dryzga (1999). Ethylene glycol developmental toxicity: unraveling the roles of glycolic acid and metabolic acidosis. *Toxicol Sci***.** 50(1): 117-126.

of maternal metabolic acidosis. Although several of the publications from 2004 and later provide additional data on comparative toxicokinetics between rats and rabbits, none of the studies establish the MOA for developmental toxicity in those species.

- The NTP Expert Panel considered that "[m]etabolism of ethylene glycol is qualitatively similar in humans, monkeys, dogs, rabbits, rats, and mice" (NTP-CERHR 2004, page II-52). However, the study by Booth et al. (2004)[23], submitted to OEHHA as one of the additional studies not considered by NTP-CERHR, reported liver slices from rabbits produced on average 10-fold less GA than that produced by liver slices from rats. With human liver, the formation of GA (glycolic acid) was not detectable using tissue from three of four human donors. A low level of GA was detected in one liver slice incubation from one of the four subjects, but only at one extended time point. Human liver tissue was the most effective at metabolizing EG to glyoxylic acid. These results imply that both rabbits and humans are likely to be less sensitive to the developmental toxicity of EG than are rodents, since exposure to GA may be lower. However, data from clinical observations in humans are not consistent with these in vitro data.

  For example, it has been reported that EG was rapidly metabolized to GA, while GA was slowly converted to oxalic acid in humans (Rosano et al., 2009 [24]; Porter, 2012[25]). Porter (2012) reported that "patients may present with low or undetectable levels of ethylene glycol but with significant levels of glycolic acid [GA]" due to oxidation of glycolic acid to oxalic acid being the rate-limiting step. Furthermore, Moreau et al. (1998)[26] reported that glycolate [the non-protonated anion form of GA] has a slow elimination rate and long half-life in EG-poisoned patients. GA has a half-life of about 10 hours in humans, while ethylene glycol, the non-toxic parent compound, has a half-life of only 6 hours (Porter, 2012). In contrast, the half-life of both GA and ethylene glycol in rats is about 1.7 hours (Hewlett et al., 1989)[27] and the half-life of ethylene glycol in rabbits is about 1.6 hours (Carney et al., 2008)[28]. No data are available on the half-life of GA in

[23] Booth, E. D., O. Dofferhoff, P. J. Boogaard and W. P. Watson (2004). Comparison of the metabolism of ethylene glycol and glycolic acid in vitro by precision-cut tissue slices from female rat, rabbit and human liver. *Xenobiotica* 34(1): 31-48.
[24] Rosano, T. G., T. A. Swift, C. J. Kranick and M. Sikirica (2009). Ethylene glycol and glycolic acid in postmortem blood from fatal poisonings. *J Anal Toxicol* 33(8): 508-513.
[25] Porter, W. H. (2012). Ethylene glycol poisoning: quintessential clinical toxicology; analytical conundrum. *Clin Chim Acta* 413(3-4): 365-377.
[26] Moreau, C. L., W. Kerns, 2nd, C. A. Tomaszewski, K. E. McMartin, S. R. Rose, M. D. Ford and J. Brent (1998). Glycolate kinetics and hemodialysis clearance in ethylene glycol poisoning. META Study Group. J *Toxicol Clin Toxicol* 36(7): 659-666.
[27] Hewlett, T. P., D. Jacobsen, T. D. Collins and K. E. McMartin (1989). Ethylene glycol and glycolate kinetics in rats and dogs. *Vet Hum Toxicol* 31(2): 116-120.
[28] Carney, E. W., B. Tornesi, D. A. Markham, R. J. Rasoulpour and N. Moore (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): 573-581.

rabbits. The substantially longer half-life of GA in humans may potentially affect their susceptibility to the embryotoxicity of GA as a result of EG exposure.

- Large interspecies variability in the developmental toxicity of EG between rodents and rabbits may result partly from some differences between placentas of rodents versus other species. The Expert Panel report in NTP-CERHR (2004) states that:

> "Mice and rats, both of which exhibit developmental toxicity after exposure to ethylene glycol, have brief gestation periods (16–22 days). In order to establish a channel of physiologic exchange between the maternal system and the developing embryos, these quickly developing species establish an early placenta (the inverted visceral yolk sac placenta) that is eventually replaced by the definitive chorioallantoic placenta. Establishment of the rodent yolk sac placenta results in formation of a vesicle that contains the embryo and is filled with exocoelomic fluid. Relative to maternal plasma, the yolk sac fluid of rats and mice concentrates weak acids, including glycolic acid. The rabbit, which has an incomplete yolk sac vesicle, does not concentrate glycolate or other weak acids in the fluid that surrounds the embryo and does not exhibit developmental toxicity after exposure to ethylene glycol. Humans never have a yolk sac placenta nor are human embryos contained in a fluid filled yolk sac cavity. In contrast to rats and mice, but similar to rabbits, the fluid that surrounds human embryos does not concentrate weak acids relative to maternal blood levels. These species differences in placentation may play a key role in the developmental toxicity of ethylene glycol, although other aspects of pharmacokinetics and pharmacodynamics may also contribute."

