OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

# Air Toxics Hot Spots Program

## Ethylene Glycol mono-n-Butyl Ether

## Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Scientific Review Panel Review Draft
November 2016

Air, Community, and Environmental Research Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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# Reference Exposure Levels

## Technical Support Document for the Derivation of Noncancer Reference Exposure Levels Appendix D1

**Prepared by the**

**Office of Environmental Health Hazard Assessment**

Lauren Zeise, Ph.D., Acting Director

**Authors**

Jianming Yang, Ph.D.

Daryn E. Dodge, Ph.D.

Rona M. Silva, Ph.D.

**Technical Reviewers**

John D. Budroe, Ph.D.

David M. Siegel, Ph.D.

Melanie A. Marty, Ph.D.

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**November 2016**

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# Ethylene Glycol mono-n-Butyl Ether

*(2-butoxyethanol; butoxyethanol; butyl cellosolve; ethylene glycol mono-n-butyl ether; butyl glycol)*

**CAS No. 111-76-2**


## 1. Summary

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 methodology for deriving acute, 8-hour and chronic Reference Exposure Levels (RELs) (OEHHA, 2008). RELs are airborne concentrations of a chemical that are not anticipated to result in adverse noncancer health effects for specified exposure durations in the general population, including sensitive subpopulations. 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.*). The methods described in the TSD were used to develop the RELs for ethylene glycol mono-n-butyl ether (EGBE) presented in this document; this document will be added to Appendix D of the TSD.

Ethylene glycol mono-n-butyl ether (EGBE), commonly called 2-butoxyethanol (2-BE), has gained widespread use in industrial and consumer applications due to its properties as a solvent. It is well-known for its hemolytic properties in rodents (*i.e.*, red blood cell (RBC) damage resulting in regenerative anemia) and the secondary effects from hemolysis including splenic congestion and liver Kupffer cell pigmentation. However, airborne exposures in humans are more often associated with eye, nose, and upper respiratory tract irritation. The critical effects of EGBE in humans resulting from short- to long-term airborne exposures are eye irritation, respiratory irritation and epithelial degeneration of upper respiratory airways. High oral doses in adult humans may result in metabolic acidosis and neurologic effects, but generally cause only minor to moderate hemolytic effects. Literature summarized and referenced in this document covers the relevant published literature for EGBE through Spring 2016.

### 1.1 EGBE Acute REL

| | |
|---|---|
| *Reference exposure level* | 4700 µg/m$^3$ (1000 parts per billion (ppb)) |
| *Critical effect(s)* | Ocular and nasal irritation (sensory irritation) |
| *Hazard index target(s)* | Eyes and respiratory system |

### 1.2 EGBE 8-Hour REL

| | |
|---|---|
| *Reference exposure level* | 164 µg/m$^3$ (34 ppb) |
| *Critical effect(s)* | Nasal hyaline degeneration of olfactory epithelium |
| *Hazard index target(s)* | Respiratory system |

### 1.3 EGBE Chronic REL

| | |
|---|---|
| *Reference exposure level* | 82 µg/m$^3$ (17 ppb) |
| *Critical effect(s)* | Nasal hyaline degeneration of olfactory epithelium |
| *Hazard index target(s)* | Respiratory system |

## List of Acronyms

| | | | |
|---|---|---|---|
| ADH | Alcohol Dehydrogenase | MCH | Mean Corpuscular Hemoglobin |
| AIC | Akaike Information Criterion | ME | 2-methoxyethanol |
| AIDS | Acquired Immune Deficiency Syndrome | MV | Minute Volume |
| | | $MV_A$ | Minute Volume for Animal |
| ALDH | Aldehyde Dehydrogenase | $MV_H$ | Minute Volume for Human |
| ARB | Air Resources Board | NOAEL | No Observed Adverse Effect Level |
| BAA | 2-butoxyacetic Acid | | |
| BAL | Butoxyacetaldehyde | NTP | National Toxicology Program |
| BCH | Basal Cell Hyperplasia | NIOSH | National Institute for Occupational Safety and Health |
| 2-BE | 2-butoxyethanol | | |
| BEG | Glucuronide conjugate of EGBE | OECD | Organisation for Economic Co-operation and Development |
| BES | Sulfate conjugate of EGBE | | |
| $BMCL_{05}$ | the 95% lower confidence interval at the 5% response rate | OEHHA | Office of Environmental Health Hazard Assessment |
| BMD | Benchmark Dose | PBPK | Physiologically Based Pharmacokinetic |
| $BMDL_{05}$ | BMD 95% lower confidence limit | PM | Particulate Matter |
| BMDS | Benchmark Dose Modelling Software | POD | Point of Departure |
| | | ppb | Parts per billion |
| BPH | Benign Prostatic Hyperplasia | ppm | Parts per million |
| BW | Bodyweight | RBC | Red Blood Cell |
| CE | Carboxylesterase | RD50 | Dose resulting in a 50% depression of respiratory rate |
| CI | Confidence Interval | | |
| CNS | Central Nervous System | REL | Reference Exposure Level |
| CTI | California Toxics Inventory | RGDR | Regional Gas Dose Ratio |
| CV | Coefficient of variation | RH | Relative Humidity |
| EE | 2-ethoxyethanol | SA | Surface Area |
| EG | Eosinophilic Globules | $SA_A$ | Surface Area for Animal |
| EGBE | Ethylene Glycol mono-n-Butyl Ether | $SA_H$ | Surface Area for Human |
| ER | Endoplasmic Reticulum | TOG | Total Organic Gas |
| EU | European Union | TSD | Technical Support Document |
| FLEC | Field and Laboratory Emission Cell | TWA | Time-weighted Average |
| | | UF | Uncertainty factor |
| GC-MS | Gas chromatography and mass spectrometry | $UF_{A-d}$ | Toxicodynamic portion of the interspecies uncertainty factor |
| GD | Gestational Day | | |
| GSD | Geometric Standard Deviation | $UF_{A-k}$ | Toxicokinetic portion of the interspecies uncertainty factor |
| HEC | Human Equivalent Concentration | | |
| Hct | Hematocrit | $UF_{H-d}$ | Toxicodynamic portion of the intraspecies uncertainty factor |
| Hgb | Hemoglobin | | |
| Ig | Immunoglobulin | $UF_{H-k}$ | Toxicokinetic portion of the intraspecies uncertainty factor |
| IP | Intraperitoneal | | |
| IV | Intravenous | $UF_L$ | LOAEL uncertainty factor |
| LC50 | Lethal concentration required to kill 50% of the population | VOC | Volatile Organic Compound |
| | | US EPA | United States Environmental Protection Agency |
| LOAEL | Lowest Observed Adverse Effect Level | | |

## 2. Physical & Chemical Properties (HSDB, 2005)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $C_4H_9$-O-$CH_2CH_2$-OH ($C_6H_{14}O_2$) |
| *Molecular weight* | 118.2 g/mol |
| *Density* | 0.90 g/cm$^3$ @ 20 °C |
| *Boiling point* | 171 °C |
| *Melting point* | -70 °C |
| *Vapor pressure* | 0.88 mm Hg @ 25°C |
| *Saturated Vapor Pressure* | 5600 mg/m$^3$ (1,160 ppm) at room temp (Corley, 1996) |
| *Odor threshold in air* | 0.48 mg/m$^3$ (0.10 ppm,; geometric mean) (AIHA, 1989) Sweet, ester-like, musty |
| *Water Solubility* | Miscible, but soluble in most organic solvents |
| *Log $K_{ow}$* | 0.81 |
| *Henry's law constant* | 2.08 × 10$^{-7}$ – 10$^{-8}$ atm-m$^3$/mole @ 25°C |
| *Flash point* | 62°C (closed cup); 70°C (open cup) |
| *Conversion factor* | 1 mg/m$^3$ = 0.207 ppm; 1 ppm = 4.83 mg/m$^3$ (at 298.26 K and 1 atm)) |

## 3. Production, Major Uses, and Occurrence

### 3.1 Production and Use

Ethylene glycol mono-butyl ether (EGBE) is a solvent with the characteristics of both alcohol and ether. As such, it is used for many applications, including as a coupling agent to stabilize immiscible ingredients. Consequently, EGBE is a high production volume chemical with estimated production at 295,000 tons in the United States in 2013 (Chinn *et al.*, 2014) (Figure 1), 161,000 tons in the European Union (EU) in 2003 (SCCP, 2007; SCHER, 2008; OECD, 2012), and up to 500,000 tons per year worldwide in 2000. In the US, specifically, production of EGBE from 2013 to 2018 is expected to increase at an average annual rate of 0.7% (Chinn *et al.*, 2014). For worldwide EGBE production estimates, 60 - 75% is for paints and coatings (Rebsdat and Mayer, 2001; SCCP, 2007) and 18% is for metal cleaners and household cleaners (NLM, 2014). Of this 18%, approximately 11% is used in detergents and cleaners, and about 0.5% is used in cosmetics and personal care products (SCCP, 2007).

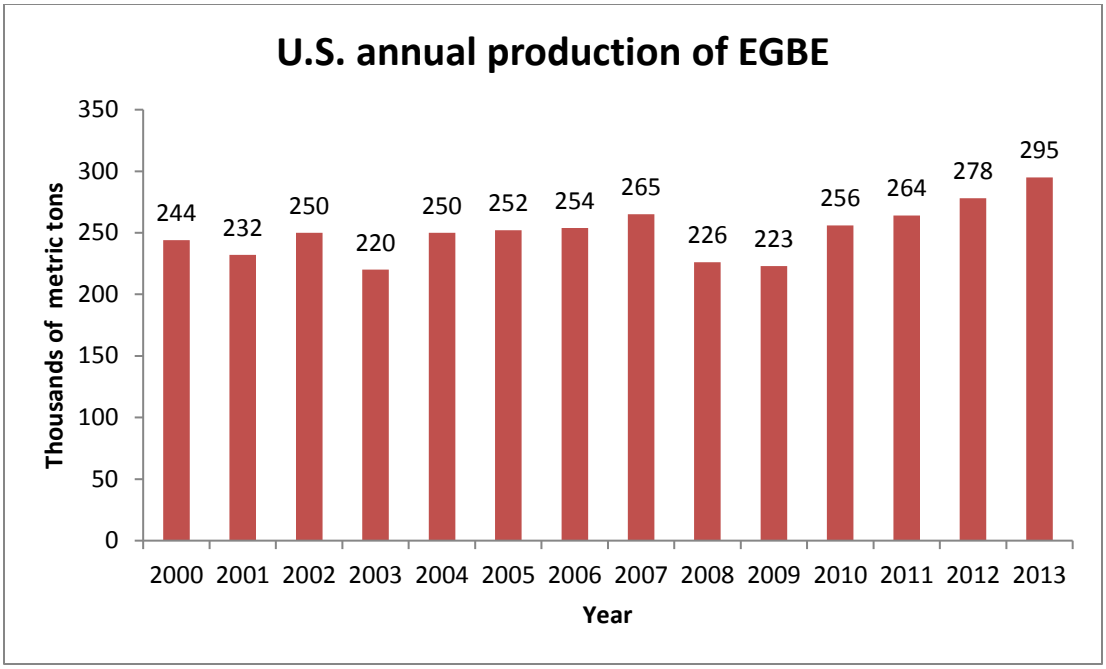


**Figure 1.** US production of EGBE from 2000 to 2013 in thousands of metric tons (Chinn *et al.*, 2014).

## 3.2 Outdoor Emissions

The California Toxics Inventory (CTI) provides emissions estimates by stationary (point and aggregated point), area-wide, on-road mobile (gasoline and diesel), off-road mobile (gasoline, diesel, and other), and natural sources. The CTI estimates total organic gas (TOG) and particulate matter (PM) for area, mobile, and natural sources. Speciated emissions for each source category are then reconciled with reported stationary point source toxics data to establish a complete inventory. Stationary sources include point source emissions estimates provided by facility operators and/or districts pursuant to the Air Toxics "Hot Spots" Program (AB 2588), and aggregated point sources estimated by the Air Resources Board (ARB) and/or districts. Area-wide sources do not have specific locations but are spread out over large areas such as emissions from consumer products and unpaved roads. Mobile sources consist of both on-road and off-road transportation sources. Natural sources such as wildfires are also included. Estimated annual EGBE emissions in California increased from 3,881 tons in 2006 to 4,363 tons in 2010 (Figure 2) (CARB, 2013).

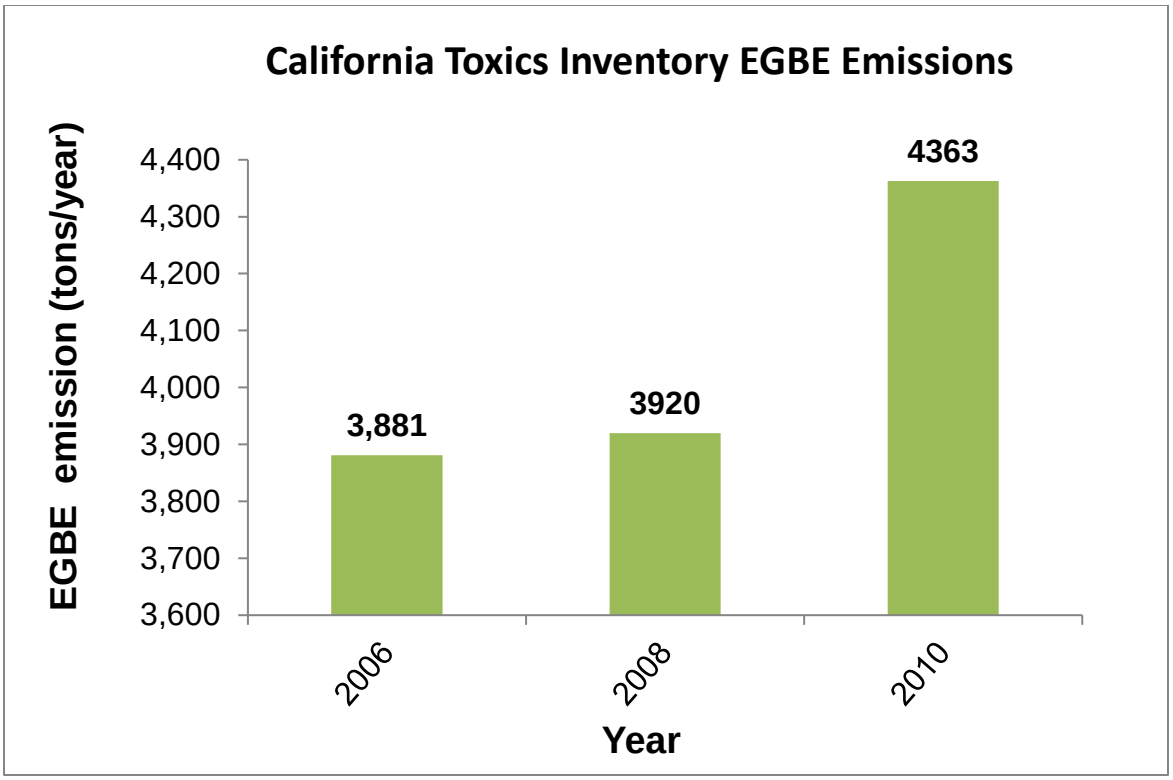


**Figure 2.** California Toxics Inventory EGBE emissions (tons/year)
Source: (CARB, 2013).

### 3.3 Occurrence in Consumer Products and Modeled Indoor Exposures

Consumer products and building materials that may contain EGBE include liquid wax and wax strippers, varnish removers and lacquers, surface cleaners and coatings, caulking products and sealants, water-based paints, resilient floorings, nail enamel removers, and permanent hair colorants (Andersen, 1996; Fang *et al.*, 1999; Zhu *et al.*, 2001; IWMB, 2003; HSDB, 2005). Investigation of 1,242 industrial and commercial cleaning agent formulas by the National Research and Safety Institute for Occupational Accidents Prevention in France, showed that 10% of the products contained between 0.2 and 80% EGBE by volume (Vincent *et al.*, 1993). Approximately 50% of the formulas for window cleaning agents, specifically, contained between 1 and 30% EGBE by volume.

Analysis of 13 glycol ether-containing consumer products purchased from local stores in Canada revealed similar results (Zhu *et al.*, 2001). Gas chromatography and mass spectrometry (GC-MS) performed on headspace samples of the purchased products showed that seven of the 13 products contained detectable levels of volatile EGBE. Five of the seven products with detectable levels of EGBE were house-cleaning agents. The concentration of EGBE ranged from 7.9 to 90.7%, when calculated as the percentage of

the area of the individual peak in the total ion chromatogram for all VOCs in the headspace (Table 1).

**Table 1. Description of consumer products containing volatilized EGBE in headspace samples.**

| Product ID # | Product Type | EGBE Concentration in Headspace (% of total VOCs)[a] |
|---|---|---|
| 1 | All-purpose cleaner | 90.70 |
| 2 | Glass and surface cleaner (clear) | 75.40 |
| 3 | Glass and surface cleaner (blue) | 13.00 |
| 4 | Antibacterial glass and surface cleaner | 9.20 |
| 5 | Lemon-fresh antibacterial spray | 7.90 |
| 6 | Nail enamel remover | 60.30 |
| 7 | Permanent hair colorant | 62.80 |

Table adapted from Zhu *et al.* (2001).

[a]The value is the percentage of area of the individual peak in the total ion chromatogram.

Subsequent GC-MS quantification of EGBE from the liquid fraction of the products showed that the EGBE concentrations ranged from 0.5 to 3.72% (Table 2). Field and laboratory emission cell (FLEC) testing data revealed emission rates from 145 to 938 $mg/m^2/hour$.

**Table 2. Concentrations, masses, and emission rates of EGBE in house-cleaning products.**

| Product ID # | EGBE Concentration (%) in Product[a] | Starting Product Mass (g)[b] | Ending Product Mass (g)[b] | Mean Emission Rate ($mg/m^2/h$)[c] | Emission Rate C.V. (%)[c] |
|---|---|---|---|---|---|
| 1 | 3.72 | 6.06 | 0.21 | 938 | 12 |
| 1[d] | 0.744[e] | 6.10 | 0.05 | 176 | 14 |
| 2 | 0.87 | 5.76 | 0.01 | 223 | 13 |
| 3 | 0.50 | 6.03 | 0.03 | 145 | 14 |
| 4 | 0.83 | 6.06 | 0.03 | 169 | 11 |
| 5 | 1.280 | 6.20 | 0.11 | 426 | 12 |

Table adapted from Zhu *et al.* (2001).
Legend: Product numbers 1, 2, 3, 4, and 5 correspond to all-purpose cleaner, glass and surface cleaner (clear), glass and surface cleaner (blue), antibacterial glass and surface cleaner, and lemon-fresh antibacterial spray, respectively. C.V. – coefficient of variation.
[a] Values measured by gas chromatography and mass spectrometry unless otherwise indicated.
[b] Values measured at the start or end of field and laboratory emission cell testing as indicated.
[c] Values calculated using measured parameters.
[d] Product 1 diluted 5 times with water.
[e] Concentration = 3.72/5.

EGBE air concentrations and inhalation exposures associated with cleaning activities using all-purpose and spray glass cleaners (Products 1, 2, 3, and 5) were estimated from these data. Air concentrations ranged from 2.8 to 62 $mg/m^3$ (0.6 to 13 ppm), based on standard product use and standard room size (volume = 17.4 $m^3$; air exchange rate = 0.5 air changes/hour). Exposures were conservatively estimated to range from 0.004 to 0.211 mg/kg bodyweight (BW)/day (Table 3).

