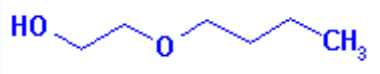
Air Toxics Hot Spots Program

Draft Noncancer Reference Exposure Levels (RELs) for Ethylene Glycol mono-n-Butyl Ether (EGBE)

Office of Environmental Health Hazard Assessment

SRP Meeting
March 4, 2016

Ethylene Glycol mono-n-Butyl Ether (EGBE)



- **Ethylene Glycol mono-n-Butyl Ether (EGBE) (CAS 111-76-2) is a solvent used in many applications (e.g. consumer products and building materials).**
- **EGBE is a high production volume chemical.**
- **Low volatility: 0.88 mm Hg @ 25°C.**
- **Induces eye, skin, respiratory system irritation and inflammation, and olfactory epithelium degeneration.**

EGBE Production and Usage

- **180,000 tons produced in the U.S. in 1992 (NTP, 2000).**
- **150,000 tons produced in the European Union (SCHER, 2008; OECD, 2012).**
- **World production is as high as 500,000 tons (Rebsdat and Mayer, 2001).**
- **Major uses: 75% for paints and coatings (Rebsdat and Mayer, 2001), 18% for metal cleaners and household cleaners (NLM, 2014).**

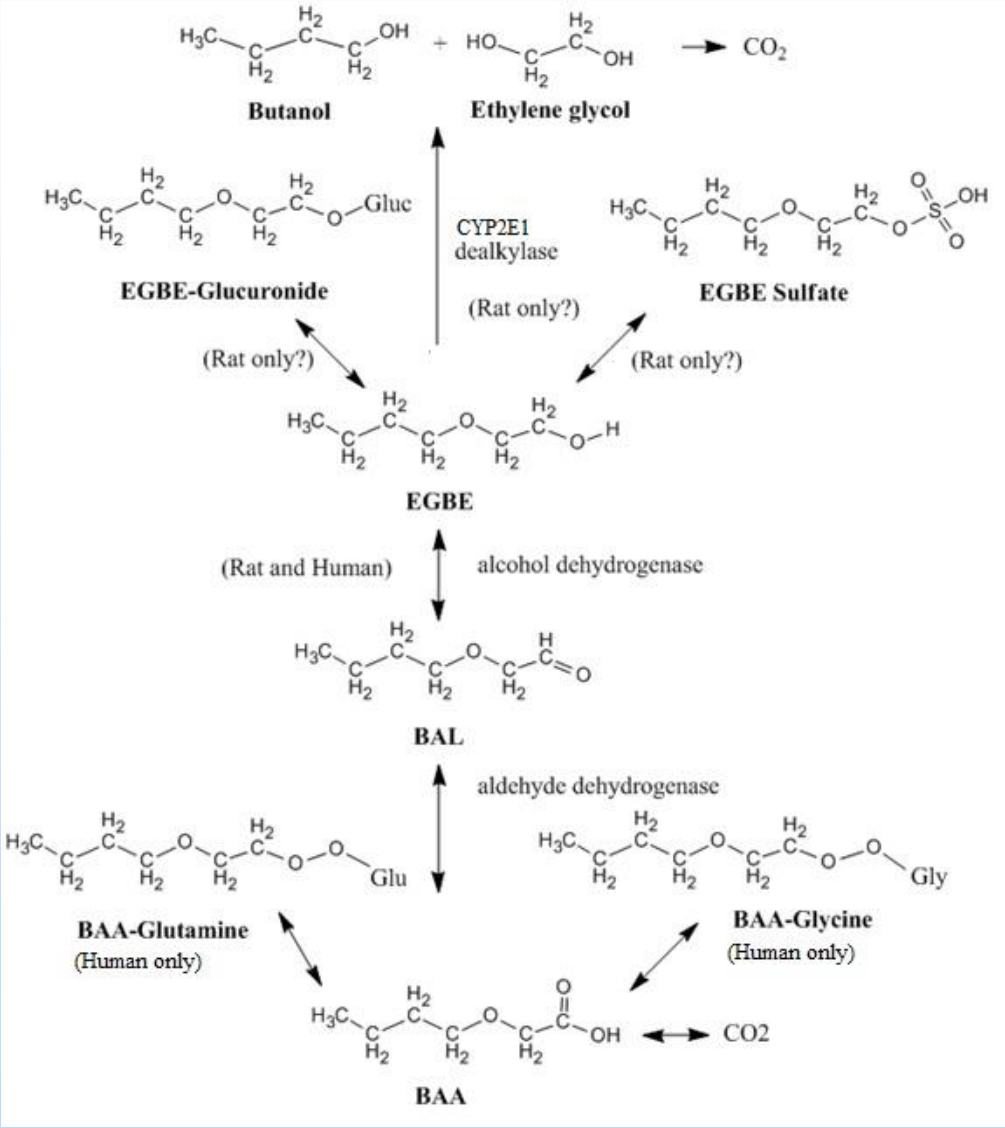
EGBE Toxicokinetics

- **Absorption: EGBE is absorbed rapidly in humans and animals following inhalation, ingestion, or dermal exposure.**
- **Distribution: EGBE is rapidly distributed to tissues in humans and rodents.**
- **Metabolism: through alcohol and aldehyde dehydrogenases. Three metabolic pathways in rats: (1) oxidized to 2-butoxyacetic acid (BAA), (2) conjugated with UDP-glucuronide acid, and (3) conjugated with the sulfate.**

EGBE Toxicokinetics

- **Elimination: Major elimination is through urine in its metabolized form of 2-butoxyacetic acid (BAA).**
- **Half-life: In human inhalation chamber studies, the EGBE elimination half-life is 40 minutes in blood and the elimination half-life of BAA in urine is approximately 6 hours.**
- **In occupational exposures, peak excretion of BAA in urine is 6 - 12 hours after exposure.**

EGBE Metabolism



EGBE Acute Reference Exposure Level (REL)

- **Based on three inhalation studies of human volunteers, LOAEL = 98 ppm (474 mg/m^3) (Carpenter et al., 1956).**