- Data analysis presented by the commenters supports this interpretation of the data. Carney et al. (2004)[29] stated that preliminary data on EG suggest that the GD 9 rabbit yolk sac is inefficient as a transporter of small molecular weight compounds. This was based on the observation that EG and glycolic acid concentrations in the yolk sac and embryo were approximately 10-fold lower than those in maternal blood, following a large oral bolus dose of EG given to rabbits on GD 9 (Carney et al., 2004, reporting unpublished data). By GD 13, however, the visceral yolk sac in rabbits is completely closed around the embryo, similar to rats at GD 7-8 (Carney et al., 2004). Carney et al. (2004) concluded that "[b]ased on the greater importance of the inverted visceral yolk sac in rats than in humans during early pregnancy, exposures found to elicit teratogenic effects solely by inhibiting or accompanying the transfer of maternal materials through the yolk sac placenta should not pose a threat to the developing human embryo."

[29] Carney, E. W., A. R. Scialli, R. E. Watson and J. M. DeSesso (2004). Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison. *Birth Defects Res C Embryo Today* 72(4): 345-360.

Despite development of the yolk sac placenta by GD 13 in rabbits, no developmental toxicity was seen in that species after exposure to EG that continued well beyond that stage (Tyl et al., 1993)[30].

This suggests that the developmental toxicity of EG may not be mediated solely by the transfer of GA from the mother through the yolk sac placenta. Carney (2011)[31] discusses ion-trapping of weak acids such as GA in the context of differential exposure of embryos across species and concomitant embryotoxicity. Structurally similar weak acids act as teratogens by accumulating in the basic milieu of the early mammalian embryo (Nau and Scott, 1986)[32]. As an example, one such weak acid, valproic acid (VA), causes neural tube defects in mice and humans, but not rabbits. Effects of VA in humans were reported at concentrations 5-10 times lower than teratogenic doses in experimental animals (Nau, 1986)[33]. VA toxicity is measured by maximal concentrations and not area under the curve (Nau et al., 1991)[34]. This is also the case for GA where its toxicity is measured by maximal concentrations. These maximal values correlate with the incidence of developmental defects in rodents where mice are the most sensitive species. This example of VA clearly indicates that developmental effects of a chemical can occur in humans, even if the chemical does not cause the same developmental effects in rabbits.

After considering the data that were not considered in the NTP-CERHR report, OEHHA found these data are consistent with and provide support for the NTP conclusion that there is clear evidence that EG causes developmental toxicity via oral dosing of laboratory rodents at relatively high doses. While some evidence not considered by NTP suggests that humans may be less sensitive to the developmental toxicity of EG compared to rodents, these data have not established that developmental toxicity from high oral exposures to EG is NOT biologically plausible in humans.

2.2 Comment:

The ACC-EGP also commented that:

> "To go from the PBPK model-predicted concentrations of GA in maternal blood to GA concentrations in the embryo, the target organ, a 0.5x factor must be applied.[4] Hence, a 20 g exposure of EG to a 58 kg pregnant woman (=350

[30] Tyl, R. W., Price, C. J., Marr, M. C., Myers, C. B., Seely, J. C., Heindel, J. J. and Schwetz, B. A. (1993). Developmental toxicity evaluation of ethylene glycol by gavage in New Zealand White Rabbits. *Fund Appl Toxicol* 20: 402-412.

[31] Carney, E.W., B. Tornesi, A.B. Liberacki, D.A. Markham, K.K. Weitz, T.M.Luders, K.G. Studniski, J.C. Blessing, R.A. Gies and R.A. Corley (2011). The Impact of dose rate on ethylene glycol developmental toxicity and pharmacokinetics in pregnant CD rats. *Toxicol Sci.* 119(1): 178-188.

[32] Nau, H. and W. J. Scott, Jr. (1986). Weak acids may act as teratogens by accumulating in the basic milieu of the early mammalian embryo. *Nature* 323(6085): 276-278.

