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

May 2018

Air, Community, and Environmental Research Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Prepared by the

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May 2018

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List of Acronyms

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

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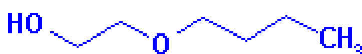
Ethylene Glycol mono-n-Butyl Ether

(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)	Hyaline degeneration of nasal 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)	Hyaline degeneration of nasal olfactory epithelium
Hazard index target(s)	Respiratory system

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 (1160 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

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). EGBE may also be found as a stabilizer in hydraulic fracturing fluids (FracFocus, 2016) and firefighting foams (Laitinen *et al.*, 2014).

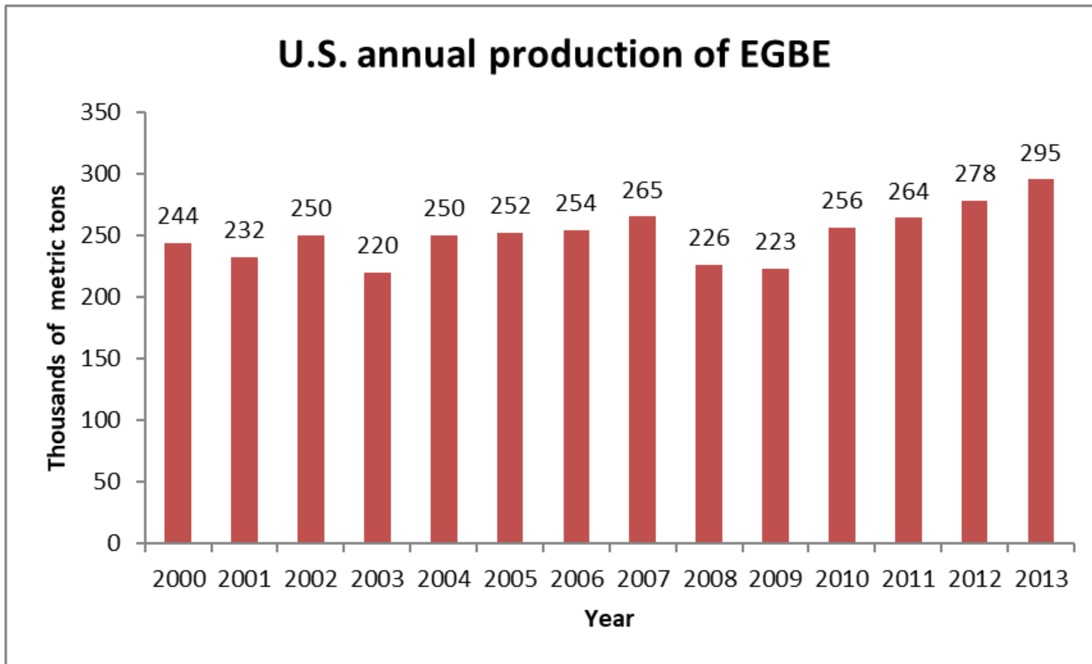


Figure 1. US production of EGBE from 2000 to 2013 in thousands of metric tons (Chinn *et al.*, 2014).

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 3881 tons in 2006 to 4363 tons in 2010 (Figure 2) (CARB, 2013).

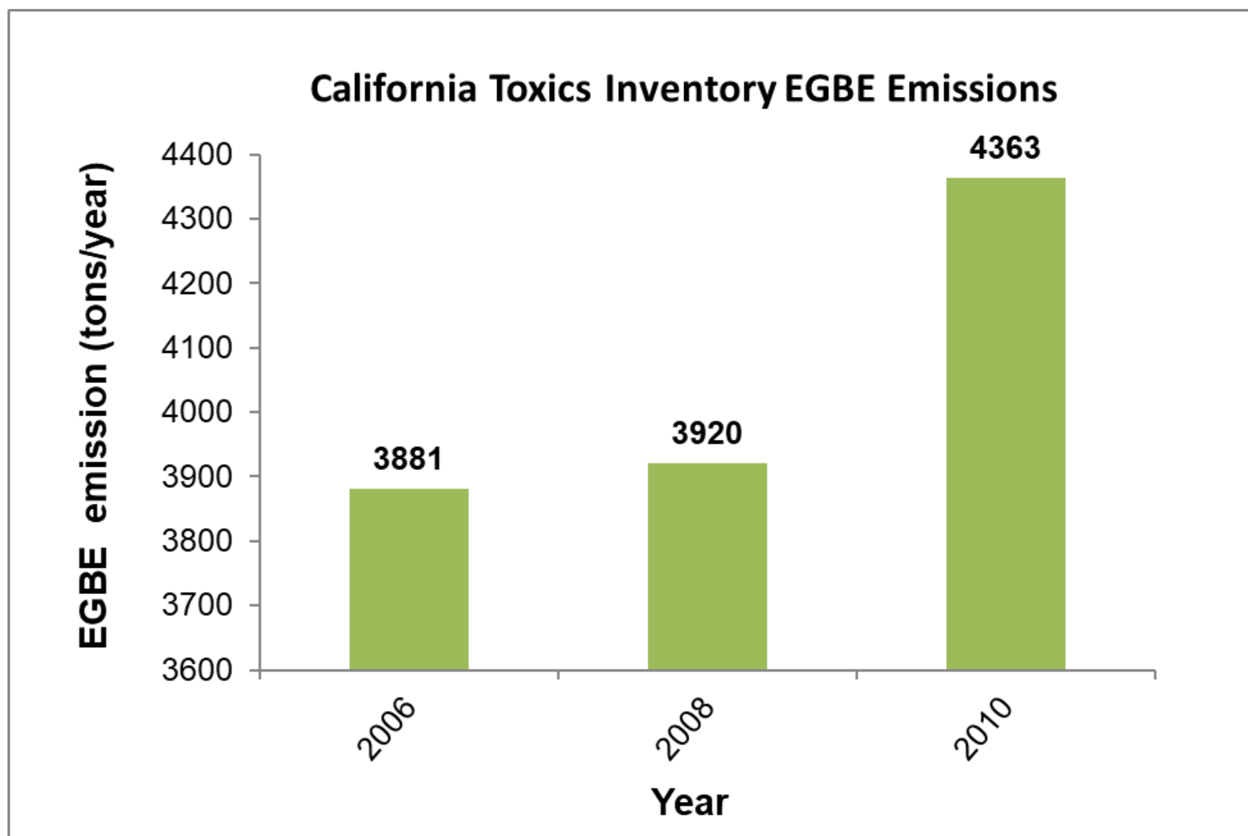


Figure 2. California Toxics Inventory EGBE emissions (tons/year)
Source: (CARB, 2013).