- **Study population: 2 to 4 human subjects per study from 3 studies.**
- **Exposure method: whole body exposure, 98, 113 and 195 ppm.**
- **Exposure duration: 8 hours (98 and 195 ppm in chamber) or 4 hours (113 ppm in room).**
- **Critical effect is ocular and nasal irritation (sensory irritation).**

EGBE Acute REL Derivation

- **Point of Departure: LOAEL, 474 mg/m^3 (98 ppm)**
- **No time adjustment**
- **LOAEL uncertainty factor (UF_L) = 10 (default)**
- **Interspecies uncertainty factor (UF_A) = 1**
- **Intraspecies toxicokinetic UF ($UF_{H\text{-}k}$) = 1 (site of action; no systemic effects)**
- **Intraspecies toxicodynamic UF ($UF_{H\text{-}d}$) = 10 (small sample size)**
- **Cumulative UF = 100**
- **Acute REL = 4.7 mg/m^3 (1 ppm)**

EGBE Chronic Toxicity

- **NTP (2000) performed a two-species, 2-year EGBE inhalation study.**
- **Animals were exposed to EGBE 6 hours/day, 5 days/week at concentrations of 0, 31, 62.5, and 125 ppm (0, 150, 302, and 604 mg/m^3) for groups of 50 Fisher 344 rats and 0, 62.5, 125, and 250 ppm (0, 302, 604, and 1,208 mg/m^3) for groups of 50 B6C3F$_1$ mice.**
- **The highest exposure was selected to produce a 10–15% depression in hematologic indices.**

EGBE Chronic Toxicity

- **Nonneoplastic effects in rats included hyaline degeneration of the olfactory epithelium and Kupffer cell pigmentation in livers.**
- **Nonneoplastic effects in mice included forestomach ulcers and epithelial hyperplasia, hematopoietic cell proliferation and hemosiderin pigmentation in the spleen, hepatic Kupffer cell pigmentation, and bone marrow hyperplasia (males only).**

EGBE Chronic REL Point of Departure choice: toxicity considerations

- **Compared to rodent studies, humans are resistant to EGBE-induced hemolysis.**
- **Hepatic Kupffer cell pigmentation is a secondary effect from EGBE's hemolytic effect and was not used as an endpoint to generate a Point of Departure (POD).**
- **Rat nasal olfactory epithelial hyaline degeneration was the most sensitive toxicity endpoint in the NTP (2000) study, and was therefore selected as the basis for a POD.**

