FINAL STATEMENT OF REASONS
TITLE 27, CALIFORNIA CODE OF REGULATIONS

PROPOSED AMENDMENT TO SECTION 25805, SPECIFIC REGULATORY LEVELS: CHEMICALS CAUSING REPRODUCTIVE TOXICITY

MAXIMUM ALLOWABLE DOSE LEVEL: ETHYLENE GLYCOL (ORAL EXPOSURE)

This is the Final Statement of Reasons for the adoption of an oral Maximum Allowable Dose Level (MADL) for ethylene glycol (EG). This chemical is known to the State of California to cause reproductive toxicity (developmental endpoint) under Proposition 65[1]. On April 8, 2016, the Office of Environmental Health Hazard Assessment (OEHHA) issued a Notice of Proposed Rulemaking to adopt the proposed level under Title 27, California Code of Regulations, section 25805(b)[2]. OEHHA proposed an oral MADL of 8,700 micrograms per day for EG. The Initial Statement of Reasons set forth the scientific basis for the proposed amendment. A public comment period was provided from April 8 to May 23, 2016. The Notice stated that a public hearing would be held only on request. No request for a public hearing was received. One written public comment was received by OEHHA.

UPDATE TO THE INITIAL STATEMENT OF REASONS

As nonsubstantive changes the Office added (ingested) after Ethylene Glycol and (oral) after 8,700 in the final regulatory text. As justification for these changes being nonsubstantive, the 45-day Notice displayed the proposed text with *ingested* following Ethylene Glycol and *oral* following 8,700. Please see below:

The proposed regulation would adopt the following MADL for oral exposure to ethylene glycol, by amending Section 25805 as follows (addition in underline):

(b) Chemical Name	Level (Micrograms/day)
Ethylene Glycol (ingested)	8,700 (oral)

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (Health and Safety Code, section 25249.5 et seq.)

[2] All further references are to sections of Title 27, California Code of Regulations, unless otherwise noted.

Throughout the Initial Statement of Reasons, it was specified that the MADL being established for Ethylene Glycol is for oral exposure. The sole commenter in this rulemaking, who wrote the following, apparently also understood this:

"In its notice dated April 9, 2016, OEHHA proposes an oral ingestion MADL of 8,700 micrograms per day."

Lastly, 'ingested' should follow Ethylene Glycol to be consistent with the same chemical listing on Title 27CCR, section 27001(c).

PEER REVIEW

On April 8, 2016, OEHHA provided the notice of proposed rulemaking and the Initial Statement of Reasons for the proposed MADL for EG to the members of the Developmental and Reproductive Toxicant Identification Committee for their review and comment as required by Section 25801(f). No comments were received from any committee members.

SUMMARY AND RESPONSE TO COMMENTS

On May 23, 2016, OEHHA received written comments from William P. Gulledge on behalf of the Ethylene Glycols (EG) Panel of the American Chemistry Council (ACC). The comments are summarized and responses are provided below.

Comment 1

The EG Panel comments state that the method of exposure in the Neeper-Bradley et al. (1995)[3] developmental toxicity study in which pregnant mice were orally gavaged with EG on gestation days 6 through 15 does not closely represent human exposure. Human exposure to EG more closely resembles *dietary* exposure rather than exposure by gavage, which amounts to a large daily bolus dose. The use of an oral developmental toxicity study using the dietary route of exposure would result in a higher MADL.

Response 1

Section 25801(a) specifies:

[3] Neeper-Bradley, T. L., Tyl, R. W., Fisher, L. C., Kubena, M. F., Vrbanic, M. A., & 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. *Toxicological Sciences*, *27*(1), 121-130.

> "The determination of whether a level of exposure to a chemical known to the state to cause reproductive toxicity has no observable effect for purposes of Section 25249.10(c) of the Act shall be based on evidence and standards of comparable scientific validity to the evidence and standards which form the scientific basis for the listing of a chemical as known to the state to cause reproductive toxicity…"

This criterion is also specified in the statute[4]. Section 25803(a)(5) further specifies that "the NOEL shall be based on the most sensitive study deemed to be of sufficient quality." The study by Neeper-Bradley et al., which is one of the studies that formed the basis for listing of EG and which provides the highest NOEL that does not exceed the lowest LOEL, meets both of these requirements.

