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TOXICOLOGICAL REVIEW

OF

ETHYLENE GLYCOL MONOBUTYL ETHER (EGBE)

NOTICE (CAS No. 111-76-2)

In Support of Summary Information on the
Integrated Risk Information System (IRIS)

APRIL 2008

U.S. Environmental Protection Agency
Washington, DC

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List of Acronyms

ADH	alcohol dehydrogenase
AIC	Akaike Information Criterion
ALD	aldehyde dehydrogenase
AUC	area under the curve
BAA	butoxyacetic acid
BAL	butoxyacetaldehyde
BMC	benchmark concentration
BMCL	benchmark concentration, 95% lower bound
BMD	benchmark dose
BMDL	benchmark dose, 95% lower bound
BMDS	benchmark dose software
BMR	benchmark response
CASRN	Chemical Abstracts Service Registry Number
CHR	contact hypersensitivity response
CI	confidence interval
Cl_S	clearance rate
con-A	concanavalin-A
C_{max}	peak concentration
CYP450	cytochrome P450
DNA	deoxyribonucleic acid
EA	ethyl acrylate
EG	ethylene glycol
EGBE	ethylene glycol monobutyl ether
EGEE	ethylene glycol ethyl ether
EGME	ethylene glycol methyl ether
EPA	U.S. Environmental Protection Agency
GD	gestation day
GFR	glomerular filtration rate
G6PD	glucose-6-phosphate dehydrogenase
GSH	glutathione
Hb	hemoglobin
Hct	hematocrit
HEC	human equivalent concentration
HED	human equivalent dose

HH	hereditary hemochromatosis
Hp	haptoglobin
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
KLH	keyhole limpet hemocyanin
LOAEL	lowest-observed-adverse-effect level
MAA	2-methoxyacetic acid
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean cell volume
ME	2-methoxyethanol
MOA	mode of action
NK	natural killer
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand white
8-OHdG	8-hydroxydeoxyguanosine
OR	osmotic resistance
OXA	oxazolone
PBPK	physiologically based pharmacokinetic
PFC	plaque-forming cell
POD	point of departure
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
ROS	reactive oxygen species
s.c.	subcutaneous
SCE	sister chromatid exchange
SD	standard deviation
$t_{½}$	half-life
TNFα	tumor necrosis factor-alpha
TNP-LPS	trinitrophenyl-lipopolysaccharide
UF	uncertainty factor
V_d	volume of distribution
WBC	white blood cell

Foreword

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to ethylene glycol monobutyl ether (EGBE). It is not intended to be a comprehensive treatise on the chemical or toxicological nature of EGBE.

The intent of Section 6, Major Conclusions in the Characterization of Hazard and Dose Response, is to present the major conclusions reached in the derivation of the reference dose, reference concentration and cancer assessment, where applicable and to characterize the overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing the quality of data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

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1. Introduction

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of ethylene glycol monobutyl ether (EGBE). IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear, presumed threshold, mode of action (MOA). The RfD, expressed in units of mg/kg-day, is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC, expressed in units of mg/m^3, is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal of entry) and effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposure (up to a lifetime), but may also be derived for acute ($\leq$ 24 hours), short-term (> 24 hours up to 30 days), and subchronic (> 30 days up to 10% of lifetime) exposure durations. All values are derived based on the assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure may be derived. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates may be derived from the application of a low-dose extrapolation procedure. If derived, the oral slope factor is an upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, an inhalation unit risk is an upper bound on the estimate of risk per $\mu g/m^3$ air breathed.

Development of these hazard identification and dose-response assessments for EGBE has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk*

Assessment (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through August 2007.

2. Chemical and Physical Information

EGBE is also known as 2-butoxyethanol. EGBE is widely used as a solvent in various applications, such as in surface coatings, spray lacquer, quick-dry lacquers, enamels, varnishes, varnish removers, latex paint, and metal cleaners and in commercially available cleaning products. EGBE has been estimated to range in concentration from 1 to 30% (volume/volume) in industrial and commercial products. The average concentration of EGBE in household products in 1977 was 2.6%. EGBE is a high production volume chemical with an estimated 390 million pounds produced in the United States in 1992 (National Toxicology Program [NTP], 2000). Some relevant physical and chemical properties of EGBE are:

CASRN:	111-76-2
Empirical formula:	C_4H_9-O-CH_2CH_2-OH
Molecular weight:	118.2
Vapor pressure:	0.88 mm Hg at 25°C (about 1200 ppm)
Water solubility:	Miscible
Log Kow:	0.81
Henry's law constant:	$2.08 \times 10^{-7} - 2.08 \times 10^{-8}$ atm-m^3/mole (25°C)
Flash point:	62°C (closed cup); 70°C (open cup)
Conversion factor:	1 ppm = 4.83 mg/m^3; 1 mg/m^3 = 0.207 ppm

EGBE exists as a colorless liquid at ambient temperature and pressure. Its evaporation rate relative to butyl acetate is 0.08; thus, it is considered a "slow evaporator." It is miscible in water and partitions about equally between phases of octanol and water. Considering the magnitude of the octanol:water partition coefficient (≈ 7 :1), it is unlikely that EGBE bioaccumulates. Based on the magnitude of the Henry's law constant, it is anticipated that partitioning of EGBE between water and air greatly favors the water phase.

3. Toxicokinetics

3.1. Absorption and Distribution

EGBE is absorbed and rapidly distributed in humans following inhalation, ingestion, or dermal exposure. Kumagai et al. (1999) examined 10 polar organic solvents, including EGBE, during short-term inhalation by humans. Four healthy male research subjects inhaled 25 ppm EGBE for 10 minutes; the mean uptake was 79.7% in the last 5 minutes of EGBE respiration.

Johanson and Boman (1991) attempted to define the relative importance of the skin to the total absorption of EGBE vapors by humans by comparing mouth-only and body-only exposures. Four research subjects were exposed to 50 ppm EGBE mouth-only for 2 hours, followed by 1 hour of no exposure, then 2 hours of 50 ppm body-only exposure (i.e., exposed in a chamber while breathing fresh air via a respirator). Blood samples were collected periodically for analysis of EGBE under the assumption that the finger-prick blood samples represented mixed arterial blood. Since the areas under the curve (AUCs) for the concentration of EGBE in the subjects' blood samples following body-only exposures were three- to fourfold greater than following mouth-only exposure, the authors concluded that the skin accounted for approximately 75% of the total uptake of EGBE in a whole-body exposure.

Corley et al. (1994) suggested that Johanson and Boman's (1991) conclusion of greater absorption of EGBE vapor through the skin than from the respiratory tract was inconsistent with the physiological differences (relative surface area, blood perfusion, barrier thickness) favoring absorption of vapors through the lungs. They reanalyzed the kinetic data of Johanson and Boman, assuming that the finger-prick blood samples represented venous blood draining the skin prior to mixing systemically. These revised calculations resulted in dermal uptake contributing no more than 22% of the total uptake of EGBE in a whole-body exposure at average temperatures and humidities (skin permeability coefficient of 3 cm/hour), assuming no clothing to hinder absorption.

To provide experimental validation of the skin's role in the uptake of EGBE vapors, Corley et al. (1997) conducted a study in which human research subjects exposed one arm to 50 ppm ^{13}C-EGBE for 2 hours. Catheters installed in the antecubital vein of the unexposed arm served as the primary site for blood collection, which was analyzed for both EGBE and 2-butoxyacetic acid (BAA). Finger-prick blood samples were collected from the exposed arm at the end of the 2-hour exposure. If Johanson and Boman's (1991) assumption that finger-prick blood samples represented systemic arterial blood was correct, then the concentrations of EGBE and BAA in the finger-prick blood samples taken from the exposed arm at the end of the 2-hour exposure should have been comparable to the corresponding catheter sample taken from the

unexposed arm. This was not the case, since the concentration of EGBE averaged nearly 1,500-fold higher in the finger-prick blood samples than in the samples collected from the unexposed arm, confirming the potential for portal of entry to have a major effect when using the finger-prick sampling technique.