EGBE-induced Chronic Toxicity Incidence (from NTP, 2000)

Endpoints	Exposure Doses (ppm) 0	31.2	62.5	125	250
Nasal Olfactory Epithelial Hyaline Degeneration					
Male Rats	13/48	21/49	23/49*	40/50***	--------
Female Rats	13/50	18/48	28/50**	40/49***	--------
Total Rats	*26/98*	*39/97**	*51/99****	*80/99****	--------
Male Mice	1/50	--------	2/50	3/48	1/48
Female Mice	6/50	--------	14/50*	11/49	12/50*
Total Mice	*7/100*	--------	*16/100**	*14/97*	*13/98*
Liver Kupffer Cell Pigmentation					
Male Rats	23/50	30/50	34/50*	42/50***	--------
Female Rats	15/50	19/50	36/50***	47/50***	--------
Total Rats	*38/100*	*49/100*	*70/100****	*89/100****	--------
Male Mice	0/50	--------	0/50	8/49**	30/49***
Female Mice	0/50	--------	5/50*	25/49***	44/50***
Total Mice	*0/100*	--------	*5/100**	*33/98****	*74/99****
Forestomach Epithelial Hyperplasia					
Male Mice	1/50	--------	7/50*	16/49***	21/48***
Female Mice	6/50	--------	27/50***	42/49***	44/50***
Total Mice	*7/100*	--------	*34/100****	*58/98****	*65/98****
Forestomach Ulcer					
Male Mice	1/50	--------	2/50	9/49**	3/48
Female Mice	1/50	--------	7/50*	13/49***	22/50***
Total Mice	*2/100*	--------	*9/100**	*22/98****	*25/98****

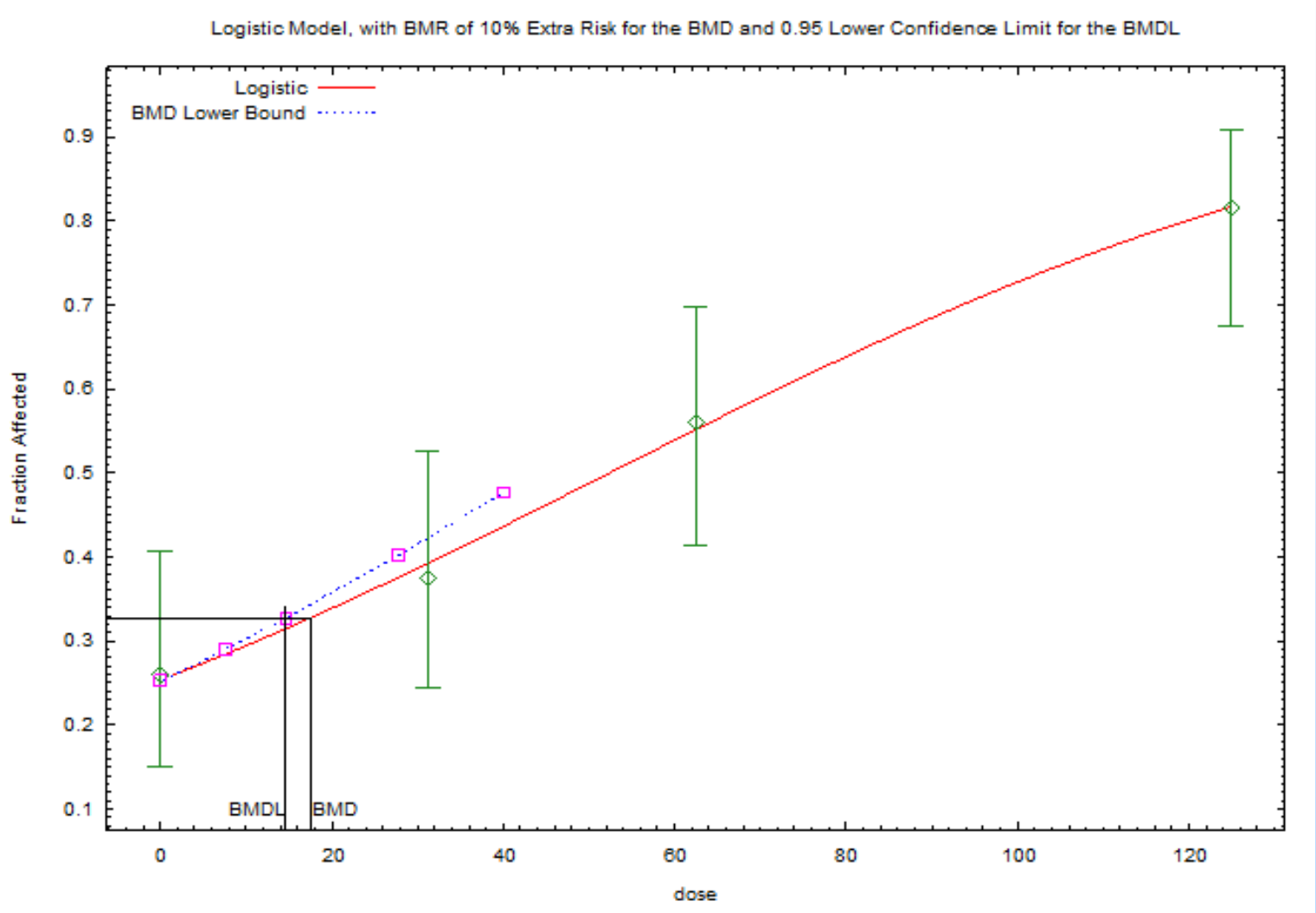
Note: Statistically significant differences compared to the control group were measured with the Chi-square (X^2) or Fisher exact test,
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (statistical analysis performed by OEHHA).