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 1242 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]	0.74[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	1.28	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	7391	4.7	5	0.002	0.006 (0.008)
			6	0.004	
3	7391	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 ≈ 7391 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- (1000 – 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 0.011 to 0.081 mg/m^3 (0.0023 to 0.017 ppm). On post-construction days 72 and 246, EGBE concentrations in the samples collected were generally lower, ranging from 0.004 to 0.046 mg/m^3 (0.00083 to 0.0095 ppm). EGBE in indoor air was thought to originate from water-based paints or adhesives (Brown, 2002).

Personal breathing zone monitoring by Vincent *et al.* (1993) demonstrated that auto cleaning workers were exposed to 8-hour time-weighted average EGBE concentrations in the range of < 0.483 to 35.4 mg/m^3 (<0.10 to 7.33 ppm) with an arithmetic mean ± SD of 11.26 ± 11.8 mg/m^3 (2.33 ± 2.44 ppm) during use of use of EGBE-containing window cleaning agents.

Indoor exposure to EGBE via inhalation and dermal routes may be more common and present a greater hazard than exposure to outdoor facility emissions of EGBE. Although the REL is designed to protect individuals living or working near facilities that may emit EGBE, it should not be overlooked that cumulative exposure to EGBE via multiple exposure sources and routes may occur (e.g., outdoor emission sources, plus dermal and inhalation exposure to consumer products containing EGBE).

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 1500-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 (USEPA, 2010).

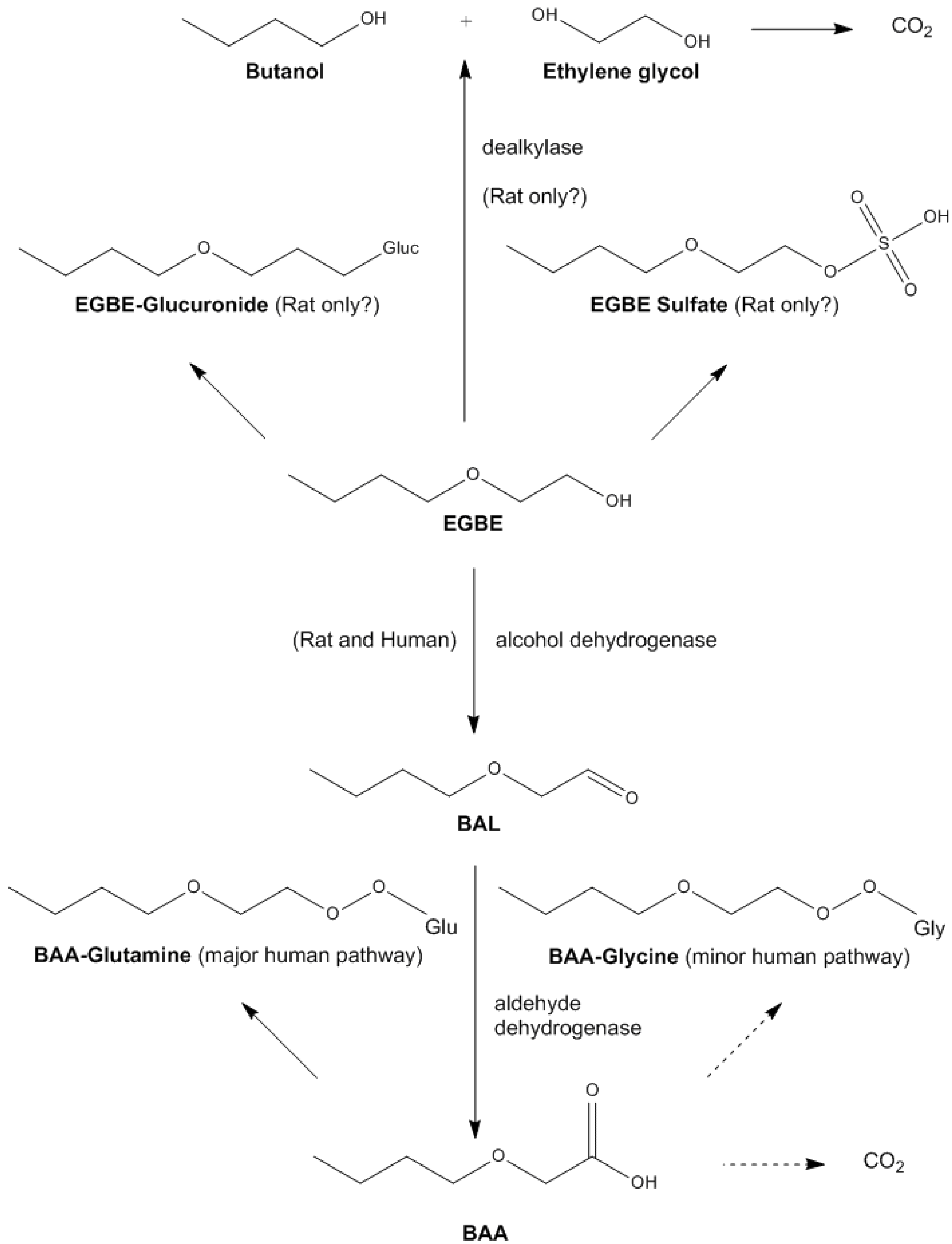


Figure 3. EGBE Metabolism in Rats and Humans. Adapted from Medinsky *et al.* (1990) and Corley *et al.* (1997). Dashed arrow indicates a theoretical (unmeasured) step in human metabolism.

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.

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, indicating that some individuals may only excrete unconjugated free BAA; 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, dermal and inhalation 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) Human, inhalation	17-55[a]	NR	NR	NR	NR	NR
Sakai *et al.* (1994) Human, inhalation	NR	44-92[b]	NR	NR	NR	NR
Rettenmeier *et al.* (1993) Human, inhalation	NR	16-64[c]	NR	NR	NR	NR
Corley *et al.* (1997) Human, dermal	33[d]	67[c]	NR	NF	[e]	NR
Kezic *et al.* (2004) Human, inhalation Human, dermal	 45[d] 42[d]	 55[b] 58[b]	NR	NR	NR	NR
Sabourin *et al.* (1992a) Rat, inhalation	43-37[f]	NR	3-10	8-16	≤10.5[g]	7
Medinsky *et al.* (1990) Rat, drinking water	50-60[f]	NR	7	10	NR	8-10
Ghanayem *et al.* (1987) Rat, oral gavage	75[f]	NR	<20	NR	[h]	NR
Sabourin *et al.* (1992b) Rat, dermal	68[f]	NR	14	5	NR	4.5
Poet *et al.* (2003) Mouse, oral gavage Mouse, IP	 37.5[i] 50.8[i]	NR	 3.3[j] 0.7-2.8[j]	NR	 7[k] 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 other adverse effects from exposure and all volunteers completed the 2-hour exposure period. 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). All volunteers completed the 2-hour exposure period. 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, at time of previous OEHHA review).