**Table 3. Estimated inhalation exposure to EGBE during cleaning activities using defined room conditions and product-use scenarios.**

| Product ID # | Amount Applied per Surface Area[a] ($mg/m^2$) | Air Concentration[b] ($mg/m^3$) | Task[c] # | Daily Average Exposure by Task[d] (mg/kg BW/day) | Daily Average Exposure by Product[e] (mg/kg BW/day) |
|---|---|---|---|---|---|
| 1 | 16,889 | 62 | 1 | 0.032 | 0.186 (0.211) |
| | | | 2 | 0.063 | |
| | | | 3 | 0.043 | |
| | | | 4 | 0.048 | |
| 2 | 7,391 | 4.7 | 5 | 0.002 | 0.006 (0.008) |
| | | | 6 | 0.004 | |
| 3 | 7,391 | 2.8 | 5 | 0.001 | 0.004 (0.004) |
| | | | 6 | 0.003 | |
| 5 | 16,889 | 25 | 1 | 0.013 | 0.075 (0.084) |
| | | | 2 | 0.025 | |
| | | | 3 | 0.017 | |
| | | | 4 | 0.019 | |

Table adapted from Zhu *et al.* (2001).
Legend: [a]For Products #1 and #5 (all-purpose spray cleaners), the authors assumed a mass of 76,000 mg product was applied to a surface area of 4.5 $m^2$ for each noted task (76,000 mg ÷ 4.5 $m^2$ ≈ 16,889 $mg/m^2$). For Products #2 and #3 (spray glass cleaners), it was assumed that a product mass of 17,000 mg was applied to a surface area of 2.3 $m^2$ for each noted task (17,000 mg ÷ 2.3 $m^2$ ≈ 7,391 $mg/m^2$).
[b] Values are 1-hour average EGBE concentrations in a "standard room" with a volume of 17.4 $m^3$ and an air exchange rate of 0.5 air changes/hr.
[c] Task 1: Clean outside of cabinets; Task 2: clean counters; Task 3: clean bathroom or other tiled or ceramic walls; Task 4: clean outside of refrigerator and other appliances; Task 5: clean inside of windows; Task 6: clean other glass surfaces such as mirrows and tables.
[d] Assuming an inhalation rate of 1.3 $m^3$/hr.
[e] Assuming an inhalation rate of 1.3 $m^3$/hr. The values in parentheses are intake when the more conservative value of 0.18 air changes/hr air change rate in the "standard room" was assumed.

Air concentration estimates from Zhu *et al.* (2001) overlapped with those from Singer *et al.* (2006). To quantify emissions and concentrations of glycol ethers from cleaning products containing EGBE, experiments were conducted by Singer *et al.* (2006) in a 50-$m^3$ chamber (ventilated at approximately 0.5 air changes/hr) designed to simulate a typical residential environment. Four cleaning products containing EGBE were applied full-strength (mass concentrations of 6 - 62 mg/ml) in countertop cleaning activities, while two of these products were diluted (53-153 g product diluted in 1 gal $H_2O$) for floor mopping activities. Countertop cleaning activities resulted in EGBE air concentrations in the first hour in the range of 0.27 to 2.3 $mg/m^3$ (0.056 to 0.48 ppm). For floor mopping activities, EGBE air concentrations in the first hour were in the range of 0.38 to 1.3 $mg/m^3$ (0.079 to 0.27 ppm). During full-strength application including rinsing with a

sponge and wiping with towels, fractional emissions (mass volatilized/dispensed) of EGBE were 50–100% with towels retained, and approximately 25–50% when towels were removed after cleaning.

### 3.4 Measured Indoor Concentrations of EGBE in Business and Residential Settings

Indoor air quality studies have measured numerous volatile organic compounds (VOCs) that humans are exposed to, often as a result of complaints of poor indoor air quality (Mendell, 1991; Daisey *et al.*, 1994; Nazaroff and Weschler, 2004). EGBE is often one of the VOCs that is investigated in these indoor air quality studies due to its frequent occurrence in cleaning products. Cleaning products that contain EGBE include all-purpose cleaners, lemon-fresh antibacterial spray, and liquid wax (Knoppel and Schauenburg, 1989; Zhu *et al.*, 2001). Use of cleaning products containing EGBE in office buildings has linked the chemical as the cause of sensory irritation and headaches in office workers (Rella *et al.*, 2012).

In a workplace air quality study of VOCs present in indoor air, an EGBE concentration (geometric mean ± geometric standard deviation (GSD)) of 0.0077 ± 0.018 mg/m$^3$ (0.0016 ± 0.0037 ppm) was recorded in 12 northern California office buildings (Daisey *et al.*, 1994). The concentration range for EGBE was < 0.0019 – 0.13 mg/m$^3$ (0.0004 - 0.027 ppm). VOC concentrations were also collected outside the buildings and used in indoor/outdoor ratios (I/O) for each VOC. For individual VOCs, the authors identified an I/O ratio >1.35 as predominantly from indoor sources, and an I/O ratio <1.35 as predominantly from outdoor sources. The I/O range for EGBE was 0.18 – 21, which suggested both indoor and outdoor sources of EGBE were present.

In a study of indoor air quality in buildings throughout the US, eight of 11 densely-occupied administrative offices (3-5 occupants/1000 ft$^2$) emitted measurable levels of EGBE (Shields *et al.*, 1996). The geometric mean ± GSD EGBE concentration was 0.001 ± 0.0032 mg/m$^3$ (0.0002 ± 0.00067 ppm) with a maximum value of 0.032 mg/m$^3$ (0.0066 ppm). A much lower EGBE detection rate of 16 out of 59 was observed in 50 telecommunication offices and nine data centers, which was attributed to lower occupancy density (<0.4 occupants/1000 ft$^2$ for telco offices; 1-4 occupants/1000 ft$^2$ for data centers). The geometric means ± GSD concentrations in telecommunication offices and data centers were 0.0001 ± 0.0007 mg/m$^3$ and 0.0002 ± 0.0003 mg/m$^3$ (0.00002 ± 0.00014 ppm and 0.00004 ± 0.000068 ppm), respectively. Maximum values of 0.033 mg/m$^3$ (0.0068 ppm) and 0.016 mg/m$^3$ (0.0033 ppm ) were recorded for telecommunication offices and data centers, respectively. Suggested indoor sources of EGBE were floor cleaners, wax strippers, varnish removers, and lacquers. Although

outdoor levels of all VOCs were also investigated near the buildings, no detectable outdoor levels of EGBE were found.

In contrast to the large office and commercial buildings investigated by other researchers, Wu *et al.* (2011) investigated the indoor air quality of 40 small- and medium-sized commercial buildings in California. Small- (1,000 – 12,000 $ft^2$) and medium-sized (12,000 – 25,000 $ft^2$) commercial buildings were defined as any low-rise building (less than four stories) with roof-top heating, ventilation, and air-conditioning units. EGBE was detected in 39 of the 40 buildings, with a geometric mean concentration of 0.00421 $mg/m^3$ (0.00087 ppm) and a range of 0.00002 to 0.356 $mg/m^3$ (0.0000041 to 0.074 ppm) (Wu *et al.*, 2011). Dental offices/health care facilities (n=4 total) had the highest mean levels among the different types of small- to medium-sized buildings examined, with a geometric mean ± GSD of 0.0186 ± 0.0105 $mg/m^3$ (0.00385 ± 0.00217 ppm) and a range of 0.0023 to 0.305 $mg/m^3$ (0.00048 to 0.063 ppm).

EGBE is also a common component of VOCs in some newly constructed homes. For example, Brown (2002) collected one or two indoor air samples each from the bedroom and living room in a new home on days 2, 19, 72, and 246 post-construction to measure levels of a number of VOCs, including EGBE. The EGBE concentration in the samples collected during post-construction days 2 and 19 ranged from 0.081 to 0.011 $mg/m^3$ (0.017 to 0.0023 ppm). On post-construction days 72 and 246, EGBE concentrations in the samples collected were generally lower, ranging from 0.046 to 0.004 $mg/m^3$ (0.0095 to 0.00083 ppm). EGBE in indoor air was thought to originate from water-based paints or adhesives (Brown, 2002).

Finally, personal breathing zone monitoring by Vincent *et al.* (1993) observed concentrations in the range of < 0.483 to 35.0 $mg/m^3$ (0.10 to 7.25 ppm) (arithmetic mean ± SD: 11.25 ± 11.79 $mg/m^3$ (2.33 ± 2.44 ppm) EGBE following daily use of EGBE-containing surface cleaners by workers to clean cars.

# 4. Toxicokinetics

## 4.1 Toxicokinetic Studies in Humans

EGBE is well-absorbed and rapidly distributed in humans following inhalation, ingestion, or dermal exposure. Inhalation studies in human volunteers found the respiratory uptake of EGBE for two hours under light physical exercise (50 watts) averaged 57% of the inspired amount and was fairly constant during the exposure period (Johanson *et al.*, 1986a). In four healthy male subjects who inhaled 121 $mg/m^3$ (25 ppm) EGBE via a mouthpiece at rest, the mean EGBE uptake was 80% in the last 5 minutes of the 10

minute respiration period (Kumagai *et al.*, 1999). The percentage of EGBE in the end-exhaled air had reached a quasi-steady-state level within the first few minutes of exposure.

Dermal studies in humans observed that airborne EGBE is also absorbed through the skin. However, respiratory uptake has been shown to be quantitatively more important than dermal uptake (Corley *et al.*, 1994; Corley *et al.*, 1997). Using a physiologically-based pharmacokinetic (PBPK) model of a whole-body human exposure scenario, Corley *et al.* (1994) calculated EGBE absorption through the skin (skin permeability coefficient of 3 cm/hr) at about 21% of the total EGBE uptake.

The same investigators (Corley *et al.*, 1997) conducted a study in humans in which one arm of each subject was exposed to 242 mg/m$^3$ (50 ppm) $^{13}C_2$-EGBE for 2 hours. Blood samples were collected from each subject by the finger-prick method from the exposed arm and by intravenous (IV) catheter from the antecubital fossa of the non-exposed arm. The concentrations of EGBE were nearly 1,500-fold higher in blood drawn from the exposed arms than from the non-exposed arms. The authors concluded that the finger-prick sampling technique overestimates systemic absorption of EGBE via the dermal route. Using the finger-prick sampling technique, Johanson and Boman (1991) had calculated a dermal EGBE uptake rate that was 2-3-fold higher than the inhalation uptake rate, suggesting dermal uptake of EGBE accounted for 75% of the total EGBE uptake from whole body exposure. Corley et al. (1997) concluded that even in a "worst-case" scenario, in which respiratory rates are lowest, no clothing is worn (100% of the body surface area is exposed), and temperatures and humidities are normal to elevated, dermal uptake of EGBE vapor should account for a maximum of only 15 - 27% of the total (inhalation + skin) uptake.

Jones and Cocker (2003) and Jones *et al.* (2003b) found a slightly lower uptake than Corley *et al.* (1997) under normal conditions of 25ºC and 40% relative humidity (RH), reporting approximately 11-12% dermal absorption after whole-body exposures of four human volunteers to 242 mg/m$^3$ (50 ppm) EGBE for two hours. Increasing the temperature to 30ºC or increasing RH to 65% resulted in little change in dermal absorption compared to normal conditions (Jones *et al.*, 2003). Additionally, wearing minimal clothing versus overalls under normal conditions did not affect the dermal absorption rate. However, the combination of high temperature (33ºC) and high RH (71%) and wearing overalls increased the proportion of EGBE dermally absorbed to 37-42%.

With respect to metabolism, EGBE is a substrate for alcohol dehydrogenase (ADH), which catalyzes the conversion of the terminal alcohol to butoxyacetaldehyde (BAL) in

humans and rodent models. Aldehyde dehydrogenase (ALDH) then rapidly converts BAL to 2-butoxyacetic acid (BAA), the predominant urinary metabolite responsible for red blood cell hemolysis in rodents (Ghanayem *et al.*, 1987b; Medinsky *et al.*, 1990; Corley *et al.*, 1997) (Figure 2). The metabolic conversion of EGBE to BAA is a saturable process demonstrating Michaelis–Menten kinetics (Gualtieri *et al.*, 2003). Prolonged EGBE elimination observed in overdose situations has been attributed to the saturable metabolic pathways. Although elimination kinetics of EGBE (and BAA) have been reported as independent of the route of exposure (Corley *et al.*, 1997), kinetics may vary in repeated inhalation exposure scenarios, due to species, sex, age, time of exposure, and/or exposure concentration (EPA, 2010).

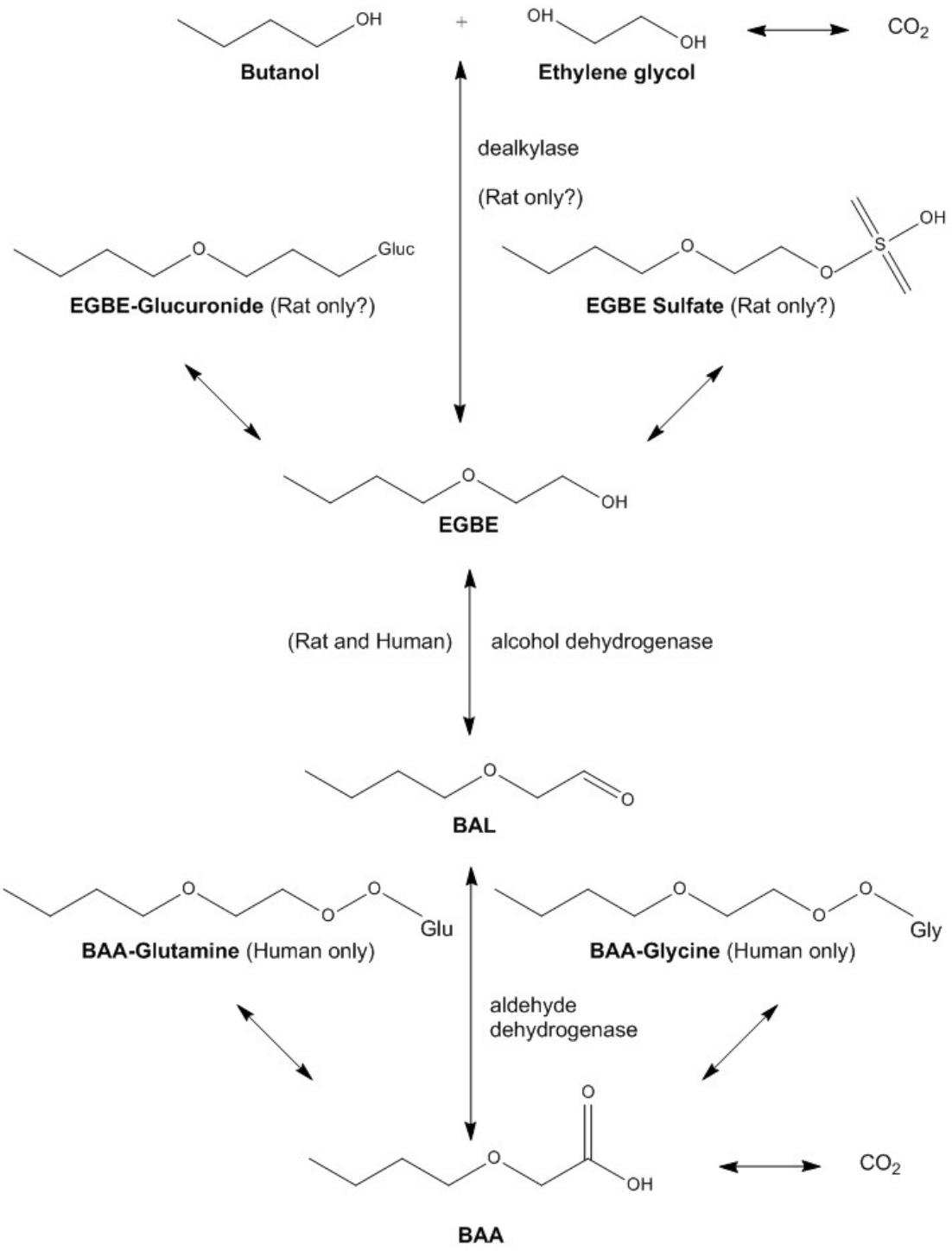


**Figure 3. EGBE Metabolism in Rats and Humans. Adapted from Medinsky *et al.* (1990) and Corley *et al.* (1997).**

PBPK modeling in workers continually exposed to EGBE suggests that its elimination from the most poorly perfused organs is rapid, and EGBE does not appear to accumulate in the body (Johanson, 1986). However, Sakai *et al.* (1994) found small

amounts of conjugated BAA in the urine of EGBE-exposed workers in the morning after a work shift, indicating slight accumulation or relatively slower elimination of the EGBE metabolites in the body.

In seven male human volunteers exposed to EGBE 97 mg/m$^3$ (20 ppm) for two hours under light physical exercise (50 watts), EGBE was removed from the blood with an average elimination half-life of approximately 40 minutes (Johanson *et al.*, 1986a). EGBE could no longer be detected in their blood 2-4 hours after the end of exposure. The major metabolite, BAA, was rapidly excreted in urine of human volunteers with a half-life of approximately 3-6 hours. Although not specifically described by the authors, the urinary BAA half-life was likely estimated based on quantitation of BAA in urine collected immediately after EGBE exposure, and from sampling at 2 hour intervals for 6 hours. Urinary excretion of EGBE was low (<0.03%) and difficult to quantify. The absorbed dose of EGBE eliminated as BAA was lower than expected, suggesting to the authors the formation of other metabolites. Following acid hydrolysis of urine samples, the amount of total uptake excreted as BAA in urine was 17 to 55%. However, the concentration of BAA in urine varied more than 10-fold among the subjects.

In six workers exposed to EGBE, Sakai *et al.* (1994) determined free and total BAA before (free BAA) and after (total BAA) acid hydrolysis of urine samples. The percentages of conjugated BAA vs. total BAA varied from 44.4% to 92.2%, with a mean of 71.1%. The concentration of total BAA in urine was linearly correlated with the worker air exposure levels of EGBE, thought to be due to use of gloves that prevented dermal absorption of EGBE.

Based on the Johanson *et al*. (1986a) findings, Rettenmeier *et al*. (1993) collected end of work-shift urine samples from six lacquerers to quantify levels of free BAA and its suspected conjugate, BAA-glutamine. EGBE was a key constituent in the lacquers used by the workers. Using high-performance liquid chromatography for analysis, a considerable fraction of total BAA, ranging from 16 to 64% (mean value 48%), was excreted in the form of BAA-glutamine. Only trace levels of these metabolites were found in pre-shift urine samples of the workers. In addition to BAA-glutamine, it has been suggested that a small percentage of the amino acid conjugate (<10%) excreted in urine may be in the form of BAA-glycine (Corley *et al.*, 1997).

The elimination of BAA, largely in the form of the glutamine conjugate, was confirmed in a dermal exposure study. Corley et al. (1997) exposed an arm of volunteers to 242 mg/m$^3$ (50 ppm) $^{13}C_2$-EGBE vapor for two hours to study the elimination kinetics of EGBE. Consistent with previous studies, metabolism and elimination of EGBE and BAA were independent of exposure route. Dermally-absorbed EGBE was primarily

eliminated as the BAA metabolite in the urine during the first 12-hour collection interval. About 67% of the total BAA excreted in the urine was in the form of the acid-labile glutamine conjugate. The remainder of the total BAA eliminated was free BAA. Unlike rodent species, no conjugates of ethylene glycol (free or acid-labile) or glycolic acid were detected in the urine.