EGBE Benchmark Dose Analysis of NTP (2000) Chronic Toxicity Data

Endpoints	$BMDL_{05}$	NOAEL	LOAEL
Nasal Olfactory Epithelial Hyaline Degeneration			
Male rats	**8.0 (Probit)**	**31.2**	**62.5**
Female rats	**7.6 (Logistic)**	**31.2**	**62.5**
Male and female rats combined	**8.2 (Probit)**	**---------**	**31.2**
Male mice	**NA**	**NE**	**62.5**
Female mice	**34.3 (LogLogistic)**	**NE**	**62.5**
Male and female rats combined	**74.2 (LogLogistic)**	**---------**	***62.5***
Liver Kupffer Cell Pigmentation			
Male rats	**5.7 (Logistic)**	**31.2**	**62.5**
Female rats	**11.6 (LogLogistic)**	**31.2**	**62.5**
Male and female rats combined	**5.5 (Logistic)**	***31.2***	***62.5***
Male mice	**97.9 (Dichotomous-Hill)**	**62.5**	**125**
Female Mice	**37.5 (LogProbit)**	**NE**	**62.5**
Male and female mice combined	**49.9 (LogProbit)**	**---------**	***62.5***
Forestomach Epithelial Hyperplasia			
Male Mice	**16.2 (Weibull)**	**NE**	**62.5**
Female Mice	**9.7 (LogProbit)**	**NE**	**62.5**
Male and female mice combined	**11.4 (Dichotomous-Hill)**	**---------**	***62.5***
Forestomach Ulcer			
Male mice	**64.5 (Dichotomous-Hill)**	**62.5**	**125**
Female Mice	**17.5 (Quantal-linear)**	**NE**	**62.5**
Male and female rats combined	**26.3 (LogLogistic)**	**---------**	***62.5***

NE: Not established; NA: Not applicable (a poor dose-response curve for BMC determination).

EGBE female rat nasal olfactory epithelial hyaline degeneration incidence: Logistic model fit



EGBE Eight-hour REL Derivation

- **Critical Effect: Nasal hyaline degeneration of female F344 rat olfactory epithelium**
- **Point of Departure: $BMDL_{05}$ = 7.6 ppm**
- **Exposure continuity: 6 hours/day, 5 days/week**
- **Exposure duration: 2 years (lifetime)**
- **Time-adjusted exposure: 2.7 ppm**

  (7.6 ppm x 6/24 x 5/7 x 20/10)
- **Human Equivalent Concentration (HEC) = Time-adjusted Exposure × Regional Gas Dose Ratio (RGDR) = 0.95 ppm, (RGDR = 0.35)**

