Responses to Public Comment on the Draft Reference Exposure Levels for Ethylene Glycol mono-n-Butyl Ether

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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On October 14, 2015, the Office of Environmental Health Hazard Assessment (OEHHA) released the draft document, *Ethylene Glycol mono-n-Butyl Ether Reference Exposure Levels: Technical Support Document for the Derivation of Noncancer Reference Exposure Levels* to solicit public comment. Responses to comments received on the draft ethylene glycol mono-n-butyl ether (EGBE) Reference Exposure Levels (RELs) are provided here.

Background

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8 hour and chronic RELs and was adopted in December 2008. The TSD presents methodology for deriving RELs. In particular, the methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to revise the acute REL, and to derive new 8-hour and chronic RELs for ethylene glycol monobutyl ether.

Comments were received from:

- American Chemistry Council Glycol Ethers Panel (ACC)

Responses to Comments Received from ACC

Overview:

ACC Comment 1:

The REL values were derived following guidelines published by OEHHA in 2008. As set forth below and in the comments attached hereto, however, the proposed RELs are not based on the best available science and should be revised.

Response to ACC Comment 1:

Following our REL guidelines (OEHHA, 2008), we believe we have used the best available risk assessment methodology and toxicity data from which to derive the EGBE RELs. In the responses to the comments below, we present in detail our reasoning for choosing the critical study for the point of departure, and for the derivation of the acute, 8-hour and chronic RELs based on the critical studies.

ACC Comment 2:

Proposed Acute REL: OEHHA bases the acute 1-hour REL value upon irritation of the respiratory system and upon a study conducted in the 1950s (Carpenter et al., 1956) whereupon test subjects were exposed to high concentrations of EGBE (113 ppm; 550 mg/m3) for 4 hours. Physiological monitoring on the test subjects was not conducted and the subjects reported eye, nose and throat irritation, altered taste and headache and nausea. No attempt was made within this experiment to discriminate between subjective effects due to the offensive odor of the chemical and true sensory irritation due to trigeminal nerve stimulation. In addition, the atmosphere the test subjects were exposed to was not characterized in terms of aerosol formation or particle size distribution. Other inhalation studies using human subjects where sensory irritation was reported were either dismissed as not being relevant (as it was not the primary purpose of the study) or not considered at all by OEHHA.

Response to ACC Comment 2:

As might be expected, the early toxicology studies generally lacked detailed methodology procedures, although the study by Carpenter et al. (1956) was likely the state-of-the-art at the time. Carpenter et al. did measure some objective symptoms during exposures to 98 ppm EGBE, including blood pressure and heart rate, three times during the exposure day. However, they reported that, “The only objective finding of

significance was the urinary excretion of butoxyacetic acid". Presumably, this means blood pressure and pulse rate were unaffected by exposure. Unlike the more recent toxicokinetic studies that OEHHA judged to be less relevant, Carpenter et al. specifically set out to describe the subjective sensations felt by the exposed subjects.

Carpenter et al. notes that, "*Human symptoms, which were secretly recorded, included nasal and ocular irritation, disagreeable metallic taste, slight increase in nasal mucous discharge, and occasional eructation*". Some subjects also experienced headache and nausea following the exposures. Carpenter then goes on to say, "*The privately recorded response of all three subjects* [inhaling 195 ppm EGBE] *included immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste*". Although the description of the odor intensity was not well-characterized from these descriptions, OEHHA believes that the level of discomfort experienced by the human subjects was clearly a LOAEL regardless of the odor intensity of the exposures.

Other human exposure studies were toxicokinetic studies that did not properly assess the subjects for sensory irritation. Additionally, OEHHA does not normally use studies, such as the toxicokinetic studies by Jones et al.(2003) and Johanson et al. (1986), with a free-standing NOAEL. (i.e., a study in which only a NOAEL, and no LOAEL, was established) for REL derivation. As noted in OEHHA's Noncancer TSD (OEHHA, 2008, page 40), *"The U.S. EPA (1994) determined that a NOAEL not associated with any biological effect identified from a study with only one dose level is unsuitable for derivation of an RfC for chronic exposure. Because there is limited availability of multi-dose studies for the variety of chemicals considered, OEHHA may use a NOAEL without an associated LOAEL identified in the same study, but only if there are no other suitable studies, and so long as the overall health hazard data (including any case reports or studies with shorter durations) for that substance are consistent with the NOAEL study".*

Because there is a more suitable study available (Carpenter et al., 1956) with a LOAEL, this is used as the key study for acute REL derivation. In addition, there is concern about the small sample size in the Jones et al. study (n=4), particularly because it is a free-standing NOAEL. A NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed population (OEHHA, 2008, page 39). This is so because only a subset of individuals from the population has been observed, and because the experiment may not have been designed to observe all adverse effects associated with the substance. Therefore, OEHHA cannot safely conclude that the study concentration or dose is not associated with any adverse effects.

Lastly, the saturation vapor pressure of EGBE is around 1000 -1200 ppm (Carpenter et al., 1956; Raymond et al., 1998). Carpenter and associates were aware of this when

they conducted their sensory exposure studies. The exposure concentrations of 98, 113 or 195 ppm used by the authors are well below the saturated vapor pressure and the EGBE was thus likely predominantly in the vapor state.

ACC Comment 3:

The acute REL for EGBE should be 5 ppm, based on the 50 ppm value from the Jones et al., 2003 study as a no observed effect concentration (NOEC) and a cumulative uncertainty factor value of 10 (derived from the intraspecies uncertainty factor for toxicodynamics (UFH-d) due to differences in response at the receptor level).

Response to ACC Comment 3:

As noted above, OEHHA believes the acute REL should be based on the LOAEL of 98 ppm identified in the Carpenter et al. (1956) study. We then applied a LOAEL-to-NOAEL UF = 10, as described in our REL Guidance (OEHHA, 2008). However, after further review, OEHHA has concluded that reducing the cumulative intraspecies uncertainty factor (UF) from 30 to 10 is more appropriate and adheres to our acute REL Guidelines (OEHHA, 2008). This would entail an intraspecies toxicokinetic UF of 1 (rather than √10 as previously proposed) and an intraspecies toxicodynamic UF of 10, for a total cumulative intraspecies UF = 10.

Previously, a toxicokinetic UF = √10 was considered by OEHHA due to concerns for bioactivation of EGBE to 2-butoxyacetic acid (BAA), the main metabolite responsible for the hemolytic action in rodents. Humans are considerably less sensitive to this particular adverse effect compared to rodents, and the acute eye and respiratory irritation is a result of direct contact of the parent compound, EGBE, onto the epithelial tissues. Sensory irritation is not expected to involve large toxicokinetic differences among individuals. Intraspecies toxicokinetic UFs greater than 1 are used for acute sensory irritants if metabolic processes also contribute to intraspecies variability. No systemic toxicity from metabolites (primarily BAA-related hemolysis) was observed during acute human exposures conducted by Carpenter et al., and *in vitro* studies have shown RBCs from children are resistant to BAA-induced hemolysis similar to RBCs from adults. Thus the toxicokinetic component of the intraspecies $UF_{H\text{-}k}$ is assigned a value = 1.

The toxicodynamic component of the intraspecies UF is assigned a value of 10 for potential exacerbation of asthma in sensitive subpopulations. Epidemiological studies suggest cleaning products, some of which include EGBE, increase the likelihood of an asthmatic episode in susceptible individuals. Thus, there is concern that EGBE may exacerbate existing asthma, particularly in children who may experience irritant-induced asthma; OEHHA views asthma as a more serious health problem in children versus

adults (OEHHA, 2001). There is also increased uncertainty in the LOAEL due to the small sample size (n=3) used in the key study, supporting the use of a $UF_{H\text{-}d}$ = 10.

ACC Comment 4:

Proposed 8-hour and Chronic RELs: OEHHA bases the 8-hour and chronic REL values on nasal hyaline degeneration of the olfactory epithelium in female rats as the critical adverse chronic effect. Given that such changes were minimal in severity and did not increase in severity with dose, are commonly present in aging rodents, and have been proposed as adaptive or protective changes, it is inappropriate to employ these as a critical chronic effect for purposes of human health risk assessment.

Response to ACC Comment 4:

A review of the literature by OEHHA indicates that nasal hyaline degeneration (i.e. formation of eosinophilic droplets) is an adverse effect indicative of cellular apoptosis. Discussion of this lesion is presented in greater detail in Response to ACC Comments #42 and #43.

ACC Comment 5:

Hematoxicity is more generally recognized and accepted as the critical adverse effect following EGBE exposures and has been employed by the U.S. EPA as well as the European Union in chronic risk assessments. The EPA Integrated Risk Information System (IRIS) reference concentration (RfC) value of 1.6 mg/m3 (0.34 ppm) should be used instead of the proposed chronic REL value. Also, as a conservative approach, this same value should be used as the 8-hour REL.

Response to ACC Comment 5:

In selecting a point of departure (POD), the hematotoxicity between species was investigated. We present considerable data that show humans are substantially less sensitive to the hemolytic effects of EGBE when compared to rodents. The Kupffer cell hemosiderin pigmentation observed in chronic rodent exposure assays is the secondary effect of RBC hemolysis. Thus, OEHHA believes a critical endpoint other than hemolysis or Kupffer cell hemosiderin pigmentation should be used for 8-hour and chronic REL derivation. OEHHA considers hyaline degeneration in nasal epithelium to be an adverse effect that is relevant to human exposure and chose this critical endpoint as the point of departure for the 8-hour and chronic RELs

Attachment A: Acute 1-Hour REL Comments

ACC Comment 6:

It is well accepted that there is difficulty in discriminating between objectionable odors (subjective symptoms) and true sensory irritation requiring trigeminal nerve stimulation (objective symptoms). Experimental methods have been developed that involve: measuring of physiological parameters (e.g., breathing rate, pulse rate, skin surface temperature and skin resistance) that are difficult if not impossible for a test subject to control; use of anosmic test subjects (people who do not have a sense of smell); and correlation of respiratory tract irritation with eye irritation (characterized by tearing and redness and measures of tear film breakup, epithelial damage and lipid layer thickness). The measurement of subjective parameters (e.g., odor intensity and “bad smell”) can be enhanced with better design of rating scales, including strength and direction of anchoring objectives (Pollack et al., 1990).