[33] Nau, H. (1986). Species differences in pharmacokinetics and drug teratogenesis. *Environ Health Perspect* 70: 113-129.

[34] Nau, H., R. S. Hauck and K. Ehlers (1991). Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. *Pharmacol Toxicol* 69(5): 310-321.

mg/kg) would result in only 1 mM GA to the embryo. Therefore, to obtain the threshold value of 4 mM GA in the embryo, four times more EG (i.e., 80 g to a 58 kg pregnant woman, or 1400 mg/kg) would be required to reach the threshold, which is a dose within the published range of minimal lethal doses for humans."[5] (ACC-EGP, p. 10)

Response:
This reasoning by ACC-EGP suggests that human exposure to EG could reach the level that produces developmental toxicity, and thus that developmental toxicity of EG observed in rodents is still biologically plausible, even though at a very high exposure level. In humans, late in the first trimester, the coelomic fluid has a lower pH than maternal serum (Carney et al., 2004)[35] suggesting a shift in pH through gestation between maternal blood and embryonic fluid, potentially making the embryo more susceptible to GA exposure.

The "0.5X factor" that ACC-EGP utilized to estimate human fetal exposure to GA was derived from ion trapping and the Henderson-Hasselbach (H-H) acid-base equation, which predicts the percentage of total GA in the free acid form that can freely diffuse across the placental membranes. However, this approach may not be valid.

As reported by Ellis-Hutchings et al. (2014)[36]

> "The data presented herein (Fig. 3A) show that the uptake of 1 mM GA into the embryo is 2.5-fold greater under more acidic culture conditions (pH 7.1 cf. pH 7.8). During the pilot study with 12 mM GA and NaG [sodium glycolate], GA reached a maximum concentration of 90 µg/mg protein in the embryo by 1 h, whereas under more alkaline conditions NaG achieved this only after 3 h (Fig. 3B). It is concluded, therefore, that sequestration of GA by the rat embryo is dependent upon the extracellular proton concentration. However, the results of the d-lactate competition study (Fig. 4) demonstrate that the disposition of GA into the embryo is not simply a matter of passive diffusion and 'ion trapping' of a weak acid across a pH gradient, rather it involves a pH-dependent specific transport mechanism with characteristics of the MCT [monocarboxylic acid transporter]. The rate of substrate transport by the MCT is not simply pH-dependent in terms of the magnitude of the trans-membrane gradient, but also its polarity."

However, the direct mechanism of MCT transport of GA has not been established and expression of its various isoforms has not yet been evaluated

[35] Carney, E. W., A. R. Scialli, R. E. Watson and J. M. DeSesso (2004). Mechanisms regulating toxicant disposition to the embryo during early pregnancy: an interspecies comparison. *Birth Defects Res C Embryo Today* 72(4): 345-360.
[36] Ellis-Hutchings, R. G., N. P. Moore, V. A. Marshall, R. J. Rasoulpour and E. W. Carney (2014). Disposition of glycolic acid into rat and rabbit embryos in vitro. *Reprod Toxicol* 46: 46-55.

mid-gestation in rodent or term human placental tissues (Ellis-Hutchings et al., 2014)[37]. Therefore, its function in either species is unclear.

Even if the pH-gradient estimation method for GA uptake into the embryo is accepted as valid, it may only be applicable for a short portion of the gestation or pregnancy period. As shown in Table 2 from the study report by Ellis-Hutchings et al. (2014) (included below), the pH gradient across the placental membranes is species- and developmental stage-dependent. The pH gradient in rodents from GD 11 onwards is qualitatively similar to that in humans during pregnancy weeks 7-14. This finding suggests that, similar to the situation in rodents, GA concentration in a human conceptus, at least during pregnancy weeks 7-14, can be higher than that in the maternal circulation.

Table 2
The pH of conceptus tissues/fluids *in vivo* at comparable stages of development in relation to that of maternal blood. Equivalence of gestation day is based on developmental staging [17]. Data represent mean ± SD.