EGBE itself has been deemed a sub-optimal marker of EGBE exposure due to its rapid metabolism and removal from venous blood (Corley *et al.*, 1997). Use of EGBE as a marker of EGBE exposure is particularly problematic in cases when a study employs 1) dermal exposures, which could create high local EGBE blood concentrations in exposed areas of the body (Corley *et al.*, 1997); or 2) measures of EGBE in breath, which may suffer from poor detection sensitivity (Jones and Cocker, 2003).

The metabolite BAA may be a practical marker for EGBE exposure. The study by Sakai *et al.* (1994) was the first to demonstrate a significant linear relationship between occupational EGBE exposure levels and conjugated or total BAA (including free BAA and conjugated BAA metabolites after acid hydrolysis) concentrations in urine. For example, based on time-weighted average (TWA) EGBE vapor concentrations in the breathing zone of six workers exposed to EGBE and their subsequent total BAA levels in urine, a total BAA concentration of 6 mg/g creatinine roughly translated to an EGBE air concentration of 1.9 mg/m$^3$ (0.4 ppm). A poorer correlation was found for EGBE exposure and the urinary concentration of free BAA. In this study, direct skin contact with liquid EGBE was considered minimal due to use of gloves by the workers.

Accordingly, Jones and Cocker (2003) proposed that total urine BAA be used as the biomarker of choice for monitoring EGBE exposure due to high variability of BAA conjugate among workers. Their research with urine from 48 occupationally-exposed workers and four chamber-exposed volunteers showed that 1) the extent of BAA conjugation in urine post EGBE exposure varied from 0 – 100% within and between individuals; 2) this variability was not related to time of day, urinary BAA concentration, or urinary pH; and 3) similar to the finding of Sakai *et al*. (1994), use of total BAA in urine as a biomarker of EGBE exposure decreased this inter-individual variability.

Emissions of EGBE from facilities that may impact surrounding communities will likely be in the gaseous or aerosol form resulting in the inhalation route as the primary route of exposure. Unlike occupational exposure situations, dermal contact with liquid EGBE is not expected to occur in exposure scenarios involving releases from industrial facilities. Nevertheless, exposure to liquid EGBE from consumer products may occur concurrently with airborne exposure from a facility source, resulting in cumulative exposure to EGBE via multiple exposure sources and routes. Therefore, additional

information on dermal absorption of EGBE in aqueous solution is included below for reference.

Unlike the occupational study by Sakai *et al.* (1994), poor correlations were found between airborne EGBE levels and urinary BAA excretion in other occupational studies due to significant skin contact with aqueous EGBE solutions. For example, Hung *et al*. (2011) investigated EGBE inhalation and dermal exposure of 80 workers. The workers were divided into three groups based on EGBE exposure: decal transfer workers (high exposure, n=31), self-adhesive decal workers (moderate exposure, n=25) and assembly workers (little or no exposure, n=24). Personal air sampling (8-hour TWA) was performed to determine EGBE air exposure, and pre- and post-shift urine samples were collected for determination of total BAA. Results showed that the decal transfer workers whose hands were in direct contact with a dilute aqueous EGBE solution were exposed to an average concentration of 8.1 mg/m$^3$ (1.7 ppm) EGBE in air. A poor correlation was observed between air levels of EGBE and post-shift total BAA levels in urine ($R^2$ = 0.0435 for Monday; $R^2$ = 0.0559 on Friday), which indicated to the authors that significant dermal uptake had occurred. Post-shift total BAA levels in urine on Monday and Friday (446.8 and 619.4 mg/g creatinine, respectively) were around 223% and 310% of the ACGIH proposed Biological Exposure Index (BEI; 200 mg/g creatinine), respectively. Employing a PBPK model that only estimates the urinary BAA concentration via whole-body exposure to airborne EGBE, only 3.7% of the increase in urinary BAA could be explained by the airborne exposure route. The authors noted that the mean pre-shift BAA level on Friday was significantly higher than that on Monday, implying accumulation of EGBE metabolites over the workweek.

Hung *et al*. (2011) also investigated exposure of 25 self-adhesive decal workers, who provided occasional assistance to the decal transfer workers, and 24 assembly workers, who acted as controls. Personal air exposure to EGBE was below the detection limit for most of the self-adhesive decal workers, so no correlation of urinary BAA level to EGBE air concentration was attempted by the authors. However, end-shift total BAA levels were found to be about 10-fold less than that of the decal transfer worker group. In the assembly workers, personal air exposure to EGBE was not detected, and no BAA was found in the urine.

Studies of dermal absorption and metabolism kinetics of EGBE were carried out in four male volunteers (Korinth *et al.*, 2007). Percutaneously penetrated EGBE was sampled and measured before it entered systemic circulation using micro-dialysis capillaries embedded under the subjects' skin. Volunteers were dermally exposed twice to 90% and 50% aqueous solutions (v/v) of EGBE for 4.5 hours. The dialysate samples were collected at 30-minute intervals during exposure. The systemic absorption of EGBE was

estimated from the concentration of free BAA in urine. A pseudo steady-state dermal absorption was reached after approximately 2 hours of exposure. The maximum dermal flux of the 50% EGBE solution was higher than that of the 90% EGBE solution (2.8 ± 0.4 and 1.9 ± 0.6 mg/ $cm^2$-hr, respectively). The more diluted EGBE solution exhibited a shorter lag time for dermal absorption: 25 versus 39 minutes. Micro-dialysis indicates that the dermal metabolism of EGBE was low; with BAA accounting for 0.03% to 1.9% of the EGBE in the same dialysate. This study demonstrated that dermal absorption of EGBE is dependent on the EGBE concentration in solution.

In another controlled human exposure study, Kezic *et al.* (2004) exposed male volunteers to EGBE via dermal and inhalation routes to compare the kinetics of urinary elimination of free and total BAA. Dermally-exposed volunteers (n=6) had a 50% aqueous solution of EGBE applied to the volar forearm for four hours. Six other male volunteers were exposed by inhalation (mouth-only) to 93 mg/$m^3$ (19 ppm) EGBE for 30 minutes. The absorbed amount of EGBE after inhalation exposure was 20.9 ± 5.0 mg, with 55 ± 21% of the total urinary excretion of BAA in the form of the conjugate. The absorbed amount of EGBE after dermal exposure was higher (567 mg), but with nearly the same proportion of BAA conjugate (58 ± 14%) excreted in urine. The urinary half-life of free and total BAA via inhalation was 3.1 and 3.4 hours, respectively. The urinary half-life of the free and total BAA following dermal exposure was 3.8 and 5.1 hours, respectively. The urinary elimination half-life of BAA was obtained from the slope of the curve of the log-linear excretion rate versus time data, if data from at least three time points were available. The authors observed that the extent of urinary BAA conjugation was highly variable between individuals, and that total BAA was a better biomarker of exposure due to reduced variation. The proportion of BAA conjugate increased in urine with time, which was consistent with the longer half-life of the conjugate compared to free BAA in urine.

### 4.2 Toxicokinetic Studies in Animals

$^{14}$C-labelled EGBE administered by gavage to rats was rapidly distributed to all tissues via the blood stream, with the highest levels of radioactivity found in the forestomach, followed by the liver, kidneys, spleen and glandular stomach (Ghanayem *et al.*, 1987b). Following subcutaneous administration, $^{14}$C-labelled EGBE in rats was also distributed widely to all tissues, but with the greatest level of radioactivity in the spleen and thymus, followed by the liver (Bartnik *et al.*, 1987).

In groups of rats inhaling 97 or 483 mg/$m^3$ (20 or 100 ppm) EGBE continuously for up to 12 days, EGBE and its metabolite BAA increased rapidly in blood during the first 1-3 days, then began to level off over the remaining days (Johanson, 1994). EGBE and BAA concentrations displayed linear kinetics, with the EGBE concentration

approximately five times higher in the 483 mg/$m^3$ (100 ppm) group compared to the 97 mg/$m^3$ (20 ppm) group. The observed urinary excretion of BAA corresponded to 64% of the calculated respiratory uptake.

In groups of rats inhaling $^{14}$C-labelled EGBE at concentrations of 20.8, 237, and 2115 mg/$m^3$ (4.3, 49, and 438 ppm), an average of 69% of the $^{14}$C-label was eliminated in urine during the 66-hour post exposure period (Sabourin *et al.*, 1992a). About 7% was metabolized and exhaled in the form of $^{14}CO_2$, and another 10-20% of the label remained in the carcass, suggesting possible binding of EGBE metabolites to tissue macromolecules. BAA was the major metabolite in urine at all exposure concentrations; although the proportion of metabolite in urine as BAA decreased with increasing dose from 43.2 to 36.6%. A minor urinary metabolite, ethylene glycol, also decreased with increasing concentration from 16.1 to 7.9%. These data indicated that metabolism of EGBE by pathways leading to ethylene glycol and BAA appears to be easily saturated. The EGBE-glucuronide conjugate (BEG) was also excreted in urine, increasing proportionally with increasing concentration from 3.4 to 10.4%. BEG elimination was also favored early during the exposures. This finding suggested to the authors that formation of BEG is favored at higher substrate concentrations (high $K_m$), but shifts to more ethylene glycol and BAA elimination as the internal EGBE concentration declines after exposure. Lesser amounts of two unknown metabolites were also detected in urine (≤10.5% of $^{14}$C-label eliminated in urine).

The elimination kinetics of EGBE and BAA in rats appear to be independent of exposure route. In a drinking water study, rats exposed to 28 to 140 mg/kg BW/day of $^{14}$C-labeled EGBE in drinking water eliminated 50-60% of the label in urine as BAA (Medinsky *et al.*, 1990). Another 10% of the label was eliminated in urine as ethylene glycol and approximately 7% was eliminated as BEG. About 8-10% of the label was removed as $^{14}CO_2$ in exhaled breath.

In rats orally administered $^{14}$C-labelled EGBE (125 mg/kg body weight), five metabolites were observed in urine in the first eight hours after treatment (Ghanayem *et al.*, 1987a). BAA and BEG were the major urinary metabolites. BAA accounted for more than 75% of the radioactivity excreted in urine, whereas BEG accounted for <20% of the radioactivity excreted in urine. A small percentage of the radioactivity in urine was the sulfate conjugate (BES), while the other minor metabolite was unidentified.

The elimination kinetics of $^{14}$C-labelled EGBE have also been investigated in dermally-exposed rats (Sabourin *et al.*, 1992b). EGBE was applied to a shaved area on the back of rats in metabolism cages for 72 hours. As with other exposure routes, BAA was the main metabolite found in urine (68% of total urine metabolites). BEG accounted for 14%

of total urine metabolites, and ethylene glycol accounted for another 5% of total urine metabolites. Approximately 4.5% of the radiolabel was exhaled as $^{14}CO_2$.

A few studies have examined the toxicokinetics of EGBE in mice. Poet *et al*. (2003) administered EGBE to mice via intraperitoneal injection (IP; 53.2 and 261 mg/kg) and oral gavage (265.2 mg/kg). BAA was the major metabolite eliminated in urine, 50.8% of the dose via IP and 37.5% of the dose via gavage. An unidentified conjugate of BAA represented 0-1.7% of the dose via IP, and about 7% of the dose via oral gavage. Very little unconjugated EGBE (<0.2%) was detected in urine. Following acid hydrolysis, 0.7-2.8% of the total dose via IP and 3.3% of the total dose via oral gavage were recovered as an EGBE conjugate presumed by the authors to be BEG.

### 4.3 Species Differences in Metabolism and Elimination of EGBE

Physiologically-based pharmacokinetic modeling of EGBE and BAA showed that even though rats metabolize EGBE and eliminate BAA faster per kilogram body weight than humans, the balance of these two processes in addition to physiological differences between species resulted in higher predicted peak blood levels as well as higher total areas under the blood concentration time curves for BAA in rats compared to humans (Corley *et al.*, 1994; Corley *et al.*, 2005). For example, the PBPK model predicted peak blood levels of BAA in male rats to be roughly twice that of humans over a range of EGBE air concentrations from 531 to 1208 mg/m$^3$ (110 to 250 ppm), and suggested that the blood concentration of BAA in humans cannot attain a level at which hemolysis can occur. In mice and female rats, the PBPK model showed peak BAA blood concentrations for air concentrations from 725 to 1208 mg/m$^3$ (150 to 250 ppm) EGBE was even greater (2-4x) compared to humans. Mice, on the other hand, eliminated both EGBE and BAA from blood faster than rats when chronically exposed to EGBE (Dill *et al.*, 1998).

In summary, while Phase I metabolism of EGBE to BAA is similar between humans and rodents, there are major differences in Phase II metabolism between the species (Table 4). Humans extensively conjugate BAA via the amino acid glutamine and probably glycine, while rats excrete mostly free BAA and a small amount as BEG. Similar to rats, mice excrete mainly free BAA in urine and a small amount (<10%) as an EGBE conjugate, possibly BEG. Ethylene glycol or glycolic acid urinary metabolites are also excreted by rats (and probably mice), but have not been found in humans.

**Table 4. Comparisons of human, rat and mouse urinary EGBE metabolites, and $^{14}C$-labelled EGBE exhaled as $^{14}CO_2$.**

| Study, Species, Exposure Route | % BAA | %Gln | %BEG | %EG | %other | $\%CO_2$ |
|---|---|---|---|---|---|---|
| Johanson *et al.* (1986)<br>Human, inhalation | 17-55[a] | NR | NR | NR | NR | NR |
| Sakai *et al.* (1994)<br>Human, inhalation | NR | 44-92[b] | NR | NR | NR | NR |
| Rettenmeier *et al.* (1993)<br>Human, inhalation | NR | 16-64[c] | NR | NR | NR | NR |
| Corley *et al.* (1997)<br>Human, dermal | 33[d] | 67[c] | NR | NF | [e] | NR |
| Kezic *et al*. (2004)<br>Human, inhalation<br>Human, dermal | <br>45[d]<br>42[d] | <br>55[b]<br>58[b] | NR | NR | NR | NR |
| Sabourin *et al.* (1992a)<br>Rat, inhalation | 43-37[f] | NR | 3-10 | 8-16 | ≤10.5[g] | 7 |
| Medinsky *et al.* (1990)<br>Rat, drinking water | 50-60[f] | NR | 7 | 10 | NR | 8-10 |
| Ghanayem *et al.* (1987)<br>Rat, oral gavage | 75[f] | NR | <20 | NR | [h] | NR |
| Sabourin *et al.* (1992b)<br>Rat, dermal | 68[f] | NR | 14 | 5 | NR | 4.5 |
| Poet *et al.* (2003)<br>Mouse, oral gavage<br>Mouse, IP | <br>37.5[i]<br>50.8[i] | NR | <br>3.3[j]<br>0.7-2.8[j] | NR | <br>7[k]<br>0-1.7[k] | NR |

Legend: Gln, BAA-glutamine conjugate; BEG, EGBE-glucuronide conjugate; EG, ethylene glycol; NF - Not found; NR – Not reported.

[a] Amount of total EGBE uptake excreted as total BAA (Free BAA + conjugated BAA)

[b] Percent of total BAA eliminated as BAA-conjugate, presumed to be BAA-glutamine

[c] Percent of total BAA eliminated as glutamine-conjugate. Corley *et al.* (1997) suggested a portion of this amino acid conjugate (<10%) is the glycine conjugate

[d] Percent of total BAA eliminated as free BAA

[e] Fraction just above detection limit eliminated as unidentified EGBE-conjugate

[f] Percent of total urinary metabolites excreted as free BAA

[g] Unidentified metabolite(s)

[h] Small, unspecified percentage eliminated as the sulfate conjugate

[i] Percent of dose excreted in urine as free BAA

[j] Percent of dose excreted in urine; EGBE conjugate presumed to be BEG

[k] Percent of dose excreted in urine; unidentified BAA-conjugate

## 4.4 Age- and Sex-Related Differences in Rodents

Age-related differences in the metabolism and clearance of EGBE have been observed in rodents. Compared to older rats (9-13 weeks old), young rats (4-5 weeks old) eliminated a larger proportion of gavage-administered EGBE as $CO_2$ in exhaled breath and excreted more EGBE metabolites (BAA, BEG, and/or BES) in the urine, resulting in lower plasma concentrations of EGBE and BAA (Ghanayem *et al.*, 1990a). Urinary excretion of BAA appeared to be impaired in older rats resulting in a larger area under the BAA time–concentration blood curve (AUC) compared to younger rats. This finding suggests older rats have a greater susceptibility to hemolysis (Ghanayem *et al.*, 1990a).

As part of a National Toxicology Program (NTP) chronic exposure study, sex and age-related differences in the toxicokinetics of EGBE were examined in rats and mice over their lifespan (Dill *et al.*, 1998). Urine and blood samples were collected periodically from the rodents during an 18-month exposure (6 hrs/day, 5 days/wk) to EGBE. A separate group of mice was exposed to EGBE only for 3 weeks when they were 19 months old. In 19-month-old mice, EGBE was rapidly cleared from the systemic circulation, exhibiting clearance profiles similar to young mice 6-7 weeks old. However, old mice eliminated the BAA metabolite from blood over 10 times more slowly than young mice after a 1-day exposure. This delayed elimination of BAA in old mice was less obvious after 3 weeks of exposure. This finding indicated that there might be other factors in addition to the age of animals, such as acute renal dysfunction due to exposure followed by rapid compensation, that could be the cause of BAA kinetic differences between young and old mice.

In rats, a sex-related difference in BAA elimination was observed, as females were about half as efficient in clearing BAA from the blood as males (Dill *et al.*, 1998). The authors suggested that the differences in renal excretion of BAA in rats were most likely responsible for the sex-dependent difference in BAA blood levels.

Overall, mice eliminated both EGBE and BAA from blood faster than rats (Dill *et al.*, 1998). However, in both species, the rates of elimination of EGBE and BAA decreased with continued exposure resulting in longer residence times in blood. The authors concluded that the elimination kinetics of EGBE and BAA following long-term exposure appear to be dependent on sex and age of the animal, but can also vary depending on the species, time of exposure, and exposure concentration.

# 5. Acute Toxicity of EGBE

## 5.1 Acute Toxicity to Adult Humans

### *5.1.1 Inhalation Exposure*

***Acute Accidental, and Incidental EGBE Inhalation Exposures***

EGBE is an irritant of the eyes and upper respiratory tract in humans. Use of cleaning products containing EGBE in office buildings specifically implicated EGBE as the cause of sensory irritation and headaches in office workers (Rella *et al.*, 2012). Although the air levels of 0.013 to 0.032 mg/m$^3$ (0.0027 to 0.0066 ppm) in the study by Rella *et al.* were well below occupational limit values, and other potential sensory irritants were present (*e.g.*, limonene, dimethylstyrene and hexanal), elimination of the cleaning products resulted in improvement of air quality and reduction of symptoms.

Accidental exposures of humans to high levels of EGBE vapors originating from misuse of concentrated EGBE cleaning products have resulted in immediate, intense eye and respiratory irritation, marked dyspnea, nausea, and faintness (Raymond *et al.*, 1998). 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/or other respiratory diseases and conditions (Bello *et al.*, 2009; Burns, 2010). Epidemiological investigations have shown an association between exposure to cleaning products and respiratory dysfunction, including exacerbation of asthma (Zock *et al.*, 2007; Siracusa *et al.*, 2013; Folletti *et al.*, 2014). Although EGBE has been implicated as a potential irritant in cleaning products that leads to respiratory problems, the presence of other VOC irritants in cleaning products and lack of quantitative assessments of exposure during cleaning activities often make it difficult to characterize the specific role of EGBE as a respiratory irritant in these products (Bello *et al.*, 2009; Bello *et al.*, 2013; Fromme *et al.*, 2013; Gerster *et al.*, 2014).