Unfortunately, no studies are available that provide all of this type of information for EGBE. Therefore, it is necessary to evaluate all of the available older studies to determine their strengths and weaknesses according to current experimental laboratory standards.

Response to ACC Comment 6:

OEHHA generally agrees with some of the ACC comments. We evaluated individual studies and compared them with each other to identify the critical study for REL derivation, in this case one of the older studies, Carpenter et al. (1956). As described in Response to Comment #2 above, this study set out to determine sensory irritant concentrations of EGBE. They also measured a few physiological parameters (blood pressure and heart rate), but apparently, these were not as sensitive an indicator of an adverse effect as sensory irritation of the nose and eyes. Later pharmacokinetic studies, namely Jones et al. (2003) and Johanson et al (1986), were not designed to determine a level of EGBE that results in sensory irritation. At best, these studies established a free-standing NOAEL that OEHHA does not use as the basis of a REL if there are more appropriate studies that establish a LOAEL (see Response to Comment #2).

ACC Comment 7:

Examination of the human studies when compared to current modern laboratory methods for respiratory tract irritation revealed that none of the available studies were designed according to current standards. However, each of these studies has strengths

and weaknesses and can be evaluated individually and collectively to provide useful information on the ability of EGBE to cause respiratory tract irritation.

Response to ACC Comment 7:

OEHHA generally agrees with this comment. We evaluated individual studies and compared them with each other to identify the study that was most appropriate for REL derivation. The Response to ACC Comments below addresses the strengths and weaknesses of the human exposure studies. In particular, Response to ACC Comment #9 describes the reasons why we did not choose the pharmacokinetic studies that the ACC identified (i.e., Johanson et al., 1986, Kumagai et al., 1999, Johanson and Boman, 1991 and Jones et al., 2003) for REL derivation.

ACC Comment 8:

The Carpenter et al., 1956 study collected only subjective symptoms without any collection of objective parameters or detailed description of the inhalation exposure atmospheres and therefore the EU Risk Assessors could not determine if the reported symptoms were due to physiological changes or simply due to “discomfort” of the test subjects to the odor and intensity of the exposures. The exposure conditions within the Carpenter et al., 1956 study were quite high (100 and 200 ppm) for a chemical with a relatively low vapor pressure (0.76 mm Hg @20° C).

Response to ACC Comment 8:

This comment was mostly addressed in Response to ACC Comment #2. The early toxicology studies generally lacked detailed methodology procedures, although the study by Carpenter et al. was likely the state-of-the-art at the time. Carpenter et al. did measure a few objective parameters during exposures to 98 ppm EGBE, including blood pressure and heart rate, three times during the exposure day. However, they reported that, “The only objective finding of significance was the urinary excretion of butoxyacetic acid”. Presumably, this means blood pressure and pulse rate were unaffected by exposure. Unlike the more recent toxicokinetic studies, Carpenter et al. specifically set out to describe the subjective sensations felt by the exposed subjects, which are likely more sensitive indicators of adverse effects than objective measures of blood pressure and pulse rate. Thus, OEHHA considers the reporting of subjective findings by Carpenter et al. better than those by later toxicokinetic studies that mainly reported some general physiological objective measures (e.g., breathing rate, pulse rate, blood pressure, skin surface temperature and skin resistance).

Carpenter et al. notes that,”*Human symptoms, which were secretly recorded, included nasal and ocular irritation, disagreeable metallic taste, slight increase in nasal mucous discharge, and occasional eructation*”. Some also experienced headache and nausea

following the exposures. Carpenter then goes on to say, "*The privately recorded response of all three subjects* [inhaling 195 ppm EGBE] *included immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste*". Although the description of the odor intensity was not well-characterized, from these descriptions OEHHA believes that the level of discomfort experienced by the human subjects was clearly a LOAEL regardless of the odor intensity of the exposures.

In terms of the saturation vapor pressure of EGBE, the concentration for aerosol formation needs to be around 1000 -1200 ppm (Carpenter et al., 1956; Raymond et al., 1998). The exposure concentrations of 98, 113 or 195 ppm in Carpenter et al., 1956 study were reasonable to identify a lowest observable adverse effect level (LOAEL), given that the predominant form of EGBE the human subjects were exposed to was in the vapor state.

ACC Comment 9:

The Johanson et al., 1986, Kumagai et al., 1999, Johanson and Boman, 1991 and Jones et al., 2003 studies are all more recent than Carpenter et al., 1956, and all of these studies collected both subjective and objective parameters of respiratory tract irritation, although this was not the primary intent of the Johanson studies. While none of these studies were designed according to current standards for collection and evaluation of subjective symptoms (odor intensity and "bad smell"), neither was the 1956 Carpenter study. However, these more recent human studies did collect objective symptoms of respiratory tract irritation in the form of physiological parameters (breathing rate, pulse rate, blood pressure, skin surface temperature and skin resistance). The EU Risk Assessors therefore concluded that the more recent human studies were of comparable quality for collection of subjective symptoms and of much better quality for collection of objective symptoms (i.e., physiological parameters) than the older Carpenter study.

Response to ACC Comment 9:

Johanson et al., 1986 studied 7 male volunteers (age ranged 21 to 38) who inhaled 20 ppm EGBE under light physical exercise (50 W) on a bicycle ergometer for 2 hours. The authors did not have the volunteers fill out a questionnaire for subjective measures of sensory irritation, or attempt to document in detail any subjective responses. The authors did report that, "None of the subjects complained of or showed any signs of adverse effects that could be related to the exposure to 2-butoxyethanol." Although the odor threshold (0.1 ppm) of EGBE is about 200 times lower than the exposure concentration, the authors did not report the odor intensity experienced by the volunteers. They did report that no effects or consistent changes occurred in the electrocardiograms, pulmonary ventilation, respiratory frequency, and heart rate. These

physiologic parameters usually reflect general body responses that are likely less sensitive as indicators of adverse effects, compared to trigeminal nerve stimulation. This is primarily why we selected the Carpenter et al. study for REL derivation.

The following summaries present the strengths and weaknesses (for use in a REL derivation) of the toxicokinetic studies highlighted in the ACC comment:

Johanson and Boman, 1991 exposed 4 male volunteers (age range 23-36, one of them was a smoker) to 50 ppm EGBE vapor for two periods of 2 hours. The first exposure was mouth-only and the second one was skin-only exposure. The authors then compared the uptake, distribution and excretion of urinary metabolites by these two routes of exposure. The authors only found small inconsistent differences in heart rates between the two routes of exposure, with mouth exposure resulting in slightly lower heart rates compared to skin-only exposure. This study did not contain any information on sensory irritation or other potential adverse effects the subjects may have experienced during EGBE exposure. Kumagai et al. (1999) determined the uptake values for 10 polar organic solvents, including EGBE, and observed the time course of chemical concentration in the exhaled air during short-term respiration. The authors exposed 4 male volunteers (3 of them were smokers) via mouthpiece to 25 ppm EGBE for 10 minutes. This toxicokinetic study was not designed to examine subjective or objective signs of sensory irritation. That said, respiratory rate and tidal volume were measured in the volunteers during the solvent exposures. Tidal volume was similar among all 10 solvents, and mean respiratory rate for nine of 10 solvents, including EGBE, were similar. The tenth solvent (iso-pentyl alcohol) caused a slightly higher respiratory rate probably related to throat irritation experienced by the volunteers. After the experiment, hematological tests (blood cell count, hematocrit, hemoglobin) and liver enzyme tests (glutamic oxaloacetic transaminase (GOT), alanine transaminase (GPT), γ-glutamyltransferase (γ-GTP)) were done; no indication of liver disorders were found.

Similar to the Johanson et al. (1986) study, Jones et al. (2003) also measured physiological responses including breathing rate, pulse rate, skin surface temperature and skin resistance (a measure of perspiration) during exposure to EGBE. However, the primary goal of the study was to investigate the effects of temperature, humidity and clothing on the whole body dermal absorption of EGBE in order to clarify some previous data and to determine the potential consequences of dermal absorption of vapors to workers. This toxicokinetic study did not investigate subjective adverse effects experienced by the exposed volunteers. In the study, four volunteers were exposed via half-face mask to 50 ppm EGBE for two hours. The authors then compared this to 8 different skin exposure scenarios and one whole body exposure condition to address skin absorption rates of EGBE under different exposure scenarios. Their results show that ‘baseline’ dermal absorption of EGBE vapor was, on average, 11% of the total absorbed dose. Higher temperature (30°C, mean 14%, $P = 0.03$) and greater humidity

(65% RH, mean 13%, P = 0.1) increased dermal absorption. The wearing of whole-body overalls did not attenuate absorption (mean 10%). No significant differences (p>0.05) in any of the physiological parameters were observed during the study. It is not clear whether the 50 ppm of EGBE for inhalation exposure is a NOEL in the Jones et al. (2003) study, as their study was not designed to identify a NOEL level of inhalation exposure. In their paper, no trigeminal nerve sensory irritation indices such as sensation on eyes and nose were reported. The physiological parameters measured, particularly pulse rate, might be better characterized as "general whole body" responses that are affected with exposure to strong irritant gases. Skin surface temperature and skin resistance parameters are usually used for dermal absorption studies, as these parameters vary depending on the body part examined.