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 10th 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 360–480 mL 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	No
Renal injury	Yes	No	No	Yes	No
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 (10th Day)	Unknown	25 (2nd Day)	Unknown	Unknown
Hemoglobin (g/dL)	9.7 (10th Day)	8.9 (2nd Day)	9.1 (2nd Day)	Unknown	10.7 (2nd 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 RD_{50} (an airborne concentration of a chemical that produces a 50% decrease in respiratory rate) of 7995 mg/m^3 (2825 ppm). The RD_{50} bioassay measures decreases in respiratory frequency in mice as a result of stimulation of the trigeminal or laryngeal nerve endings. This RD_{50} needed to be extrapolated because the authors were unable to generate EGBE concentrations that were adequately high to directly determine the RD_{50}. Although not specified by the authors, this could be a result of the exposures reaching the saturated vapor pressure, about 2830 to 4528 mg/m^3 (1000 to 1600 ppm) depending on the temperature and humidity, prior to reaching the RD_{50}. EGBE was categorized as a weak sensory irritant by Kane *et al.* (1980) when compared to the RD_{50} 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 2415 mg/m^3 and 3864 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 2415 mg/m^3 (500 ppm) for 4 or 8 hours (n = 6/group) resulted in only one death. Exposure to 3864 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 3800-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, 2526, or 4188 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 4188 mg/m^3 (867 ppm) group died within 24 hours of exposure. The estimated LC_{50} was 2348 mg/m^3 (486 ppm) for males and 2174 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 1183 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 1183 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 3057 mg/m^3 (633 ppm; 5 males) and 3338 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 3212 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 (Boatman *et al.*, 2014).

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 (MCV) 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 1932 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/m³ (140 – 1400 ppm) for 10 min	RD_{50}: 7995 mg/m³ (2825 ppm).
Dodd *et al.* (1983)	Rats (n = 6/sex/group) 6-7 wks old	976, 2526, or 4188 mg/m³ (202, 523, or 867 ppm) for 4 hrs	LC_{50}: 2347 mg/m³ (486 ppm) for males and 2174 mg/m³ (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/m³ (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/m³ (633 ppm; males) or 3338 mg/m³ (691 ppm; females) for 1 hr	LC_{50}: > 3057 mg/m³ (633 ppm) for males and > 3338 mg/m³ (691 ppm) for females. No mortality or clinical signs of toxicity.

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

Reference	Species	Exposure	Results
Carpenter *et al.* (1956)	Mice n = 15/sex/group 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) ↑ RBC osmotic fragility at all EGBE exposures
	Rats n= 5 – 6/group 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”: 5-6 week-old rats: NOAEL 604 mg/m^3 (125 ppm) LOAEL 1208 mg/m^3 (250 ppm)
	Guinea pigs n = 2 per group age not stated	3212 mg/m^3 (665 ppm) for 8 hrs	No effect on RBC osmotic fragility or hemoglobinuria
	Rabbits n = 2-4/group 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 n = 1 - 2/group 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. 1860 mg/m^3 (385 ppm) - Emesis, weakness, ↑ RBC osmotic fragility, ↓ RBC count and Hgb. 2980 mg/m^3 (617 ppm) - Emesis, extreme weakness, death at end of day 2.
	Monkeys n = 1 - 2/group age not stated	966 mg/m^3 (200 ppm) for 7 hrs or 1014 mg/m^3 (210 ppm) for 7 hrs/day, 5 days/wk	No ↑ RBC osmotic fragility or hemoglobinuria for 7 hours exposure. On 4th day of exposure to 1014 mg/m^3 (210 ppm) - ↑ osmotic fragility

Legend: BWG – Body weight gain; Hgb – Hemoglobin; MCH – Mean corpuscular Hgb; NRBC – Nucleated red blood cell; RBC – Red blood cell; LC_{50}: lethal concentration required to kill 50% of the population; RD_{50} - an airborne concentration of a chemical that produces a 50% decrease in respiratory rate.

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. According to Corley (1997), 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's (2002) comparisons of rat (n = 4 - 10) and human (n = 4 - 14) RBCs exposed to BAA for four hours showed that the latter required a 100-fold greater BAA concentration (10 mM) to develop the same pre-hemolytic changes in red cell deformability, osmotic fragility, and sodium content as observed in the former at 0.1 mM BAA. An additional survey of blood samples from children and adults demonstrated that a four-hour RBC exposure to 10 mM BAA was insufficient to produce hemolysis (n = 97) or increased MCV (n = 65) relative to controls. 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.

A cross-sectional occupational study that investigated changes in nine erythroid parameters of workers exposed to EGBE (Haufroid *et al.*, 1997) 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 free 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.

For the erythroid parameters, Hct decreased (p = 0.03) 3.3% from 45.5 ± 2.7% in controls to 43.9 ± 2.1% in exposed workers, while MCH concentrations increased (p = 0.02) 2.1% from 32.9 ± 1.1 g/dL in controls to 33.6 ± 0.9 g/dL in exposed workers (Haufroid *et al.*, 1997). Reduced Hct occurs due to a reduction in RBC volume in blood. However, the researchers did not find an associated reduction in RBC count in the exposed workers. The authors speculated that the MCH concentration, which is a measure of the average Hgb concentration in RBCs, could reflect early RBC membrane damage that reduced the surface area of RBCs relative to their volume. However, no difference in osmotic fragility of RBC membranes was found between controls and

exposed workers. The osmotic resistance test determines the NaCl concentration corresponding to 50% hemolysis of RBCs. [OEHHA notes that Hct and MCH concentration of exposed workers were within the normal clinical range: Hct - 40.7%-50.3% for adult males; MCH concentration - 32-36 g/dL for all adults (Medlineplus, 2017)]. No significant effects were found for other erythroid parameters in exposed workers, including Hgb concentration, MCV, MCH, haptoglobin percentage, and reticulocytes percentage. In addition, no correlation was found between any of the erythroid parameters and end-shift urinary free BAA concentration. The authors concluded that changes in Hct and MCH concentration could reflect early markers of erythroid toxicity, but this would need to be confirmed in further studies.

In another occupational study, investigators evaluated the hematological and immunological 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-exposed occupations. No other details about the controls were available. Significant exposure to other organic solvents detected 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. 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. Regarding the hematological findings, the authors concluded that the workers showed slight-to-negligible decreases in RBC counts (p = 0.068, from $5.1 \times 10^6 \pm 0.5 \times 10^6$ in controls to $4.5 \times 10^6 \pm 0.5 \times 10^6$ in exposed workers) and Hgb concentrations (p = 0.102, from 15.2 ± 0.8 g/dL in controls to 14.4 ± 0.9 g/dL in exposed workers) compared to controls. No other erythroid parameters were measured.