Species	Gestation day	pH_{blood}	$pH_{conceptus}$	Conceptus tissue	Reference
Rabbit	9	7.45	7.2	YSCF	Carney et al. [14]
Mouse	9	7.26 ± 0.03	7.64 ± 0.07	Embryo	Nau and Scott [28]
Mouse	9	7.27 ± 0.10	7.65 ± 0.02	Embryo	Collins et al. [17]
Rat	11	7.44 ± 0.04	7.61 ± 0.08	Embryo	Nau and Scott [28]
Rat	11	7.33 ± 0.10	7.66 ± 0.05	Embryo	Collins et al. [17]
Monkey	24–29	7.33 ± 0.04	7.35 ± 0.18	Embryo	Collins et al. [17]
Mouse	11	7.24 ± 0.06	7.22 ± 0.06	Embryo	Collins et al. [17]
Rat	12	7.51 ± 0.03	7.44 ± 0.04	Embryo	Collins et al. [16]
Rat	13	7.53 ± 0.04	7.31 ± 0.05	Embryo	Collins et al. [16]
Rat	13	7.44 ± 0.07	7.27 ± 0.10	Embryo	Collins et al. [17]
Monkey	30–31	7.33 ± 0.04	7.15 ± 0.15	Embryo	Collins et al. [17]
Mouse	12	7.30 ± 0.10	7.12 ± 0.10	Embryo	Collins et al. [17]
Rat	14	7.47 ± 0.05	7.05 ± 0.08	Embryo	Nau and Scott [28]
Rat	14	7.47 ± 0.03	7.11 ± 0.03	Embryo	Collins et al. [16]
Rat	14	7.44 ± 0.07	7.01 ± 0.09	Embryo	Collins et al. [17]
Monkey	36–37	7.35 ± 0.04	6.74 ± 0.29	Embryo	Collins et al. [17]
Human	7–14 wk	7.38	7.18	Coelomic fluid	Jauniaux et al. [18]

(From Ellis-Hutchings et al., 2014)

2.3 Comment:

The Risk Assessment Report by Environment Canada in 2010[38] should be included as supportive evidence. This report concluded 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. (Submitted by ACC-EGP (pages10-12); CSPA and PETRA support ACC-EGP.)

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

[38] Environment Canada (2010). Priority Substances List Assessment Report: Follow-up to the State of Science Report, 2000. Ethylene Glycol. Available at http://www.ec.gc.ca/lcpe-cepa/documents/substances/eg/eg_draft-eng.pdf

Response:
The risk assessment report by Environment Canada (2010) is an update to a report by Health Canada[39] that was cited in the NTP-CERHR 2004 monograph. The findings of the updated report are consistent with those of NTP, the authoritative body for Proposition 65, and are expressly presented in the report as such. This document provides no additional information relevant to consideration of EG for listing under Proposition 65 as known to cause reproductive toxicity, and thus does not provide evidence that the criteria in Section 25306(g)(2) are not met.

3. Comments related to limiting the scope of the listing.

Comment:
Dermal or inhalation exposures to EG are of negligible concern. (Submitted by ACC-EGP (pages 12-13); CSPA and PETRA support the comments by ACC-EGP).

If EG is included on the list of chemicals known to the state to cause reproductive toxicity (developmental toxicity endpoint) under Proposition 65, EG should be listed only for the oral exposure route. (Submitted by WIMA and ACMI (page 1); ITIC (page 1))

Response:
As discussed above, NTP concluded that there is clear evidence that EG causes adverse developmental effects in laboratory animals after high oral doses.

With regard to the developmental toxicity of EG via dermal exposure, NTP-CERHR reviewed one prenatal developmental toxicity study in mice (Tyl et al., 1995)[40] that found no maternal or developmental toxicity following dermal exposure to undiluted EG at 3,549 mg/kg-day. For inhalation exposure, the Expert Panel report in the NTP-CERHR monograph concluded that there were insufficient data to determine whether EG causes developmental toxicity in rodents.

OEHHA reviewed the studies published in 2004 and later, which were submitted by commenters, specifically that by Saghir et al. (2010)[41], as well as those by Hess et al. (2004)[42] and Upadhyay et al. (2008)[43]. The findings from these

[39] Health Canada (2000). Priority substances list–State of the science report for ethylene glycol. Available at http://hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/ethylene_glycol/index-eng.php.
[40]Tyl, R. W., Fisher, L. C., Kubena, M. F., Vrbanic, M. A. and Losco, P. E. (1995). Assessment of the developmental toxicity of ethylene glycol applied cutaneously to CD-1 mice. *Fundam Appl Toxicol* 27: 155-66.
[41]Saghir, S.A., M.J. Bartels and W.M. Snellings. (2010). Dermal penetration of ethylene glycol through human skin in vitro. *Int JToxicol* 29(3): 268-276.
[42]Hess, R., M.J. Bartels and L.H. Pottenger. (2004). Ethylene glycol: an estimate of tolerable levels of exposure based on a review of animal and human data. *Arch Toxicol* 78: 671–680.

studies demonstrate that exposure to EG via the dermal or inhalation route result in very low levels of GA internally.