3.2. Metabolism and Elimination

The metabolism of EGBE has been studied extensively in rodents, particularly in rats, and the large body of literature on this subject has been thoroughly reviewed (Commonwealth of Australia, 1996; European Centre for Ecotoxicology and Toxicology of Chemicals, 1994). Proposed pathways for the metabolism of EGBE in rats and humans are presented in Figure 3-1. The principal products from metabolic processes in rats or humans are butoxyacetaldehyde (BAL) and its corresponding carboxylic acid, BAA, and ethylene glycol (EG).

The two main oxidative pathways of EGBE metabolism observed in rats are via alcohol dehydrogenase (ADH) and O-dealkylation by a cytochrome P450 (CYP450) dealkylase (CYP 2E1). Because BAA is excreted in the urine of both rats and humans following EGBE exposure, it has been suggested that the production of BAA through the formation of BAL by ADH would be applicable to both rats and humans (Corley et al, 1997; Medinsky et al., 1990). However, the other proposed metabolic pathways of EGBE may only be applicable to rats, since the metabolites of these pathways (i.e., EG, EGBE glucuronide, and EGBE sulfate) have been observed in the urine of rats (Bartnik et al., 1987; Ghanayem et al., 1987a), but not in humans (Corley et al., 1997). In addition, Corley et al. (1997) confirmed an observation of Rettenmeier et al. (1993) that approximately two-thirds of the BAA formed by humans is conjugated with glutamine and, to a lesser extent, glycine. The BAA-glutamine and BAA-glycine conjugation pathways have not been detected in the rat.

Carpenter et al. (1956) first identified BAA as the metabolite responsible for the hemolytic toxicity of EGBE by incubating BAA with whole blood from a variety of species. Blood from rats, mice, and rabbits was more rapidly hemolyzed than blood from humans, monkeys, dogs, or guinea pigs when incubated in vitro at 37.5°C with a saline solution of 0.1% of the sodium salt of BAA. These results correlated well with osmotic fragility studies using blood from these same species following in vivo inhalation exposures to EGBE. In contrast, a much higher concentration (2.5%) of EGBE was required to produce a similar degree of hemolysis in vitro. Subsequent investigations have shown that hemolytic blood concentrations of BAA can be produced following oral or dermal administration or inhalation of EGBE.

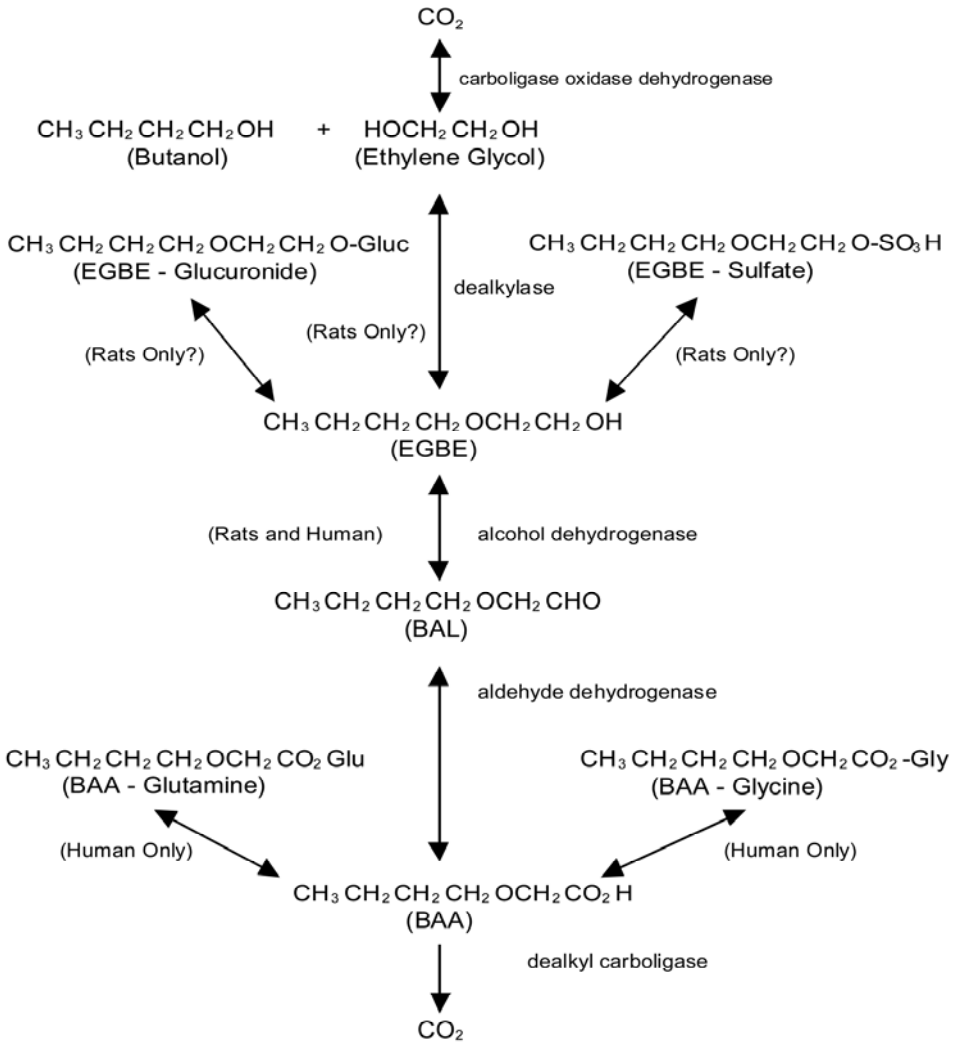

Figure 3-1. Proposed metabolic scheme of EGBE in rats and humans.

Source: Adapted from Medinsky et al. (1990) and Corley et al. (1997).

The uptake and metabolism of EGBE is essentially linear following a 6-hour inhalation exposure of up to 438 ppm, a concentration that causes mortality in animals (Sabourin et al., 1992a). BAA is the primary metabolite in rats following drinking water (Medinsky et al., 1990) and inhalation (Dill et al., 1998) exposures. EGBE is eliminated primarily as BAA in urine. Lesser amounts of the glucuronide and sulfate conjugates of EGBE have been observed in the urine of rats (Bartnik et al., 1987; Ghanayem et al., 1987a) but not humans (Corley et al., 1997). No significant differences in the urinary levels of BAA were found following administration of equivalent doses of EGBE dermally or in drinking water (Shyr et al., 1993; Sabourin et al., 1992b; Medinsky et al., 1990). Corley et al. (1997) reported that the elimination kinetics of EGBE and BAA appear to be independent of the route of exposure. Elimination of EGBE and BAA following repeated inhalation exposure appears to be dependent on species, gender, age, time of exposure, and exposure concentration (NTP, 2000; Dill et al., 1998).