OEHHA's reasoning for not using these toxicokinetic studies as the basis of the acute REL derivation (i.e., used as the point of departure) is as follows:

1) Examining only physiological factors, many of which are likely less sensitive endpoints compared to subjective responses, may overestimate the NOAEL and miss the most sensitive endpoint (i.e., sensory irritation).
2) These toxicokinetic studies only used one exposure concentration with no apparent adverse effects on the human subjects. As such, they are free-standing NOAELs. Our revised TSD Noncancer REL guidance (OEHHA, 2008) notes that, "*OEHHA may use a NOAEL without an associated LOAEL identified in the same study (a free-standing NOAEL), but only if there are no other suitable studies, and so long as the overall health hazard data (including any case reports or studies with shorter durations) for that substance are consistent with the NOAEL study*". OEHHA guidance does not recommend using a NOAEL and a LOAEL from different studies, and that a free-standing NOAEL not be used as the basis of a REL if a more suitable study (e.g., a study with a LOAEL) exists. Thus, we base the proposed acute REL on the LOAEL of 98 ppm determined in the Carpenter et al. study, which OEHHA believes is a more suitable study.
3) There is concern about the small sample sizes in studies that have free-standing NOAELs, particularly for the Jones et al. (2003) study (n=4). As noted in the OEHHA Noncancer TSD (OEHHA, 2008, page 39), "*A NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed population. This is so because only a subset of individuals from the population has been observed, and because the experiment may not have been designed to observe all adverse effects associated with the substance"*. Therefore, OEHHA cannot safely conclude that the singled-dose studies exposing only a few human subjects are not associated with any adverse effects.

The EU (2008) chose an acute NOAEL of 50 ppm, based on the Jones et al. (2003) study. The EU reports: "*In a recent study (Jones et al., 2003) no signs of irritation were reported after exposure to 50 ppm EGBE. The published study paper does not mention if these signs were checked except [for] the recording of the physiological changes. However, the author did indicate in a written communication that the volunteers were asked to report any adverse effects and none were reported (Jones, personal communication)".*

OEHHA generally does not rely on written communications that have not been peer-reviewed for the basis of a REL. Additionally, for the reasons stated above, including less sensitive measures of adverse effects, no peer-reviewed assessment of sensory irritation, use of only one exposure level that resulted in free-standing NOAELs, and small sample sizes of the free-standing NOAELs, OEHHA chose a sensory irritation study that established a LOAEL (Carpenter et al., 1956).

ACC Comment 10:

In addition, the more recent studies were conducted at lower exposure concentrations (20 to 50 ppm) with well-characterized test atmospheres and in an exposure range where aerosol production would not be expected to occur.

Response to ACC Comment 10:

A summary of the toxicokinetic studies that exposed human subjects to 20-50 ppm EGBE is presented above in Response to ACC Comment #9. The saturated vapor pressure of EGBE is around 1000-1200 ppm, presumably at room temperature. Carpenter et al. (1956) was well-aware of this limitation, as reported in their study, when they exposed human subjects to concentrations of 98-195 ppm EGBE. This concentration range is well below the saturation vapor concentration. Thus, exposures were likely predominantly to the vapor form of EGBE.

ACC Comment 11

The Carpenter et al., 1956 study should not have been chosen as the basis for setting the 1-hour REL because it has the same shortcomings in terms of collecting and reporting of subjective symptoms (when compared to current standards) as the more current studies, yet is remarkably deficient in the collection of objective symptoms (physiological parameters) and characterization of the test atmospheres.

Response to ACC Comment 11:

This comment was largely addressed in Response to ACC Comment #2 and #8. As might be expected, the early toxicology studies generally lacked detailed methodology

procedures, although the study by Carpenter et al. was likely the state-of-the-art at the time. Carpenter et al. did measure some objective symptoms during exposures to 98 ppm EGBE including blood pressure and heart rate of the human volunteers. However, they reported that, "*The only objective finding of significance was the urinary excretion of butoxyacetic acid*". Presumably, this means blood pressure and pulse rate were unaffected by exposure. Unlike the more recent toxicokinetic studies, Carpenter et al. specifically set out to describe the subjective sensations felt by the exposed subjects. Thus, OEHHA considers the reporting of subjective findings by Carpenter et al. less deficient than those by Jones et al. (2003).

Finally, Carpenter et al. describes the method of vapor generation for the animal exposures, which is presumably the same as that used for the human exposures: "*Solvent was delivered at a constant rate by means of a displacement-type proportioning pump, originally described by Irish and Adams (15), or by a motor-driven syringe into an electrically heated tubular Pyrex evaporator, such as described by Carpenter and co-workers (16). The resulting vapor-air mixtures were conducted to the chambers under slight negative pressure at a rate to provide a theoretical turnover of chamber air every three to five minutes*". Later, Carpenter et al. writes, "*The concentration of butyl Cellosolve vapor in the exposure chambers were checked four times daily with a portable 50 cm Zeiss interferometer. The instrument was calibrated against a bichromate oxidation method, adapted from the procedure described by Werner and Mitchell*…" From the method of vapor generation described and the observation by Carpenter et al. that the saturated vapor pressure of EGBE is about 1000 ppm, it is likely that the predominant form of EGBE the human subjects were exposed to was in the vapor state.

Although the method used for vapor generation and measurement is primitive by today's standards, OEHHA nevertheless believes that the Carpenter et al. study is the correct study for the basis of setting the acute REL.

ACC Comment 12

In addition, the newer studies characterize the respiratory irritation potential in the lower exposure range, at exposure concentrations more likely to be encountered through the use of EGBE-containing consumer products. Studies evaluating the lower exposure concentrations should not be ignored. The argument by OEHHA that the Johanson studies were primarily designed to investigate pharmacokinetic parameters is correct but is not a reason to ignore the other data (e.g., physiological parameters) that were collected within those studies as part of the study design.

Response to ACC Comment 12:

OEHHA summarized the Johanson studies that were pertinent to the REL document. However, OEHHA has added text in the acute derivation section why these studies (and Jones et al. (2003)) were not used as the basis of the acute REL.

Our revised TSD Noncancer REL guidance (OEHHA, 2008) notes that, "*OEHHA may use a NOAEL without an associated LOAEL identified in the same study, but only if there are no other suitable studies, and so long as the overall health hazard data (including any case reports or studies with shorter durations) for that substance are consistent with the NOAEL study".*

Our new guidance does not recommend using a free-standing NOAEL, which is what the Johanson studies contain, as the basis of a REL if a more suitable study (e.g., a study with a LOAEL) exists. The result is that we base the proposed acute REL on the LOAEL of 98 ppm determined in the Carpenter et al. study, which OEHHA believes is a more suitable study for the basis of the acute REL.

ACC Comment 13

In fact, OEHHA considered the Johanson study for the 2008 acute 1-hr REL, which was set at 2.8 ppm.

Response to ACC Comment 13:

The 2008 acute REL for EGBE is being revised as noted in the Background section on the first page. In addition, new 8-hour and chronic RELs are being proposed as part of the revised methodology for deriving RELs. In particular, the methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations.

The acute REL for EGBE currently in place uses a NOAEL and a LOAEL from different studies: the NOAEL is 20 ppm from Johanson et al (1986) and the LOAEL is 113 ppm from Carpenter et al. (1956). Thus, the point of departure for REL derivation is 20 ppm.

Our revised TSD Noncancer REL guidance (OEHHA, 2008) notes that, "*OEHHA may use a NOAEL without an associated LOAEL identified in the same study, but only if there are no other suitable studies, and so long as the overall health hazard data (including any case reports or studies with shorter durations) for that substance are consistent with the NOAEL study".*

As presented, our new guidance does not recommend using a NOAEL and a LOAEL from different studies, and that a free-standing NOAEL not be used as the basis of a REL if a more suitable study (e.g., a study with a LOAEL) exists. The result is that we

base the proposed acute REL on the LOAEL of 98 ppm determined in the Carpenter et al. study, which OEHHA believes is a more suitable study for the basis of the acute REL.

ACC Comment 14:

The Jones et al., 2003 study was designed primarily to study dermal absorption, yet the use of face masks to supply fresh air in half of the exposures while breathing EGBE concentrations of 50 ppm in the other half of the exposures, along with measuring physiological parameters during all of the exposures, suggests that this study should be selected as providing the no observed effect concentration (NOEC) for respiratory tract irritation for EGBE.

Response to ACC Comment 14:

This comment was addressed in Response to Comment #2 and #9. Please refer to these sections above.

ACC Comment 15

In addition, the evidence provided within the EU Risk Assessment for EGBE clearly states that none of the test subjects [in Jones et al., 2003] reported adverse subjective symptoms (e.g., bad smell or odor intensity).

Response to ACC Comment 15:

The ACC appears to be referring to the EU Risk Assessors contacting of the study authors in Jones et al. to obtain the details of adverse subjective effects. A similar comment is contained in ACC Comment #9 above. As presented in the EU Risk Assessment (Page 160 of Part II, Human Health), 50 ppm EGBE exposures reportedly did not cause adverse subjective symptoms within the test subjects in the Jones et al. (2003) study. The report was specifically a toxicokinetic study to estimate the fraction of EGBE that is dermally absorbed into the bloodstream under varying conditions of temperature, humidity and amount of clothing worn.