EGBE Eight-hour REL Derivation

- **Interspecies toxicokinetic uncertainty factor ($UF_{A\text{-}k}$) = 1**
- **Interspecies toxicodynamic uncertainty factor ($UF_{A\text{-}d}$) = $\sqrt{10}$**
- **Intraspecies toxicokinetic UF ($UF_{H\text{-}k}$) = $\sqrt{10}$**
- **Intraspecies toxicodynamic UF ($UF_{H\text{-}d}$) = $\sqrt{10}$**
- **Cumulative UF = 30**
- **Eight-hour REL = 0.15 mg/m^3 (0.032 ppm)**

EGBE Chronic REL Derivation

- **Critical Effect: Nasal hyaline degeneration of female F344 rat olfactory epithelium**
- **Point of Departure: $BMDL_{05}$ = 7.6 ppm**
- **Exposure continuity: 6 hours/day, 5 days/week**
- **Exposure duration: 2 years (lifetime)**
- **Time-adjusted exposure: 1.357 ppm**

  (7.6 ppm x 6/24 x 5/7)
- **Human Equivalent Concentration (HEC) = 0.475 ppm (gas with extrathoracic respiratory effects, RGDR = 0.35)**

EGBE Chronic REL Derivation

- **Interspecies toxicokinetic uncertainty factor ($UF_{A\text{-}k}$) = 1**
- **Interspecies toxicodynamic uncertainty factor ($UF_{A\text{-}d}$) = $\sqrt{10}$**
- **Intraspecies toxicokinetic UF ($UF_{H\text{-}k}$) = $\sqrt{10}$**
- **Intraspecies toxicodynamic UF ($UF_{H\text{-}d}$) = $\sqrt{10}$**
- **Cumulative UF = 30**
- **Chronic REL = 0.077 mg/m^3 (0.016 ppm)**

EGBE REL Summary

- **Proposed EGBE RELs**

Acute: 4.7 mg/m^3 (1 ppm)

8-Hour: 0.15 mg/m^3 (0.032 ppm)

Chronic: 0.077 mg/m^3 (0.016 ppm)

Public Comments

EGBE Comments and Responses

We received comments on EGBE from

- **Jonathon Busch on behalf of the Glycol Ethers Panel of the American Chemistry Council**

EGBE Comments and Responses
Acute REL

- **Comment: OEHHA Acute REL based on subjective measures of sensory irritation (Carpenter et al., 1956); no attempt made to discriminate between subjective effects due to offensive odor and true sensory irritation due to trigeminal nerve stimulation**

Response:

- **Carpenter et al. specifically set out to describe the subjective sensations felt by the exposed subjects. Subjects independently reported immediate sensory irritation with exposure. Some reported headache and nausea following exposure.**
- **Odor intensity was not well characterized in the study. However, OEHHA believes the level of discomfort experienced by subjects was clearly a LOAEL regardless of trigeminal nerve effects or odor intensity.**

EGBE Comments and Responses
Acute REL

- **Comment: Physiological monitoring of test subjects, which are more objective measures of exposure, was not conducted by Carpenter et al. (1956). More recent pharmacokinetic studies performed physiological tests and should be the basis of the Acute REL**

Response:

- **Carpenter et al. did measure some objective indices such as blood pressure and heart rate, that were apparently unremarkable with exposure to EGBE**
- **OEHHA did not use the pharmacokinetic studies (mainly Jones et al., 2003 and Johanson et al., 1986) as the basis of the REL for several reasons:**
  - **Carpenter et al. specifically set out to describe the subjective sensations felt by the exposed subjects; the pharmacokinetic studies did not**

EGBE Comments and Responses
Acute REL

Response Continued:

- **The pharmacokinetic studies found no effect of EGBE on physiological parameters (breathing rate, pulse rate, blood pressure, skin surface temp, skin resistance) – establishing only a "free-standing NOAEL"**
- **OEHHA does not use free-standing NOAELs as the basis of RELs if a more relevant study (with a LOAEL) is available**
- **Sensory irritation is likely a more sensitive indicator of effects than physiological parameters used; no sensory irritation tests were performed in the pharmacokinetic studies**
- **Only one concentration of EGBE examined (20 or 50 ppm) by the pharmacokinetic studies**

EGBE Comments and Responses
Acute REL

- **Comment: The acute REL for EGBE should be 5 ppm, based on the 50 ppm no observed effect concentration from Jones et al. (2003) and a cumulative intraspecies uncertainty factor value of 10 (10 for UFH-d and 1 for UFH-k) instead of 30.**

Response:

- **OEHHA believes the LOAEL of 98 ppm (Carpenter et al., 1956) is the most appropriate point of departure for the REL**
- **However, OEHHA concurs that the total intraspecies UF be reduced from 30 to 10.**
  - **Toxicodynamic UF stays at 10 for potential exacerbation of asthma in sensitive subpopulations**
  - **Toxicokinetic UF changed from 3 to 1: Use UFH-k of 1 for direct-acting sensory irritants (per Guidelines)**

EGBE Comments and Responses
Acute REL

- **Comment: The chamber atmosphere in Carpenter et al. study was not characterized in terms of aerosol formation and particle size**

Response:

- **Carpenter et al. (1956) was aware of the EGBE saturation vapor pressure (1000 – 1200 ppm). The exposure concentrations of 98, 113, and 195 ppm for the sensory irritation study are well below the saturation VP and likely predominantly in the vapor state when heated to vaporization for the exposures**

EGBE Comments and Responses
Acute REL

- **Comment: Kane et al. (1980) RD50 study in mice is 2825 ppm. As proposed by Alarie, 0.1 × RD50 (283 ppm) would cause definitive but tolerable sensory irritation in humans**

Response:

- **OEHHA added summary of the RD50 study**
- **Kane et al. could not reach the RD50, so it was extrapolated to 2825 ppm**
- **EGBE may not be an ideal solvent to extrapolate to a safe level using a factor of 0.1: 195 ppm in Carpenter study was considered too high for comfort by exposed subjects**

EGBE Comments and Responses
Acute REL

- **Comment: 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.**

Response:

- **No specific studies have shown EGBE alone to cause an asthmatic episode; however, irritants can trigger asthma exacerbation.**
- **OEHHA views asthma as a more serious disease in children; epidemiological studies suggest cleaning products, including EGBE, increase the likelihood of an asthma episode**

EGBE Comments and Responses
Acute REL

- **Comment: The commenter challenged several references used by OEHHA in support of EGBE contributing to potential asthmatic reactions in people with asthma.**

Response:

- **OEHHA removed some references that were less relevant (Burns, 2010; Bonisch et al., 2012; Burge, 2010; Burge et al., 2012) and replaced them with other references (Siracusa et al., 2013; Folletti et al., 2014; Zock et al., 2007) more supportive of the statement that EGBE is a possible contributor in cleaning agents to exacerbation of asthma.**

EGBE Comments and Responses
Acute REL

Response continued:

- **In response to the comment that OEHHA did not make a strong case for EGBE possibly contributing to asthmatic reactions in people with asthma, OEHHA revised the paragraph to include:**

"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)".

EGBE Comments and Responses
Acute REL

- **Comment: The European Union (EU, 2008) does not classify EGBE as a respiratory irritant**

Response:

- **OEHHA has been unable to find a similar statement made by the EU.**
- **The EU sets forth in their 2008 document an 8-hour TWA occupational standard of 12 mg/m3 (2.5 ppm) for eye and respiratory irritation.**

EGBE Comments and Responses
Acute REL

- **Comment: Appearance of cherry angiomas following acute high exposure to EGBE in Raymond (1998) report actually occur naturally with age**

Response:

- **OEHHA added "Cherry angiomas can appear spontaneously usually after age 50, but have been observed in workers following exposure to other irritating gases".**

EGBE Comments and Responses 8-Hour and Chronic RELs

- **Comment: Hematoxicity is recognized and accepted as the critical adverse effect following EGBE exposure and is used by U.S. EPA as the critical endpoint in their reference concentration (RfC) derivation. OEHHA should use this as the critical endpoint for 8-hr and chronic RELS.**

Response:

- **Hemolysis endpoints for REL derivation were previously considered and rejected by the SRP**
- **OEHHA presents considerable evidence that shows humans are substantially less sensitive to the hemolytic effects of EGBE compared to rodents**
- **OEHHA considers hyaline degeneration in nasal epithelium to be an adverse effect that is relevant to human exposure.**

EGBE Comments and Responses 8-Hour and Chronic RELs

- **Comment: The Agency for Toxic Substances and Disease Registry proposed a Minimal Risk Level of 0.2 ppm (1.0 mg/m^3) for chronic (≥ 365 days) human exposure, derived from a NOAEL value of 0.6 ppm for decreased corpuscular hemoglobin concentrations in male workers (Haufroid et al., 1997).**