Percutaneous absorption of EGBE in rats is rapid and produces measured blood levels of BAA sufficient to produce hemolysis (Bartnik et al., 1987). Metabolism, disposition, and pharmacokinetic studies in male F344 rats conducted by Corley et al. (1994) produced hemolytic blood concentrations of BAA (0.5 mM) following a single oral dose of 126 mg/kg of ^{14}C-labeled EGBE. Using their physiologically based pharmacokinetic (PBPK) model, they predicted that such hemolytic blood concentrations would also be produced in rats following a single 6-hour EGBE inhalation exposure greater than 200 ppm. A report that evaluated the NTP (2000) inhalation bioassay suggests that BAA blood concentrations in rats exceeded 0.5 mM (approximately 67 μg BAA/g blood), following exposure to 62.5 ppm EGBE for both 1-day and 12-month exposure durations (Dill et al., 1998).

The metabolic basis for the hematotoxicity of EGBE was studied in male F344 rats by using pyrazole and cyanamide as metabolic inhibitors of ADH and aldehyde dehydrogenase (ALD), respectively (Ghanayem et al., 1987b). Male F344 rats, 9 to 13 weeks old, were pretreated with pyrazole or cyanamide followed by administration of 500 mg/kg EGBE by gavage. The use of pyrazole protected rats from EGBE-induced hematotoxicity and resulted in a 10-fold lower ratio of BAA to conjugated EGBE excreted in urine. Cyanamide treatment significantly reduced the hematotoxic response in a manner similar to that of pyrazole; it also resulted in a high mortality rate in rats given cyanamide and EGBE. This effect was possibly due to the increased levels of unmetabolized BAL; the effect was not observed in animals treated with cyanamide or EGBE alone. Pyrazole completely blocked the increase in spleen weight to body weight ratios seen in EGBE-treated animals. Gavage administration of either BAL or BAA at equivalent molar doses to 125 mg/kg EGBE produced identical increased spleen to body weight ratios and identical increases in free hemoglobin (Hb) levels in plasma. Pretreatment of rats with cyanamide prior to administration of BAL provided significant protection against BAL-induced hematotoxicity. These studies confirm the central role of BAA in the hematotoxic response elicited in rats.

Green et al. (2002) explored reasons that female mice develop marked hyperkeratosis in the forestomach when given oral doses (1/day for 10 days) of either EGBE or BAA. Irritation from the carboxylic acid BAA is hypothesized to cause cell damage followed by cell proliferation and eventually the observed hyperkeratosis. Their studies examined the activity and localization of ADH and ALD, the principal enzymes involved in the metabolism of EGBE in the stomach tissues of mice and rats, the localization of these enzymes in a human stomach sample, and whole body autoradiography of mice exposed to radiolabeled EGBE (see Section 4.4.1). Tissue homogenates were prepared from female B6C3F1 mice (n = 30) and rats (n = 10; gender and species not specified) and centrifuged at 41,000 × g, with the supernatants used to examine the metabolism of EGBE by ADH and ALD. The stomachs were separated into fore and glandular sections and used to measure the metabolism of EGBE to BAL and BAL to BAA by

ADH and ALD, respectively. A marked species difference in ALD activity was observed between rats and mice:

Rats: K_m = 0.29 mM

V_{max} = 1.627 nmol/minute per mg protein, forestomach, K_m = 0.73 mM

V_{max} = 2.170 nmol/minute per mg protein, glandular stomach

Mice: K_m = 46.59 mM

V_{max} = 17.094 nmol/minute per mg protein, forestomach, K_m = 87.01 mM

V_{max} = 13.986 nmol/minute per mg protein, glandular stomach

K_m values were up to one order of magnitude greater in mice compared to rats. Based upon the K_m and V_{max} values reported, while the mouse ALD enzyme has a lower affinity than the rat enzyme for EGBE, the mouse enzyme has a much greater capacity to metabolize EGBE to the intermediate without becoming saturated. The fact that EGBE is held in the forestomach, along with the information that rates for the ADH enzyme were of the same order of magnitude for rats and mice, suggests that mice are capable of generating more BAA in the forestomach than rats for the same dose and exposure duration.

Green et al. (2002) also examined the distribution of ALD and ADH in rat, mouse, and human stomach tissue sample from a single individual, using histochemical staining. The stratified squamous epithelium of the forestomach of both rats and mice contained the highest staining intensity for ALD and ADH. These enzymes were found throughout the mucosa in the human stomach tissue sample; the highest concentration was found in the mucus-producing cells at the surface. Data indicates that the distribution of these enzymes in humans is more closely comparable to that found in the rodent glandular stomach than in the rodent forestomach. This finding, combined with the difference in ALD and ADH activity between mouse and rat forestomach, suggests that humans are at much lower risk for the tissue irritation seen in the mouse forestomach.

The effects of age, dose, and metabolic inhibitors on the toxicokinetics of EGBE were studied in male F344 rats (Ghanayem et al., 1990). Rats aged 3–4 months and 12–13 months were administered a single gavage dose of 31.2, 62.5, or 125 mg/kg EGBE. Pretreatments included pyrazole, cyanamide, or probenecid, an inhibitor of renal anion transport. Toxicokinetic parameters for EGBE, including AUC, maximum plasma concentration (C_{max}), and clearance rate (Cl_S), were dose dependent; AUC and C_{max} increased and Cl_S decreased as dose levels increased. Other measured parameters were unaffected by dose. Age had no effect on half-life ($t_{½}$), volume of distribution (V_d), or $C1_S$ of EGBE, but C_{max} and AUC increased with age. As expected from previous studies, inhibition of EGBE metabolism by either pyrazole or cyanamide resulted in significantly increased $t_{½}$ and AUC, as well as decreased Cl_S. BAA toxicokinetics were also altered by dose and age, as well as by administration of metabolic inhibitors. Statistically significant, slight increases in C_{max}, AUC, and $t_{½}$ were seen at higher doses; these results were more pronounced in older rats. Probenecid pretreatment at EGBE dose levels of 31.2

and 62.5 mg/kg produced no changes in the measured toxicokinetic parameters for EGBE. Pretreatment produced two- to threefold increases in AUC, and two- to sixfold increases in $t_{½}$ for BAA. These results indicate that renal organic acid transport is vital to renal elimination of BAA. The increased C_{max}, AUC, and $t_{½}$ in older versus younger rats may be due to differences in relative contributions of the two primary metabolic pathways previously discussed, or to compromised renal clearance.

For humans, the elimination kinetics of EGBE and BAA appear to be independent of the route of exposure. The $t_{½}$ in humans for the elimination of EGBE and BAA averaged 0.66 and 3.27 hours, respectively. For whole-body exposures under exercise conditions, the elimination $t_{½}$ for EGBE and BAA were 0.66 and 4 hours, respectively (Johanson and Johnsson, 1991; Johanson, 1986). For dermal exposure to neat liquids, the $t_{½}$ for elimination of EGBE and BAA were 1.3 and 3.1 hours, respectively (Johanson et al., 1988). For dermal exposure to vapors, the elimination $t_{½}$ for EGBE was 0.53–0.6 hour.

Haufroid et al. (1997) conducted a study on 31 male workers exposed to low levels of EGBE in a beverage package production plant. The average airborne EGBE exposure concentration was 2.91 ± 1.30 mg/m^3 (0.59 ± 0.27 ppm). Post-shift urine samples showed an average BAA concentration of 10.4 mg/g creatinine. One exposed individual who exhibited a very low urinary BAA excretion was found to possess a genetic polymorphism for CYP 2E1 that produced increased oxidative activity. However, the researchers did not measure BAA conjugated to glutamine, an alternative pathway for BAA excretion in humans. Further investigations on the influence of genetic polymorphisms for CYP 2E1 on urinary BAA excretion rate are needed before conclusions can be drawn.