The published study contains no description of subjective measures, including sensory irritation and rating of odor intensity. Thus, the finding of no adverse effects relies on a written statement sent to the EU (EU, 2008) by the author(s). In the published study, no changes in physiological parameters, including breathing rate, pulse rate, skin surface temperature and skin resistance (a measure of perspiration) were affected by the EGBE exposure. As noted earlier, OEHHA cannot rely on an unpublished written account for derivation of the acute REL, the physiological measures used are relatively insensitive compared to measures of sensory irritation near the threshold for trigeminal nerve

stimulation, and the Jones et al. report, at best, represents a free-standing NOAEL that OEHHA does not use if a suitable study with a LOAEL exists (OEHHA, 2008, page 40). Thus, OEHHA based the acute REL on the Carpenter et al. (1956) study in which a LOAEL was determined.

ACC Comment 16

OEHHA cites the Johanson, 1986 publication that references the physiologically-based pharmacokinetic (PBPK) model developed for humans, but does not reference the Johanson, et al. 1986 publication that contains the detailed methodology and data from the human studies. The 2008 REL for EGBE (with a value of 2.8 ppm) references the human PBPK paper (Toxicokinetics of inhaled 2-butoxyethanol (ethylene glycol monobutyl ether) in man. Scand J Work Environ Health 1986; 12:594-602).

Response to ACC Comment 16:

As the ACC points out, the Johanson et al. (1986) study is indeed the toxicokinetic study that provides the detailed methodology for exposure of human subjects to 20 ppm EGBE. The Johanson (1986) paper is a physiological-based pharmacokinetic modeling (PBPK) study. In this paper, *in vitro* rat data are extrapolated to man *in vivo*, and concentration-time curves are generated by computer simulation. The outcome of the simulation is then compared with the human toxicokinetic exposure results that were generated in the Johanson et al. (1986) study. The reference to the Johanson (1986) study in Section 5.1 will be changed to the Johanson et al. (1986) report in which the human exposure to EGBE is the primary focus of the paper.

ACC Comment 17

OEHHA does not include Johanson and Boman, 1991 in the reference list for the 2015 draft REL document.

Response to ACC Comment 17:

This reference was inadvertently left out of the reference list, even though it is summarized in Section 4.1. It has now been added. The Johanson and Boman (1991) study compared the uptake of EGBE by mouth only and skin only in human subjects exposed to 50 ppm for periods of two hours each. This reference does not contain any information on sensory irritation or other potential adverse effects the subjects may have experienced during EGBE exposure. What the authors observed was that dermal uptake via chamber exposure in subjects wearing only shorts accounted for 75% of the total uptake, suggesting that workers exposed to EGBE vapors may not be adequately protected by using a respiratory protection mask alone. However, other studies (Jones

et al., 2003) have since found dermal uptake of EGBE to be considerably less in proportion to inhalation exposure.

ACC Comment 18

The EU Risk Assessment concluded that the NOEC for human respiratory tract irritation should be based upon the Jones et al., 2003 study where exposures to 50 ppm EGBE in a well-characterized and controlled atmosphere did not result in any changes in objective or subjective parameters of respiratory tract irritation. Although the original 2003 publication did not report the occurrence of subjective symptoms (the report only details a lack of changes in physiological parameters), the EU Risk Assessors contacted the study authors to obtain the details of adverse subjective effects. As detailed in the EU Risk Assessment (Page 160 of Part II, Human Health), 50 ppm EGBE exposures did not cause adverse subjective symptoms within the test subjects in the Jones et al., 2003 study.

Response to ACC Comment 18:

This comment is similar to Comments in #9 and #15 above. Other human studies, including Jones et al., did not properly assess the subjects for sensory irritation. OEHHA does not normally use a study (i.e., the Jones et al., 2003 study) with a free-standing NOAEL (i.e., a study in which only a NOAEL, and no LOAEL, was established) as the key study to base a REL on. As noted in previous responses, in OEHHA's Noncancer TSD (OEHHA, 2008, page 40), we clearly state that a free-standing NOAEL is generally not useful for derivation of a REL.

ACC Comment 19

The EU Risk Assessment also contains information that supports the data obtained in the more recent human studies. The Kane et al., 1980 study using male Swiss-Webster mice provides an RD50 value for an EGBE concentration associated with a 50% decrease in respiratory rate. The basis for this animal test, as noted in the OEHHA Technical Document, is that when a mouse is exposed to an irritant, the decrease in respiratory rate is proportional to the concentration of the chemical. The value obtained when EGBE is tested within this test system is 2825 ppm (confidence limits = 1695 to 7278 ppm). The 2825 ppm value is well in excess of the saturated vapor concentration. The criteria for evaluating the test data from these studies (Alarie et al., 1995) dictates that an exposure concentration of 0.01 times the RD50 (28 ppm for EGBE) would cause minimal or no sensory irritation and 0.1 times the RD50 (280 ppm) would be expected to cause definitive but tolerable sensory irritation (EU Risk Assessment 2008). These values correspond very well with the human data reported in the more recent human

studies (Johanson et al., 1986, Kumagai et al., 1999, Johanson and Boman, 1991 and Jones et al., 2003).

Response to ACC Comment 19:

OEHHA will include a summary of the EGBE RD50 findings in mice by Kane et al. (1980). This RD50 study shows that the authors were unable to generate a high enough EGBE concentration to directly determine the RD50. Thus, they extrapolated to the RD50 from the respiratory depression data they had. This could be a result of the exposures reaching the saturated vapor pressure (about 1000 to 1600 ppm, depending on the temperature and humidity) before reaching the RD50. The study by Alarie et al. (1995) observed that for a series of homologous nonreactive solvents, there is an excellent correlation between the log RD50 values obtained in mice and the log P (vapor pressure). Several other physicochemical properties of solvents also correlated well with the RD50. However, EGBE falls just outside of the upper 95% PI (prediction interval) for this correlation suggesting EGBE may not be an ideal solvent to extrapolate from the RD50 to safe acute exposure levels using the 0.01 and 0.1 factors described in the ACC's comment. For example, RD50 × 0.1 (280 ppm) is stated to cause definitive but tolerable sensory irritation. Carpenter et al. (1956) and the Mellon Institute 1955 industry report summarized in the EU 2008 Risk Assessment observed that exposure of three individuals to 200 ppm EGBE for 2 to 4 hours resulted in immediate sensory irritation, severe gastrointestinal effects (e.g., vomiting) and CNS effects. The subjects exposed in the Carpenter study agreed that 195 ppm EGBE was too high for comfort. This description of toxic effects suggests occupational exposure at this level of EGBE would be intolerable for many workers.

Additionally, Kane et al. (1980) also describes a 0.001 factor × RD50 as being a "safe" level with no effect. This would result in an exposure level of 2.8 ppm for EGBE, and is closer to what OEHHA would estimate for an acute REL. Considering that the RD50 for EGBE may overestimate "safe" levels for humans using the safety factors described by Kane et al., the proposed acute REL for EGBE (0.33 ppm) appears to be reasonably close.

Finally, Kane et al. presents the RD50 concentration-response relationship for EGBE in a figure (Figure 2 of the study). At the lowest dose examined, the data shows there is a 20% reduction in respiratory rate at about 140 ppm in the mice. This finding suggests sensory irritation is present in the exposed mice at this concentration. This is in the same concentration range (98 to 195 ppm) where human subjects experienced sensory irritation in the Carpenter et al. (1956) study.

ACC Comment 20

Furthermore, OEHHA toxicologists have previously published on the usefulness of this mouse sensory irritation assay and the correlation between setting REL values and the RD50 values derived from these studies (Kuwabara, et al., 2007).

Response to ACC Comment 20:

The OEHHA Hot Spots guidelines have been updated since the Kuwabara report was published to account for increased sensitivity of sensitive populations, including children. Therefore, the equation derived to estimate an acute noncancer REL from an RD50 value is not applicable anymore. However, the equation to estimate the LOAEL from an RD50 value can still be used. In the Kuwabara et al. report, a strong correlation (R^2=0.80) was found between RD50s and LOAELs for 25 chemicals with eye or respiratory irritation responses in humans. Using linear least squares regression analysis for log RD50 vs. log LOAEL, the equation derived was:

$$\text{Log } RD_{50} = 1.16(\log \text{LOAEL}) + 0.77$$

Using the EGBE RD50 of 2825 ppm, the human LOAEL is calculated to be 205 ppm. This calculated human LOAEL is about double the EGBE LOAEL of 98 ppm determined by Carpenter et al. (1956) in their human exposure study. The calculated LOAEL of 205 ppm may be overestimated, considering EGBE does not fit the RD50 and vapor pressure correlation well (see Response to ACC Comment 19). Nevertheless, applying a 10-fold LOAEL-to-NOAEL uncertainty factor results in a human NOAEL of 20.5 ppm. This is in the range of the EGBE concentrations used in the human toxicokinetic studies where less sensitive measures for adverse effects were used.

OEHHA notes that, following our REL guidance (OEHHA, 2008), it is more appropriate to use human exposure data when available, rather than animal exposure data, to derive a REL value. Kuwabara et al. (2007) states that the RD50 test is a good starting point for setting exposure standards for acute airborne irritants. They also state that their equation to calculate human LOAELs from mouse RD50 data can be applicable where there is a lack of human exposure studies. Thus, these findings can be used as support for the acute REL derived from the human exposure studies, but not as the basis of a REL since there is human exposure data available.

ACC Comment 21

For the reasons stated above, OEHHA should use the Jones et al., 2003 study as the definitive study as it provides the best experimental data supporting a NOEC of 50 ppm for respiratory tract irritation for EGBE in humans. This would also allow for the

elimination of the “LOAEL Uncertainty Factor” of 10 currently included within the calculations.

Response to ACC Comment 21:

This comment is similar to Comments in #2, #9 and #15. Please refer to Response to Comments #2, #9 or #15 above.