Response:

- **Changes in hematology values (hematocrit and MCHC) of the occupational study were significant (p = 0.02 or 0.03), but within the range of normal values**
- **No significant changes were found in other erythroid parameters**
- **ATSDR called Haufroid value a NOAEL (essentially a free-standing NOAEL**
- **OEHHA notes that additional occupational studies are needed to confirm this effect**

EGBE Comments and Responses 8-Hour and Chronic RELs

- **Comment: The European Union (2006) concluded hemolysis was the critical effect for EGBE exposure in rodents. No other lesions were identified attributable to EGBE.**

Response:

- ***In vivo* and *in vitro* studies show humans to be relatively insensitive to the hemolytic effects of EGBE compared to rodents. Case studies in which humans ingested large quantities of EGBE products did produce mild to moderate hemolysis, but also lead to other more serious effects, including metabolic acidosis and coma.**
- **OEHHA considers hyaline degeneration in nasal olfactory epithelium to be a relevant critical effect in chronic rodent exposure studies**

EGBE Comments and Responses
8-Hour and Chronic RELs

- **Comment: Nasal hyaline degeneration (i.e., formation of eosinophilic globules) showed minimal changes in severity, did not increase in severity with dose, is commonly present in aging rodents, and has been proposed as adaptive or protective changes (i.e., not a true adverse effect).**

Response

- **OEHHA agrees that there were minimal changes in severity of this lesion with increasing dose, and the lesions are found in aging rats. However, the incidence of the lesion clearly increased with increasing dose.**
- **Presence of eosinophilic globules with hyaline degeneration has been shown to be linked with increased apoptosis in several tissues.**

EGBE Comments and Responses 8-Hour and Chronic RELs

- **Comment: The National Toxicology Program does not consider hyaline droplet accumulation of the nasal epithelium to be a degenerative change; the lesion is proposed to have an adaptive/protective role.**

➢ **Response: The conclusion by NTP (2015) does not appear to have considered that:**

1) data have been published on these lesions in tumor and benign tissues

2) new data from multiple studies showed a universal link between eosinophilic globules (EG) from various tissues and increased apoptosis

3) perturbations in the frequency of apoptotic events result in disease

EGBE Comments and Responses
8-Hour and Chronic RELs

Response Continued:

- **The NTP conclusion relies on previous data by Buckley et al. (1985) that hyaline degeneration is an adaptive response, yet the authors note that the true nature of the lesion "has yet to be determined"**
- **New research suggests that hyaline degeneration represents stages of cell injury and death related to condensation of cellular constituents, blebbing, auto- and hetero-phagocytosis, and intracellular accumulation of plasma proteins.**

EGBE Comments and Responses
8-Hour and Chronic RELs

Response Continued:

- **Monticello et al. (1990) noted "eosinophilic globules often exhibit massively dilated cisternae of the rough ER".**
- **Schönthal (2012) says lumenal dilation of the endoplasmic reticulum (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) stated in their study of 24 tumor types that the role of the ER in apoptosis is related to proteolysis and solubilization of cytoskeletal proteins, and they observed eosinophilic globules often in or around the ER of dying cells.**

EGBE Comments and Responses 8-Hour and Chronic RELs

Response Continued:

- **Papadimitriou et al. (2000) observed that:**
  - **Eosinophilic globules occurred almost exclusively in areas of apoptosis and sometimes contained pyknotic nuclear fragments**
  - **Exhibited the same ultrastructural features irrespective of tumor type or location**
  - **Occurred in cells exhibiting intense blebbing**
  - **Stained positively for plasma proteins and occurred in cells with increased membrane permeability**

EGBE Comments and Responses 8-Hour and Chronic RELs

Response Continued:

- **Dikov et al. (2007) looked at quantitative and qualitative differences between normal and pathologic gastrointestinal (GI) epithelium from a series of 2,230 biopsies.**
  - **They found eosinophilic globules were very rare (1.1%) in normal tissues, occurring almost exclusively in areas of apoptosis and sometimes containing pyknotic nuclear fragments**

In conclusion, OEHHA believes these data show more convincingly that the lesion is representative of adverse/degenerative processes, and that the 8-hour and chronic RELs can use the lesion as a critical adverse effect.