Measured EGBE concentrations ranging from 62.8 to 816 mg/m$^3$ (13 to 169 ppm) near silk screening equipment have resulted in complaints of odor and sensory irritation during use (Kullman, 1987). Raymond *et al.* (1998) reported that long-term effects of high acute accidental EGBE exposures (approximately 41.4 – 62.1 mg/m$^3$; 200 – 300 ppm) included recurrent eye and respiratory irritation, dry cough, and headache eight months post-exposure, and new cherry angiomas 4 – 60 months post exposure. The appearance of cherry angiomas was reported in 6 of 7 workers (mean age: 36 yrs) four months following the high acute EGBE exposure. Cherry angiomas can appear spontaneously, usually after age 50, but have been observed in workers following exposure to other irritating gases (*e.g.,* mustard gas). The authors suggested cherry

angiomas may represent, in some persons, a nonspecific response of exposure to noxious agents.

***Acute EGBE Inhalation Chamber Studies***

In a chamber study conducted to investigate the toxicokinetics of EGBE, seven healthy male adults were exposed to 97 mg/m$^3$ (20 ppm) EGBE for 2 hours during light exercise on a bicycle ergometer (Johanson *et al.*, 1986a). There were reportedly no complaints or any other adverse effects from exposure. No changes in pulmonary ventilation, respiratory frequency or heart rate were seen, but the study was not designed to collect detailed information on potential sensory irritant effects. In another toxicokinetic study, whole body 2-hour exposure of four volunteers to 237 mg/m$^3$ (49 ppm) EGBE did not result in physiological changes in breathing rate, pulse rate, skin surface temperature or skin resistance (Jones and Cocker, 2003; Jones *et al.*, 2003b). Although an odor was noted upon entering the chamber, and some volunteers found it initially unpleasant, perception of the smell diminished over time during exposure (electronic communication from K. Jones, 2005).

In whole-body chamber studies conducted by Carpenter *et al.* (1956), human volunteers were exposed to 473 mg/m$^3$ (98 ppm; two men and one woman) or 942 mg/m$^3$ (195 ppm; two men and two women) EGBE for a total of 8 hours. Even at the lower exposure level, eye, nose, and throat irritation, taste disturbances, headache, and nausea were reported by the human volunteers. Two men exposed to 546 mg/m$^3$ (113 ppm) EGBE for 4 hours reported similar effects. RBC osmotic fragility and urinalysis were normal in the human subjects during and after exposure.

### *5.1.2 High-dose Oral, Intentional Exposure*

In separate case reports, two women who ingested large amounts of window cleaner (containing about 12% EGBE; dose range 391 – 933 mg/kg) showed severe respiratory effects including pulmonary edema and increased respiration rate (20 breaths/minute versus adults normal range: 12 – 18 breaths/minute) that required a ventilator (Rambourg-Schepens *et al.*, 1988; Gijsenbergh *et al.*, 1989). After exposure to approximately 45 g EGBE, one 50-year-old woman experienced moderate hemoglobinuria on the third day post-exposure, which lasted until the sixth day, inducing progressive erythropenia (RBC 3 x10$^{12}$/L, hematocrit (Hct) 28.6%, hemoglobin (Hgb) 9.7 g/L on the 10$^{th}$ day) (Rambourg-Schepens *et al.*, 1988). Another 23-year-old woman, who had ingested approximately 25 – 30 g EGBE, experienced a fall in Hgb from 11.9 g/dL on admission to 8.9 g/dL on the second day, together with the appearance of hematuria (Gijsenbergh *et al.*, 1989). Both patients recovered and were discharged in good condition after 8 to 10 days.

One 53-year-old patient was admitted to the intensive care unit after attempting suicide with ingestion of 500 ml of a house cleaning fluid (Bauer *et al.*, 1992). The cleaning fluid's composition included 2.5% ethanol, 9.1% (45.5 g) EGBE, and traces of diethylene glycol monoethyl ether, which was determined by gas-chromatography. The patient was comatose (Glasgow Coma Score 5/15) with metabolic acidosis, shock (blood pressure 60/30 mmHg), and non-cardiogenic pulmonary edema confirmed by a hemodynamic study. Physical and laboratory exams found crackling sounds in both lungs and a transient polyuria (2500 ml urine in 2 hours), respectively. No blood ethanol could be detected, but the serum EGBE concentration was 0.00528 mg/L. No EGBE was found in gastric lavage juice or urine. This patient was an alcohol abuser, exhibited neurosis, and had a history of trichloroethylene ingestion (14 and 4 weeks before that event). The patient had undergone vascular surgery in the past. This patient's outcome was marked by a dramatic improvement of respiratory function within five days. Acidosis and hypoxemia were corrected in 4 hours; shock was stabilized in 12 hours. By 36 hours after admission, biologic data showed a non-hemolytic hypochromic anemia (Hct: 25% with thrombopenia (platelet count: 85 000)). The patient was discharged and had fully recovered after 15 days. The author concluded that acute poisoning by EGBE could cause not only hematologic, neurologic, renal, and metabolic disturbances, but also severe acute and transient respiratory failure, the mechanism of which is unknown (Bauer *et al.*, 1992).

A case report by Gualtieri *et al.* (2003) described an 18-year-old male who ingested 360–480ml of a glass cleaner which contained 22% EGBE and then again ingested approximately 480 ml of the same cleaner 10 days later. Approximately 10 hours after the first ingestion, the patient developed severe central nervous system (CNS) depression, metabolic acidosis, hematuria, and mild elevation of hepatic enzymes. He was treated initially with ethanol therapy but continued to deteriorate and was started on hemodialysis. The highest BAA and EGBE serum concentrations noted after the first ingestion were 4.86 and 0.00038 mmol/L, respectively, from a sample collected approximately 16 hours post-ingestion and 7 hours prior to hemodialysis. Within four hours after the second ingestion, the patient again received ethanol and hemodialysis treatments. During his second hospitalization, the patient did not develop severe CNS depression or profound metabolic acidosis. The highest BAA and EGBE serum concentrations noted after his second ingestion were 2.07 and 0.108 mmol/L, respectively, collected approximately 22 hours post-ingestion and 2 hours after the start of hemodialysis. Neither episode produced clinically significant hemolytic anemia, oxaluria, ethylene glycol production, or renal failure.

Lastly, Hung *et al.* (2010). reported that a 53-year old worker co-ingested an unknown quantity of ethanol and 150–250 mL of 99% EGBE, which resulted in rapid obtundation (altered level of consciousness), severe airway edema, hypotension, and prolonged acidosis despite the co-ingestion of ethanol and the administration of a loading dose of fomepizole, an alcohol dehydrogenase inhibitor. Following hemodialysis, the patient recovered without apparent sequelae. The authors concluded that alcohol dehydrogenase inhibitors may not be adequate to prevent acidosis for significant EGBE ingestions and hemodialysis treatment may be necessary. A summary of EGBE poisoning cases is presented in Table 5 below.

**Table 5: Synopsis of EGBE poisoning cases.**

| | Rambourg-Schepens *et al.*, 1988* | Gijsenbergh *et al.*, 1989* | Bauer *et al.*, 1992* | Gualtieri *et al.* (2003) | Hung *et al.*, 2010 |
|---|---|---|---|---|---|
| Sex | Female | Female | Male | Male | Unknown |
| Age (years) | 50 | 23 | 53 | 18 | 53 |
| Ingested Dose (g) | 45 | 25 - 30 | 45 | 80 - 100 | 135-225 |
| CNS depression | Yes | Yes | Yes | Yes | Yes |
| Lung injury | No | No | Yes | No | Yes |
| Liver injury | No | No | Yes | Yes | |
| Renal injury | Yes | No | No | Yes | |
| pH | 7.23 | 7.08 | 7.05 | 7.34 | 7.16 |
| $HCO_3^-$ (mmol/L) | 5 | 2.4 | 5.6 | 19.5 | 21 |
| Hematocrit (%) | 28.6 ($10^{th}$ Day) | Unknown | 25 ($2^{nd}$ Day) | Unknown | Unknown |
| Hemoglobin (g/dL) | 9.7 ($10^{th}$ Day) | 8.9 ($2^{nd}$ Day) | 9.1 ($2^{nd}$ Day) | Unknown | 10.7 ($2^{nd}$ Day) |
| Outcome | Discharged | Discharged | Discharged | Discharged | Discharged |

* Adapted from Bauer *et al.* (1992) to include data from Gualtieri *et al.* (2003) and Hung *et al.* (2010).

## 5.2 Acute Toxicity to Infants and Children

No studies of children exposed to airborne EGBE were located. However, acute ingestions of EGBE in cleaning solutions by 24 children (aged from 7 months to 9 years) from a regional poison control center have been reviewed (Dean and Krenzelok, 1992). These reports included the ingestion of 5-300 ml of liquid glass cleaning products containing 0.5 to 9.9% EGBE. All ingestions were reported within 5 minutes of ingestion,

and all 24 children, including two children who ingested more than 15 ml of EGBE-containing glass/window cleaners, were hospitalized for 24 hours following gastric emptying and gastric lavage. The children were asymptomatic both at the time the ingestions were reported and 24 hours later. The five-month retrospective review of the two hospitalized children who ingested >15 m EGBE failed to find symptoms consistent with hemolysis, nervous system depression, acidosis, or renal compromise.

## 5.3 Acute and Subacute Toxicity to Experimental Animals

### *5.3.1 Acute and Subacute Studies*

Kane *et al.* (1980) exposed male Swiss Webster (outbred) mice (n = 4/group; age and weight not stated) to EGBE vapor for 10 minutes over a concentration range of approximately 676 – 6762 mg/m$^3$ (140 – 1400 ppm). They estimated an RD50 (an airborne concentration of a chemical that produces a 50% decrease in respiratory rate) of 7,995 mg/m$^3$ (2825 ppm). The RD50 bioassay measures decreases in respiratory frequency in mice as a result of stimulation of the trigeminal or laryngeal nerve endings. This RD50 needed to be extrapolated because the authors were unable to generate EGBE concentrations that were adequately high to directly determine the RD50. Although not specified by the authors, this could be a result of the exposures reaching the saturated vapor pressure, about 2,830 to 4,528 mg/m$^3$ (1,000 to 1,600 ppm) depending on the temperature and humidity, prior to reaching the RD50. EGBE was categorized as a weak sensory irritant by Kane *et al.* (1980) when compared to the RD50 of potent sensory irritants such as chlorine, acrolein, formaldehyde and toluene diisocyanate.

In range finding inhalation studies conducted by Carpenter *et al.* (1956) as a guide for subsequent 30-day exposure trials (discussed in Section 6.3), an unspecified strain of rats were exposed to a range of EGBE concentrations for 4 to 8 hrs/day, for up to 6 days. Mortality and hemoglobinuria were the endpoints assessed. Hemoglobinuria was used as the basis of the NOAEL and LOAEL (604 and 1208 mg/m$^3$, 125 and 250 ppm EGBE, respectively) when it was observed in young female rats (n = 6/group; 5 – 6 weeks of age; 88 – 104 g) exposed 8 hrs/day for 6 days. In older female rats (age not specified) weighing 140-160 gms, 8-hour exposures to 1208 mg/m$^3$ (250 ppm) EGBE for four days resulted in hemoglobinuria and mortality (n = 1 of 5). In rats about 1 yr old, one 7-hour exposure to 1811 mg/m$^3$ (375 ppm) EGBE resulted in hemoglobinuria and mortality in all 23 exposed female rats (250-330 g),and in 11 of 13 exposed male rats (380-500 g). At higher EGBE concentrations of 2,415 mg/m$^3$ and 3,864 mg/m$^3$ (500 and 800 ppm), 6 week-old female rats weighing 100-130 g exhibited hemoglobinuria, but were more resistant to the lethal effects of EGBE compared to the 1-yr olds. Exposure to 2,415 mg/m$^3$ (500 ppm) for 4 or 8 hours (n = 6/group) resulted in only one death.

Exposure to 3,864 mg/m$^3$ (800 ppm) resulted in no mortality with 4 hours exposure, and 50% mortality (n = 3 of 6) with 8 hours exposure.

Dodd *et al.* (1983) performed a comprehensive study on the effects of EGBE vapor inhalation in 6 – 7-week old Fischer 344 (inbred) rats. Acute, 9-day, and 90-day (discussed in Section 6.3) exposure experiments were performed in a 3,800-liter chamber for 4 hours on one day, 6 hrs/day for 9 days, and 6 hrs/day, 5 days/wk for 13 weeks, respectively. Biological endpoints including RBC Hgb, mean corpuscular hemoglobin (MCH) concentration and numbers of nucleated RBCs, reticulocytes, and lymphocytes were assessed.

In acute experiments, male and female rats (n = 6/sex/group) were exposed by inhalation to EGBE for 4 hours at concentrations of 976, 2,526, or 4,188 mg/m$^3$ (202, 523, or 867 ppm, respectively). There was no control group. All EGBE exposed rats exhibited loss of coordination, rapid shallow breathing, and red discharge from the urogenital region. All of the rats in the 4,188 mg/m$^3$ (867 ppm) group died within 24 hours of exposure. The estimated $LC_{50}$ was 2,348 mg/m$^3$ (486 ppm) for males and 2,174 mg/m$^3$ (450 ppm) for females (Dodd *et al.*, 1983).

In 9-day (6 hrs/day, 5 days/wk) experiments, rats (n = 8/sex/group) were exposed to EGBE concentrations of 0, 97, 415, or 1,183 mg/m$^3$ (0, 20, 86 or 245 ppm, respectively). An additional 8 rats/sex/group were assigned to the control and highest EGBE exposure groups and allowed a 14-day recovery following the ninth exposure day. The authors found EGBE exposure significantly ($p \leq 0.05$) affected hematological parameters and body/organ weights. Male and female rats from the 1,183 mg/m$^3$ (245 ppm) group had reduced RBC counts, Hgb and MCH concentrations, and BW gains, and increased nucleated RBCs, reticulocytes, lymphocytes, and liver weights relative to control when necropsied immediately after the 9-day exposure. A 14-day post-exposure recovery resulted in substantial reversal of the affected blood parameters. Similar hematologic effects were observed in the 415 mg/m$^3$ (86 ppm) group, but not in the 97 mg/m$^3$ (20 ppm) group. The authors reported a No Observed Adverse Effect Level (NOAEL) and a Lowest Observed Adverse Effect Level (LOAEL) of 97 and 415 mg/m$^3$ (20 and 86 ppm), respectively, based on an anemia endpoint (Dodd *et al.*, 1983).

Whole body inhalation exposure of 400 – 500 g, 5-week old Hartley albino guinea pigs for 1 hour to EGBE at 3,057 mg/m$^3$ (633 ppm; 5 males) and 3,338 mg/m$^3$ (691 ppm; 5 females) resulted in no mortality or clinical signs of toxicity immediately or up to 14 days following exposure (Gingell *et al.*, 1998). Eight-hour exposures of male guinea pigs to 3,212 mg/m$^3$ (665 ppm) EGBE by a different group did not result in increased osmotic

fragility or hemoglobinuria (Carpenter *et al.*, 1956). No information was provided regarding the guinea pig strain, age, or BW for the 8-hour study.

### *5.3.2 Species Differences*

Substantial species differences exist among experimental animals in their acute/sub-acute responses to EGBE. In sensitive mammalian species, hemolytic anemia and increased RBC osmotic fragility are primary toxic endpoints of EGBE exposure.

According to the study by Carpenter *et al.*(1956), hemolytic responses were observed in highly susceptible species including rats, mice and rabbits, but not humans, monkeys, dogs and guinea pigs. Some of the responses reported by Carpenter *et al*. (1956) have been observed in at least one other *in vivo* study (Ghanayem and Sullivan, 1993) and two *in vitro* studies (Corley *et al.*, 1994; Udden, 2002). In their comparison of hematological parameters in rats and guinea pigs, Ghanayem and Sullivan noted that a single gavage administration of EGBE to rats at 250 mg/kg caused an early increase (1 hour post-treatment) in mean corpuscular volume and Hct, which declined over a 24 hour period. This was associated with hemolysis and a decline in Hgb and RBC numbers. However, the same treatment in guinea pigs had no similar effect (Ghanayem and Sullivan, 1993).

Species comparisons by Carpenter *et al*. (1956) were primarily made using results of separate exposures for each tested species. However, simultaneous chamber exposures of six rats and two men to EGBE (546 mg/m$^3$ ,113 ppm) for four hours showed humans to be insensitive to hemolytic endpoints at this dose level when compared to rats (Carpenter *et al.*, 1956). No differences in pre- and post-exposure RBC fragility were observed in the men, but according to the authors, RBC fragility in rats “rose appreciably.” These rat responses were not quantified in the text.

In 30-day exposures in C3H mice (7 hrs/day/ 5 days/wk), hemoglobinuria was observed after the first 7-hour exposure to 966 mg/m$^3$ (200 ppm) (n= 9 of 60) and 1,932 mg/m$^3$ (400 ppm) (n = 26 of 60) EGBE (Carpenter *et al.*, 1956). This effect was not apparent after the third 7-hour exposure. No hemoglobinuria was observed in mice exposed to 483 mg/m$^3$ (100 ppm) for 7 hours. However, RBC osmotic fragility was noted at all three concentrations after the first 7-hour exposure.

Rabbits exposed to 604 or 952 mg/m$^3$ (125 or 197 ppm) EGBE for 7 hours were reported to have significantly increased RBC osmotic fragility at 604 mg/m$^3$ (125 ppm) but no hemoglobinuria at concentrations up to 952 mg/m$^3$ (197 ppm) (Carpenter *et al.*,

1956). (Animal numbers were not stated, the *p*-value was not defined, and it was unclear from the text that a control group was included.)

Experiments in dogs suggested that concentrations up to 966 mg/m$^3$ (200 ppm) may have no adverse effects in the short term, while those at 1860 mg/m$^3$ (385 ppm) and above may be lethal. Dogs (n = 1/sex) exposed to 966 mg/m$^3$ (200 ppm) EGBE intermittently for 7 hrs/day showed no apparent toxic manifestations during the first two weeks of exposure. Toxic manifestations were observed during the first week of exposure in dogs (n = 1/sex) breathing 1860 mg/m$^3$ (385 ppm) EGBE 7 hrs/day. Responses included emesis, generalized weakness, increased RBC osmotic fragility, and in the male only, significantly decreased RBC count and Hgb levels. (It is unknown whether statistical tests were done for RBC fragility, and for the last two endpoints, no *p*-values were given.) The female dog died after the 8th day. Daily 7-hour exposures of 2702 mg/m$^3$ (617 ppm) EGBE in one female dog resulted in emesis and extreme weakness during the first two days, with death occurring near the end of the second day (Carpenter *et al.*, 1956).

Monkeys(n = 1/sex; strain not identified) exposed to 966 mg/m$^3$ (200 ppm) EGBE for 7 hours did not result in increased osmotic RBC fragility or hemoglobinuria. However, a rhesus monkey (sex unstated) exposed to 1014 mg/m$^3$ (210 ppm) EGBE for 30 days (7 hrs/day, 5 days/wk) exhibited transient RBC osmotic fragility after the fourth exposure.

Table 6 presents a summary of the *in vivo* findings from acute and subacute studies with animals.