Hung *et al.* (2011) analyzed the Hgb concentration in the blood of 80 bicycle manufacturing workers at two Taiwanese factories. 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) was similar or slightly higher than the self-adhesive decal workers (28%, 7 of 25) and the assembly workers (21%, 5 of 24). However, the difference between the assembly workers and the decal transfer workers was not statistically significant (X^2 test, $p = 0.4319$).

Taken together, these three occupational studies show slight changes in some hematological parameters suggestive of increased hemolysis in workers chronically exposed to EGBE. In most cases, the measured hematological parameters investigated in these studies were not statistically different ($p < 0.05$) between exposed and control groups, and in all cases, none of the group mean parameters were outside of normal ranges expected in the human population. Only the study by Haufroid *et al*. (1997) provided a comprehensive analysis of RBCs normally performed in complete blood count (CBC) tests. CBC tests provide a more complete evaluation of the condition of the RBCs.

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 1100 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 difference in the geometric mean EGBE indoor air concentrations was noted between the controls (3×10^{-3} mg/m^3; 6.21×10^{-4} ppm; 95% confidence interval (CI) 3×10^{-4} – 2.96×10^{-2} mg/m^3, 6.21×10^{-5} – 6.13×10^{-3} ppm) and

the cases (3×10^{-3} mg/m^3, 0.64 ppm; 95% CI 7.6×10^{-4} - 1.27×10^{-4} mg/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 1932 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 89th exposure, and, in all instances, was normal after a 17-hour rest. At 1932 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 – 2087 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 ≥1517 and ≥980 mg/m^3 (≥314 and ≥203 ppm), respectively. However, at concentrations ≥1517 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 ≥1517 mg/m^3 (≥314 ppm),

but at these concentrations, females appeared more susceptible to the effects of EGBE, with 100% mortality at 1517 and 2087 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, 1517, or 2386 mg/m^3 (0, 54, 107, 203, 376, or 494 ppm, respectively) for 30 days did not show evidence of 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 1517 mg/m^3 (376 ppm) or higher (Carpenter *et al.*, 1956).

Several experiments in dogs were performed by 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 1860 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 8th (female) and 28th (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 1014 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 4th, 14th, and 30th 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 hemolysis. ↑ kidney weights at ≥517 mg/m^3 (≥107 ppm). Lung congestion and kidney swelling in 3 animals that died at ≥1517 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	1860 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 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 Hgb; 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 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 1208 (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 1208 mg/m^3 (125 and 250 ppm) concentrations (NTP, 2000). Anemia was observed following 3, 6, or 12 months of exposure at 604 or 1208 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 1208 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 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) (USEPA, 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 9.2.

7. Immune Effects and Asthma Risk

Effects of EGBE exposure on the immune system have been examined in several rodent studies (Exon *et al.*, 1991; Smialowicz *et al.*, 1992; Singh *et al.*, 2001; Chereshnev *et al.*, 2014) and are summarized in Table 9 below.

In subchronic exposures by Exon *et al.* (1991), adult male and female Sprague-Dawley rats (n = 6/sex/group) were exposed to EGBE in drinking water for 21 days at concentrations of 0, 2000, or 6000 ppm for males and 0, 1600, or 4800 ppm for females. These concentrations corresponded to exposures (arithmetic mean ± standard error of the mean) of 0, 180 ± 8, or 506 ± 10 mg/kg/day (average 0, 62, or 170 mg/rat/day) in males and 0, 204 ± 3, or 444 ± 15 mg/kg/day (average 0, 46, or 99 mg/rat/day) in females. Significant ($p \leq 0.05$) effects relative to controls included 1) increased BW-normalized thymus weights in 1600 ppm (46 mg/day) females; 2) increased BW-normalized liver weights in 2000 ppm (62 mg/day) males; 3) decreased BWs in 6000 ppm males,1600 ppm females, and 4800 ppm females (170, 46, and 99 mg/day, respectively); and 4) increased cytotoxic action by natural killer (NK) cells in 2000 ppm males and 1600 ppm females (62 and 46 mg/day, respectively). NK cells are lymphocytes (white blood cells) that are able to rapidly and non-specifically recognize and respond to stressed and/or infected cells that need to be eliminated. Based upon their experimental results, Exon *et al.* (1991) suggested that EGBE may produce stronger immunomodulatory effects on females versus males, and at lower versus higher concentrations.

Smialowicz *et al.* (1992) examined the effects of EGBE on the plaque-forming cell (PFC) response[1] to trinitrophenyl-lipopolysaccharide (TNP-LPS)[2]. Male Fischer 344 rats (8 – 10 weeks old; n = 6/group) were immunized with TNP-LPS (20 µg endotoxin/rat) and exposed by gavage 4 and 28 hours later to EGBE in distilled water at 0, 50, 100, or 200 mg/kg. Three days following immunization, PFC responses were measured. Results indicated that the 200 mg EGBE/kg dose was toxic as two of the six rats died over the course of the 3-day study. At the time of the PFC assay, one rat had died and another was moribund. The four remaining rats had significantly ($p < 0.05$) decreased antibody titers relative to controls. Citing previous research (Carpenter *et al.*, 1956; Tyler, 1984; Ghanayem *et al.*, 1987), the authors stated that the deaths were likely due to age-related sensitivity to hemolysis and secondary effects like prostration and death. They also speculated that the increased mortality at 200 mg EGBE/kg/day might have resulted from a synergistic or additive effect of EGBE on LPS-induced toxicity, reasoning that the low level of endotoxin used to immunize the rats was well tolerated by rats given glycol ethers other than BE. Because no significant effects were observed at EBGE doses that were not overtly toxic, the authors concluded that EGBE-induced hematotoxicity was not related to immunotoxicity in rats.

Two studies by Singh *et al.* (2001; 2002) suggest that EGBE may have immunomodulatory effects in dermally exposed mice. Singh *et al*. (2001) reported that female BALB/c mice (≥6 weeks of age; n = 5/treatment group) exposed topically to EGBE exhibited significantly ($p < 0.05$) suppressed T-cell immunity and enhanced splenic cellularity. T-cells are thymus-derived lymphocytes that participate primarily in cell-mediated immune responses by activating phagocytes (cells that ingest harmful/foreign particles, and dead/dying cells), other T-cells, and the release of cytokines (chemical messengers). In the experiments by Singh *et al.* (2001), EGBE was applied to a shaven area of the back in 25 µL aliquots, at a dose rate of 0, 100, 500, 1000, or 1500 mg EGBE/kg/day, for 4 consecutive days. Assessment of immune parameters including B- and T-cell function and proliferation, NK and T-killer cell cytotoxic activity, and lymphoid organ cellularity and weights began 24 hours after the last exposure.