Scientific literature supports that high oral exposures (large bolus doses) to EG occur in humans. Multiple clinical and case studies report high levels of EG exposure in a number of patients via large amounts of EG ingestion[5,6,7,8,9,10,11,12] which demonstrate the potential for human exposures of this type. Even dietary exposure to EG in humans may be more analogous to bolus exposure than to dietary exposures in rodents. Humans ingest quantities of different foods and beverages at different times of day, rather than ingesting exactly the same food

[4] Health and Safety Code section 25249.10(c)
[5] Czyewska, S., R. Winnicka, J. Rzepecki, Z. Kolacinski, P. Politanski, J. Sawicka and A. Krakowiak (2013). "[Acute ethylene glycol poisoning among patients of Nofer Institute of Occupational Medicine in Lodz, Toxicology Unit, hospitalized in the years 2000-2009]." Przegl Lek **70**(8): 500-505.
[6] Gomolka, E., M. Kapala and P. Hydzik (2013). "[Confirmed poisonings with ethylene glycol and methanol in south Poland in the years 2010-2012 based on results from toxicological laboratories in Krakow and Sosnowiec]." Przegl Lek **70**(8): 506-510.
[7] Cantrell, L. and J. Lucas (2014). "Suicide by non-pharmaceutical poisons in San Diego County." Clin Toxicol (Phila) **52**(3): 171-175.
[8] Schoen, J. C., M. R. Cain, J. A. Robinson, B. M. Schiltz and M. S. Mannenbach (2016). "Adolescent Presents With Altered Mental Status and Elevated Anion Gap After Suicide Attempt by Ethylene Glycol Ingestion." Pediatr Emerg Care.
[9] Szmigielska, A., H. Szymanik-Grzelak, E. Kuzma-Mroczkowska and M. Roszkowska-Blaim (2015). "Hemodiafiltration efficacy in treatment of methanol and ethylene glycol poisoning in a 2-year-old girl." Dev Period Med **19**(2): 174-177.
[10] Tanasescu, A., R. A. Macovei and M. S. Tudosie (2014). "Outcome of patients in acute poisoning with ethylene glycol--factors which may have influence on evolution." J Med Life **7 Spec No. 3**: 81-86.
[11] Thanacoody, R. H., C. Gilfillan, S. M. Bradberry, J. Davies, G. Jackson, A. J. Vale, J. P. Thompson, M. Eddleston and S. H. Thomas (2016). "Management of poisoning with ethylene glycol and methanol in the UK: a prospective study conducted by the National Poisons Information Service (NPIS)." Clin Toxicol (Phila) **54**(2): 134-140.
[12] Viinamaki, J., A. Sajantila and I. Ojanpera (2015). "Ethylene Glycol and Metabolite Concentrations in Fatal Ethylene Glycol Poisonings." J Anal Toxicol **39**(6): 481-485

throughout the entire day as is the case in rodent diet studies. Ingestion at one meal of a food or beverage that contains EG may constitute a bolus dose of the chemical.

Comment 2

The proposed MADL does not adjust for the differences in pharmacokinetics in humans compared to rodents. The proximate developmental toxicant is glycolic acid, a metabolite of EG. Developmental toxicity occurs in rodents only at doses that saturate the metabolism of glycolic acid, resulting in a sharp increase in peak levels of glycolic acid (Carney et al., 2008; Carney et al., 2011)[13,14]. In comparison to the high dose testing in rodents, saturation of metabolism is unlikely to occur in humans exposed to EG in consumer products or through workplace exposure.

Response 2

The pharmacokinetics of EG in humans and rodents are very similar. As OEHHA noted in the Initial Statement of Reasons[15], no human data relevant for establishing a MADL based on the developmental effects of EG were identified. The MADL is based on studies in mice, which were the most sensitive species tested. With regard to doses that saturate the metabolism of glycolic acid (GA), OEHHA concurs with NTP-CERHR's findings that "EG may adversely affect human development if oral exposures are sufficiently high", and the statement 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" (NTP-CERHR 2004)[16]. The commenter's statement that "saturation of metabolism is unlikely to occur in humans exposed to EG in consumer products or through workplace exposure" appears to be based on the assumption that people will not orally ingest a large amount of EG in consumer products or through workplace exposure.

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

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

[15] Available at http://oehha.ca.gov/proposition-65/crnr/notice-proposed-rulemaking-amendment-section-25805-specific-regulatory-levels

[16] National Toxicology Program. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Ethylene Glycol. NTP CERHR MON. 2004 Jan;(11):1-III36. PubMed PMID: 16015391.