**Table 6. Summary of acute and subacute EGBE inhalation exposure studies in animals.**

| Reference | Species | Exposure | Results |
|---|---|---|---|
| Kane *et al.* (1980) | Mice (n = 4/group) Age not indicated | 676 – 6762 mg/m$^3$ (140 – 1400 ppm) for 10 min | RD50 (50% depression in respiratory rate) at 7995 mg/m$^3$ (2825 ppm). |
| Dodd *et al.* (1983) | Rats (n = 6/sex/group) 6-7 wks old | 976, 2526, or 4188 mg/m$^3$ (202, 523, or 867 ppm) for 4 hrs | LC50: 2347 mg/m$^3$ (486 ppm) for males and 2174 mg/m$^3$ (450 ppm) for females. Other findings include ↓ coordination, ↑ rate of shallow breathing, and red urogenital discharge in all rats. Death at highest concentration. |
| | Rats n = 8/sex/group 6-7 wks old | 0, 97, 415, or 1183 mg/m$^3$ (0, 20, 86 or 245 ppm), for 9 days (6 hrs/day, 5 days/wk) | Transiently ↓ RBCs, Hgb, MCH and BWGs, and ↑ reticulocytes, lymphocytes, NRBCs, and liver weights at the two highest exposure concentrations.) |
| Gingell *et al.* (1998) | Guinea pigs n = 5/sex/group 5 wks old | 3057 mg/m$^3$ (633 ppm; males) or 3338 mg/m$^3$ (691 ppm; females) for 1 hr | LC50: > 3057 mg/m$^3$ (633 ppm) for males and > 3338 mg/m$^3$ (691 ppm) for females. No mortality or clinical signs of toxicity. |

Legend: BWG – Body weight gain; Hgb – Hemoglobin; MCH – Mean corpuscular Hgb; NRBC – Nucleated red blood cell; RBC – Red blood cell; LC50: lethal concentration required to kill 50% of the population.

**Table 6. Summary of acute and subacute EGBE inhalation exposure studies in animals (continued).**

| Reference | Species | Exposure | Results |
|---|---|---|---|
| Carpenter *et al.* (1956) | Mice<br>n = 15/sex/group<br>age not stated | 0, 483, 966, or 1932 mg/m$^3$ (0, 100, 200, or 400 ppm) for 7 hrs | Hemoglobinuria at 966 and 1932 mg/m$^3$ (200 and 400 ppm)<br>↑ RBC osmotic fragility at all EGBE exposures |
| | Rats<br>n= 5 – 6/group<br>ages variable | 604, 1208, 1811, 2415 or 3864 mg/m$^3$ (125, 250, 375, 500 or 800 ppm) for 4 to 8 hrs | Hemoglobinuria in all groups. Death at >250 ppm (1208 mg/m$^3$) in 5-6-week old rats and >125 ppm (604 mg/m$^3$) in "older rats":<br>5-6 week-old rats:<br>NOAEL 604 mg/m$^3$ (125 ppm)<br>LOAEL 1208 mg/m$^3$ (250 ppm) |
| | Guinea pigs<br>n = 2 per group<br>age not stated | 3212 mg/m$^3$ (665 ppm) for 8 hrs | No effect on RBC osmotic fragility or hemoglobinuria |
| | Rabbits<br>n = 2-4/group<br>age not stated | 604 or 952 mg/m$^3$ (125 or 197 ppm) for 7 hrs | ↑ RBC osmotic fragility at 604 mg/m$^3$ (125 ppm), but no hemoglobinuria up to 952 mg/m$^3$ (197 ppm) |
| | Dogs<br>n = 1 - 2/group<br>age not stated | 966, 1860 or 2980 mg/m$^3$ (200, 385 or 617 ppm) for for 1 to 5 days (7 hrs/day) | 966 mg/m$^3$ (200 ppm) - No effect.<br>1860 mg/m$^3$ (385 ppm) - Emesis, weakness, ↑ RBC osmotic fragility, ↓ RBC count and Hgb.<br>2980 mg/m$^3$ (617 ppm) - Emesis, extreme weakness, death at end of day 2. |
| | Monkeys<br>n = 1 - 2/group<br>age not stated | 966 mg/m$^3$ (200 ppm) for 7 hrs or<br>1014 mg/m$^3$ (210 ppm) for 7 hrs/day, 5 days/wk | No ↑ RBC osmotic fragility or hemoglobinuria for 7 hours exposure.<br>On 4$^{th}$ day of exposure to 1014 mg/m$^3$ (210 ppm) - ↑ osmotic fragility |

Legend: Hgb – Hemoglobin;RBC – Red blood cell

### *5.3.3 In Vitro Studies*

*In vitro* studies have shown considerably less risk of hemolysis in human RBCs compared to rat RBCs when blood is incubated with BAA (Corley *et al.*, 1994; Udden, 2002). After comparing *in vivo* and *in vitro* studies for several species, including monkeys, Carpenter *et al.* (1956) stated "the *in vivo* response of erythrocytes [RBCs] to butyl Cellosolve [EGBE] is more closely correlated to the *in vitro* response to sodium butoxyacetate [BAA] than to the *in vitro* response to butyl Cellosolve. This suggests that [BAA] is more directly responsible for *in vivo* RBC fragility or hemolysis than is [EGBE]."

PBPK modeling for exposures to saturated atmospheres of EGBE showed that the maximum blood concentration (up to 195 µM) of BAA is below that needed to produce hemolysis in humans. An *in vitro* no effect level of 2 mM BAA was observed by Corley *et al.* (1997) when BAA was incubated for 4 hours with "normal" human blood and blood from humans susceptible to hemolysis. These *in vitro* studies with human blood suggested that pre-hemolytic changes (*i.e.* increased osmotic fragility) are not reached until concentrations are well above 5 mM in 4-hour incubations (Udden, Corley's personal communication). The resistance of RBCs in healthy adults to the hemolytic effects of BAA *in vitro* extends to RBCs from elderly individuals, children, and individuals with sickle cell disease or hereditary spherocytosis (Udden and Patton, 1994; Udden, 2002). Given these cumulative findings by Corley, Udden, and their respective colleagues, human inhalation exposure to EGBE is unlikely to reach a concentration that will cause hemolysis. At the same time, human RBC resistance to hemolysis may be overwhelmed by high oral exposures in individuals who ingest high amounts of EGBE, as previous studies have shown it to have some hematoxicity with intakes ranging from 25 – 225 g (Carpenter *et al.*, 1956; Gijsenbergh *et al.*, 1989; Raymond *et al.*, 1998), with at least one case of hemoglobinuria (Gijsenbergh *et al.*, 1989).

Some chemicals have a protective effect, known as heteroprotection, against the hemolytic effects of EGBE exposure. For example, research by Palkar *et al.* (2007) showed that a priming dose of phenylhydrazine may protect rats from the hemolytic and lethal effects of BAA. The authors stated that this heteroprotection may be due to phenylhydrazine-treated rats having lower renal and hepatic BAA levels and approximately 3-fold higher urinary excretion of BAA compared to control rats. However, hepatic ADH and ALDH activities were unaltered, indicating that bioactivation of EGBE to BAA was unaffected by phenylhydrazine. Instead, higher erythropoietin levels, reticulocyte count, and resiliency of RBCs in phenylhydrazine-primed rats indicated that newly formed RBCs were resistant to the hemolytic action of BAA (Palkar *et al.*, 2007).

Young rodent RBCs have been found to be less sensitive to BAA than older RBCs (Ghanayem *et al.*, 1992).

# 6. Chronic Toxicity of EGBE

## 6.1 Chronic Toxicity to Adult Humans

### *6.1.1 Occupational Inhalation Exposure*

Occupations in the US that lead to personal exposures to EGBE above the NIOSH REL of 24 mg/m$^3$ (5 ppm) have been reported by ATSDR (1998). These include silk screening and printing press operations, furniture production and asbestos/mastic removal. Other sources of exposure include spray painting operations, specialty chemical production, and paint formulating. The occupational studies summarized below investigated the effects of EGBE, primarily on erythroid endpoints.

One cross-sectional study (Haufroid *et al.*, 1997) reported significant effects in some of the measured erythroid parameters. The study included 31 male workers (22 - 45 years old) who had been employed for 1 to 6 years in a beverage packing plant. These workers were exposed at a mean concentration (± SD) of 2.91 ± 1.30 mg/m$^3$ (0.59 ± 0.27 ppm) EGBE in varnish or during external décor production. (It was not stated whether the mean and SD were geometric or arithmetic.) Co-exposure to methyl ethyl ketone was also reported. The control group was comprised of 21 workers who were working in a shop or administrative section of the plant and not occupationally exposed to EGBE. There was a reasonably good correlation between the EGBE concentration in air and post-shift BAA in urine (average 10.4 mg/g creatinine; r = 0.55; $p$ = 0.0012), which was thought to be related to prevention of dermal absorption through use of gloves. Slight but significant effects on Hct (a 3.3% decrease, $p$ = 0.03) and MCH concentrations (a 2.1% increase, $p$ = 0.02) relative to controls suggested RBC membrane damage in exposed workers, but no significant effects were found for other erythroid parameters (*e.g.*, RBC number, Hgb, mean corpuscular volume, MCH, haptoglobin, and reticulocytes). The US Environmental Protection Agency (US EPA) (2010) noted that Hct and MCH concentrations in exposed workers reported by Haufroid *et al.* (1997) were still within normal clinical ranges.

In another occupational study, investigators evaluated the hematological status of nine parquet floorers exposed to a mean 8-hour concentration of 24.6 mg/m$^3$ (5.1 ppm) EGBE (max: 350 mg/m$^3$ (72 ppm) by personal air sampling) (Denkhaus *et al.*, 1986). The control group consisted of nine healthy age-matched volunteers (age 25 – 56 years, average 37.9) from non-EGBE-exposed occupations. No other details about the controls were available. An active "personal air" sampling technique was applied (pump: Compur 4900; UL SKC, USA), using NIOSH charcoal tubes (100 + 50 mg), which were

changed every hour during an 8-hour working period. The workers (age range = 25 – 58 years) had all been occupationally exposed to mixtures of organic solvents for an average of 18.9 years. Detected organic solvents in the air and in the blood samples included 1-butanol, iso-butanol, EGBE, 2-ethoxyethanol, 2-methoxyethynol, toluene, m-xylene, 2-butanone, and 2-hexanone. The workers' RBC counts showed a slight but non-significant ($p > 0.05$) decrease, and their Hgb concentrations were unaffected.

Hung *et al.* (2011) analyzed the Hgb concentration in the blood of 80 bicycle factory workers. These workers were divided into three groups based on EGBE exposure: decal transfer workers (high exposure, n=31), self-adhesive decal workers (moderate exposure, n=25) and assembly workers (little or no exposure, n=24). Based on personal air sampling (8-hour TWA), the decal transfer workers were exposed to an average concentration of 8.1 mg/m$^3$ (1.7 ppm) EGBE in air. A poor correlation was observed between air levels of EGBE and post-shift total BAA levels in urine due to considerable dermal absorption via direct contact on their hands with a dilute aqueous solution of EGBE. Only 3.7% of the increase in urinary BAA could be explained by airborne EGBE exposure. In the self-adhesive workers with only occasional inhalation and dermal EGBE exposure, end-shift total BAA levels were found to be about 10-fold less than that of the decal transfer worker group. In the assembly workers, personal air exposure to EGBE was not detected, and no BAA was found in the urine. Hgb test results showed assembly workers (24 females, no males) had a slightly higher mean Hgb concentration (8.02 ± 0.16 mmol/l) compared to decal transfer workers (7.72 ± 0.19 mmol/l; 30 females, one male) and the self-adhesive decal workers (7.80±0.19 mmol/l; 24 females, one male). However, no statistical difference was found between the assembly workers and the decal transfer workers (Mann Whitney U test, $p = 0.2731$). Normal levels of Hgb for females and males were regarded as 7.4-9.9 mmol/L and 8.3-10.9 mmol/L, respectively. The percentage of below-normal Hgb levels in the decal transfer group (29%, 9 of 31) appeared higher than the self-adhesive decal workers (28%, 7 of 25) and the assembly workers (21%, 5 of 24). However, the difference was not statistically significant ($X^2$ test, $p = 0.4319$).

These studies showing slight changes in some hematological parameters with exposure to EGBE were not considered suitable by OEHHA to develop RELs because the changes were either non-significant or values were within normal ranges.

### *6.1.2 Exposure and Asthma Risk*

As noted in Section 3 of this document, EGBE is used in a variety of industrial and consumer products, including cleaning products. Exposure to substances in the workplace has been estimated to cause about 10% of all cases of adult-onset asthma (Blanc and Toren, 1999). A prospective study of 6,837 participants from 13 countries in the EU found the population-attributable risk for adult asthma due to occupational exposures ranged from 10% to 25%, equivalent to an incidence of new-onset occupational asthma of 250–300 cases per million people per year. Asthma risk was also reported to be increased in participants who reported an acute symptomatic inhalation event such as fire smoke exposure, mixing cleaning products, or chemical spills (RR = 3.3, 95% CI 1.0–11.1, p = 0.051) (Kogevinas *et al.*, 2007). Cleaning workers have been described as an exposure group at high risk of developing occupational asthma and asthma-like symptoms (Kogevinas *et al.*, 2007). However, the determination of which health hazards are associated with exposure to cleaning agents is a complex issue, and the contribution of sensitization to specific agents or exposure to irritants in the pathogenesis of respiratory symptoms associated with cleaning is unclear (Quirce and Barranco, 2010). A European Academy of Allergy and Clinical Immunology task force consensus statement indicated cleaning sprays, bleach, ammonia, disinfectants, mixing products, and specific job tasks have been identified as specific causes and/or triggers of asthma (Siracusa *et al.*, 2013). Siracusa *et al.* (2013) did not indicate that cleaning products containing glycol ethers (including EGBE) were specifically included as asthmagens in their assessment.

## 6.2 Chronic Toxicity to Infants and Children

Choi *et al.* (2010) conducted a case-control study of exposure to common household chemicals and the resulting prevalence of allergic airway disease in Swedish pre-school age children. Cases (n = 198) were defined, through a baseline questionnaire or a follow-up questionnaire (done 1.5-years after the baseline questionnaire), as children 3-8 years of age who were reported to have at least two symptoms of wheezing, rhinitis, or eczema without a cold during the preceding 12 months. Controls (n = 202) were randomly identified from 1,100 symptom-free children from local primary care clinics. Air and dust samples were collected from the bedrooms of the houses where the cases and controls lived and analyzed for several classes of VOCs, including glycols and glycol ethers.

Of the original population of cases and controls, 18 cases and 9 controls were found to have EGBE indoor air concentrations greater than the EGBE functional detection limit (not specified). No significant differences in the geometric mean EGBE indoor air concentrations were noted between the controls ($3 \times 10^{-3}$ mg/m$^3$; $6.21 \times 10^{-4}$ ppm; 95%

confidence interval (CI) $3 \times 10^{-4} – 2.96 \times 10^{-2}$ mg/m$^3$, $6.21 \times 10^{-5} – 6.13 \times 10^{-3}$ ppm) and the cases (3.11 µg/m$^3$, 0.64 ppm; 95% CI 0.76-12.67 µg/m$^3$, 0.16-2.62 ppm).

## 6.3 Chronic Toxicity to Experimental Animals

The principal toxic effect of exposure to EGBE in sensitive species is reversible hemolytic anemia. In rodents, the primary effect on the hematologic system was anemia characterized as macrocytic (rat), normocytic (mouse), normochromic, and regenerative in exposed rats and mice (NTP, 2000). More generally, EGBE also causes irritation and damage to epithelial tissues at portal of entry sites (*i.e*., eyes and respiratory airways).

In a series of experiments by Carpenter *et al.* (Carpenter *et al.*, 1956), both rodent (mice, rats, and guinea pigs) and non-rodent (rabbits, dogs, and monkeys) species were exposed to EGBE via inhalation for 7 hrs/day, 5 days/wk for up to 90 days. The authors did not indicate which statistical methods were used, and in many cases, it was unclear whether the reported biological responses were statistically significant.

Groups of male mice (n = 10 - 15 /group) exposed to 0, 541, 966, or 1,932 mg/m$^3$ (0, 112, 200, or 400 ppm) EGBE for 30, 60, or 90 days exhibited RBC fragility at all concentrations. Fragility appeared to be as great after the first exposure as it was after 89$^{th}$ exposure, and, in all instances, was normal after a 17-hour rest. At 1,932 mg/m$^3$ (400 ppm), liver weights normalized to BW were significantly ($p < 0.05$) decreased relative to controls after 30 exposure days and significantly increased after 60 or 90 exposure days. Normalized liver weights of mice exposed at this concentration for 90 days and allowed a 42-day rest period prior to necropsy were not significantly different from controls. Transient hemoglobinuria was also observed at the highest concentration. However, no mortality occurred, and no gross pathology of organs was observed 42 days after cessation of exposure (Carpenter *et al.*, 1956).

Male and female Sherman rats (n = 15/sex/group; 140 – 190 g) were exposed to EGBE at concentrations ranging from 0 – 2,087 mg/m$^3$ (0 – 432 ppm) for 30 days (6 weeks). A dose-dependent increase in RBC osmotic fragility was observed at all exposure levels. At 517 and 980 mg/m$^3$ (107 and 203 ppm), “significant [$p < 0.05$] increases” in liver weights (normalized to BW) were observed in male and female rats compared to controls. Normalized kidney weights were significantly ($p < 0.05$) increased relative to controls at the 517 mg/m$^3$ (107 ppm) exposure concentration, and hemoglobinuria was evident at concentrations ≥980 mg/m$^3$ (203 ppm). Liver and kidney weight data were not provided for groups exposed at ≥1,517 and ≥980 mg/m$^3$ (≥314 and ≥203 ppm), respectively. However, at concentrations ≥1,517 mg/m$^3$ (≥314 ppm), cloudy swelling of the liver was noted upon histological examination. Gross pathological

findings at the same concentrations included hemorrhage of the lungs and congestion of the lungs and abdominal viscera. Deaths also occurred at ≥1,517 mg/m$^3$ (≥314 ppm), but at these concentrations, females appeared more susceptible to the effects of EGBE, with 100% mortality at 1,517 and 2,087 mg/m$^3$ (314 and 432 ppm) in contrast to 0% and 80% mortality in males, respectively (Carpenter *et al.*, 1956).

Male guinea pigs (n = 10/group; 435 – 580 g; age and strain not stated) exposed to EGBE at 0, 261, 517, 980, 1,517, or 2,386 mg/m$^3$ (0, 54, 107, 203, 376, or 494 ppm, respectively) for 30 days did not show evidence of RBC hemolysis at any concentration. BW-normalized kidney weights were significantly ($p < 0.05$) increased relative to controls at (≥517 mg/m$^3$) (≥107 ppm), but no significant effects were observed with respect to liver weights. Lung congestion and kidney swelling were the only findings among the three animals that died at 1,517 mg/m$^3$ (376 ppm) or higher (Carpenter *et al.*, 1956).

Several experiments in dogs were performed by Carpenter *et al.* (Carpenter *et al.*, 1956). In one experiment, Basenji dogs from the same litter (n = 1/sex/group; age not stated) were exposed to EGBE at 0 or 966 mg/m$^3$ (0 or 200 ppm) for 31 days. RBC osmotic fragility, compared to similar control dogs, increased slightly (not statistically significant) in the EGBE-exposed male and female.

A separate inhalation experiment by the same authors, exposed male and female wire-haired terrier littermates (n = 1/sex; age = 8 months) to EGBE at 483 mg/m$^3$ (100 ppm) for 90 days. Hematological parameters were tested before exposure, for use as the baseline control, and after 90 days of exposure. Midway through the 90-day exposure, transient doubling of the leucocyte count was observed in both dogs. By the end of the exposure period, the leucocyte count in the female returned to baseline, while that in the male remained 50% higher than the pre-exposure level. Hct values in males decreased from 43% packed RBC volume before the first exposure to 34.5% after 90-days exposure.