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[1] The PFC response measures antibody-producing B-cells and is an indicator of B-cell function and proliferation. B-cells are bone marrow-derived lymphocytes. Unlike NK cells and T-cells, B-cells express B-cell receptors on their cell membranes that allow them to bind directly to specific antigens (molecules that induce immune reactions) on the surface of a pathogen (*e.g.* bacteria or virus) to neutralize it and initiate an antibody response. Antibodies also neutralize pathogens by binding specifically to surface antigens. The PFC assay allows the enumeration, in agar, of cells producing antibodies to foreign (*e.g.* sheep) or autologous RBCs.

[2] TNP-LPS is an immunogen that induces the secretion of antibodies by B-cells. TNP-LPS is formed by the conjugation of the trinitrophenyl hapten to lipopolysaccharide (endotoxin) from the cell wall of gram-negative bacteria.

In comparison to controls, mice exposed to EGBE at 500 or 1000 mg/kg/day had significantly ($p < 0.05$) decreased T-cell responses (>30% reduction) to Concanavalin A, a chemical that stimulates lymphocyte mitosis, and significantly decreased ($p < 0.05$) lymphoproliferative responses in a one-way mixed-lymphocyte reaction[3]. The proliferative responses of “responder” splenocytes from female BALB/c mice exposed to EGBE at 500 or 1000 mg/kg/day were reduced by 55% and 56%, respectively, when compared to associated vehicle controls (-EGBE, + “stimulator” splenocytes). Given that the mixed lymphocyte assay produced significant results and primarily measured responses of T-cells with surface CD4 proteins, while the T-killer cytotoxicity assay produced no significant results and measured the function of T-cells with CD8 surface proteins, the authors speculated that EGBE might have a stronger effect on the $CD4^+$ T-cell subtype.

Statistically significant ($p < 0.05$) effects at the high dose were limited to increased spleen-to-BW ratio and spleen leukocyte cellularity. These effects were not observed at lower doses. According to the authors, splenomegaly and congestion of the spleen have been reported in association with and secondary to hemolytic anemia in EGBE-exposed rats; therefore, the increased spleen weights observed at the high dose may have been related to RBC sequestration in the spleen following acute hemolysis. No measures of hemolysis were reported in the study by Singh *et al.* (2001). The authors estimated that dermal absorption of 1000 or 1500 mg EGBE/kg/day for 4 days would correspond to a maximum total absorbed EGBE dose of 20 – 30 mg, given 25% absorption. Extrapolating to a 70-kg human, the authors estimated immunomodulatory effects would occur at 28 – 105 g EGBE, quantities more likely to be relevant in cases of deliberate oral consumption of EGBE-containing cleaning products, such as that reported by Rambourg-Schepens *et al.* (1988).

A subsequent study by Singh *et al.* (2002) reported the effects of EGBE on the contact hypersensitivity response (CHR)[4], an inflammatory skin reaction that occurs in

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[3] The mixed-lymphocyte reaction assay is generally done *ex-vivo* with two lymphocyte populations that are from the same species but genetically distinct. In the one-way assay, “responder” cells are expected to proliferate in the presence of “stimulator” cells that have a different set of surface antigens. “Stimulator” cells are rendered non-proliferative, so only the “responder” cell responses are measured. The assay performed by Singh *et al*. (2001) looked at native BALB/c mouse “responder” splenocyte responses to “stimulator” C57Bl/6 mouse splenocytes. Both splenocyte populations were extracted from mice exposed to EGBE as described above.

[4] Development of contact sensitivity occurs in several stages. During the first topical exposure to a specific chemical antigen, dendritic cells of the skin (e.g. epidermal Langerhans cells and dermal dendritic cells) acquire the antigen, travel to regional lymph nodes, and activate antigen-specific T-cells. These antigen-specific T-cells then proliferate as 1) effector CD4+ T-helper cells and CD8+ T-killer cells which mount an immune response to the antigen (e.g. localized inflammation), or 2) as long-lived memory T-cells, which are relatively inactive in the skin until subsequent antigen exposures. These steps, from the initial antigen exposure to the resulting T-cell responses, mark the sensitization phase of the CHR. Upon subsequent exposure to the same antigen, the challenge phase of the CHR, the immune response is stronger and faster due in part to the presence of memory T-cells.

individuals sensitized to and subsequently challenged by a specific chemical antigen. In the study by Singh *et al.* (2002), the antigen was oxazolone (OXA), a small molecule xenobiotic irritant that binds to endogenous proteins and stimulates an immune response. Female mice (6-10 weeks old; n = 5/group) were sensitized with a 25-µL aliquot of 2% (weight/vol) OXA in vehicle (4:1 acetone and olive oil) applied on a shaved back. Five days after sensitization, mice were challenged with a non-irritating dose (20 µL; 0.5% weight/vol) of OXA in vehicle placed on the dorsal and ventral sides of the right ear. The CHR, measured as an inflammatory change in ear thickness, was tested prior to and 24 hours post challenge and compared among treated and control animals. A negative control group (-EGBE, -OXA) and a vehicle control group (-EGBE, +OXA) were included in the comparisons.

Along with the OXA sensitization and challenge, several EGBE exposure protocols were tested to determine the role of the exposure route, dose, time and metabolites in the OXA-induced CHR. These exposure protocols are summarized in Table 9 (page 52), below.

Results reported by Singh *et al.* (2002) showed that 1) dermal exposure to 4 mg EGBE (but not 0.25, 1, or 16 mg EGBE) at the time of OXA sensitization and/or challenge resulted in a lesser CHR than observed with corresponding vehicle controls; 2) effects were induced by EGBE and not BAA; 3) EGBE suppression of the CHR during the challenge phase was dependent upon dermal exposure immediately after OXA challenge (not 1 – 10 hours before/after challenge); and 4) suppression of the CHR was not related to expression of major histocompatibility complex (MHC) class II[5] molecules on the surface of Langerhans cells or protein synthesis by epidermal cells.

The authors stated that it was unlikely that systemic absorption of EGBE was related to the observed immune suppressive effects. They reasoned that oral EGBE exposures had no effect on the CHR; therefore, EBGE may be cleared rapidly from systemic circulation before it has time to distribute to and bioaccumulate at the site of the measured hypersensitivity response (right ear). The authors were also careful to state that it is unclear what effect topically applied EGBE may have on *in vivo* protein synthesis and MHC class II expression, but that EGBE cytotoxicity may play a significant role in its immunomodulatory effects. They speculated that EGBE might alter sensitization to OXA or activation of the CHR by affecting allergen delivery or other aspects of the immune response at the site of OXA sensitization/challenge.