Products including anti-freeze, hydraulic brake fluids and de-icing solutions contain EG and high dose ingestion of such products can occur, at levels associated with saturating pharmacokinetics[17]. In 2007, the Association of Poison Control Centers[18] reported 5,731 EG exposures, 84% of which were unintentional. In a study by Rosano et al. (2009)[19], the authors reported high levels of glycolic acid in postmortem circulation in 12 EG poisonings, where the levels of EG were low or undetectable. This is attributed to the rapid metabolism of EG coupled with the relatively long half-life and slow elimination rate of GA. In clinical studies such as that by Gabow et al. (1986)[20], three patients with severe EG poisoning were examined and all had highly elevated GA levels. In this study, after ingestion of 207 mL of lamp oil with high concentrations of EG, a 41-year-old patient showed clinically significant amounts of only organic acid metabolites of EG, one of which was a high level of glycolic acid. Preventative measures in this patient and two other EG-exposed patients by inhibition of the metabolism of EG with alcohol, which competitively inhibits alcohol dehydrogenase and consequently reduces formation of GA, prevented major organ damage.

Comment 3:

The commenter states that the NOEL used for this proposed MADL comes from a developmental toxicity study in mice, yet the mouse may not be the most relevant species for purposes of estimating a MADL for humans. The commenter cites a conclusion by Carney et al. (2008)[21] that the rabbit may be the most scientifically appropriate model for estimating the potential risk of developmental toxicity to humans based on the negligible role of the rabbit visceral yolk sac in placental transfer (humans lack a visceral yolk sac) and similar rates of EG metabolism and extraembryonic fluid turnover. Using the mouse study as the basis for the proposed MADL, even though the rabbit study may be more scientifically appropriate, is conservative because the NOEL in the

[17] Rosano TG, Swift TA, Kranick CJ, Sikirica M. Ethylene glycol and glycolic acid in postmortem blood from fatal poisonings. J Anal Toxicol. 2009 Oct;33(8):508-13. PubMed PMID: 19874660.

[18] A.C Bronstein, D.A. Spyker, L.R. Cantilena, Jr., J.L. Green, B.H. Rumach, and S.E. Heard. 2007 annual report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 25th Annual Report. Clin. Toxicol. **46:** 927-1057 (2008).

[19] Rosano TG, Swift TA, Kranick CJ, Sikirica M. Ethylene glycol and glycolic acid in postmortem blood from fatal poisonings. J Anal Toxicol. 2009 Oct;33(8):508-13. PubMed PMID: 19874660.

[20] Gabow PA, Clay K, Sullivan JB, Lepoff R. Organic acids in ethylene glycol intoxication. Ann Intern Med. 1986 Jul;105(1):16-20. PubMed PMID: 3717806.

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

mouse study (150 mg/kg bw/day) was more than 13 times lower than the NOEL in the rabbit study (>2000 mg/kg bw/day).

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

Response 3

All these points were addressed in OEHHA's response to comments from the ACC Ethylene Glycols Panel on the listing of ethylene glycol under Proposition 65[22]. As noted in those responses, OEHHA reviewed the 11

[22] Available at http://oehha.ca.gov/media/downloads/proposition-65/crnr/comments/061815egnoilresponsetocomments.pdf .

publications[23,24,25,26,27,28,29,30,31,32,33] cited by the ACC Ethylene Glycols Panel as not having been considered by NTP. Of the 11 publications, four are 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) of EG. After detailed consideration of those data that were not considered in the NTP-CERHR report, OEHHA found those 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

[23] 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.
[24] 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.
[25] 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.
[26] 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.
[27] Carney, E.W. (2011). Ethylene Glycol. In Reproductive and Developmental Toxicology (Ramesh C. Gupta, ed.). Elsevier Inc. pp. 607-615.
[28] 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.
[29] 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.
[30] 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.
[31] 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
[32] Saghir S.A., Bartels MJ, Snellings WM (2010). Dermal penetration of ethylene glycol through human skin in vitro. Int J Toxico 29: 268-76.
[33] 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.

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 do not provide a sufficient basis for making a quantitative adjustment to the rodent NOEL based on differences in the dose-response relationships in rodents and humans.

The Panel's assertion that the sufficiency of evidence criteria for listing were not met was addressed in the responses to the Panel's comments on the listing of EG, and is not relevant to establishment of a MADL for EG.

The NTP-CERHR Monograph (NTP-CERHR, 2004) identified the studies considered by NTP to be of sufficient quality for identifying EG as causing developmental toxicity. OEHHA considers those studies to be of sufficient quality for use in determining a MADL. The study by Neeper-Bradly et al. (1995)[34] provides a LOEL and a NOEL of 500 and 150 mg/kg-day, respectively, for the developmental toxicity of EG and was deemed to be the most sensitive study.