After consideration of this information and the findings of the authoritative body, OEHHA is listing EG (ingested) as causing reproductive toxicity (developmental endpoint) under Proposition 65.

4. Other comments that do not pertain to the Notice of Intent to List (NOIL)

Some of the comments submitted are not relevant to the NOIL EG or raise issues that do not pertain to the listing process, and these comments are addressed very briefly here:

- *WIMA and ACMI commented that a scientifically unsupported listing of EG will jeopardize WIMA's and CMI's certification programs, which incorporate federal requirements for EG-containing products.*

  The scientific support for listing EG is discussed extensively below. If, for any listed chemical, federal law governs warnings in a manner that preempts state authority, an exemption from the warning requirement under Proposition 65 is expressly provided on that basis[44].

- *WIMA and ACMI further commented that listing EG will not promote meaningful Proposition 65 warnings and will increase frivolous litigation.*

  EG must be added to the Proposition 65 list if the relevant statutory[45] and regulatory[46] provisions are met. Once the chemical is listed, OEHHA can assist businesses in complying with the statute's requirements by developing a Maximum Allowable Dose Level[47], or evaluating requests for safe use determinations[48].

- *OWI submitted comments opposing listing of EG as causing reproductive toxicity, based on the evidence of male and female reproductive toxicity discussed by NTP-CERHR.*

---

[43]Upadhyay, S., J. Carstens, D. Klein, T.H. Faller, S. Halbach, W. Kirchinger, W. Kessler, G. A. Csanady, J.G. Filser. (2008). Inhalation and epidermal exposure of volunteers to ethylene glycol: Kinetics of absorption, urinary excretion, and metabolism to glycolate and oxalate. *Toxicology Letters* 178: 131–141.
[44] Health and Safety Code section 25249.10(a).
[45] Health and Safety Code section 25249.8
[46] Section 25306
[47] Sections 25803-25805
[48] Section 25204

As stated in the NOIL[49], OEHHA is listing EG under Proposition 65 as known to cause reproductive toxicity on the basis of developmental toxicity and not on the basis of male or female reproductive toxicity.

- *PETRA commented that the maximum allowable dose level (MADL) for EG should be 3,500 micrograms per day (µg/day), nominally based on developmental toxicity but applying the assumed body weight of 70 kg that is pertinent only to male reproductive toxicity[50].*

  Development of a MADL occurs subsequent to listing of a chemical as known to cause reproductive toxicity under Proposition 65. Thus, this comment does not pertain to the listing of EG under Proposition 65. Further, as noted above, EG is being listed as causing reproductive toxicity solely on the basis of developmental toxicity. If OEHHA proceeds with development of a MADL for EG, it will be based on data pertinent to developmental toxicity and the process applicable to developmental toxicity will be followed. There will be opportunity for public comment on the MADL during that process.

- *PETRA also provided data on migration of EG from their products and commented that those data document that PETRA products should be excluded from warnings under Proposition 65.*

  The levels of exposure to a chemical that will result from the use of any specific products are not relevant to whether the chemical meets the criteria for listing. Such data are relevant to determinations of the applicability of the warning requirement, subsequent to listing of the chemical.

- *CSPA commented that industry has taken action voluntarily to reduce the possibility of ingestion of certain products containing EG by adding a bitterant to antifreeze.*

  OEHHA acknowledges this responsible act by CSPA to help protect pets, wildlife and children attracted to antifreeze because of its sweet taste. However, this has no relevance to whether EG meets the criteria for listing.

- *Unifi commented that the toxicity data collected on EG by OEHHA specifically states that it is tied to oral consumption. Polyester fiber and yarn products do not contain EG in a form that is available for consumption and those products are not intended to be a food source for humans or animals. These products therefore present no reasonable risk of EG exposure through normal, intended use.*

---

[49] Available at http://www.oehha.ca.gov/prop65/CRNR_notices/admin_listing/intent_to_list/041114NOILethyleneglycol.html

[50] Section 25803(8)(b)

As noted above in the response to comments by PETRA, the levels of exposure to a chemical that will result from the use of any specific products are not relevant to whether the chemical meets the criteria for listing. Such data are relevant to determinations of the applicability of the warning requirement, subsequent to listing of the chemical.