Johanson and Johnsson (1991) analyzed venous blood samples collected at 0, 2, 4, and 6 hours from exposure from five healthy, male research subjects exposed to 20 ppm EGBE via inhalation for 2 hours during light physical exercise on a bicycle ergometer. Blood samples were analyzed for BAA concentrations. An average peak blood concentration of 45 µM BAA was reached 2–4 hours after exposure. The range of concentration was from 36 to 60 µM. The average $t_{½}$ for elimination of BAA from blood was 4.3 hour, with a range from 1.7 to 9.6 hours, suggesting that blood levels of BAA would probably not increase following prolonged occupational exposures to concentrations of EGBE vapor at or below existing occupational exposure limits of 20–25 ppm. Thus, blood levels would not reach those shown to cause adverse effects in vitro. The average renal clearance of BAA was 23–39 mL/minute, which was only about one-third of the glomerular filtration rate (GFR). The authors suggested that the low clearance of BAA relative to the GFR could have been related to the binding of BAA to proteins in blood or to a low efficiency in renal tubular secretion. The low pKa of 3.5 estimated by the researchers indicates that tubular reabsorption is unlikely, because more than 99% of the BAA in normal human urine (pH ~6) is ionized. The V_d averaged 15 L (range 6.5–25 L) based on whole

blood measurements, and was approximately equal to the volume of extracellular water (13–16 L), a further indication of binding of BAA to blood proteins.

Laitinen (1998) reported BAA levels in a study of eight silkscreen printers (gender not specified) exposed to a mixture of EGBE and 2-butoxyethylacetate. Daily mean 8-hour air concentrations ranged from 0.1 to 0.6 ppm during a 5-day period. Urine samples from these workers contained 75 mg BAA/g creatinine immediately after the work shift, and 58 mg/g creatinine the following morning, 14 to 16 hours post-shift. Laitinen et al. (1998) reported similar post-shift urinary levels of 60 mg BAA/g creatinine in another group of 37 male and 15 female silkscreen workers exposed to 5 ppm EGBE and its acetate for one 8-hour workday.

Deisinger and Boatman (2004) determined the extent of the in vivo formation of BAL and BAA from EGBE and their elimination kinetics from blood, liver, and forestomach of mice. Male and female B6C3F1 mice (n = 4/gender/time point) were administered oral doses of 600 mg/kg EGBE dissolved in distilled water. At 5, 15, 45, and 90 minutes following the dose, blood, liver, and forestomach tissues, along with forestomach contents, were collected and processed to determine EGBE, BAL, and BAA concentrations in the samples. High EGBE concentrations were measured at all time points; maximum concentrations occurred 5 minutes after dosing, with a mean of 123 mM in females and 129 mM in males. EGBE levels in blood and liver were also at maximum concentrations at 5 minutes post-dosing, but at levels that were roughly 50-fold lower than in the forestomach. BAA concentrations in all organs were substantial in the 5-minute samples, and concentrations continued to increase until leveling off in the 45- and 90-minute samples. Concentrations of BAA measured in the forestomach were lower than concentrations in blood and liver tissues. Furthermore, BAA was found to be associated with forestomach tissues, rather than forestomach contents. BAL levels were highest in the initial samples, 5 minutes post-dose, then declined. Levels of BAL measured in the forestomach were 10-fold to 100-fold lower than the parent compound or carboxylic acid metabolite. No differences between male and female mice were apparent in the parent compound or BAA organ concentrations at comparable time points following dosing, but the BAL concentrations were up to twofold greater at some time points in the liver and forestomach of female mice compared to male mice.

Dill et al. (1998) reported on toxicokinetic findings collected from exposures carried out in male and female F344 rats and male and female B6C3F1 mice as part of the 2-year EGBE inhalation toxicity and carcinogenicity study conducted by the NTP (2000). Blood samples were collected from some of these animals post- exposure (i.e., after the daily 6-hour exposure) after 1 day, 2 weeks, and 3, 6, 12, and 18 months of exposure to target EGBE concentrations of 0 and 31.2 ppm (rats only) and 62.5, 125, or 250 ppm (mice only) by whole-body inhalation and assayed for EGBE. Post-exposure time points varied from 10 to 720 minutes following 1 day, 2 weeks, and 3 and 6 months and varied from 10 to 2,880 minutes following 12 months. Post-exposure 16-hour urine samples were collected after 2 weeks and 3, 6, 12, and 18 months of

exposure and assayed for BAA. In addition, a separate set of aged mice were kept in the control chamber and exposed to EGBE for 3 weeks when they were approximately 19 months old. Post-exposure blood samples were collected after 1 day and 3 weeks of exposure; 16-hour urine samples were collected after 2 weeks of exposure. Overall, mice eliminated both EGBE and BAA from blood faster than rats: for example after the 1-day exposure, $t_{½}$ for rats was less than 10 minutes, and $t_{½}$ for mice was less than 5 minutes. In contrast, the rate of BAA elimination from blood decreased as the exposure concentration increased. As exposure continued, the rates of elimination for both EGBE and BAA decreased in both species, resulting in longer residence times in the blood. At 1 day post-exposure, $t_{½}$ in male rats was 9.4 minutes, and at 18 months post-exposure $t_{½}$ was 15.8 minutes. Female rats were significantly less efficient in clearing BAA from their blood than males, possibly as a result of reduced renal clearance in female rats. The aged mice were observed to eliminate BAA from blood >10 times slower than young mice after 1-day of exposure, but this difference was less obvious after 3 weeks of exposure. These findings provide evidence that the elimination kinetics of EGBE and BAA following repeated inhalation exposure to EGBE appears to be dependent on various factors, including species, gender, age, time of exposure, and exposure concentration.

Using rate constants derived from mouse stomach fractions (Green et al., 2002) and making several assumptions about the use of these enzyme activity data, Corley et al. (2005a) estimated that 250 ppm EGBE would result in peak C_{max} concentrations of 7 µM EGBE, 0.5 µM BAL, and 3,250 µM BAA in liver tissue of male mice at the end of a 6-hour inhalation exposure. The model includes the metabolism of EGBE to BAL via ALD, and the subsequent metabolism of BAL to BAA via ADH in both the liver and forestomach. The model predicts that the concentrations of BAL in gastrointestinal tract tissues of male and female mice at 5 minutes postdosing, the time of maximal concentration, would be 18 and 33 µM, respectively, following oral gavage exposure to 600 mg/kg EGBE. This compares well with the levels of BAL actually observed in forestomach tissue of male and female mice at 5 minutes post-dosing, 19 and 33 µM, respectively, following oral gavage exposure to EGBE at 600 mg/kg (Deisinger and Boatman, 2004).

4. Hazard Identification

4.1. Studies in Humans: Epidemiology, Case Reports, Clinical Controls

Carpenter et al. (1956) conducted three controlled inhalation studies. In the first study, a group of two men and six rats were exposed simultaneously for 4 hours to an EGBE concentration of 113 ppm in a 1,250-cubic foot room. Effects observed in humans included nasal and ocular irritation, a metallic taste, and belching. Erythrocyte osmotic fragility did not change for the men, yet rose appreciably for the rats. In a second study, a group of two men, one woman, and three rats were exposed to 195 ppm EGBE for two 4-hour periods, separated by a 30-minute recess, in a 6.5-cubic foot room. There was no change in the subjects' blood pressure, erythrocyte fragility, or pulse rate. They experienced nose and throat irritation, followed by ocular irritation and disturbed taste; one subject reported a headache. In the rats, an increase in erythrocyte fragility values was noted. In the third study, two men and two women were exposed for 8-hours to a 100 ppm EGBE concentration. No changes in blood pressure, erythrocyte fragility, or pulse rate were observed. Again, nasal and throat irritation followed by ocular irritation and a disturbing metallic taste were experienced. Two subjects reported headaches.