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. The subsequent study by Carney et al. (2011)[35] re-confirmed the developmental effects of EG in rats at a dose of 1000 mg/kg-day.

Regarding the placental transfer of EG in rabbits and rodents and how conservative the study in rodents vs rabbits was, NTP-CERHR (2004) also discussed oral dosing with EG in drinking water causing no developmental toxicity in rabbits at a dose of 2,000 mg/kg-day, a dose which resulted in maternal deaths and whole litter loss. The NTP-CERHR report states that

> "[t]he 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."

[34] Neeper-Bradley, T. L., Tyl, R. W., Fisher, L. C., Kubena, M. F., Vrbanic, M. A., & 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. *Toxicological Sciences*, *27*(1), 121-130.
[35] 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.

An *in vitro* study using rabbit embryo cultures by Carney et al. (2008)[36] 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 embryos to GA, due to the structural characteristics of rabbit placenta and other factors. A further study by Ellis-Hutchings et al. (2014)[37] 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) 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."

However, the structure of the placenta is not the only factor contributing to fetal exposure to GA. Regarding the yolk sac and placental transfer, pH is a crucial component driving exposure of the fetus/embryo to GA. In humans, late in the first trimester, the coelomic fluid has a lower pH than maternal serum[38] suggesting a shift in pH through gestation between maternal blood and embryonic fluid that may make the embryo more susceptible to GA exposure. Data presented in Table 2 (reproduced from Ellis-Hutchings et al., 2014)[39] suggest that at different periods in gestation the rabbit yolk sac would represent a pH gradient more similar to that which occurs in mice, supporting the relevance of the mouse as a model for humans. In addition, regardless of the differences in species placentation during the most commonly chosen test period of GD9, there are multiple other gestation periods where human and rodent placental structure is more similar than are human and rabbit placental structures.

[36] 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.
[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] 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.
[39] 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.

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]

EHHA does not consider the rabbit study[40] identified by the commenter to be more scientifically appropriate than the mouse study by Neeper-Bradley et al. (1990)[41], for several reasons. First, there are neither pharmacokinetic data nor an established MOA in the rabbit. In contrast, there are many pharmacokinetic studies in mice showing the similarities between rodent and human metabolism. Second, the rabbit is not the most sensitive test species. Third, weak organic acids are known to cause developmental toxicity and a given compound may cause a specific type of developmental toxicity in one species and not the other (also as concluded by the NTP-CERHR). For example, valproic acid causes spina bifida (neural tube defect) in humans that is very difficult to produce in the rat, rabbit, and monkey; however, it is producible in the mouse[42], despite the assertion that the rabbit may be a more "scientifically appropriate" species model for humans. Moreover, it has been suggested that most well-documented human teratogens are weak acids[43].

[40] 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.
[41] Neeper-Bradley, T. L., Tyl, R. W., Fisher, L. C., Kubena, M. F., Vrbanic, M. A., & 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. *Toxicological Sciences*, *27*(1), 121-130.
[42] 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.
[43] Scott WJ, Collins MD, Nau H. Pharmacokinetic determinants of embryotoxicity in rats associated with organic acids. *Environmental Health Perspectives*. 1994;102(Suppl 11):97-101.

ALTERNATIVES DETERMINATION

In accordance with Government Code section 11346.9(a)(4), OEHHA has, throughout the adoption process of this regulation, considered available alternatives to determine whether any alternative would be more cost effective in carrying out the purpose for which the regulation was proposed, or would be as cost effective and less burdensome to affected private persons than the proposed action.

OEHHA has determined that no reasonable alternative considered by OEHHA or that has otherwise been identified or brought to the attention of OEHHA would either be more effective in carrying out the purpose for which the action is proposed, or would be as effective and less burdensome to affected private persons, or would be more cost-effective to affected private persons and equally effective in implementing the statutory policy or other provision of law than the proposed regulation.

LOCAL MANDATE DETERMINATION

OEHHA has determined this regulatory action will not impose a mandate on local agencies or school districts nor does it require reimbursement by the State pursuant to Part 7 (commencing with Section 17500) of Division 4 of the Government Code. OEHHA has also determined that no nondiscretionary costs or savings to local agencies or school districts will result from this regulatory action. Proposition 65 provides an express exemption from the warning requirement and discharge prohibition for all state and local agencies. Thus, these regulations do not impose any mandate on local agencies or school districts.