In high-exposure, short-term, repeated inhalation experiments, two Basenji hybrid dogs (n = 1/sex) were exposed to EGBE at 1,860 mg/m$^3$ (385 ppm) for 27 – 28 exposures. No controls were used. Both dogs exhibited nasal and ocular infection, generalized weakness, apathy, anorexia, emesis and death following the 8$^{th}$ (female) and 28$^{th}$ (male) exposures. It was of note that in an RBC osmotic fragility test using varying degrees of saline concentration, the fragility value of the male dog RBCs reached a maximum of 0.54-0.42% saline (saline concentrations eliciting initial and complete hemolysis, respectively) in *7* days and fell to 0.32-0.20% saline after *27* days. The authors stated this demonstrated that “all susceptible RBCs had been removed from

this animal's blood stream" (Carpenter *et al.*, 1956). Although RBC fragility was not discussed for the female, it was reported that she exhibited severe hemorrhage of the lung, and congestion of the lung, kidneys and liver.

Two monkeys (n = 1/sex; age and strain not stated) were also exposed to 483 mg/m$^3$ (100 ppm) EGBE for 90 days. Transient RBC osmotic fragility was observed in both monkeys, with a greater response in females versus males. However, by the end of the exposure period, RBCs returned to "normal." The authors did not mention controls. Pulmonary tuberculosis was also found in both monkeys at autopsy, at a level that may have obscured EGBE-related effects. Tuberculosis has been shown to contribute to decreased RBC osmotic resistance (Marks *et al.*, 2002; Reddy *et al.*, 2012). However, no other noteworthy histopathological findings were reported. A separate study with one rhesus monkey (age not stated) exposed to 1,014 mg/m$^3$ (210 ppm) EGBE for 30 days resulted in transiently increased RBC fragility (RBCs returned to baseline overnight.), a quadrupled level of plasma fibrinogen, and a 50% decreased RBC count and Hgb level after the 4$^{th}$, 14$^{th}$, and 30$^{th}$ exposure, respectively. Emesis was observed four times during the latter part of the exposure period, and a suggestion of pulmonary tuberculosis was reported at autopsy. In this study, pre-exposure hematological values served as controls (Carpenter *et al.*, 1956).

In a 90-day inhalation experiment by Dodd *et al.* (1983), Fischer 344 rats (16 rats/sex/group; 6 – 7 weeks old) were exposed for 13 weeks (6 hrs/day, 5 days/wk) to EGBE at target concentrations of 0, 24, 121, or 372 mg/m$^3$ (0, 5, 25, or 77 ppm, respectively). A subset of six rats/sex/group was killed after 6 weeks of exposure for hematologic evaluation only. Significantly decreased RBC (13% below control, $p < 0.01$) and slightly decreased Hgb (4.5% below control, not statistically significant) concentrations were reported, accompanied by increased MCH (11% above control) in 372 mg/m$^3$- (77 ppm) exposed females after 6 weeks. At the end of the exposure period, RBC and MCH levels in these females were still significantly different from control (7% lower; $p < 0.01$, and 4% higher; $p < 0.001$, respectively). The only significant hematological finding in males was a 5% decrease in MCH relative to controls, which occurred in the 372 mg/m$^3$ (77 ppm) exposed group. The severity of RBC depression in this study was not increased compared to the 9-day study (discussed in Section 5.3.2). There were no significant biological effects in rats exposed subchronically at the 24 mg/m$^3$ (5 ppm) EGBE concentration. Therefore, NOAEL and LOAEL values of 121 and 372 mg/m$^3$ (25 and 77 ppm), respectively, are appropriate for anemia in male and female rats from this study.

Chronic/subchronic EGBE toxicity studies by Carpenter *et al.* (1956) and Dodd *et al.* (1983) are summarized in Table 7.

**Table 7. Summary of chronic/subchronic EGBE inhalation studies by Carpenter *et al.* (1956) and Dodd *et al.* (1983).**

| Reference | Species | Exposure | Results |
|---|---|---|---|
| Carpenter *et al.* (1956)[a] | Male mice n = 10-15/group | 0, 541, 966, or 1932 mg/m$^3$ (0, 112, 200, or 400 ppm) for 30 –90 days | Transient RBC osmotic fragility in all EGBE-exposed groups. Transient hemoglobinuria and liver weight changes at 1932 mg/m$^3$ (400 ppm). |
| | Rats n= 15/sex/group | 0, 261, 517, 981, 1517, or 2087 mg/m$^3$ (0, 54, 107, 203, 314, or 432 ppm) for 30 days | RBC osmotic fragility in all EGBE-exposed groups. At 517 mg/m$^3$ (107 ppm), ↑ kidney and liver weights. At ≥980 mg/m$^3$ (203 ppm), hemoglobinuria, ↑ liver weights. Deaths at ≥1517 mg/m$^3$ (314 ppm). |
| | Male guinea pigs n = 10/group | 0, 261, 517, 981, 1517, or 2386 mg/m$^3$ (0, 54, 107, 203, 376, or 494 ppm) for 30 days | No effect on RBC hemolysis. ↑ kidney weights at ≥517 mg/m$^3$ (≥107 ppm). Lung congestion and kidney swelling in 3 animals that died at ≥1,517 mg/m$^3$ (≥376 ppm). |

Legend: Hct – Hematocrit; RBC – Red blood cell; WBC – White blood cell (leukocyte).
[a]Animals were exposed 7 hrs/day, 5 days/wk for up to 90 days.

**Table 7. Summary of chronic/subchronic EGBE inhalation studies by Carpenter *et al.* (1956) and Dodd *et al.* (1983) (continued).**

| | | | |
|---|---|---|---|
| Carpenter *et al.* (1956)[a] | Dogs n = 1/sex/group | 0 or 966 mg/m$^3$ (0 or 200 ppm) for 31 days | RBC osmotic fragility (not statistically significant) |
| | Dogs n = 1/sex | 483 mg/m$^3$ (100 ppm) for 90 days | ↑ WBCs in both dogs midway through the exposure period, with the female's returning to baseline and the male's remaining ~50% higher at the end of exposure. In males, ↓ Hct after 90-days. |
| | Dogs n = 1/sex | 1859.55 mg/m$^3$ (385 ppm) for 27 – 28 days | In both dogs, nasal and ocular infection, generalized weakness, apathy, anorexia, emesis and death. Temporally variable RBC osmotic fragility in the male. |
| | Monkeys n = 1/sex | 483 mg/m$^3$ (100 ppm) for 90 days | Transiently ↑ RBC osmotic fragility. Pulmonary tuberculosis. |
| | Monkey n = 1 | 1014.3 mg/m$^3$ (210 ppm) EGBE for 30 days | Emesis, ↑ plasma fibrinogen, ↓ RBCs, ↓ Hgb, and transient RBC fragility at various timepoints. Suggestion of pulmonary tuberculosis |
| Dodd *et al.* (1983) | Rats n=16/sex/group | 0, 24, 121, or 372 mg/m$^3$ (0, 5, 25, or 77 ppm) for 13 weeks (6 hrs/day, 5 days/wk) | ↓ RBCs and Hgb, and ↑ MCH in 372 mg/m$^3$- (77 ppm-) exposed females after 6 weeks. RBC and MCH responses remained until the end of the exposure period, but decreased in magnitude. In males, ↓ MCH at the highest exposure concentration. |

Legend: Hgb – Hemoglobin; MCH – Mean corpuscular hemoglobin; RBC – Red blood cell;
[a]Animals were exposed 7 hrs/day, 5 days/wk for up to 90 days.

Subsequently, NTP (2000) conducted a 14-week whole-body EGBE inhalation exposure study in Fischer 344 rats and B6C3F$_1$ mice. Exposure (6 hrs/day, 5 days/wk) to 150, 302, 604, 1208, or 2415 mg/m$^3$ (31, 62.5, 125, 250, or 500 ppm) EGBE resulted in clinical findings that included abnormal breathing, pallor, red urine stains, nasal and eye discharge, lethargy, and increased salivation and/or lacrimation primarily at the three highest concentrations in rats, and at the highest concentration in mice. The most

pronounced effect was concentration-related hemolytic anemia in male rats and mice exposed to 604 mg/m$^3$ (125 ppm) or above and, to a greater extent, in all exposed groups of female rats and mice. Exposure-related increases in the incidences of forestomach inflammation and epithelial hyperplasia, bone marrow hyperplasia (rats only), Kupffer cell pigmentation of the liver, splenic hematopoietic cell proliferation, and renal tubule pigmentation were observed in male and/or female rats and mice surviving to the end of the study. The latter three effects were secondary to red cell hemolysis and regenerative anemia, with female rats showing the greatest sensitivity. Statistically significant increases in Kupffer cell pigmentation and bone marrow hyperplasia were apparent in female rats at concentrations as low as 302 mg/m$^3$ (62.5 ppm).

In the following NTP 2-year study, Fischer 344 rats and B6C3F$_1$ mice were exposed to 0, 151 (rats only), 302, 604, or 1,208 (mice only) mg/m$^3$ (0, 31.2, 62.5, 125, and 250 ppm) EGBE via inhalation for 6-hrs/day, 5 days/wk. In rats, anemia occurred in females starting at 151 mg/m$^3$ (31.2 ppm), and in males starting at 302 mg/m$^3$ (62.5 ppm). The anemia was considered mild and persisted with no apparent progression or amelioration of severity from 3 months to 12 months (final blood collection). Incidences of hyaline degeneration of the olfactory epithelium were increased in 302 or 604 mg/m$^3$ (62.5 or 125 ppm) groups of both sexes, although the severity of this lesion was minimal (incidence presented in Table 8).

In mice, survival of males was reduced at 604 and 1,208 mg/m$^3$ (125 and 250 ppm) concentrations (NTP, 2000). Anemia was observed following 3, 6, or 12 months of exposure at 604 or 1,208 mg/m$^3$ (125 or 250 ppm) in both male and female mice. Incidences of forestomach ulcer and hyperplasia, and nasal hyaline degeneration of olfactory and respiratory epithelia were increased in all exposed female mice. In male mice, there was an increased incidence of forestomach ulcer at 604 mg/m$^3$ (125 ppm). All groups of exposed males showed increased incidence of forestomach hyperplasia. A mouse urologic infection syndrome was apparent in males, and appeared to be exacerbated by EGBE exposure at the 604 and 1,208 mg/m$^3$ (125 and 250 ppm) concentrations. Effects secondary to hemolysis were also observed including splenic congestion and hemosiderin deposition in Kupffer cells of the liver in both rats and mice (incidence presented in Table 8). The principal non-cancer toxic endpoints not linked to RBC hemolysis were nasal olfactory epithelial lesions (hyaline degeneration), forestomach epithelial hyperplasia, and forestomach ulcers (incidences presented in Table 8).

**Table 8. Incidence of nasal olfactory epithelial hyaline degeneration, liver Kupffer cell pigmentation, forestomach epithelial hyperplasia and ulcer in rats and mice following 2-year EGBE inhalation study (NTP, 2000)**

| Endpoints | Exposure Doses mg/m$^3$ (ppm) | | | | | Trend test *p*-value |
|---|---|---|---|---|---|---|
| | 0 | 151 (31.2) | 302 (62.5) | 604 (125) | 1208 (250) | |
| **Nasal Olfactory Epithelial Hyaline Degeneration** | | | | | | |
| Male Rats | 13/48 | 21/49 | 23/49$^{*}$ | 40/50$^{***}$ | -------- | <0.0001 |
| Female Rats | 13/50 | 18/48 | 28/50$^{**}$ | 40/49$^{***}$ | -------- | <0.0001 |
| *Total Rats* | *26/98* | *39/97*$^{*}$ | *51/99*$^{***}$ | *80/99*$^{***}$ | -------- | *<0.0001* |
| Male Mice | 1/50 | -------- | 2/50 | 3/48 | 1/48 | 0.5074 |
| Female Mice | 6/50 | -------- | 14/50* | 11/49 | 12/50 | 0.1532 |
| *Total Mice* | *7/100* | -------- | *16/100** | *14/97* | *13/98* | *0.1743* |
| **Liver Kupffer Cell Pigmentation** | | | | | | |
| Male Rats | 23/50 | 30/50 | 34/50$^{*}$ | 42/50$^{***}$ | -------- | <0.0001 |
| Female Rats | 15/50 | 19/50 | 36/50$^{***}$ | 47/50$^{***}$ | -------- | <0.0001 |
| *Total Rats* | *38/100* | *49/100* | *70/100*$^{***}$ | *89/100*$^{***}$ | -------- | *<0.0001* |
| Male Mice | 0/50 | -------- | 0/50 | 8/49$^{**}$ | 30/49$^{***}$ | <0.0001 |
| Female Mice | 0/50 | -------- | 5/50$^{*}$ | 25/49$^{***}$ | 44/50$^{***}$ | <0.0001 |
| *Total Mice* | *0/100* | -------- | *5/100*$^{*}$ | *33/98*$^{***}$ | *74/99*$^{***}$ | *<0.0001* |
| **Forestomach Epithelial Hyperplasia** | | | | | | |
| Male Mice | 1/50 | -------- | 7/50$^{*}$ | 16/49$^{***}$ | 21/48$^{***}$ | <0.0001 |
| Female Mice | 6/50 | -------- | 27/50$^{***}$ | 42/49$^{***}$ | 44/50$^{***}$ | <0.0001 |
| *Total Mice* | *7/100* | -------- | *34/100*$^{***}$ | *58/98*$^{***}$ | *65/98*$^{***}$ | *<0.0001* |
| **Forestomach Ulcer** | | | | | | |
| Male Mice | 1/50 | -------- | 2/50 | 9/49$^{**}$ | 3/48 | 0.1324 |
| Female Mice | 1/50 | -------- | 7/50$^{*}$ | 13/49$^{***}$ | 22/50$^{***}$ | <0.0001 |
| *Total Mice* | *2/100* | -------- | *9/100*$^{*}$ | *22/98*$^{***}$ | *25/98*$^{***}$ | *<0.0001* |

Note: Statistically significant differences compared to the control group were measured with the Fisher exact test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (statistical analysis performed by OEHHA). Trend test incorporated in BMDS software (version 2.6) (EPA, 2015).

Chronic contact irritation by EGBE, and in particular the EGBE metabolites BAA and 2-butoxyacetaldehyde, has been implicated in the damage to the forestomach in mice (Green *et al.*, 2002; Poet *et al.*, 2003). Metabolism of EGBE by ADH to BAA in the

rodent forestomach is thought to play a role in the development of epithelial hyperplasia and ulcers. A similar mechanism of action in rat and mouse nasal olfactory epithelium also likely occurs (Gift, 2005). Intravenous, oral, and inhalation studies have shown accumulation of EGBE and BAA in the mouse forestomach (Boatman *et al.*, 2004). Thus, systemic blood circulation, grooming of contaminated fur, and clearance of mucus from the respiratory tract are all factors in the accumulation of EGBE in the forestomach (NTP, 2000). For the development of the chronic REL, we focus on the respiratory endpoints (upper respiratory tract irritation and nasal hyaline degeneration of the olfactory epithelium) due to their greater relevance for human exposure. Details for selecting the endpoint to derive the 8-hour and chronic RELs for EGBE are provided in Section 8.2.

# 7. Developmental and Reproductive Effects

EGBE is not listed as a developmental or reproductive toxicant under California Proposition 65 (OEHHA, 2016). Unlike some structurally-similar glycol ethers listed under Proposition 65, EGBE exposure did not cause significant effects in the male reproductive organs, including testes (Dodd *et al.*, 1983; NTP, 2000). Quantitative Structure Toxicity Relationship (QSTR) models have also predicted that EGBE has no developmental toxicity (Ruiz *et al.*, 2011).

The following studies in animals have been conducted to investigate the effects of EGBE on the female reproductive system and the embryo.

In an inhalation study, EGBE was vaporized at doses of 0, 725, 966 mg/m$^3$ (0, 150, 200 ppm) and administered to approximately 15 pregnant SD rats in each exposure group (except control; n = 34) for 7 hrs/day on gestational days (GD) 7 – 15. Dams were sacrificed on GD 20, and data were analyzed on a litter basis. Some hematuria was observed on the first day of exposure in the group exposed to 966 mg/m$^3$ (200 ppm) EGBE, but no increase in congenital defects was observed at that concentration. No other adverse effects were observed in the dams or the pups in either treatment group. The number of resorptions and fetal weights, and the incidence of malformations did not differ from the controls (Nelson *et al.*, 1984).

In another inhalation study of developmental toxicity, female Fisher 344 rats (n = 36/group) and female New Zealand White rabbits (n = 24/group) were exposed to EGBE vapors at 0, 121, 242, 483, or 966 mg/m$^3$ (0, 25, 50, 100, or 200 ppm, respectively) for 6 hrs/day on GD 6-15 for rats and GD 6-18 for rabbits (Tyl *et al.*, 1984). In rats, maternal toxicity included evidence of anemia, and significantly ($p < 0.05$) decreased BW gain and food consumption relative to controls in the 483 and 966 mg/m$^3$

(100 and 200 ppm) groups. Embryotoxicity included, at the highest concentration (966 mg/m$^3$; 200 ppm), significantly decreased numbers of viable implantations and percent live fetuses per litter, and significantly increased numbers of totally resorbed litters. At 483 and 966 mg/m$^3$ (100 and 200 ppm), significantly delayed skeletal ossification in offspring was observed. In rabbits, toxicity included maternal deaths, spontaneous abortions and significantly decreased BW at 966 mg/m$^3$ (200 ppm) relative to control, while hematological parameters were normal. Embryotoxicity was indicated by significantly reduced gravid uterine weight and a significant concomitant reduction in total and viable implantations at 966 mg/m$^3$ (200 ppm).

In a two-generation reproductive toxicity study, performed in accordance with NTP's Continuous Breeding Protocol, 11-week old outbred Swiss CD-1 mice of both sexes (n= 13-20/sex/group) were exposed to EGBE in drinking water available *ad libitum* at concentrations of 0 (distilled water), 0.5, 1, or 2% (weight/vol) (Heindel *et al.*, 1989; Heindel *et al.*, 1990; EPA, 2010). Using average fluid consumption and mean BW data from adult male mice, the authors estimated that at these concentrations, animals received 0, 700, 1300, or 2100 mg EGBE/kg BW-day, respectively. However, these data were not shown, and neither were corresponding data for adult females or weaned but sexually immature offspring, so it was unclear to OEHHA that the dose estimates were accurate for all animals. The study consisted of four separate, step-wise experiments including a dose-setting phase (not discussed here), a continuous breeding phase, a crossover breeding phase in which exposures were halted, and an offspring assessment phase, as prescribed in the NTP protocol. Results showed that EGBE exposure produced significant ($p < 0.05$) changes in BWs and organ weights relative to control. Decreased BWs and increased kidney and/or liver weights were observed in parental mice and their offspring at non-lethal doses (nominal 700 mg/kg BW-day). At the same time, this reproductive study had several issues which ultimately undermined the ability of the authors to make solid conclusions regarding the reproductive and developmental toxicity of EGBE. These included, but were not limited to:

1) two out of three EGBE exposure doses that resulted in excessive maternal toxicity;
2) no reported gross, histopathological, or weight analysis of female reproductive organs despite signs that they appeared more sensitive than males (which was confirmed in the crossover breeding phase); and
3) limited assessment of biological endpoints from offspring that died before birth or lived through the end of the offspring reproductive assessment phase.