ACC Comment 22

In addition, the use of an intraspecies uncertainty factor for toxicokinetic (UFH-k) of √10 as a default is not supported by the 2008 Technical Guidance Document for setting RELs (OEHHA, 2008). The Technical Guidance Document states “*The toxicokinetic uncertainty factor is meant to cover differences in humans in disposition of the toxicant (absorption, distribution, metabolism, and elimination), while the toxicodynamic uncertainty factor is meant to account for differences in response at the receptor level*.” (Page 12 of 131). However, when the effect of concern is respiratory tract irritation through trigeminal nerve stimulation (Technical Guidance Document Page 75), the effect is related to the exposure concentration of the chemical in the inspired air, not to the “…*differences in humans in disposition of the toxicant (absorption, distribution, metabolism, and elimination)*….” What happens to the chemical once it is absorbed within the body is irrelevant to the air concentration required to stimulate the trigeminal nerve endings within the respiratory tract.

Response to ACC Comment 22:

OEHHA has considered this line of reasoning presented by the ACC and has revised the REL document to use an intraspecies toxicokinetic uncertainty factor of 1 rather than a √10. As discussed in Response to Comment #3 above, a toxicokinetic UF = √10 was previously considered by OEHHA due to concerns for bioactivation of EGBE to 2-butoxyacetic acid (BAA), the main metabolite responsible for the hemolytic action in rodents. Humans are considerably less sensitive to this particular adverse effect compared to rodents, and the acute eye and respiratory irritation is a result of direct contact of the parent compound, EGBE, onto the epithelial tissues. Sensory irritation is not expected to involve large toxicokinetic differences among individuals. Intraspecies toxicokinetic UFs greater than 1 are used for acute sensory irritants if metabolic processes also contribute to intraspecies variability. No action from metabolites (primarily BAA-related hemolysis) was observed during acute human exposures conducted by Carpenter et al., and *in vitro* studies have shown RBCs from children are resistant to BAA-induced hemolysis similar to RBCs from adults. Thus the toxicokinetic component of the intraspecies $UF_{H\text{-}k}$ is assigned a value = 1.

ACC Comment 23

In addition, the Technical Guidance Document states "*If the irritation reaction is a function of the concentration, then the fact that children have higher breathing rates than adults should not influence the health impact of a particular concentration. There is no evidence that infants and children have different or more irritation receptors than adults. Therefore, OEHHA has not assumed that children are more sensitive than adults to the sensory effects of eye, nasal or respiratory irritants. However, it must be considered that many irritants, especially those that are chemically reactive, may have the potential to exacerbate or induce asthma, which is a special concern for children's health*." (Page 76). This reasoning suggests that if the respiratory tract irritation of EGBE through trigeminal nerve stimulation is related to the concentration of the chemical in the inspired air, then no additional uncertainty factors are necessary to include the pediatric population. In addition, since EGBE is not chemically reactive and has not been demonstrated to exacerbate or induce asthma, there is no logical reason to include an intraspecies uncertainty factor to protect children.

The acute REL for EGBE should be 5 ppm, based on the 50 ppm value from the Jones et al., 2003 study as a NOEC and a cumulative uncertainty factor value of 10 (derived from the Intraspecies uncertainty factor for toxicodynamics (UFH-d) due to differences in response at the receptor level).

Response to ACC Comment 23:

We agree with the ACC that a $UF_{H\text{-}d}$ of 10 be applied for REL derivation to address the human variation in the intraspecies toxicodynamic response to respiratory irritants. However, we disagree with the ACC regarding EGBE's potential for exacerbation of asthma in children (OEHHA, 2008, page 48). Epidemiological studies suggest cleaning products, including EGBE, increase the likelihood of an asthmatic episode in susceptible individuals. Thus, there is concern that EGBE may exacerbate existing asthma, particularly in children who may have more serious consequences from an asthma exacerbation than adults; OEHHA views asthma as a more serious disease in children (OEHHA, 2001). The $UF_{H\text{-}d}$ = 10 incorporates sensitive subpopulations such as children with asthma. There is also increased uncertainty in the LOAEL due to the small sample size (n=3) used in the key study, supporting the use of a $UF_{H\text{-}d}$ = 10.

However, small sample sizes (n=4) are primarily a concern for the human EGBE physiology study (Jones et al., 2003; Jones and Cocker, 2003) in which a free-standing NOAEL were observed. This is, in part, why OEHHA does not use this study as the basis for the acute REL. A NOAEL could be associated with a substantial (1-20%) but undetected incidence of adverse effects among the exposed population (OEHHA, 2008, page 39). This is because only a subset of individuals from the population has been

observed, and because the experiment may not have been designed to observe all adverse effects associated with the substance. Therefore, OEHHA cannot safely conclude that the study concentration or dose is not associated with any adverse effects.

ACC Comment 24

Finally, statements made in Section 5.1 – Acute Toxicity to Adult Humans – regarding EGBE and respiratory irritation/diseases are not supported by the cited references. While some of the references provide support for the argument that general irritants can exacerbate asthma symptoms and link these to adverse respiratory effects and others indicate exposure to EGBE during cleaner use, none of the references cited link these two together. A detailed review of each cited reference is contained in Table 1.

Response to ACC Comment 24:

In response to the ACCs comment, the first paragraph in Section 5.1 has been revised. See Response to Comments 27-34 below for details on these revisions.

ACC Comment 25

While the EU at one time classified EGBE as a respiratory irritant, this classification was deleted in 2000 because there were no data to support this classification (ECB, 2000). Furthermore, the 2008 EU risk assessment (EU, 2008) concluded that animal studies do not show any signs of significant respiratory irritation and there are no human data indicating such effects.

Response to ACC Comment 25:.

For occupational inhalation exposure of EGBE, the EU (2008) sets forth an 8-hr TWA of 12 mg/m^3 (2.5 ppm) for eye and respiratory irritation. From this statement in the EU report, it appears that the EU regards EGBE as an eye and respiratory irritant.

The ACC comment suggesting EGBE is not a sensory irritant also seems at odds with the support they give for the RD50 study by Kane et al. (1980) in Comment 19. However, Kane et al. does refer to EGBE as a weak respiratory irritant in comparison with potent respiratory irritants (including formaldehyde, acrolein, chlorine and toluene diisocyanate) they had tested in earlier studies.

ACC Comment 26

OEHHA states that “EGBE exposure should be considered a potential etiologic agent in case of respiratory system diseases and other related conditions.” Kimber and Pieters (2012), however, indicate that experimental studies are needed to investigate these

claims and that it is “premature to conclude that exposure to glycol ether encourages allergic sensitization, or has contributed to the increased prevalence of allergy and asthma.”

Response to ACC Comment 26:

OEHHA is not claiming that recurrent exposure to EGBE may lead to sensitization and asthma. However, there is indirect evidence that EGBE exposure can cause exacerbation of asthma in individuals already afflicted with the disease.

ACC comments on OEHHA Summaries in Section 5.1

ACC Comment 27

Raymond 1998: While the statements attributed [by OEHHA] to this publication are accurately reported, they do not take into account that these effects [primarily findings of dermal cherry angiomas] have never been reported elsewhere and do occur spontaneously with age, albeit usually over 50. The authors acknowledge that their inability to exclude this is a major weakness of their report. This study is primarily to report these dermal findings and that EGBE is a known dermal irritant.

Response to ACC Comment 27:

It is inaccurate for the ACC to describe this report as primarily concerned with the dermal findings of workers exposed to high airborne levels of EGBE. The sensory irritant and CNS effects of high EGBE exposure were also a primary finding of this report and discussed in detail. However, OEHHA has revised the EGBE REL document to include clarifying language about the appearance of cherry angiomas following high acute exposure to EGBE:

“The appearance of cherry angiomas were also reported to occur in 6 of 7 workers (mean age: 36 yrs) 4 months following the high acute EGBE exposure (Raymond et al. 1998). Cherry angiomas can appear spontaneously usually after age 50, but have been observed in workers following exposure to other irritating gases. The authors suggested cherry angiomas may represent a nonspecific response in some persons to inhalation of noxious agents.”

ACC Comment 28

Kullman 1987: The reference does not contain the information stated in the draft REL document. [i.e., There was no mention in this report of the workers complaining of sensory irritation.]

Response to ACC Comment 28:

The NIOSH investigators in the Kullman report undertook the study precisely because of complaints of odor and irritation primarily due to airborne EGBE emissions. On page 2 in the Introduction, Kullman states, "*The request, submitted by Dalb management, cited employee concerns related to odors/irritations from silkscreening operations where EGBE is used*." No revision of the brief summary of the Kullman report is necessary in the OEHHA REL document.

ACC Comment 29

Bello et al., 2009: The reference provides no evidence to support the statement [that respiratory irritation due to EGBE exposure could trigger asthmatic episodes in people with asthma and also pose risks for people with chronic obstructive pulmonary disease, emphysema and other respiratory diseases and conditions] and no indication of any link between EGBE exposure and adverse effects on the respiratory system.

Response to ACC Comment 29:

The statement in the EGBE REL document being challenged by the ACC is: "*Respiratory irritation due to EGBE exposure could trigger asthmatic episodes in people with asthma and also pose risks for people with chronic obstructive pulmonary disease, emphysema and other respiratory diseases and conditions (Bello et al., 2009; Burns, 2010)*."

Bello et al. (2009) noted in the Background section that, "*Results from epidemiological investigations support the hypothesis that exposure to cleaning products is related to the development and/or exacerbation of respiratory symptoms, including asthma*." Bello et al. then cites 8 studies to support this assertion. Bello et al. (2009) in their study characterized the exposures to cleaning agents during cleaning tasks by hospital workers. Their results showed that EGBE was one of the most frequently used solvents and had the highest concentrations in the bulk products investigated. OEHHA supports the authors' contention that cleaning agents, including EGBE, have been identified as an occupational risk due to increased incidence of reported respiratory effects (including asthma and asthma-like effects). Therefore, no revision to the REL summary will be made.