There are a number of case reports of acute ingestion of EGBE, consisting primarily of accidental or intentional ingestion. Bauer et al. (1992) reported the effects of acute ingestion of 500 mL of window cleaner containing 9.1% EGBE and 2.5% ethanol by a 53-year-old alcoholic male. He was comatose with metabolic acidosis, shock and noncardiogenic pulmonary edema when brought to a hospital, approximately 10 hours after ingestion. He had increased heart rate, decreased blood pressure, and transient polyuria and hypoxemia. Hypochromic anemia was evident with an Hb concentration of 9.1 g/100 mL, a hematocrit (Hct) of 25%, and thrombocytopenia. The patient recovered and was discharged after 15 days.

Gijsenbergh et al. (1989) reported that a 23-year-old woman weighing 64 kg ingested approximately 25–30 g of EGBE (~400–500 mg/kg) and ethanol (~4:1 ratio) as a window cleaner in an apparent suicide attempt. She was comatose when admitted to the hospital, exhibiting dilated pupils, obstructive respiration, and metabolic acidosis, including depression of blood Hb concentration and hematuria. The presence of EGBE in the blood and dialysis fluid was confirmed. Treatment consisted of supportive therapy, forced diuresis, bicarbonate administration, and hemodialysis. Her Hb concentration fell from 11.9 g Hb/100 mL upon admission to 8.9 g Hb/100 mL. She was discharged after 8 days.

Gualideri et al. (2003, 1995) reported a case of a suicide attempt with an industrial-strength window cleaner. The 18-year-old male weighed 71 kg; he consumed between 360 and

480 mL of a concentrated glass cleaner that contained 22% EGBE, a dose equivalent to 1,131–1,509 mg/kg. He was admitted to the hospital with no abnormalities other than epigastric discomfort within 3 hours post-ingestion. Approximately 10 hours post-admission, the patient was noticeably lethargic, weak, and hyperventilating, symptoms consistent with the onset of metabolic acidosis. BAA was measured; the highest serum concentration found was 4.86 mmol/L, collected approximately 16 hours post-ingestion. The patient was transferred to a tertiary care hospital where hemodialysis was initiated at approximately 24 hours post-ingestion. Ethanol therapy was started 30 minutes later. Treatment also consisted of intravenous (i.v.) doses of 100 mg thiamine and 50 mg folic acid every 12 hours and 50 mg pyridoxine every 6 hours. Following 4 hours of dialysis, the patient was alert and remained hemodynamically stable. Ten days after discharge, the patient was readmitted following a second ingestion of 480 mL of the same cleaner, a EGBE dose equivalent to 1,509 mg/kg. Treatment included ethanol therapy and hemodialysis, and was initiated within a few hours of ingestion to control the metabolic acidosis. Due to this early treatment, ethanol therapy had an impact on the disposition of EGBE and BAA. As with the first episode, metabolic acidosis was manifested. This high-dose oral ingestion was nearly 1.1–1.5 g EGBE/kg body weight. The highest serum BAA concentration was 2.07 mmol/L, collected 22 hours post-ingestion. No evidence of hemolysis or renal abnormalities was detected.

A 50-year-old woman ingested approximately 250–500 mL of a window cleaner containing 12% EGBE, representing ~30–60 mL, in an apparent suicide attempt (Rambourg-Schepens et al., 1988). She was diagnosed with metabolic acidosis, hypokalemia, a rise in serum creatinine level, and a marked increase in urinary excretion of oxalate crystals. Moderate hemoglobinuria appeared on the third day post-exposure, and a progressive erythropenia was noted. In the absence of more complete hematologic details from this and other similar case studies, it is not possible to determine whether these effects were due to hemolysis or other factors related to the profound blood chemistry changes observed. The clinical status improved gradually and the patient was discharged on the tenth day.

Burkhart and Donovan (1998) summarized the case of a 19-year-old male who ingested 20–30 ounces, or ~590–885 mL, of a product that contained 25–35% EGBE, an exposure equivalent to ~177–265 mL, estimated at >3,000 mg/kg, along with 15–25% propylene glycol, 5–10% monoethanolamine, and 1–3% potassium hydroxide. On his arrival at the hospital 3.5 hours after ingestion, the patient was deeply comatose with severe hypotension. Hematuria developed on the second day, with no evidence of renal or hepatic toxicity; however, pulmonary toxicity consisting of severe aspiration pneumonia was present. The patient had a significant recovery, despite severe neurologic deficits that were slow to resolve.

Osterhoudt (2002) reported on a 16-month-old girl who ingested an unknown amount of cleaning solution containing EGBE (10–30%), monoethanolamine (5–10%), alkoxylated linear alcohols (1–5%), ethylenediaminetetraacetic acid (1–5%), and potassium hydroxide (1–5%).

Metabolic acidosis was manifest, and a single dose (15 mg/kg) of the ALD inhibitor fomepizole was administered. Within 2 hours, the metabolic acidosis was completely resolved, and there was no evidence of alkaline mucosal injury, hepatic or renal dysfunction, or hemolysis.

Dean and Krenzelok (1991) reported that 24 children, aged 7 months to 9 years, were observed subsequent to oral ingestion of at least 5 mL of glass window cleaner containing EGBE in the 0.5–9.9% range. Two children drank more than 15 mL, and were treated by gastric lavage. No symptoms of EGBE poisoning, such as metabolic acidosis, and no hemolysis were observed in any of the children.

Raymond et al. (1998) reported on seven clerical workers who were evaluated 8 months after they entered a file room where the supervisor believed that EGBE had been applied overnight to strip the floor. Exact details of the product used were unknown, but based on containers found and exposure symptoms of noted intense eye and respiratory irritation, marked dyspnea, nausea, and faintness, the authors suggested that they were exposed to EGBE concentrations of 200–300 ppm. Of major concern were skin spots—cherry angiomas—that appeared between 4 and 22 weeks after exposure in six of the seven workers. All workers continued to experience recurrent eye and tracheobronchial irritation; four had a dry cough. Workplace air sampling conducted by a certified industrial hygienist one week after the floor stripping found no detectable EGBE, although traces (0.1–0.2 ppm) of formaldehyde were identified. Five years after the exposure, four of the workers who could be contacted reported that they continued to have outbreaks of new cherry angiomas. It should be noted that no other studies linking EGBE exposure to outbreaks of cherry angiomas are available in the literature. The authors included the observation that, since this report, they had seen three patients who they believe were also exposed to EGBE vapor in an unrelated incident, and who did not develop any skin spots. Cherry angiomas are the most common cutaneous vascular lesion; they are benign and formed by a proliferation of dilated venules. The spots occur more frequently with increasing age but can appear in younger individuals. There are reports in the literature of cherry angiomas appearing following individual exposure to other chemicals, such as bromides (Cohen et al., 2001), glutaraldehyde (Raymond et al., 1998), and sulfur mustard gas (Firooz et al., 1999).