Deaths in mid- and high-dose parental (filial generation 0; $F_0$) females in the continuous breeding phase equated to mortality of 30% (6/20) and 65% (13/20), respectively. In the crossover breeding phase, 7/20 females previously exposed at 1,300 mg/kg BW-day

appeared to have died prematurely. This represents a 35% mortality in the group, similar to that noted in the continuous breeding experiment. In contrast, no deaths were reported in $F_0$ males. These results suggested that the nominal 1,300 and 2,100 mg/kg BW/day doses may have been too high for assessing reproductive/developmental toxicity of EGBE in $F_0$ mice and their offspring. According to US EPA guidelines for developmental toxicity assessments (1991), the high dose should produce no more than 10% mortality in dams; otherwise, resulting responses [in dams and/or offspring] may be difficult to interpret and of limited value.

In an oral gavage study (Wier *et al.*, 1987), random-bred, virus-antibody-free CD- 1 pregnant mice were exposed to EGBE at doses of 0, 350, 650, 1,000, 1,500, and 2,000 mg/kg-day (6 animals per group) during GD 8-14 and sacrificed at GD18. Hemolytic effects in the dams were observed starting at 650 mg/kg-day. At 1,500 mg/kg-day and 2,000 mg/kg-day, the maternal mortality rate was 3/6 and 6/6 (50% and 100%), respectively. Increased resorption rates ($p \leq 0.05$, compared to the control) and a reduced number of viable fetuses were observed at exposures of 1,000 and 1,500 mg/kg-day. Four (all in the same litter) of 43 fetuses (9%) at 1,000 mg/kg-day and one of 25 fetuses (4%) at 1,500 mg/kg-day had cleft palates. For this study, the NOAEL for maternal toxicity was 350 mg/kg-day and the NOAEL for developmental toxicity was 650 mg/kg-day (Wier *et al.*, 1987; SCCP, 2007). Since only some of the offspring of pregnant mice exposed to very large doses of EGBE by gavage had cleft palates, it was concluded by EPA (2010) and ATSDR (1998) that EGBE was not significantly toxic to the reproductive organs of adult males or females, or to the developing fetuses of laboratory animals.

# 8. Derivation of Reference Exposure Levels

## 8.1 EGBE Acute Reference Exposure Level

| | |
|---|---|
| *Study* | Carpenter *et al.*, 1956 |
| *Study population* | 2 to 4 human subjects per study |
| *Exposure method* | Whole body exposure, 474, 546 and 942 mg/m$^3$ (98, 113 and 195 ppm) |
| *Exposure duration* | 8 hours, 474 and 942 mg/m$^3$ (98 and 195 ppm) in chamber or 4 hours, 546 mg/m$^3$ (113 ppm) in room |
| *Critical effects* | Subjective ocular and respiratory irritation |
| *LOAEL* | 473 mg/m$^3$ (98 ppm) |
| *NOAEL* | None |
| *Time- adjusted exposure* | None |
| *Human equivalent concentration (HEC)* | None |
| *LOAEL uncertainty factor ($UF_L$)* | 10 |
| *Subchronic uncertainty factor ($UF_S$)* | N/A |
| *Interspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{A-k}$)* | 1 |
| *Toxicodynamic ($UF_{A-d}$)* | 1 |
| *Intraspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{H-k}$)* | 1 (site of action; no systemic effects) |
| *Toxicodynamic ($UF_{H-d}$)* | 10 (potential asthma exacerbation in children; small sample size) |
| *Cumulative uncertainty factor* | 100 |
| *Reference Exposure Level* | 4700 µg/m$^3$ (1000 parts per billion (ppb)) |

RELs are based on the most sensitive and relevant health effects reported in the medical and toxicological literature. Acute RELs are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008). The acute EGBE REL is based on three whole-body human exposure studies of small sample size (n=2 to 4) (Carpenter *et al.*, 1956). These studies identified a LOAEL of 473 mg/m$^3$ (98 ppm), based on subjective sensory irritation. The response at 473 mg/m$^3$ (98 ppm) was reported to be nearly as great as that elicited at 942 mg/m$^3$ (195 ppm), which included immediate onset of nasal and throat irritation, followed by ocular irritation. Supporting studies (Johanson, 1986; Johanson *et al.*, 1986a; Jones *et al.*, 2003b) in which volunteers were exposed to lower concentrations of 97 mg/m$^3$ (20 ppm) and 237 mg/m$^3$ (49 ppm) examined some physiological responses during exposure but did not find obvious health effects. However, these studies were primarily toxicokinetic

studies that were not designed for a detailed analysis of acute sensory irritant effects or for a dose-response assessment (*i.e.*, both studies used a single dose exposure concentration).

For the acute REL derivation, the critical effects of trigeminal-mediated sensory irritation are usually a concentration-dependent response. Thus, no time-adjustment to the exposure was applied. Since these studies were conducted in humans, no interspecies UFs are required. However, a $UF_L$ of 10 to account for extrapolation from a LOAEL to a NOAEL was applied.

The toxicokinetic component of the intraspecies $UF_{H-k}$ is assigned a value of one. Chemicals that result in eye and upper respiratory sensory irritation are not predicted to be substantially different in children compared to adults when dosimetric adjustments are made (OEHHA, 2008). An intraspecies toxicokinetic of one ($UF_{H-k}$ = 1) is applied to acute sensory irritants if metabolic processes do not contribute to intraspecies variability. No systemic toxicity from metabolites (primarily BAA-related hemolysis) was observed during acute human exposures conducted by Carpenter *et al.*. *In vitro* studies have shown RBCs from children are similarly resistant to BAA-induced hemolysis as RBCs from adults.

The toxicodynamic component of the intraspecies $UF_{H-d}$ is assigned a value of 10 for potential exacerbation of asthma in sensitive subpopulations. In addition, the small sample size in the critical study (n= 3 - 4) warrants a larger intraspecies uncertainty factor. Epidemiological studies suggest cleaning products, including those products that utilize EGBE, increase the likelihood of an asthmatic episode in susceptible individuals (Bello *et al.*, 2009; Bello *et al.*, 2013; Fromme *et al.*, 2013; Gerster *et al.*, 2014). Although there is no direct evidence that EGBE by itself can exacerbate asthma, the respiratory irritation induced by inhaled EGBE may lead to an asthmatic reaction, particularly in children who may experience irritant-induced asthma; OEHHA views asthma as a more serious health problem in children than in adults (OEHHA, 2001). Thus, the cumulative UF is 100 and the acute REL is 4.7 $mg/m^3$ (1 ppm).

An acute animal exposure study was not chosen for the derivation of the acute REL. The LOAEL and NOAEL for the most sensitive endpoint, RBC hemolysis, in a subacute EGBE inhalation rat study (9 days total exposure; 5 days, two days of no exposure, then 4 days, 6 hrs/day) were 415 and 97 $mg/m^3$ (86 ppm and 20 ppm), respectively (Dodd *et al.*, 1983). This data set was not used to develop an acute REL because humans tend to be resistant to the hematological effects of EGBE and, as discussed above, the use of human toxicity data to develop a REL is preferred when possible over animal data

(OEHHA, 2008). Further, the multi-day exposure study design is not particularly amenable to estimating an acute REL, which is meant for infrequent 1-hour exposures.

More recent human exposure studies (primarily Jones *et al.* (2003) and Johanson *et al.* (1986a)) were also not used for derivation of the acute REL. There are several reasons why OEHHA staff decided not to use these studies as the point of departure (POD) for the acute REL derivation:

1. Physiological factors (e.g., breathing rate, pulse rate, skin surface temperature and skin resistance) may be less sensitive endpoints compared to subjective responses. These may overestimate the NOAEL and miss the most sensitive endpoint (*i.e.*, sensory irritation).

2. The toxicokinetic studies, mainly Jones *et al.* (2003) and Johanson *et al.* (1986a), used only one exposure concentration and produced no apparent adverse effects on the human subjects. As such, they are free-standing NOAELs. Our revised Noncancer REL TSD 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.*" In other words, OEHHA guidance does not recommend using a NOAEL and a LOAEL from different studies, or a free-standing NOAEL 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 473 mg/m$^3$ (98 ppm) determined in the Carpenter *et al.* (1956) study.

3. The studies that have free-standing NOAELs have small sample sizes, particularly the Jones *et al.* 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, single-dose studies exposing only a few human subjects may easily miss adverse effects that would be apparent in larger groups of exposed individuals.

The Carpenter *et al.* (1956) study, upon which the acute REL is based, does have several limitations compared to the more recent toxicological studies that were considered. These limitations include 1) unknown purity of the EGBE used to generate

the exposure atmosphere; 2) potential presence of other irritant gases in the exposure chamber; 3) use of a gas interferometer to estimate EGBE exposure concentrations; and 4) unknown variability in the EGBE exposure concentrations over time.

The purity of the EGBE used in the human and animal exposures was not stated by Carpenter *et al.* (1956), so the quantity and types of impurities in the EGBE solution are unknown. Toxicological and pharmacokinetic studies conducted since the 1980s generally used EGBE with a purity of >99%. Impurities in purified EGBE may include 2-butoxyethoxyethanol (≤ 0.3% w/w), 1,2-ethanediol (≤ 0.3-0.5% w/w), 1-butanol (≤ 0.1-0.2% w/w) and water (< 0.1-0.2% w/w) (EU, 2006). Some of these impurities may also be sensory irritants. Current EGBE preparations frequently include an additive (0.008-0.012% w/w 2,6-bis(1,1-dimethylethyl)-4-methylphenol) to prevent the formation of peroxides. If no additive was in the formulation used by Carpenter *et al.,* some level of peroxides may have been present in the test substance.

Although EGBE exposure can result in eye and respiratory tract irritation, the contribution of other potentially irritant gases in the Carpenter *et al*. (1956) study is possible. Unfortunately, case reports of EGBE sensory irritation resulting from occupational exposure (Kullman, 1987; Raymond *et al.*, 1998) are complicated by unknown exposure concentrations and potential co-exposure to other irritating gases. There are currently no other controlled human studies that estimated air concentrations of EGBE that resulted in sensory irritation. In rodent studies in which the purity of the EGBE used was stated (reagent quality or >99% purity), sensory irritation was apparent in the form of abnormal breathing, eye and nasal discharge (NTP, 2000) and respiratory depression (Kane *et al.*, 1980).

Another potential limitation of the Carpenter *et al.* (1956) study is the method of analysis, a gas interferometer, used to estimate the EGBE concentration in exposure chambers. Interferometers have a number of different applications, but in gas interferometry the instrument can measure the difference in refractivity between a standard gas of known refractivity and a mixture of some contaminating gas or vapor (Patty, 1939). With knowledge of the refractivities of both the standard gas (*i.e.*, usually air without airborne contaminants) and the contaminating vapor (*i.e.*, EGBE, in this case), the concentration of a contaminant gas can be estimated. Drawbacks with this instrumentation include high price, difficulties of calibration, and necessity for gas concentrations to be greater than the expected measurement error for a particular gas. Advantages for the use of gas interferometers include quick analysis of gas concentrations and accuracy (once calibration of the instrument has been mastered by the recorder). More recently, other forms of analysis (infrared spectrophotometry; flame ionization detector; gas chromatography) are used for measurement of EGBE in

exposure chambers. Gas interferometry has some current use in the form of Fourier transform infrared spectroscopy for the measurement of toxic gases and vapors in the environment and in the workplace (Xiao and Levine, 1993; Schafer *et al.*, 1994).

Even if it is assumed that the Carpenter et al (1956) study was not hindered by the use of a noise-limited and difficult-to-calibrate analytical device, the study is still limited by unknown variability of EGBE chamber concentrations. The EGBE concentrations were analyzed four times during each exposure, but standard curves and the chamber measurement variability were not presented in the report.

Given the limitations of the Carpenter *et al.* (1956) study, we compared the inhalation toxicity of the EGBE used by Carpenter *et al.* against other toxicity studies in which better analysis of gas concentrations and EGBE purity (*i.e.*, >99%) are presented. Comparisons are presented below in Table 9.

In the Carpenter *et al.* (1956) study, the critical hematological endpoint examined was hemoglobinuria. The NOAEL and LOAEL for this endpoint in both rats and mice were about 483 and 966 mg/m$^3$ (100 and 200 ppm), respectively, with 7-hour acute exposure to EGBE (Table 9). Similar values were obtained with repeated exposures (7 hrs/day, 5 days/wk) for up to 30 days. However, the test for osmotic fragility of RBCs with a range of saline solution concentrations following the acute exposures resulted in a lower NOAEL and LOAEL of 155 and 299 mg/m$^3$ (32 and 62 ppm), respectively.

In the rodent studies by Tyl *et al.* (1984), NTP (2000) and Dodd *et al.* (1983) the critical endpoint was primarily hemolytic anemia, a hematological endpoint determined in blood samples. Repeated exposure protocols yielded NOAELs and LOAELs for this endpoint in the range of 121 – 302 mg/m$^3$ (25 - 62.5 ppm) and 372 – 604 mg/m$^3$ (77 - 125 ppm), respectively. In these studies, a number of blood parameters were usually affected, including reduced RBC counts and reduced Hct.

**Table 9. Comparison of NOAELs and LOAELs for hematological endpoints in rodent EGBE exposure studies**

| Study | Species EGBE Exposure Duration | Hematological Endpoint | NOAEL mg/m$^3$ (ppm) | LOAEL mg/m$^3$ (ppm) |
|---|---|---|---|---|
| Carpenter *et al.* (1956) | Rats/mice 7 hrs | Hemoglobinuria | 483 – 517 (100 -107) | 966 – 980 (200 -203) |
| | Rats 7 hrs/day × 5 days/wk × 30 times | Hemoglobinuria | 517 (107) | 980 (203) |
| | Mice 7 hrs/day × 5 days/wk × 30 times | Hemoglobinuria | 541 (112) | 966 (200) |
| | Rats 4 hrs | RBC osmotic fragility | 155 (32) | 299 (62) |
| Tyl *et al.* (1984) | Rats 6 hrs/day on days 6-15 of gestation | Hemolytic anemia | 242 (50) | 483 (100) |
| | | Hemoglobinuria | 242 (50) | 483 (100) |
| | | RBC osmotic fragility | 966 (200) | nd |
| NTP (2000) | Rats/mice 6 hrs/day, 5 days/wk for 14 wks | Hemolytic anemia | 302 (62.5) | 604 (125) |
| Dodd *et al.* (1983) | Rats 6 hrs/day, 5 days/wk for 9 days | Hemolytic anemia | 97 (20) | 415 (86) |
| | Rats 6 hrs/day, 5 days/wk for 90 days | Hemolytic anemia | 121 (25) | 372 (77) |
| | | RBC osmotic fragility | 121 (25) | 372 (77) |

The 2-fold higher NOAELs and LOAELs mostly observed in the Carpenter *et al.* (1956) study compared to the more recent studies may, in part, be a result of increased sensitivity in measuring hematological endpoints in the later studies. However, lower purity of EGBE and/or lower sensitivity of measurement instrumentation could also be reasons for the higher NOAEL and LOAEL values of the study by Carpenter *et al.* (1956). The relevance of the hematological endpoints for human sensory irritation is

unclear; although one might assume that the LOAEL (and NOAEL) for sensory irritation may actually occur at lower concentrations using the better methodology applied in later studies. This discrepancy is addressed using a full 10-fold LOAEL-to-NOAEL uncertainty factor, although uncertainty factors in general are meant to address these types of methodological uncertainties.

Basing a REL on a free-standing NOAEL of a different human chamber exposure study using analytical grade EGBE did not result in an appreciably different value, as presented below:

For a comparison acute REL, the free-standing NOAEL of 97 mg/m$^3$ (20 ppm) based on subjective remarks made by volunteers in the Johanson *et al.* (1986a) toxicokinetic study is used as a POD. This study is better supported than the Jones *et al.* (2003b) study in which a free-standing NOAEL of 242 mg/m$^3$ (50 ppm) was observed. Unlike the Jones *et al.* (2003b) study, Johanson *et al.* (1986a) had reported 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 of EGBE should have been apparent to the subjects (but was not described). Also, Johanson *et al.* (1986a) had a greater number of subjects participating in their study (n=7) compared to the Jones *et al.* (2003b) study (n=4). No time extrapolation from the 2-hour exposures to 1 hour was applied since sensory irritation is usually a concentration-dependent response. Applying the same intraspecies UF = 10 (10 for toxicokinetic $UF_{H\text{-}k}$ and 1 for toxicodynamic $UF_{H\text{-}d}$) to the POD as that used for the acute REL derivation results in an acute REL of 9.7 mg/m$^3$ (2 ppm), which is approximately twice the REL value of 4.8 mg/m$^3$ (1 ppm) based on the Carpenter *et al.* (1956) study. Given that the human studies with a single exposure and small sample size could easily miss an adverse effect, the more health protective acute REL resulting from the Carpenter *et al*. study was selected.

## 8.2 EGBE 8-Hour Reference Exposure Level

The 8-hour REL is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Noncancer REL TSD (OEHHA, 2008).

| | |
|---|---|
| *Study* | NTP, 2000 |
| *Study population* | Rats (50 animals/group/gender) |
| *Exposure method* | Discontinuous whole-body inhalation exposure to 0, 151, 302, or 604 mg/m$^3$ (0, 31.2, 62.5, or 125 ppm) |
| *Critical effects* | Nasal hyaline degeneration of olfactory epithelium |
| *LOAEL* | 151 mg/m$^3$ (31.2 ppm) |
| *NOAEL* | Not observed |
| *$BMCL_{05}$* | 39.4 mg/m$^3$ (8.16 ppm),; Probit model from male and female rats |
| *Exposure continuity* | 6 hrs/day, 5 days/wk |
| *Exposure duration* | 2 years |
| *Time-adjusted exposure* | 14.1 mg/m$^3$ (2.91 ppm) ( = 8.16 ppm x 6/24 x 5/7 x 20/10) |
| *Human Equivalent Concentration (HEC)* | 4.93 mg/m$^3$ (1.02 ppm) (gas with extra-thoracic respiratory effects, RGDR = 0.35) |
| *LOAEL uncertainty factor* | 1 (with use of a $BMCL_{05}$) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{A-k}$)* | 1 |
| *Toxicodynamic ($UF_{A-d}$)* | √10 |
| *Intraspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{H-k}$)* | √10 |
| *Toxicodynamic ($UF_{H-d}$)* | √10 |
| *Cumulative uncertainty factor* | 30 |
| *Reference Exposure Level* | 164 µg/m$^3$ (34 ppb) |

Note: Time-adjusted Exposure: The POD is first adjusted to a 24-hour continuous exposure (6/24 hours x 5/7 days per week), then multiplied by 2 (20m$^3$/10m$^3$) to represent an active worker breathing half the volume of air breathed in a 24-hour period during an 8-hour work day. HEC = Time-adjusted Exposure × the Regional Gas Dose Ratio (RGDR). RGDR = ($MV_A/MV_H$) / ($SA_A/SA_H$); MV is Minute Volume = inhaled volume × respiratory rate, and SA is surface area for the lung region of concern ($_A$ and $_H$ represent animal and human respectively). Gas with extra-thoracic respiratory effects, RGDR = 0.35, $MV_A$ = 0.38 m$^3$/day, $MV_H$ = 14.48 m$^3$/day, $SA_A$ = 15 cm$^2$, $SA_H$ = 200 cm$^2$ (OEHHA, 2008).

In the key study (NTP, 2000), rats and mice subjected to a whole-body inhalation exposure of 0, 151, 302, or 604 mg/m$^3$ (0, 31.2, 62.5, or 125 ppm) for two years displayed nasal olfactory epithelial hyaline degeneration, liver Kupffer cell pigmentation, and forestomach epithelial hyperplasia and ulcers in both species. This study was chosen because it used a lifetime inhalation exposure, and provided the most sensitive toxicity endpoint not dependent upon hemolytic anemia (Humans are more resistant to the hematological effects of EGBE compared to rodents).