[5] MHC II molecules are found on cells that collect and present Ags (e.g. OXA) and interact with CD4+ T-helper II cells to modulate immune responses. Singh et al. (2002) cited previous studies showing that topical xenobiotic application could lead to CHR suppression via inhibition of protein synthesis and reduced expression of major histocompatibility complex (MHC) class II molecules (Blaylock et al., 1991; Blaylock et al., 1993).

Although the immunosuppression reported by Singh *et al.* (2002) was shown to be due to EGBE, and hemolysis due to BAA, it may be helpful to understand how these two endpoints compare with respect to the EGBE concentrations required to produce significant effects. The 4 mg EGBE/20 µL concentration at which *in vivo* immunosuppression was observed (Singh *et al.*, 2002) is equivalent to 1690 mM EGBE (4 mg EGBE/20 µL = 200,000 mg EGBE/L; 200,000 mg EGBE/L ÷ 118,200 mg EGBE/mol x 1000 mM/M). *In vitro* human RBC lysis was reported at EGBE concentrations ranging from 125 to 200 mM (Udden, 2002), and at least one case report (Gijsenbergh *et al.*, 1989) discussed signs of hemolysis (*i.e.* decreased Hgb levels) in a patient ingesting 423 mM (25 g EGBE/500 mL = 50,000 mg/L. 50,000 mg/L x mol/118,000 mg EGBE = 0.423 M = 423 mM EGBE) to 507 mM EGBE (30 g EGBE/500 mL = 60,000 mg/L. 60,000 mg/L x mol/118,000 mg EGBE = 0.507 M = 507 mM EGBE). Assuming 20% absorption (Singh *et al.*, 2001), dermal exposure to 1690 mM EGBE would result in an absorbed EGBE concentration of 338 mM. Despite that, an absorbed EGBE concentration of 338 mM could be hemolytic in humans, and mice are much more sensitive than humans are to the hemolytic effects of EGBE (via its metabolism to BAA), hemolytic endpoints were neither measured nor reported by Singh *et al.* (2002).

One study has investigated the intersection of immunotoxicity and hemolysis. To create an animal model of hemolytic anemia for immune studies, Chereshnev *et al.* (2014) exposed 4-month old male and female white rats (n = 20/treatment group; 150 – 200 g BW) to EGBE at 0 or 20 mg EGBE/kg BW by a single IP injection. The number of rats/sex was not stated. Each rat received 0 or 4 mg EGBE in 1 mL of injectant (0 or 4000 ppm). On day 10 post exposure, animals were euthanized for analysis of peripheral blood indices (RBC number, percent reticulocytes (immature RBCs), and Hgb concentration), blood serum agglutination, and histopathology of lymphoid tissues including the spleen, thymus, and intestinal Peyer's patches[6].

Although no early deaths were reported by Chereshnev *et al.* (2014), comparisons between pre- and post-exposure blood indices of EGBE-exposed rats showed significant ($p < 0.05$) decreases in RBC numbers and Hgb concentrations (28% and 31% change, respectively). These losses may have been offset by the significant ($p < 0.05$) increase (190% change from pre-exposure levels) in the percentage of reticulocytes. No significant ($p < 0.05$) changes were reported for control animals. Agglutination tests were negative for controls and positive for EGBE-exposed rats. These latter results suggested that EGBE-exposed rats developed antibodies against their own RBCs, an autoimmune response. Histopathology of EGBE exposed rats

[6] Peyer's patches are lymphoid follicles in the wall of the small intestine that contain macrophages, dendritic cells, B- and T-cells involved in the development of immunity to antigens in the intestinal tract.

revealed signs of thymic involution, a progressive atrophy of the thymus that occurs normally with age and anomalously with stress. Lymphocyte death was observed in the cortical (outer) and medullary (middle) layers of the thymus, with large numbers of mast cells, pronounced cortical lymphocyte death in 40% of the animals, substitution of lymphoid with adipose tissue in 20% of the animals, and cystic medullary thymic cells in 20% of the animals. Splenic tissues of EGBE-exposed rats showed similar lymphoid tissue destruction, as the volume of splenic white pulp (lymphatic tissue) was roughly half of that expected for a healthy rat, and significantly ($p < 0.05$) lower than control-exposed rats. Splenic red pulp, a tissue with venous sinuses and different types of red and white blood cells, was characterized by hemosiderosis, cellular depletion, and sinusoidal congestion in 80 – 100% in EGBE-exposed animals. Intestinal Peyer's patches in EGBE-exposed animals were reported as excessively collagenized with destruction of lymphocytes. While histopathology of control rats was noted as fitting the species norm, that of EGBE-exposed rats was likened by the authors to stress-induced immunosuppression. Given their blood and tissue findings, the authors loosely speculated that EGBE may act indirectly as a hapten that stimulates antibody-driven hemolysis, and directly by increasing production of hypothalamic corticotropin-releasing hormone, a neurotransmitter involved in the stress response. No experiments were performed to test the action of EGBE as a hapten or on hypothalamic corticotropin-releasing hormone.

Overall, results showed that EGBE immunomodulatory effects include enhanced NK cell activity, increased autoimmune responses, lymphoid tissue destruction in rats, decreased $CD4^+$ T-cell proliferation, and T-cell-mediated inflammation in mice (Singh *et al.*, 2001). No significant B-cell effects were observed in these studies, and no reports regarding EGBE immunolomodulatory effects in humans were identified by OEHHA.

Although not discussed by Singh *et al.* (2001; 2002), EGBE-induced T-cell suppression may have implications for people with asthma. The results of this study suggested that $CD4^+$ T-cells, which play a large role in asthma, had significantly ($p < 0.05$) impaired proliferation and function when exposed to EGBE (Singh *et al.*, 2001). This impairment may have been related to EGBE's ability to decrease the CHR to OXA . Although the CHR and asthma can be linked by shared cellular and molecular players, it is difficult to predict exactly how EGBE exposure would alter the development of asthma or the biological processes that lead to an asthmatic episode and its resolution. Asthma is a heterogeneous disease, with different cell types driving pathology in different patients. In addition, $CD4^+$ T-cells can be further divided into separate subtypes with varying and sometimes opposing functions. For example, a large body of literature suggests that $CD4^+$ T-helper cells can initiate and maintain key pathophysiological features of asthma, such as allergic sensitization, eosinophil survival, and the development of airway hyperresponsiveness, through the production of cytokines like IL-4, IL-5, and IL-13,

respectively. At the same time, research also shows that CD4$^+$ T-regulatory cells can prevent allergic activation of dendritic cells in the airways, development of airway inflammation, and hyperresponsiveness through the production of anti-inflammatory cytokines and expression of inhibitory molecules (Lloyd and Hessel, 2010).

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 6837 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 complex, 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.

Table 9. Summary of EGBE immunotoxicity studies.