ACC Comment 30

Burns, 2010: This review contains no data to support the conclusions stated and should not be used to set or justify regulatory values.

Response to ACC Comment 30:

As in the previous comment, the ACC is challenging the same statement in the EGBE REL that, "*Respiratory irritation due to EGBE exposure could trigger asthmatic episodes in people with asthma and also pose risks for people with chronic obstructive pulmonary disease, emphysema and other respiratory diseases and conditions (Bello et al., 2009; Burns, 2010)*."

In this brief review of the current toxicity information on EGBE, Burns notes in her summary (Page 3) that, "*EGBE is also a well-recognized irritant that affects the respiratory tract and the eyes. It can exacerbate asthma and many other respiratory diseases (e.g., emphysema, chronic obstructive pulmonary disease, upper and lower respiratory infections)*". Although Burns notes that other Agencies (OEHHA, IARC) found EGBE to have irritant effects, the author does not provide or summarize the epidemiological studies (similar to what Bello et al. (2009) had done) that have associated cleaning products (including EGBE) with respiratory effects, such as exacerbation of asthma. Because this report does not contain any documentation supporting the statement in question, OEHHA will remove this reference.

ACC Comment 31

Bonisch 2012: The inclusion of this reference is not clear. It makes no mention of glycol ethers other than the mention through cross reference to another paper, and there is no mention of EGBE. The paper could be regarded as showing that complex VOC compositions can be linked to adverse respiratory effects but the reference provides no evidence to support the statement in Section 5.1.

Response to ACC Comment 31:

The ACC is suggesting that the statement in the EGBE REL document, "*No direct studies were located with EGBE alone causing an asthmatic episode, in part because EGBE is often present in air together with other VOCs that increase allergic airway inflammation (Bonisch et al., 2012)*" cannot really be attributed to Bonisch et al. (2012). No statement similar to this could be located by OEHHA in Bonisch et al. A more appropriate reference would be Bello et al. (2009) in which they state that, "*Due to the lack of systematic occupational hygiene analysis and workplace exposure data, it is not clear which cleaning-related exposures induce or aggravate asthma and other respiratory effects*." Bello et al. then goes on to conclude in their study that EGBE was one of the most frequent solvents in the products studied, and that it had the highest concentrations in the bulk products. OEHHA will replace the Bonisch et al. reference with the Bello et al. reference.

ACC Comment 32

Burge, 2010: The reference provides no evidence to support the statement that EGBE exposure should be considered a potential etiological agent in case of respiratory diseases and other related conditions.

Response to ACC Comment 32:

The ACC is referring to the sentence, "EGBE exposure should be considered a potential etiologic agent in case of respiratory system diseases and other related conditions (Burge, 2010; Burge et al., 2012; Melchior Gerster et al., 2014)."

Burge (2010) is a summary of recent developments in occupational asthma and does not discuss in detail the various agents that may result in sensitization and asthma.

OEHHA has removed the Burge (2010) reference and revised the end of this paragraph to say: "Epidemiological investigations have shown an association between exposure to cleaning products and respiratory dysfunction, including exacerbation of asthma (Siracusa et al., 2013; Folletti et al., 2014; Zock et al., 2007). Although EGBE has been implicated as a potential irritant in cleaning products that lead to respiratory problems, the presence of EGBE in mixtures with other VOC irritants and the lack of quantitative assessments of exposure during cleaning activities makes it difficult to identify EGBE's role as a respiratory irritant in these products (Gerster et al., 2014; Bello et al., 2013; Fromme et al., 2013; Bello et al. 2009)".

ACC Comment 33

Burge, 2012: If EGBE were established as a respiratory irritant, this paper could be used to support an argument that respiratory irritants can cause irritant induced asthma. However, EGBE is not an established irritant and this reference provides no evidence to support the statement in the draft REL document.

Response to ACC Comment 33:

See Response to ACC Comment 32. Burge et al. (2012) is primarily a case study involving exposure to chemicals other than EGBE. OEHHA will remove this reference and revise the section as shown above in the Response to Comment 32.

ACC Comment 34

Gerster, 2014: The publication is an exposure study. The justification given for the work is that EGBE is an irritant. However, the references cited only show that EGBE is a skin and eye irritant. The reference provides no direct or indirect evidence to support the statement in the draft REL document.

Response to ACC Comment 34:

See Response to ACC Comment 32. OEHHA revised the end of the paragraph so that the Gerster et al. (2014) reference is now a pertinent reference to include.

Attachment B: 8-Hour and Chronic REL Comments

ACC Comment 35:

The toxicological database for EGBE is complete and includes extensive and reliable information on the toxicokinetics, acute and chronic toxicity, genotoxicity, carcinogenicity and reproductive and developmental toxicity of this important solvent (Carpenter et al., 1956; ATSDR, 1998; Gift, 2005; U.S.)(EPA, 2010). Exposure to EGBE by differing routes of exposure produces a consistent hemolytic response in sensitive laboratory species that can be characterized clinically by the appearance of hemoglobinuria and pathologically by changes in a number of blood parameters including depressed hemoglobin and erythrocyte counts, hemoglobinuria, and increased erythrocyte fragility.

Response to ACC Comment 35:

OEHHA partially agrees with these statements and major findings for EGBE toxicity, although the toxicological database for EGBE could be improved and updated. The human toxicological database, in particular, would be improved by more recent acute exposure studies specifically designed to examine sensory irritant and odor ratings to establish both a NOAEL and LOAEL. Such information may well help reduce the existing uncertainty for sensory irritation in humans, since the critical study by Carpenter et al. (1956) only observed a LOAEL, and not a NOAEL.

ACC Comment 36:

Carpenter et al. (1956) were the first to show that the agent responsible for the hemolytic toxicity of EGBE was the acid metabolite, 2-butoxyacetic acid (BAA). In *in vitro* studies, BAA rapidly hydrolyses blood from sensitive species such as rats, mice and rabbits versus less sensitive species such as dogs, humans or guinea pigs. The human erythrocyte is far less sensitive to the hemolytic effects of EGBE/BAA (Ghanayem and Sullivan, 1993; Udden, 1996; 2000). The blood from suspected sensitive subpopulations, including the aged and those predisposed to hemolytic disorders was shown to be unaffected by incubations with 2 mM concentrations of BAA for up to 4 hours (Udden and Patton, 1994a; 1994b). Although slight, pre-hemolytic effects corresponding to similar changes in rat erythrocytes were observed with human blood but at 150-fold higher concentrations (Udden, 2002).

Response to ACC Comment 36:

OEHHA generally agrees with this ACC summary of this literature. We have included summaries of these key studies in the REL document as well. This information assisted OEHHA in determining that a REL based on a rodent hemolysis-related endpoint was not appropriate.

ACC Comment 37:

The Agency for Toxic Substances and Disease Registry (ATSDR) has reviewed the toxicology of EGBE (ATSDR, 1998) and has proposed Minimal Risk Levels (MRLs) for EGBE for human exposures based on differing exposure durations (Table 1). An inhalation MRL for intermediate duration exposures in humans (15-364 days) of 3 ppm (14.5 mg/m^3) was derived from a subchronic (90-day) inhalation toxicity study in rats with hematological effects noted as the most sensitive indicator of toxicity (Dodd et al., 1983). A MRL of 0.2 ppm (1.0 mg/m^3) for chronic (≥ 365 days) human exposures was derived from a NOAEL value of 0.6 ppm for decreased corpuscular hemoglobin concentrations in male workers (ATSDR, 1998).

Response to ACC Comment 37:

ATSDR defines MRLs as "…*an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure.* ATSDR (1998) based their intermediate MRL on hemolysis in rats. OEHHA does not believe the hemolysis endpoint observed in rodents is relevant for derivation of the RELs for human exposure. The chronic MRL is based on a human occupational study by Haufroid et al. (1997). OEHHA summarized this study in the EGBE REL document and determined that additional studies are needed to confirm if the slight decrease in hematocrit and the slight increase in mean corpuscular hemoglobin concentration actually represent an early marker for EGBE toxicity. The erythroid changes were still within normal clinical ranges and no significant effect was found for other erythroid parameters examined.

In their report, the ATSDR observed that, "*Two small but statistically significant differences in hematology values were observed: a significant decrease (p=0.03) in hematocrit values (exposed: 43.9% ± 2.1, range: 39.9-50.7, controls: 45.5% ± 2.7, range: 40.6-50.4) and a significant (p=0.02) increase in mean corpuscular hemoglobin concentration (exposed: 33.6 g/dL ± 0.9, range: 31.8-35.6; controls: 32.9 g/dL ±1.1, range: 31.1-35.6). ATSDR considers these differences to be consistent with hemolysis observed in animal studies, and they may be early indicators of potential adverse effects in humans. However, because the changes in both hematocrit and mean corpuscular hemoglobin concentration were in the range of normal clinical values, the*

effect was considered a NOAEL." A total uncertainty factor of 3, for human variability, was used in the MRL derivation. As a general policy, OEHHA avoids basing RELs on free-standing NOAELs, as the ATSDR has done. We also use a UF = 10 to 30 for overall human variability, unless the human study used was in a sensitive subpopulation.