A cross section of 31 male workers, aged 22 to 45, employed for 1–6 years, who were exposed to low levels of EGBE in a beverage packing production plant were monitored by Haufroid et al. (1997). The effect of external EGBE exposure and internal BAA levels on erythrocyte lineage were investigated: red blood cell (RBC) count, Hb, Hct, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), haptoglobin (Hp), reticulocyte count, and osmotic resistance (OR), a measure of osmotic fragility. Also studied were serum glutamic-oxaloacetic and glutamic-pyruvic transaminases and renal creatinine and urinary retinol binding protein parameters. The average airborne concentration of EGBE was 2.91 mg/m^3, or 0.6 ppm (standard deviation [SD] of ±1.30 mg/m^3 or 0.27 ppm). In addition, there was coexposure to methyl ethyl ketone. Single

determinations of BAA in post-shift urine samples were used to assess exposure to low levels of EGBE. No differences were observed for RBC counts, Hb, MCV, MCH, Hp, reticulocyte count, and OR between exposed and control workers. The only statistically significant change observed in exposed workers when compared with a matched control group (n = 21) was a 3.3% decrease in Hct ($p = 0.03$) and a 2.1% increase in MCHC ($p = 0.02$). The implications of these small erythroid effects are unclear. Both values are within their corresponding normal clinical ranges and, given that no statistically significant changes were observed in other erythroid parameters, they do not appear to be related to the more severe adverse effects observed in laboratory animals. Furthermore, no correlation was found between any of the nine erythroid parameters measured and the parameters of internal exposure. No significant differences were observed in hepatic and renal biomarkers.

Several human studies investigated the dermal absorption of EGBE. Jakasa et al. (2004) dermally exposed six male research subjects, ages 22–55, to 50%, 90%, or neat EGBE for 4 hours on the forearm over an area of 40 cm^2. The dermal absorption of EGBE from aqueous solutions was markedly higher than from neat EGBE. In Jones et al. (2003), four research subjects were exposed via inhalation of 50 ppm EGBE for 2 hours on nine separate occasions, with each occasion separated by 3 weeks, at varying temperatures and humidity levels. Results show that "baseline" dermal contribution to total body absorption of EGBE vapor in appropriately dressed workers was, on average, 11%. Higher temperature (30°C, mean 14%, $p = 0.03$) and greater humidity (65% relative humidity, mean 13%, $p = 0.1$) both increased dermal absorption. The wearing of whole-body overalls did not attenuate absorption (mean 10%). By combining several factors together in the industrial scenario, dermal absorption of vapors was reported to be significantly increased to a mean value of 39% of the total absorbed dose.

4.2. Subchronic and Chronic Studies and Cancer Bioassays in Animals: Oral and Inhalation

4.2.1. Subchronic Studies

4.2.1.1. Oral

A number of subchronic studies by the oral gavage route of exposure have been conducted. Krasavage (1986) conducted a toxicity study using groups of 10 COBS CD (SD)BR adult male rats treated by gavage with 222, 443, or 885 mg/kg-day undiluted EGBE 5 days/week for 6 weeks. Endpoints evaluated throughout the study included body weight, food consumption, clinical signs, and survival. Hematology and serum clinical chemistry parameters were determined after the last treatment. Dose-related changes were observed in the RBC counts of all treatment groups, including statistically significant decreases in RBC count and Hb concentration, and a statistically significant increase in MCH. Statistically significant

hematological changes occurring at 443 and 885 mg/kg-day were increased MCV and decreased MCHC. The increased MCV at higher doses is likely due to both an increase in MCV and in the number of larger reticulocytes in the circulation following the erythropoietic response (NTP, 2000). Based on decreased RBC count and trends in Hb and other hematological endpoints, the lowest-observed-adverse-effect level (LOAEL) was determined to be 222 mg/kg-day, the lowest dose tested. A no-observed-adverse-effect level (NOAEL) was not identified.

Nagano et al. (1979) performed a toxicity study in male JCL/ICR mice (five/group) using gavage doses of 0, 357, 714, or 1430 mg/kg-day EGBE 5 days/week for 5 weeks. Parameters evaluated at the end of the study were hematology (RBC and white blood cell [WBC] counts, MCV, and Hb), absolute and relative weights of testes, and testicular histology. Mean RBC counts were significantly lower than the control values in the 357 and 714 mg/kg-day groups, but WBC counts were not affected. All animals in the 1,430 mg/kg-day group died before examinations were performed; mortality was not observed in the lower dose groups, and no differences in testes weights or histology were found. The LOAEL for this study, based on the reduced RBC count, was 357 mg/kg-day. A NOAEL was not determined.

Siesky et al. (2002) investigated whether subchronic exposure to EGBE in F344 male rats, 8–10 weeks old, and B6C3F1 male mice, 8–10 weeks old, produced an increase in oxidative damage and deoxyribonucleic acid (DNA) synthesis in endothelial cells and hepatocytes in the mouse liver, the putative cancer targets. Mice (60/group) were treated via gavage with doses of 0, 225, 450, and 900 mg/kg-day and rats (20/group) with 0, 225, and 450 mg/kg-day for 90 days. A dose-related increase in hemolysis was observed in both rats and mice. An increase in the percentage of iron-stained Kupffer cells was observed following 450 and 900 mg/kg in mice and 225 and 450 mg/kg in rats. An increase in oxidative damage, as measured by 8-hydroxydeoxyguanosine (8-OHdG) levels, was seen in mouse livers at 450 and 900 mg/kg-day after 7 or 90 days, while no increase was seen in rat livers at any dose or time point examined. Vitamin E levels were reduced by all doses of EGBE in the mouse and rat liver (statistically significant at 7 and 90 days in both mice and rats); however, the basal level of vitamin E was ~2.5-fold higher in rat than in mouse liver. The LOAEL for this study was 450 mg/kg-day in mice and 225 mg/kg-day in rats, based on the percentage of iron-stained Kupffer cells. The NOAEL was 225 mg/kg-day in mice, and a NOAEL was not determined in rats.

NTP (1993) performed a 13-week toxicity study in F344 rats and B6C3F1 mice, where groups of 10 animals/gender/species received EGBE in drinking water at doses of 0, 750, 1500, 3000, 4500, and 6000 ppm in rats and 0, 750, 1,500, 3,000, 4,500, and 6,000 ppm in mice. The corresponding doses in mg/kg-day, based on measured drinking water consumption were: 0, 69, 129, 281, 367, or 452 mg/kg-day in male rats; 0, 82, 151, 304, 363, or 470 mg/kg-day in female rats; 0, 118, 223, 553, 676, or 694 mg/kg-day in male mice; and 0, 185, 370, 676, 861, or 1,306 mg/kg-day in female mice. Due to a dose-related decrease in water consumption in the 2-week studies, the test chemical was administered at a constant concentration (ppm) in the 13-

week studies rather than on a mg/kg body weight basis. Hematology was performed on rats but not on mice. Complete histological exams were performed on all control animals and all animals in the highest dose group. Vaginal cytology and sperm indices were evaluated in rats and mice from the control and three highest dose groups. Hematologic changes in both genders persisting until or developing by 13 weeks included dose-related indications of mild-to-moderate anemia. Portions of the hematologic results from the NTP 13-week rat drinking-water study are presented in Table 4-1. The various results shown in this table are indicative of the various degrees of hemolysis caused by exposure to increasing concentrations of EGBE. Overall, the dose-related increase in MCV is indicative of erythrocyte swelling that would be expected to precede cell lysis and an increase in the number of reticulocytes. Deficits in RBCs as a result of lysis manifest through clear dose-related decreases in the measures of both RBC count and Hb concentration. Hct would be expected to decrease but did not. The increases noted both in reticulocytes (young RBCs) and, at higher doses, in nucleated erythrocytes (immature and prematurely released blood cells) are homeostatic responses that would be anticipated to occur as the lysed blood cells are being replaced. More specifically, male rats evaluated at 13 weeks showed significantly reduced RBC counts at ≥ 281 mg/kg-day and reduced Hb concentration, reduced platelets, and increased bone marrow cellularity at ≥ 367 mg/kg-day. These data also suggest that female rats are more sensitive to the effects from EGBE, since several statistically significant effects occurred at the 750-ppm concentration, the lowest level tested in females; males did not show statistically significant effects until two dose levels higher, 3,000 ppm. In addition, the degree to which these various measures were affected was somewhat greater in females than males, (indicated as percent control in the tables), particularly at the three highest exposure concentrations.