Reference	Species	Exposure	Results
Exon *et al.* (1991)	Male and female rats n = 6/sex/group	0, 2000, or 6000 ppm (0, 180 ± 8, or 506 ± 10 mg/kg/day) for males, and 0, 1600, or 4800 ppm (0, 204 ± 3, or 444 ± 15 mg/kg/day) for females via drinking water for 21 days.	Males: ↑ BW-normalized liver weights (2000 ppm only), and ↓ BWs (6000 ppm only). Females: ↑ BW-normalized thymus weights (1600 ppm only), and ↓ BWs (1600 and 4800 ppm). Males and Females: ↑ cytotoxic action by NK cells from males and at 2000 and 1600 ppm, respectively.
Smialowicz *et al.* (1992)	Male rats n = 6/group	TNP-LPS immunization (20 µg endotoxin/rat) followed 4 and 28 hours later by EGBE gavage at 0, 50, 100, or 200 mg/kg	At 200 mg EGBE/kg/day, ↓ decreased Ab titres, moribund condition, and death
Singh *et al.* (2001)	Female BALB/c mice n = 5/treatment group	Dermal application of 0, 100, 500, 1000, or 1500 mg/kg/day, for 4 consecutive days	At 500 or 1000 mg/kg/day, ↓ T-cell mitosis and lymphoproliferative responses in a one-way mixed-lymphocyte reaction. At 1500 mg EGBE/kg/day, ↑ spleen-to-BW ratio and spleen leukocyte cellularity.

Legend: Ab – Antibody; BW – Body weight; NK – Natural Killer; TNP-LPS - trinitrophenyl-lipopolysaccharide

Table 9. Summary of EGBE immunotoxicity studies (continued).

Reference	Species	Exposure	Results
Singh *et al.* (2002)	Female mice n = 5/group	Dermal sensitization with 2% OXA followed 5 days later by dermal OXA challenge[a]. Oral gavage with EGBE at 0, 50, 150, or 400 mg EGBE/kg/day in 0.1 to 0.15 mL) for 10 consecutive days, with OXA sensitization occurring on 10th day of EGBE treatment.	No significant ($p < 0.05$) effects on the CHR at 24 hours post challenge.
		Topical application of 0, 0.25, 1, 4, or 16 mg EGBE/ear immediately after OXA challenge.	At 4 mg, ↓ CHR 24 hours post challenge.
		Topical application of 0 or 4 mg EGBE/ear immediately after OXA sensitization, challenge, or both.	For 4 mg exposures given immediately after sensitization or challenge, 18% ↓ CHR relative to controls at 24 hours post challenge.[b] For 4 mg exposures given immediately after sensitization and challenge, 22% ↓ CHR relative to controls at 24 hours post challenge.
		Topical application of 0 or 4 mg EGBE/ear at 0 (T_0), 1, 3, 6, or 10 hour(s) before or after OXA challenge.	For 4 mg exposures at the T_0 timepoint, ↓ CHR.
		Topical application of 0, 2, 4, or 8 mg BAA/ear immediately after OXA challenge.	No significant ($p < 0.05$) effects on the CHR at 24 hours post challenge.
		Gavage with or without 4-methylpyrazole (MP) from sensitization to challenge, and topical application of 0 or 4 mg EGBE/ear immediately after OXA challenge	MP gavage + 4 mg EGBE resulted in ↓ CHRs relative to EGBE-exposed mice that did not get the MP treatment.

Legend: CHR - contact hypersensitivity response; OXA – oxazolone. ↓ - statistically significant ($p < 0.05$) difference due to a decrease [in a specific biological endpoint].
[a]The OXA protocol was repeated for all following exposures on this page. [b]Results were the same irrespective of whether EGBE was applied after sensitization or after challenge with OXA.

Table 9. Summary of EGBE immunotoxicity studies (continued).

Reference	Species	Exposure	Results
Chereshnev *et al.* (2014)	Male and female rats n= 20/group[b]	0 or 20 mg/kg BW (0 or 4000 ppm per animal) by IP	At 4000 ppm, ↓ RBCs and Hgb, ↑ reticulocytes, positive hemagglutination test, and lymphocyte death in the spleen, thymus, and intestinal Peyer's patches.

Legend: Hgb – hemoglobin; IP – Intraperitoneal injection; RBC – Red blood cell

[b] Number of rats/sex/group not stated.

8. 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; USEPA, 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 (This 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 1300 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 1300 and 2100 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, 1000, 1500, and 2000 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 1500 mg/kg-day and 2000 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 1000 and 1500 mg/kg-day. Four (all in the same litter) of 43 fetuses (9%) at 1000 mg/kg-day and one of 25 fetuses (4%) at 1500 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.

9. Derivation of Reference Exposure Levels

A dominant toxic endpoint in rodents following inhalation of EGBE is hemolysis. Nevertheless, the RELs derived below did not use this lesion as a critical endpoint for human exposure to EGBE. PBPK modeling in rodents and humans show that following EGBE inhalation, peak blood levels of BAA (the primary metabolite responsible for hemolysis) and AUC of blood BAA are greater in rodents than in humans (Corley *et al.*, 1994; Corley *et al.*, 2005). Peak blood levels of BAA can be 2-4 times greater in rodents than in humans for the same exposure to EGBE. In addition, *in vitro* hemolysis studies show that human RBCs are roughly 16 – 100 times more resistant to hemolysis compared to rat RBCs. Ghanayem (1989) showed that approximately 0.5 mM BAA was required to produce slight hemolysis of rat RBCs, whereas a similar level of slight hemolysis of human RBCs required 8 mM BAA. Udden (2002) reported that a 100-fold greater concentration of BAA is required *in vitro* to result in early hemolytic changes to human erythrocytes compared to rats. When incorporated into PBPK models, the low blood levels of BAA and high resistance to hemolysis indicated that even saturated air concentrations of EGBE could not produce high enough concentrations of BAA in humans to cause hemolysis (Corley *et al.*, 1994; Corley *et al.*, 2005). This finding is supported by acute human chamber EGBE exposure studies in which hemolysis was not found (Carpenter *et al.*, 1956).

Challenging this evidence is the finding that urinary levels of free BAA in humans can vary up to 10-fold. An occupational study by Jones and Cocker (2003) found a fraction of individuals that did not appear to have conjugated BAA in the urine. This would indicate a subset of the human population may have higher blood levels of free BAA, and thus may be more susceptible to hemolysis. However, no investigations have been identified that determine if there is an association between individuals who are non-BAA conjugators and high levels of BAA in their blood. Other data suggesting hematological sensitivity to EGBE come from occupational studies, where some measured RBC parameters in EGBE-exposed workers showed minor, but consistent, changes (Denkhaus *et al.*, 1986; Haufroid *et al.*, 1997; Hung *et al.*, 2011). The most comprehensive study examining RBCs in exposed workers observed changes in Hct percent and MCH concentration (p = 0.02 - 0.03) when compared to a control group (Haufroid *et al.*, 1997). However, this finding conflicted with a lack of statistically significant changes in RBC count, Hgb concentration, MCH mass, and osmotic resistance. The authors did not specify if some workers may have been more susceptible to RBC changes suggestive of hemolysis.