ACC Comment 38:

In a first review of the toxicology of EGBE and to provide summary information for the Integrated Risk Information System (IRIS), the U.S. EPA determined red blood cell hemolysis to be the critical endpoint of concern for setting an RfC value (Table 1) (EPA, 1999). An RfC value of 13 mg/m^3 (2.7 ppm) for chronic lifetime exposure was derived based on an LOAEL of 31 ppm for hematotoxicity in female rats and after applying PBPK and BMC/D methods to derive a human equivalent concentration (HEC). In a second review of EGBE for IRIS, hemosiderin deposition in the liver of male rats, secondary to hemolysis, was chosen as the most relevant and sensitive adverse effect. Again, using combined PBPK and BMC/D methods to derive a HEC, an RfC value of 1.6 mg/m^3 (0.33 ppm) was determined (EPA, 2010).

Response to ACC Comment 38:

Due to the considerable sensitivity differences for red blood cell lysis and hemolysis-related adverse effects between rodents and humans, OEHHA does not believe this endpoint observed is relevant for derivation of the RELs. Other critical endpoints unrelated to hemolysis are observed (nasal epithelial lesions, forestomach lesions) and at roughly the same dose levels as hemolysis.

ACC Comment 39:

A complete and detailed hazard and risk characterization for EGBE was conducted in the European Union (EU, 2006). It was concluded that hemolysis was the most critical key effect in rodents and LOAEL / NOAEL values based on hematotoxicity in rodents were used for risk characterizations. For all repeated-dose risk calculations, hematotoxicity was chosen as the critical effect since no other lesions were identified that could be specifically attributable to treatment with EGBE. A PBPK model was used to calculate equivalent human internal doses as Cmax values for 2-butoxyacetic acid (BAA) similar to the methodology employed by the EPA (EPA, 1999). An inhalation human equivalent concentration (HEC) LOAEL of 98 ppm (474 mg/m^3) was calculated as that Cmax value for BAA in blood as that producing effects in rats. Given significant dermal absorption following exposure to EGBE vapors, combined inhalation and dermal exposures were used to derive final MOS (Margin of Safety) values. In the case of all worker and consumer exposure scenarios identified in the EU risk report; all calculated

MOS values were in excess of 1 leading to the overall conclusion that "There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already" (see Table 1).

Response to ACC Comment 39:

As noted earlier, OEHHA believes humans are relatively insensitive to the hemolysis endpoint compared to rodents, with other acute and chronic adverse effects occurring in humans prior to a mild hemolytic effect occurring. Case studies suggest hemolysis in humans occur largely due to heroic oral doses of EGBE, which result in other, more serious, adverse effects (e.g., metabolic acidosis, coma).

ACC Comment 40:

The 8-hour and chronic REL values proposed by OEHHA are based on an increased incidence, but not severity, of a common rat nasal lesion identified as hyaline degeneration of the olfactory epithelium but also referred to as epithelial hyalinosis, eosinophilic globules, or intracytoplasmic hyaline droplets (Harkema *et al.*, 2006). This effect (in either the rat or mouse) was not accompanied by any significant increases in other non-neoplastic nasal lesions such as cell death, epithelial hyperplasia, metaplasia or atrophy. The use of this effect as the critical endpoint leads to calculated REL values 10x to more than 100x lower than corresponding exposure limits calculated by other means (Table 1).

Response to ACC Comment 40:

The incidence of hyaline degeneration of the olfactory epithelium was increased with a similar dose-response pattern as liver Kupffer cell pigmentation, a hemolysis-related lesion. Differences in OEHHA's EGBE health values compared to health values from other organizations are not so much due to choice of critical endpoint from the chronic study as they are with the derivation from the point of departure. OEHHA employs uncertainty factors to protect sensitive subpopulations (OEHHA, 2008). Evidence for human variability in a response to a chemical insult is often 10-fold or greater.

For example, US EPA bases its RfC, analogous to OEHHA's chronic REL, on hemosiderin deposition in male rats. A BMCL10 of 133 µmol hour/L for hemosiderin staining in liver of male rats chronically exposed to EGBE was used as the point of departure to calculate the RfC. A human PBPK model (Corley et al., 1997) was used to back-calculate to a HEC (human equivalent concentration) of 16 mg/m^3 (3.4 ppm) for the BMCL HEC. US EPA then applied a UF of 10 to account for the uncertainty associated with the variability of the human response (UFH) to the effects of EGBE,

resulting in an RfC of 1.6 mg/m^3. The NOAEL and LOAEL in male rats for this endpoint was 31 and 52.5 ppm (EPA, 2010).

OEHHA based the chronic REL on nasal hyaline degeneration (NOAEL, not observed, LOAEL 31 ppm) and deriving a BMCL05 of 7.6 ppm. OEHHA uses the 5% response rate for chronic RELs because the lower 95% confidence bound on the BMC05 (the 5% response rate) typically appears equivalent for risk assessment purposes to a NOAEL in well-designed and conducted animal studies (OEHHA, 2008, page 43). The HEC is then applied, using the Regional Gas Dose Ratio (RGDR= 0.35) for gases with extrathoracic respiratory effects. The HEC-adjusted value is then 0.475 ppm. Following time adjustment and application of a total UF=30 (3 for interspecies and 10 for intraspecies), the chronic REL is 0.077 mg/m^3 (0.016 ppm). The REL is roughly 20-fold lower than the US EPA RfC.

ACC Comment 41:

Minimal effects noted in the olfactory epithelium of rats do not represent an adverse toxicological endpoint.

For the purpose of deriving 8-hour and chronic REL values for EGBE, OEHHA has chosen nasal hyaline degeneration of the olfactory epithelium in female rats following 2-year chronic, whole-body inhalation exposure to EGBE as the critical adverse effect (OEHHA, 2015). As indicated in Tables 2 and 3, the incidence of this effect does increase with increasing exposure concentration, but does not increase significantly with regard to severity. This effect, although present in all control as well as exposed animals, is not accompanied by any significant increase in more adverse nasal effects such as cell death, epithelial hyperplasia, metaplasia or atrophy (NTP, 2000). In this regard, only three female rats from the highest exposure concentration displayed minimal (level 2) severity ratings for this effect but were free of any other nasal effects such as hyperplasia, metaplasia or atrophy (see Table 3) (NTP, 2000).

Response to ACC Comment 41:

OEHHA agrees with this interpretation of the findings. However, as detailed below in Response to ACC Comment #43, there is considerable published information on eosinophilic globule lesions in tumor and benign tissues, and data from multiple studies showed a universal link between eosinophilic globules from various tissues and increased apoptosis. Perturbations in the frequency of apoptotic events result in disease, suggesting it is a degenerative change. Therefore OEHHA believes nasal hyaline degeneration is indicative of an adverse effect and can be used as the point of departure for 8-hour and chronic REL derivation.

ACC Comment 42:

Hyaline degeneration (hyaline droplet accumulation) of the olfactory epithelium is a commonly observed and non-specific epithelial change in the nasal epithelium of both mice and rats often appearing at increased rates in aging rats and mice (Harkema et al., 2006) and is the most common age-related change in the nasal passages of aging rats (St. Clair and Morgan, 1992). It has been proposed to serve an adaptive or protective role (Buckley et al., 1985)(EU, 2006). According to the National Toxicology Program (NTP, Miller and Cesta, 2015), hyaline droplet accumulation of the nasal epithelium is not considered a degenerative change and any concurrent cellular degeneration should be diagnosed and graded separately. The initial assessment of hyaline droplet accumulation of the olfactory epithelium of rats and mice by the NTP (NTP, 2000)as minimal and having a proposed adaptive/protective role was confirmed in subsequent reviews by the EPA (EPA, 1999; 2010)and by the EU (EU, 2006).

Response to ACC Comment 42:

OEHHA has included the references listed in the ACC comments in the document. However, we do not agree with all of the noted findings. Buckley et al. (1985), similarly cited across several publications in support of the “adaptive” nature of hyaline droplets (also known as hyaline degeneration, hyaline/eosinophilic globules), offered a speculative hypothesis as to the true globule nature, which was neither substantiated by originally reported results nor corroborated sufficiently in following reviews of EGBE literature by the NTP (2000), EU (2006), and US EPA (1999; 2010). Furthermore, the conclusion by NTP (2015) that accumulation of eosinophilic globules (EG) in the nasal epithelium was not a degenerative change does not appear to have considered that 1) data have been published on EG lesions in tumor and benign tissues, 2) new data from multiple studies showed a universal link between EG from various tissues and increased apoptosis, and 3) perturbations in the frequency of apoptotic events result in disease.