Statistically significant hematologic effects in female rats at week 13 included reduced RBC counts and Hb concentrations at ≥ 82 mg/kg-day and increased reticulocytes, decreased platelets, and increased bone marrow cellularity at approximately 304 mg/kg-day, all being indicative of hemolysis. There were no histopathological changes in the testes or epididymis at ≥ 129 mg/kg-day.

Table 4-2 shows that liver lesions, including cytoplasmic alterations, hepatocellular degeneration, and pigmentation were observed in the mid- and high-dose groups (129, 281, 367, and 452 mg/kg-day for males and 151, 304, 363, and 470 mg/kg-day for females; statistics not reported).). As with the hematologic effects, these effects appeared to be more severe in females than in males. Cytoplasmic alterations of liver hepatocytes, consisting of hepatocytes staining more eosinophilic and lacking the basophilic granularity of the cytoplasm present in hepatocytes from control animals, were observed in the lowest-dose groups tested (69 mg/kg-day for males and 82 mg/kg-day for females). The lack of cytoplasmic granularity or “ground-glass” appearance of the hepatocytes suggests that this response was not due to enzyme induction (Greaves, 2000). The cytoplasmic alterations were judged to increase in severity in both genders, but especially in females; as the doses increased with the severity in the two highest dose groups

Table 4-1. Hematology and hemosiderin data from the 13-week drinking-water exposure to EGBE in F344 rats.

Endpoint*	Control	750 ppm (69 mg/kg-day)	1,500 ppm (129 mg/kg-day)	3,000 ppm (281 mg/kg-day)	4,500 ppm (367 mg/kg-day)	6,000 ppm (452 mg/kg-day)
Hct (%)						
Males	44.8 ± 0.8	45.0 ± 0.6 (100)	44.7 ± 0.4 (99)	44.1 ± 0.7 (98)	42.3 ± 0.6** (94)	43.4 ± 0.4 (97)
Females	44.8 ± 0.6	43.2 ± 0.8 (96)	42.8 ± 0.7 (95)	43.6 ± 0.7 (97)	44.4 ± 0.7 (99)	46.1 ± 0.7 (103)
Hb (g/dL)						
Males	15.0 ± 0.2	15.2 ± 0.1 (101)	14.9 ± 0.1 (99)	14.6 ± 0.1 (97)	14.0 ± 0.1*** (93)	13.7 ± 0.2*** (91)
Females	14.9 ± 0.2	14.4 ± 0.2** (97)	13.9 ± 0.2§ (93)	14.2 ± 0.2§ (95)	14.0 ± 0.2*** (94)	13.4 ± 0.2*** (90)
Erythrocytes (106/µL)						
Males	8.64 ± 0.15	8.74 ± 0.10 (101)	8.54 ± 0.09 (99)	8.11 ± 0.12** (94)	7.48 ± 0.12*** (86)	7.18 ± 0.12*** (83)
Females	8.15 ± 0.09	7.59 ± 0.15*** (93)	7.09 ± 0.14*** (87)	7.00 ± 0.12*** (86)	6.80 ± 0.11*** (83)	6.58 ± 0.14*** (81)
Reticulocytes (106/µL)						
Males	0.14 ± 0.03	0.24 ± 0.06	0.15 ± 0.02	0.18 ± 0.02	0.22 ± 0.05	0.46 ± 0.07***
Females	0.12 ± 0.02	0.17 ± 0.03	0.19 ± 0.03	0.28 ± 0.03***	0.28 ± 0.05***	0.27 ± 0.05***
Nucleated erythrocytes (103/µL)						
Males	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.04 ± 0.02***
Females	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.05 ± 0.02	0.10 ± 0.03**	0.16 ± 0.04***
MCV (fL)						
Males	52.0 ± 0.4	51.5 ± 0.3 (99)	52.3 ± 0.4 (100)	54.4 ± 0.3*** (105)	56.7 ± 0.5*** (109)	60.6 ± 1.1*** (116)
Females	54.8 ± 0.3	57.0 ± 0.4*** (104)	60.5 ± 0.4*** (110)	62.4 ± 0.6*** (114)	65.3 ± 0.6*** (119)	70.1 ± 0.9*** (128)
MCH (pg)						
Males	17.4 ± 0.2	17.4 ± 0.1	17.5 ± 0.2	18.0 ± 0.2**	18.7 ± 0.3***	19.1 ± 0.3***
Females	18.3 ± 0.2	18.9 ± 0.2	19.7 ± 0.2	20.2 ± 0.3***	20.6 ± 0.2***	20.4 ± 0.1***
Hemosiderin (incidence)						
Males	0/10	0/10	0/10	0/10	0/10	7/10
Females	0/10	0/10	2/10	10/10	10/10	10/10

* Values listed are mean ± standard error at various EGBE concentrations in ppm for the 13-week time point. Percent of control values in parentheses.
** Statistically significant difference, $p \leq 0.05$.
*** Statistically significant difference, $p \leq 0.01$.

Table 4-2. Incidence[*] and severity of selected histopathological lesions from the 13-week drinking water exposure to EGBE in F344 rats.

	Control	750 ppm (69 mg/kg-d)	1500 ppm (129 mg/kg-d)	3000 ppm (281 mg/kg-d)	4500 ppm (367 mg/kg-d)	6000 ppm (452 mg/kg-d)
Rat						
N	10	10	10	10	10	10
Liver cytoplasmic alterations (NR)						
Males	0	4 (1.0)	8 (1.0)	7 (1.1)	10 (2.0)	10 (1.8)
Females	0	5 (1.4)	9 (2.0)	10 (2.2)	10 (3.0)	10 (3.0)
Hepatocellular degeneration (NR)						
Males	0	0	0	8 (1.0)	8 (1.0)	8 (1.0)
Females	0	0	0	10 (1.3)	10 (1.3)	10 (1.1)
Kupffer cell pigmentation (NR)						
Males	0	0	0	0	0	7 (1.0)
Females	0	0	2 (1.0)	10 (1.2)	10 (1.9)	10 (1.9)
Mouse						
N	10	10	10	10	10	10
Relative kidney weight (right) (mg organ wt/g body wt)						
Females (only)	6.33 ± 0.10	7.69 ± 0.14***	8.06 ± 0.29***	7.47 ± 0.19***	7.55 ± 0.18***	8.21± 0.26***
Necropsy body weight (g) §						
Females (only)	31.1 ± 0.7	31.8 ± 0.8	30.9 ± 1.5	28.0 ± 0.7**	28.4 ± 0.5**	27.8 ± 0.9***

[*] Incidences represent the number of animals with lesions. Average severity (in parentheses) is based on the number of animals with lesions.
1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
** $p \leq 0.05$
*** $p \leq 0.01$
NR = Statistics not reported
§ mean ± standard error

Source: NTP (1993)

being judged as “moderate.” Liver pigmentation, colored brown to green and staining strongly positive for iron (indicative of hemosiderin accumulation), was noted in the cytoplasm of Kupffer cells in both genders of rats. In females, liver pigmentation was noted in 0/10 controls and 0/10 at 82 mg/kg-day, 2/10 with a severity grade of 1 (minimal) at 151 mg/kg-day, and 10/10 in the three highest dose levels; the severities increased from a numerical grade of 1.2 in the 304 mg/kg-day group to 1.9 in both upper two dose groups. In males, the hemosiderin pigmentation was noted in animals exposed to the highest dose only (452 mg/kg-day) at an incidence of 7/10 and a severity rating of 1 (minimal). No hepatic pigmentation was reported in the mice exposed for 13 weeks.The hematological (decreased RBC count and Hb) and hepatic changes were dose-related; 69–82 mg/kg-day was considered a LOAEL. A NOAEL was not identified.