Lastly, case reports of large intentional oral intakes of cleaners that contain EGBE or coingestion of ethanol and EGBE have resulted in hemoglobinuria and low levels of Hct and Hgb in some cases, but changes in some RBC parameters lasted only a short time

period (no blood transfusion needed), and recovery occurred after a couple of days (Rambourg-Schepens *et al.*, 1988; Gijsenbergh *et al.*, 1989). Differences between RBC effects by route of exposure is likely due to dose rate differences. A large oral bolus dose of EGBE will yield a higher peak blood level of BAA than the same amount of EGBE in air taken in over a several-hour exposure. Additionally, other effects resulting from intentional oral intake of EGBE were more serious, including metabolic acidosis, severe CNS depression, and liver injury.

In conclusion, the weight of evidence currently does not support a REL based on hemolysis when other endpoints, including acute sensory irritation and chronic nasal epithelial degeneration, are more sensitive indicators of injury resulting from inhalation of EGBE. Given human RBC resistance to lysis, even if a subset of the population was found to produce higher blood levels of free BAA upon exposure to EGBE, it is very unlikely that the level of BAA would be sufficient to produce hemolysis unless the EGBE dose or dose rate is unusually high (*e.g.,* intentinal ingestion of bolus amounts). However, future research may help clarify whether a subset of the human population may be more susceptible to hemolysis due to differences in EBGE metabolism. OEHHA could then revisit the RELs to confirm if they are still protective of sensitive individuals in the general population.

9.1 EGBE Acute Reference Exposure Level

Study	Carpenter *et al.*, 1956
Study population	2 to 4 human subjects per study
Exposure method	Whole body exposure, 473, 546 and 942 mg/m^3 (98, 113 and 195 ppm)
Exposure duration	8 hours, 473 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
LOAEL uncertainty factor (UF_L)	10
Subchronic uncertainty factor (UF_S)	N/A
<u>Interspecies uncertainty factor</u>	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	1
<u>Intraspecies uncertainty factor</u>	
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, 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 10.

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 10). 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 10. 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-k} and 1 for toxicodynamic UF_{H-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. Finally, the small difference in derived values (about 2-fold) between the Carpenter *et al*. study and the Johanson *et al*. study also underscores that the use of Carpenter *et al*. for REL derivation is reasonable.

9.2 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	Hyaline degeneration of nasal 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
<u>Interspecies uncertainty factor</u>	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10
<u>Intraspecies uncertainty factor</u>	
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 (USEPA, 2015). The calculated $BMCL_{05}$ values, and corresponding NOAEL and LOAEL values are listed in Table 11. 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 11. 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 11: $BMCL_{05}$, 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/m^3 (ppm) (BMD model)	NOAEL mg/m^3 (ppm)	LOAEL mg/m^3 (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 (USEPA, 2015); NE, Not established.

Of the chronic effects noted in rats and mice in Table 11, 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 response/change 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; USEPA, 1999; EU, 2006; USEPA, 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 2230 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 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 11). 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 12. 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 12 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 12. 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 (USEPA, 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.

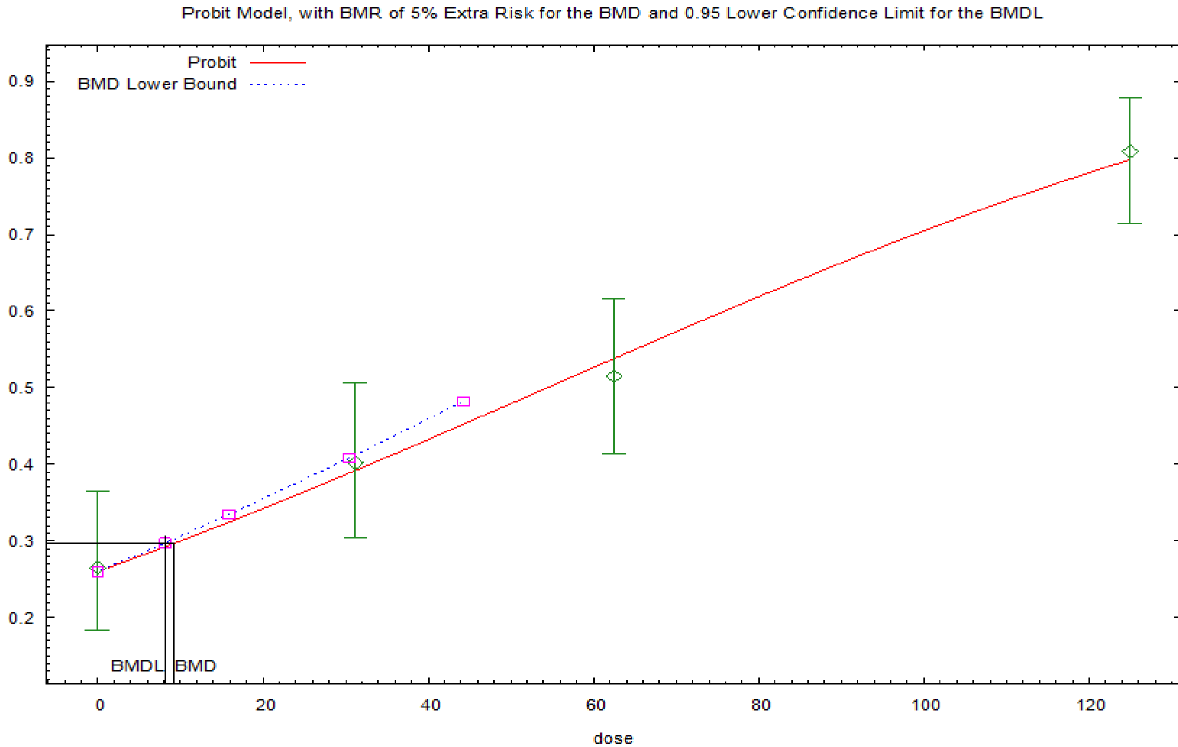

Figure 4. Dichotomous Probit model fit to nasal olfactory epithelium incidences in male and female rats after inhalation exposure to EGBE for 2 years (NTP, 2000)

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).

9.3 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	Hyaline degeneration of nasal 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
<u>Interspecies uncertainty factor</u>	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10
<u>Intraspecies uncertainty factor</u>	
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).

10. 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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