Buckley et al. (1985) reported effects of dimethylamine (DMA) in Fischer 344 (F-344) rats and $B6C3F_1$ mice following one year of inhalation exposure at a target concentration of 0, 10, 50, or 175 ppm. Male and female rodents (95/sex/species/treatment group), 6-10 weeks old, were exposed in whole-body chambers for 6 hr/day, 5 days/week for 12 months and sacrificed at 6 or 12 months post exposure. Severe lesions were reported in the respiratory and olfactory epithelia of mice and rats exposed to the highest DMA concentration (175 ppm) for 12 months. In the respiratory epithelium, clearly adverse lesions including but not limited to destruction of the anterior portions of the naso- and maxilloturbinates and fenestration of the nasal septum were noted. Concentration-dependent destruction and variable vacuolization of

1087 the olfactory epithelium was observed with degeneration of olfactory sensory cells and
1088 nerves. Epithelial damage and atrophy of olfactory nerves in the lamina propria were
1089 accompanied by accumulation of EG in markedly hypertropic sustentacular cells of the
1090 olfactory epithelium. EG were also observed in the overlying airway suggesting that they
1091 may be secretory products of the sustentacular cells. Although regeneration of the
1092 sensory cells is possible, sustained atrophy and loss were observed in this study and
1093 related (by the authors) to repeated exposures.
1094
1095 EG in the respiratory and olfactory epithelia were morphologically similar. Despite that
1096 these lesions were collocated with severe adverse intransient lesions, the authors
1097 stated that they may "represent a defensive response by the production of surface
1098 secretions over the olfactory epithelium." However, no other analyses were performed
1099 to determine the mechanisms behind globule formation, or the relationship of the
1100 globules to the destructive effects observed in the nose. Instead, the authors stated,
1101 that the nature of the globules "has yet to be determined." Despite this uncertainty,
1102 subsequent reviews by NTP (2000; 2015), EPA (1999; 2010), and European Union
1103 (2006) commonly propagated the unsubstantiated idea that EG were indicative of an
1104 adaptive response.
1105
1106 NTP (2000) described EGBE exposure-related increases in the incidence of EG in the
1107 olfactory epithelium of male and female rats, and in the olfactory and respiratory
1108 epithelia of female mice, but no statistical analyses were reported on the incidence or
1109 severity of the observed EG in the olfactory and/or respiratory epithelial cells, and no
1110 rubric was provided to explain the subjective, ordinal severity scores (minimal/1, mild/2,
1111 moderate/3, and severe/4). No supplementary work was done by NTP to characterize
1112 the eosinophilic lesions, which in their study, were not reported to be associated with
1113 additional nasal perturbations. U.S. EPA (2010) more comprehensively analyzed the
1114 NTP histopathology data for EGBE, performing the first-reported statistical analysis of
1115 the incidence of EG in the olfactory epithelium of rats and mice. No mention was made
1116 of globules in the respiratory epithelium.
1117
1118 Given that the effect is likely related to a continuum of changes known to represent an
1119 established adverse effect, and that some female rats (n = 3/49) in the NTP EGBE
1120 study (2000), which as a group appear to be more sensitive to EGBE than exposed
1121 males of the genus, exhibited increased EG severity at the 125 ppm concentration
1122 compared to controls, OEHHA stands by its use of epithelial globule formation as the
1123 critical endpoint for REL development.

ACC Comment 43:

Thus, the use of nasal hyaline degeneration of the olfactory epithelium in female rats by OEHHA is inconsistent with other expert hazard assessments performed by the EPA and the European Union and represents an unsupported interpretation of a minimal and presumed non-adverse adaptive response.

Response to ACC Comment 43:

While the use of nasal hyaline degeneration of the olfactory epithelium in female rats by OEHHA differs from other expert hazard assessments (EPA, 1999; NTP, 2000; EU, 2006; EPA, 2010), new information undocumented in reviews by NTP, US EPA, and EU, supports our interpretation that this lesion is indicative of an adverse response to toxicant exposures. As a whole, new research suggests that hyaline degeneration, also known as formation of EG, represents stages of cell injury and death related to condensation of cellular constituents, blebbing, auto- and hetero-phagocytosis, and intracellular accumulation of plasma proteins.

One of the references (Monticello et al. 1990) cited by NTP (2015) states that cells with eosinophilic globules often “exhibit massively dilated cisternae of the rough endoplasmic reticulum.” Similar swelling of the smooth ER in cells of the nasal mucosa was noted by Lewis and colleagues (1994), who observed increased numbers of globules and decreased P-450 enzymes in CDF(F344)/CrlBR rats exposed to cigarette smoke for 32 weeks versus those exposed for 4 weeks. According to Schönthal (2012), lumenal dilation of the ER appears to be a coping mechanism for increased crowding of proteinaceous constituents resulting from accumulation of un- or mis-folded proteins. ER stress can result in either adaptation to and neutralization of stress, or activation of pro-apoptotic pathways and eventual cell death. Papadimitriou et al. (2000) have stated that the role of the ER in apoptosis is related to proteolysis and solubilization of cytoskeletal proteins, and they observed EG often in or around the ER of dying cells. Their review of literature on over 50 benign and malignant conditions containing globules, and research on 80 tumor cases (24 tumor types) containing EG led them to hypothesize that all EG reflect stages of cell injury often related to apoptosis.

Microscopic evaluations revealed that EG 1) occurred almost exclusively in areas of apoptosis and sometimes contained pyknotic nuclear fragments; 2) exhibited the same ultrastructural features irrespective of tumor type or location; 3) occurred in cells exhibiting intense blebbing; and 4) stained positively for plasma proteins and occurred in cells with increased membrane permeability. Intracellular globules were linked to dense networks of fibrin fibrils which crossed through the cells and into the extracellular matrix. Extracellular EG were also shown to be linked to the extracellular matrix by

fibrils suggesting a process of remodeling. Given their findings, Papadimitriou et al. (2000) hypothesized that the globules are not specific to any tumor type but represent a degenerative process leading to apoptosis, which is common to all cell types. The authors also recognized that although the concept of apoptosis doesn't generally allow for outward leakage of intracellular constituents, condensation of the cell with the observed cross-linking of the cytoskeleton maintains internal contents *in situ* preventing the random release of contents that leads to inflammation in necrosis. Influx and accumulation of plasma proteins with anti-protease activity would also inhibit inflammatory responses that can occur with organelle and lysosomal enzyme release. Linking of the intracellular globules to the extracellular matrix allows for their incorporation into the matrix, which accounts for the final disposal of apoptotic cell remnants.

Dikov et al. (2007) studied quantitative and qualitative differences between normal and pathologic gastrointestinal (GI) epithelia from a series of 2,230 biopsies. EG were rarely found in normal tissues (1.1% incidence). In comparison, EG frequency was higher in tissues with non-ischemic inflammation (gastritis, duodenitis, and colitis; $p = 0.007$), circulatory disorders/ischemic injury (acute edema and congestion, pericarcinomatous mucosa, ischemic colitis; $p < 0.0001$), and ulcerous edges ($p < 0.0001$). Their incidence in benign regenerative cell proliferation lesions (e.g. hyperplastic polyps, or focal foveolar hyperplasia), adenomatous polyps, and adenocarcinomas was also higher than in normal tissues ($p < 0.05$).

In contrast to reviews by the NTP (2000; 2015), U.S. EPA (1999; 2010), and the EU (2006), which primarily relied upon supposition by Buckley (1985) as to the adaptive nature of EG, these articles show more convincingly that the lesions are representative of adverse/degenerative processes.

ACC Comment 44:

As a conservative approach, OEHHA should adapt the approach used by the EPA in the most recently derived IRIS RfC value for EGBE (EPA, 2010). The IRIS RfC value of 1.6 mg/m^3 (0.34 ppm) is recommended to be used as the chronic REL value. In this case, the critical effect was identified as increased hemosiderin staining in the liver of male F344 rats. A BMCL10 value of 133 µmol-hour/L (AUC) for hemosiderin staining in liver of male rats chronically exposed to EGBE was calculated. Assuming a 24 hour/day exposure, the PBPK model of Corley *et al.* (1997) was used to calculate the HEC value of 16 mg/m^3 with the final RfC value derived from applying an uncertainty factor of 10. As a conservative approach, the 8-hour REL for EGBE is recommended to be assigned the same value as the chronic REL.

Response to ACC Comment 44:

Increased deposition of hemosiderin, a pigment product of red blood cell lysis (hemolysis), was considered previously and rejected by the Scientific Review Panel (SRP) as a critical endpoint for REL development. This is primarily because toxicant-induced hemolysis is not a sensitive endpoints in humans and is generally preceded in humans by much more sensitive and severe endpoints when orally absorbed at high concentrations (e.g. metabolic acidosis) as discussed in Section 4.1 of U.S. EPA's most recent EGBE toxicological review (2010).

Research by Udden (2000; 2002) demonstrated that human red blood cells (erythrocytes) are more resistant to EGBE-induced hemolysis than rats. Briefly, in 2000, he reported that rat erythrocytes exposed *in vitro* for 30 minutes to the principal metabolite of EGBE, butoxyacetic acid (BAA; at 1.0 or 2.0 mM), exhibited the same morphological features as those exposed *in vivo* (at 125 or 250 mg/kg/rat via gavage) to EGBE and examined up to four hours later. Rather than having a normal bi-concave disk shape, exposed cells were sphere-shaped (spherocytic), cup- or coffee bean-shaped (stomatocytic), or thorny (echinocytic), indicating a developing hemolytic state. Human erythrocytes exposed to up to 2.0 mM BAA exhibited none of these morphological anomalies. Additional work, summarized in 2002, which compared sub-hemolytic effects of 10 mM and 0.1 mM BAA in human and rat erythrocytes, respectively, showed that even at a 100x greater concentration not likely to occur with normal use of EGBE-containing products, human erythrocytes were much less affected by exposure than rat cells. These findings are also discussed by US EPA (2010).

According to an essay by Cunningham (2002), research by Udden (2000) indicates that EGBE-induced hemolysis in humans is unlikely to cause the hepatic iron accumulation and resulting effects (e.g. oxidative stress and Kupffer cell activation) observed in rodents. This is due in part to a higher basal level of Vitamin E (tocopherol) in humans, which acts against the oxidant effects of iron. When quantified using high performance liquid chromatography (HPLC), hepatic tocopherol levels in mice and rats were approximately 2 and 4.5 nmol/g liver, respectively (Siesky et al., 2002). In humans, levels were approximately 100x greater (450 nmol/g liver; (Rocchi et al., 1997)). Overall, these studies suggest that hemosiderin accumulation in the liver, from EGBE-induced hemolysis, is not likely to be a biologically significant effect in humans at normal exposure concentrations. Indeed, several case reports (Dean and Krenzelok, 1992; Osterhoudt, 2002; Gualtieri et al., 2003) of accidental or intentional acute EGBE ingestion summarized by US EPA (2010) showed no evidence of hemolysis despite high exposure doses.

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