Exposure doses and related toxicity endpoints are listed in Table 8 of Section 6.3. Benchmark dose analysis was performed using Benchmark Dose Modeling Software (BMDS) version 2.6 (EPA, 2015). The calculated $BMCL_{05}$ values, and corresponding NOAEL and LOAEL values are listed in Table 10. Because dose-responses were not noted for nasal hyaline degeneration in male or female mice, or for forestomach ulcers in male mice, specifically (Table 8), associated BMCL data were excluded from Table 10. We are using the $BMCL_{05}$ values as the POD for REL derivation. For each endpoint, the $BMCL_{05}$ is derived from the models that provided the best visual and statistical fit to the data, particularly in the low dose region of the dose-response curve where the $BMCL_{05}$ resides. Following US EPA guidelines, the model with the lowest Akaike Information Criterion (AIC) was chosen in instances where various model fits to the data were similar.

**Table 10: $BMCL_{05}$, NOAEL and LOAEL values for nasal olfactory epithelial hyaline degeneration, liver Kupffer cell pigmentation, and forestomach ulcers in rats and mice, and epithelial hyperplasia in mice exposed to EGBE by inhalation for two years (NTP, 2000)**

| Endpoints | $BMCL_{05}$ mg/m³ (ppm) (BMD model) | NOAEL mg/m³ (ppm) | LOAEL mg/m³ (ppm) |
|---|---|---|---|
| **Nasal Olfactory Epithelial Hyaline Degeneration** | | | |
| Male rats | 39 (8.0) (Probit) | 151 (31.2) | 302 (62.5) |
| Female rats | 37 (7.6) (Logistic) | 151 (31.2) | 302 62.5) |
| ***Male and female rats combined*** | ***40 (8.2) (Probit)*** | ***NE*** | ***151 (31.2)*** |
| **Liver Kupffer Cell Pigmentation** | | | |
| Male rats | 28 (5.7) (Logistic) | 151 (31.2) | 302 (62.5) |
| Female rats | 56 (11.6) (LogLogistic) | 151 (31.2) | 302 (62.5) |
| *Male and female rats combined* | *27 (5.5) (Logistic)* | 151 (31.2) | *302 (62.5)* |
| Male mice | 354 (73.2) (LogProbit) | 302 (62.5) | 604 (125) |
| Female Mice | 181 (37.5) (LogProbit) | NE | 302 (62.5) |
| *Male and female mice combined* | *241 (49.9) (LogProbit)* | *NE* | *302 (62.5)* |
| **Forestomach Epithelial Hyperplasia** | | | |
| Male Mice | 78 (16.2) (Weibull) | NE | 302 (62.5) |
| Female Mice | 47 (9.7) (LogProbit) | NE | 302 (62.5) |
| *Male and female mice combined* | *55 11.4 (Dichotomous-Hill)* | *NE* | *302 (62.5)* |
| **Forestomach Ulcer** | | | |
| Female Mice | 85 (17.5) (Quantal-linear) | NE | 302 (62.5) |
| *Male and female mice combined* | *127 (26.3) (LogLogistic)* | *NE* | *302 (62.5)* |

Note: $BMCL_{05}$ is based on dichotomous models (model shown in parenthesis) with best visual and statistical fit (EPA, 2015); NE, Not established.

Of the chronic effects noted in rats and mice in Table 10, hyaline degeneration of the olfactory epithelium is more analogous to what would occur with human exposure to EGBE than the other lesions. The primary cause of the nasal lesions is likely to be direct EGBE irritation through the inhalation route (NTP, 2000). We are focusing on the regional responses/changes in the nose and upper respiratory tract, which is the most sensitive endpoint, and is more consistent with the acute inhalation effect of EGBE in humans (Carpenter *et al.*, 1956).

Hyaline degeneration of the olfactory epithelium often appears at increased rates in aging rats and mice. Other entities establishing health values have based their hazard assessments on hematological endpoints rather than nasal hyaline degeneration of the olfactory epithelium in rats (ATSDR, 1998; EPA, 1999; EU, 2006; EPA, 2010). However, OEHHA here considers information not discussed in the reviews by others that supports our interpretation that this lesion is indicative of an adverse response to toxicant exposures. This additional information suggests that hyaline degeneration, also known as formation of eosinophilic globules (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.

Perturbations in the frequency of apoptotic events result in disease, suggesting EG formation is a degenerative change. Previous research in F-344 rats and $B6C3F_1$ mice by Buckley *et al.* (1985) showed increased incidence of EG in combination with other adverse pathologies such as destruction of the naso- and maxillo-turbinates after exposure to dimethylamine. Monticello *et al.* (1990) stated that cells with EG often "exhibit massively dilated cisternae of the rough endoplasmic reticulum [ER]". 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 and neutralization of stress or activation of pro-apoptotic pathways and eventual cell death.

Papadimitriou *et al.* (2000) 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 research on 80 tumor cases (24 tumor types) containing EG led them to hypothesize that all EG reflect stages of cell injury related to apoptosis.

Microscopic observations 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. No research was found by OEHHA that linked EG and fibrosis (*e.g.*, by imaging, laboratory or lung function tests, and/or histology). 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 does not 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 and 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. Eosinophilic globules 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 ($p < 0.05$).

Since EG formation is a marker of stress/injury that could lead to apoptosis and is likely related to a continuum of changes known to represent an established adverse effect, OEHHA believes that olfactory hyaline degeneration hallmarked by EG formation is an appropriate choice as the critical endpoint for REL development.

Although liver Kupffer cell pigmentation in rats would provide a slightly lower $BMCL_{05}$, this effect is secondary to RBC hemolysis, which is not considered by OEHHA to be relevant for EGBE REL derivation in humans. Regarding the forestomach effects in mice, humans do not have a similar organ, but it is conceivable that EGBE could irritate

the lining of the esophagus or stomach in humans via incidental or intentional ingestion. However, this endpoint in mice was not as sensitive as hyaline degeneration of the olfactory epithelium in rats (Table 10). Since this document is focusing on inhalation REL development, we are selecting nasal olfactory epithelium hyaline degeneration in rats as an endpoint to derive 8-hour and chronic RELs.

Logistic regression was performed by OEHHA to determine the relationship among rat sex, EGBE exposure concentration, and incidence of olfactory epithelial hyaline degeneration. A Wald test indicated that sex was not a significant factor for nasal olfactory epithelial hyaline degeneration in rats (Wald $X^2 = 0.20$; $p = 0.65$). Therefore, combining male and female rats for $BMCL_{05}$ estimation is applicable for the nasal endpoint in Table 10. In addition, the combined LOAEL of 151 mg/m$^3$ (31.2 ppm) for male and female rats is smaller than the LOAEL for males or females alone (302 mg/m$^3$; 62.5 ppm). Table 11 lists the Benchmark Dose (BMD), BMD 95% lower confidence limit ($BMDL_{05}$), AIC and goodness-of-fit P-values for the several dichotomous models fit to male and female rat combined incidences of nasal olfactory epithelial hyaline degeneration. Figure 4 provides a graphic display of the dichotomous probit model fit to male and female rat nasal olfactory epithelium lesion incidence data.

**Table 11. BMDS dichotomous models fit to incidence of hyaline degeneration of the olfactory epithelium in male and female rats after inhalation exposure to EGBE for 2 years (NTP, 2000)**

| Model Name | BMD $mg/m^3$ (ppm) | $BMDL_{05}$ $mg/m^3$ (ppm) | Goodness-of-fit p-value | AIC | Scaled residual |
|---|---|---|---|---|---|
| Multistage | 27.0703 (5.6046) | 21.9928 (4.5534) | 0.1972 | 485.392 | 0.549 |
| Gamma | 79.0087 (16.3579) | 26.0642 (5.3963) | 0.4104 | 484.765 | 0.522 |
| Logistic | 46.0019 (9.5242) | 40.0318 (8.2882) | 0.8236 | 482.479 | 0.115 |
| LogLogistic | 93.8720 (19.4352) | 40.8242 (8.4522) | 0.2900 | 485.205 | 0.704 |
| LogProbit | 101.6430 (21.0441) | 48.4367 (10.0283) | 0.2441 | 485.441 | 0.726 |
| **Probit** | **44.9094 (9.2980)** | **39.4300 (8.1636)** | **0.8492** | **482.417** | **0.109** |
| Weibull | 71.9713 (14.9009) | 27.2101 (5.6336) | 0.4890 | 484.567 | -0.158 |
| Quantal-Linear | 27.0703 (5.6046) | 21.9928 (4.5534) | 0.1972 | 485.392 | 0.549 |

Note: Results are from benchmark dose analysis using BMDS version 2.6 (EPA, 2015). We selected the best available model based on a smaller AIC and larger goodness-of-fit P-value among the different models. In this case, the Probit model (bold) was the most appropriate model. AIC = Akaike Information Criterion. Scaled residual is for the dose group nearest the BMD.

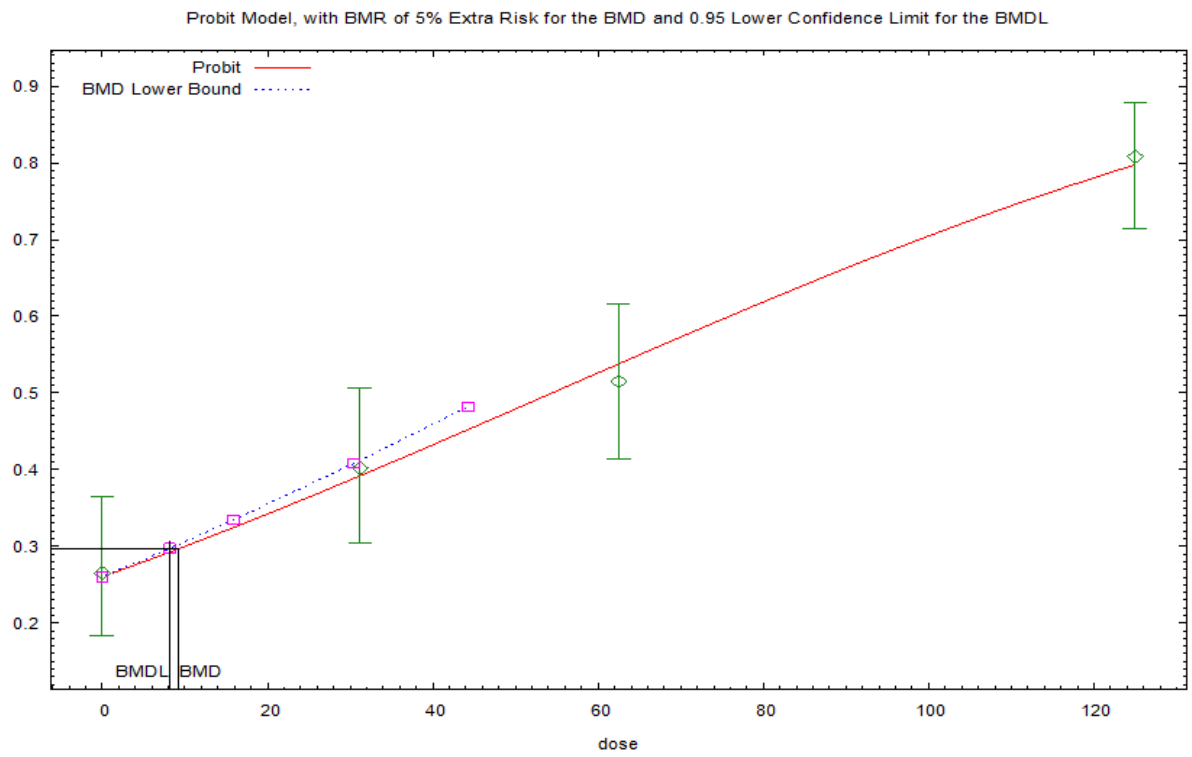


**Figure 4.** Dichotomous Probit model fit to nasal olfactory epithelium incidences in male and female rats after inhalation exposure to EGBE for 2 years (NTP, 2000)

The point of departure (the $BMCL_{05}$) was adjusted for 8-hour exposures, seven days/wk. The assumption is that the rats show both mixed active and inactive periods during exposure, and a time adjustment is made to simulate an active 8-hour working period during which the off-site worker is exposed. The concentration is first adjusted down to 24-hour continuous exposure (6/24 hours × 5/7 days per week), then multiplied by 2 ($20m^3/10m^3$) to represent an active individual breathing half the air breathed in a day during an active working 8-hour period when exposure occurs, compared to what a resident would breathe over a 24-hour period.

Adjustments for differences in MV and for relative areas of human and rat extra-thoracic regions of the respiratory tract resulted in a human equivalent concentration of 14.15 $mg/m^3$ (2.9 ppm) (OEHHA, 2008). We used an interspecies UF = √10. This was composed of a toxicokinetic UF of 1 because we utilized the HEC dosimetric adjustment and the toxicological endpoint is a port of entry effect. We retained a UF of √10 to account for interspecies tissue sensitivity differences. The intraspecies toxicokinetic and toxicodynamic UFs were both assigned √10. No additional adjustment was made for early life exposures, since the effect of concern is at the portal of entry and thus age-related differences in toxicokinetics do not likely influence response. The cumulative UF

was 30 which results in an 8-hour REL of 0.165 mg/m$^3$ (0.034 ppm) and this value is just slightly lower than EGBE's odor threshold 0.483 mg/m$^3$ (0.10 ppm).

## 8.3 EGBE Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | NTP, 2000 |
| *Study population* | Rats (50 animals/group/gender) |
| *Exposure method* | Discontinuous whole-body inhalation exposure of 0, 151, 302, or 604 mg/m$^3$ (0, 31.2, 62.5,or 125 ppm) |
| *Critical effects* | Nasal hyaline degeneration of olfactory epithelium |
| *LOAEL* | 151 mg/m$^3$ (31.2 ppm) |
| *NOAEL* | Not observed |
| *$BMC_{05}$* | 39.4 mg/m$^3$ (8.16 ppm; Probit model from male and female rats) |
| *Exposure continuity* | 6 hrs/day, 5 days/wk |
| *Exposure duration* | 2 years |
| *Time-adjusted exposure* | 7.04 mg/m$^3$ (1.46 ppm) (ppm = 8.16 ppm x 6/24 x 5/7) |
| *Human Equivalent Concentration* | 2.46 mg/m$^3$ (0.510 ppm; gas with extra-thoracic respiratory effects, RGDR = 0.35) |
| *LOAEL uncertainty factor* | NA |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{A-k}$)* | 1 |
| *Toxicodynamic ($UF_{A-d}$)* | √10 |
| *Intraspecies uncertainty factor* | |
| *Toxicokinetic ($UF_{H-k}$)* | √10 |
| *Toxicodynamic ($UF_{H-d}$)* | √10 |
| *Cumulative uncertainty factor* | 30 |
| *Reference Exposure Level* | 82 µg/m$^3$ (17 ppb) |

The chronic REL is based on the same study as the 8-hour REL (NTP, 2000) and uses the same benchmark dose analysis with a POD of 40 mg/m$^3$ (8.2 ppm). In this instance the time adjusted exposure reflects conversion of an intermittent to a continuous exposure. The same uncertainty factors apply to give a cumulative UF of 30 and a chronic REL of 83 µg/m$^3$ (17 ppb).

Occupational exposure limits for EGBE have been established by various agencies in the US NIOSH based an 8-hour TWA Recommended Exposure Limit of 24 mg/m$^3$ (5

ppm) on tissue irritation, CNS depression, and adverse effects on the blood and hematopoietic systems. Both the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) established a TWA of 120 mg/m$^3$ (25 ppm, based on the risk of hematologic and other systemic effects associated with exposure to EGBE. These values were established more than 20 years ago (NIOSH, 1992).

## 9. Evidence for Differential Sensitivity of Children

No human inhalation studies were found that addressed differential sensitivity of children relative to adults exposed to EGBE in terms of eye and upper respiratory irritation. In experimental animals, no evidence was found for differential sensitivity in developmental studies, as both maternal toxicity and fetotoxicity occurred at similar exposure concentrations. Regarding the hemolytic action of EGBE, an animal oral gavage study found that adult (9-13 weeks) male rats (12/12, 100%) were more sensitive to the hemolytic effects of EGBE at 125 mg/kg in water than young (4-5 weeks) male rats (1/11, 9.1%) (Ghanayem *et al.*, 1987). In humans, *in vitro* studies in RBCs from children and healthy adults showed no difference in their resistance to the hemolytic effects of BAA (Udden, 1994; Udden, 2002). Due to the sensory irritant action of EGBE exposure, asthmatics including children may be more sensitive to EGBE exposure compared to the general population. Otherwise, there is currently insufficient evidence to consider EGBE a chemical for which children are more sensitive compared to the general population.

Several epidemiological studies indicate that indoor factors might cause asthma in childhood. The most consistent finding for induction of asthma in childhood is related to exposure to environmental tobacco smoke, and living in homes close to busy roads or homes damp with visible molds. More research is needed to clarify the potential risk for exposure to volatile and semi-volatile organics due to renovation activities or cleaning (Heinrich, 2011). Further study is needed to identify whether EGBE contributes to increased childhood asthma in the home environment.

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# 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

## January, 2016

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 $\sqrt{10}$ as previously proposed) and an intraspecies toxicodynamic UF of 10, for a total cumulative intraspecies UF = 10.

Previously, a toxicokinetic UF = $\sqrt{10}$ 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 $UF_{H-d}$ = 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 $UF_{H-d}$ 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 $UF_{H-d}$ = 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 $UF_{H-d}$ = 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

the olfactory epithelium was observed with degeneration of olfactory sensory cells and nerves. Epithelial damage and atrophy of olfactory nerves in the lamina propria were accompanied by accumulation of EG in markedly hypertropic sustentacular cells of the olfactory epithelium. EG were also observed in the overlying airway suggesting that they may be secretory products of the sustentacular cells. Although regeneration of the sensory cells is possible, sustained atrophy and loss were observed in this study and related (by the authors) to repeated exposures.

EG in the respiratory and olfactory epithelia were morphologically similar. Despite that these lesions were collocated with severe adverse intransient lesions, the authors stated that they may "represent a defensive response by the production of surface secretions over the olfactory epithelium." However, no other analyses were performed to determine the mechanisms behind globule formation, or the relationship of the globules to the destructive effects observed in the nose. Instead, the authors stated, that the nature of the globules "has yet to be determined." Despite this uncertainty, subsequent reviews by NTP (2000; 2015), EPA (1999; 2010), and European Union (2006) commonly propagated the unsubstantiated idea that EG were indicative of an adaptive response.

NTP (2000) described EGBE exposure-related increases in the incidence of EG in the olfactory epithelium of male and female rats, and in the olfactory and respiratory epithelia of female mice, but no statistical analyses were reported on the incidence or severity of the observed EG in the olfactory and/or respiratory epithelial cells, and no rubric was provided to explain the subjective, ordinal severity scores (minimal/1, mild/2, moderate/3, and severe/4). No supplementary work was done by NTP to characterize the eosinophilic lesions, which in their study, were not reported to be associated with additional nasal perturbations. U.S. EPA (2010) more comprehensively analyzed the NTP histopathology data for EGBE, performing the first-reported statistical analysis of the incidence of EG in the olfactory epithelium of rats and mice. No mention was made of globules in the respiratory epithelium.

Given that the effect is likely related to a continuum of changes known to represent an established adverse effect, and that some female rats (n = 3/49) in the NTP EGBE study (2000), which as a group appear to be more sensitive to EGBE than exposed males of the genus, exhibited increased EG severity at the 125 ppm concentration compared to controls, OEHHA stands by its use of epithelial globule formation as the 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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