Female mice showed statistically significant reductions in body weight gain starting at 3000 ppm, and statistically significant increases in relative kidney weight at all doses. Changes at the higher doses followed the reductions in body weight at those dose levels, while the increases at lower doses (750 and 1500 ppm) were due to increased absolute kidney weights at those doses. Body weight reduction followed the decreased water consumption data. No histopathologic changes were noted at any dose level, even though relative kidney weights showed a statistically significantly increase at 750 and 1500 ppm in the absence of reduction in body weight gain.

Keith et al. (1996) administered EGBE at 120 mg/kg-day for 120 days by gavage to transgenic FVB/N mice (25 mice/gender/group) carrying the v-Ha-ras oncogene and observed the animals for an additional 120 days. EGBE did not induce increases in tumors at any site.

4.2.1.2. Inhalation

Wistar-derived rats (23 animals/group, gender not specified) were exposed to 0, 135, or 320 ppm EGBE for 7 hours/day, 5 days/week for 5 weeks (Werner et al., 1943a). Hematologic endpoints — RBC, WBC, differential, and reticulocyte counts and Hb concentration — were evaluated. Exposure to 320 ppm EGBE resulted in an increased percentage of circulating immature granulocytes, a decreased Hb concentration and RBC count, and an increased reticulocyte count. These hematologic changes were not severe; they were reversed 3 weeks after discontinuing exposure. No effect on the WBC count was observed. In another study, Werner et al. (1943b) exposed groups of two dogs of unspecified strain to subchronic inhalation doses of 0 or 415 ppm EGBE for 7 hours/day, 5 days/week for 12 weeks. Necropsies were performed 5 weeks post-exposure; hematologic parameters were examined before, during, and after the exposure. No statistical analysis was presented. The authors concluded that exposure of dogs to EGBE vapors resulted in decreased Hb concentration and RBC count with increased hypochromia, polychromatophilia, and microcytosis. These hematologic effects were not severe; they were reversed 5 weeks after the end of exposure.

Carpenter et al. (1956) studied the hemolytic effects of EGBE vapor inhalation in rats, mice, dogs, and monkeys, in addition to humans. An unspecified strain of rats (15/gender/group) was exposed via inhalation to 54, 107, 203, 314, or 432 ppm EGBE 7 hours/day, 5 days/week for 6 weeks. Erythrocyte osmotic fragility was observed in rats immediately after a single 7-hour exposure to 107 ppm or higher. Osmotic fragility in females exceeded that for males. In almost all cases, these high fragility values returned to normal after the rats rested overnight. In the same study, the authors exposed groups of 10 male C3H mice to 100, 200, or 400 ppm EGBE 7 hours/day for 30, 60, or 90 days. An increase in erythrocyte osmotic fragility occurred at all concentrations and was consistent throughout the exposures. In all instances, erythrocyte osmotic fragility was normal after a 17-hour rest period. The LOAELs for these rat and mouse studies were 54 and 100 ppm, respectively. No NOAELs were reported. The authors reported slight increases in erythrocyte osmotic fragility for a male and a female dog (basenji hybrids) exposed to 200 ppm EGBE for 31 days (7 hours/day). RBC counts and Hb concentrations were slightly decreased in the female. Erythrocyte permeability, as determined by radioiodine uptake, was increased in both genders, but was not statistically different when compared with control values. A female dog succumbed after 8 days of inhalation exposure to 385 ppm of EGBE (7 hours/day). Symptoms included loss of weight, transitory increases in erythrocyte osmotic fragility, nasal and ocular infection, weakness, apathy, anorexia, and increased WBC count. Necropsy of this animal revealed severe congestion and hemorrhage of the lungs and congestion of the liver and both kidneys. In addition, a severe subcapsular hemorrhage was found in one adrenal gland. A male dog survived after 28 days of inhalation exposure to 385 ppm of EGBE for 7 hours/day. Effects in the male were similar to the female, but developed more slowly. At autopsy, congestion of the kidneys was not observed. In studies on male and female monkeys, occasional rises in erythrocyte osmotic fragility were reported that were more frequent in the female than in the male following 90-day inhalation exposure to 100 ppm of EGBE.

A 90-day subchronic inhalation study was performed using F344 rats (16/gender/group) exposed to EGBE for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, and 77 ppm (Dodd et al., 1983). After 31 completed exposures (6 weeks), the 77 ppm female rats had slight but statistically significant decreases in RBC counts (13% below control value) and Hb concentrations, accompanied by an 11% increase above the control value in MCH. The 77 ppm males exhibited slight (5%) but statistically significant decreases in RBC counts and Hb concentration that were accompanied by increases in MCH. At the end of the 90-day study (66 exposures), the hematologic effects seen in the 77 ppm exposed animals had either lessened or returned to the ranges of control values and were no longer statistically significant. The NOAEL was determined to be 25 ppm, and the LOAEL was 77 ppm.

In the subchronic portion of the inhalation NTP (2000) study, F344 rats and B6C3F1 mice (10/gender) were exposed to EGBE concentrations of 0, 31, 62.5, 125, 250, and 500 ppm (0, 150, 302, 604, 1,208, and 2,416 mg/m^3) for 6 hours/day, 5 days/week for 14 weeks.

Hematologic and hemosiderin staining results are presented in Table 4-3 and Table 4-4. These results are indicative of the various degrees of hemolysis caused by exposure to increasing concentrations of EGBE. Both rat genders exhibited clinical signs at the three highest doses, consistent with the hemolytic effects of EGBE, including: (1) deficits in RBCs as a result of lysis manifestation through the clear dose-related decrease in Hct, a finding consistent with decreases noted for both RBC count and Hb concentrations; and (2) increases in both reticulocytes and nucleated erythrocytes at higher doses, homeostatic responses that would be anticipated to occur as the lysed blood cells are being replaced. Female rats may be somewhat more sensitive: several statistically significant effects occur at the 31 ppm level in females, as opposed to a single parameter for males. In addition, the degree to which these various measures are affected is somewhat greater in females than males, indicated as percent control, particularly at the three highest concentrations. Hematologic evaluation showed mild-to-moderate regenerative anemia at all concentrations in females and at the three highest concentrations in males. Exposure-related trends were noted for reticulocyte count, RBC count, MCV, Hb concentration, and Hct. Liver-to-body-weight ratios increased significantly in males at the two highest concentrations and in females at the highest concentration. Histopathologic effects at concentrations in excess of 62.5 ppm for male rats and 31 ppm for females consisted of excessive splenic congestion in the form of extramedullary hematopoiesis, hemosiderin accumulation in Kupffer cells, liver necrosis, centrilobular hepatocellular degeneration, renal tubular degeneration, intracytoplasmic Hb and hemosiderin deposition, and bone marrow hyperplasia. In addition, five moribund female rats were sacrificed from the highest concentrations, and one from the 250 ppm group. The LOAEL for hematological alterations was 31 ppm for female rats and 62.5 ppm for male rats. The 31 ppm exposure level was considered a NOAEL for male rats.

The mice exposed via the inhalation route exhibited clinical signs consistent with the hemolytic effects of EGBE at the two highest concentrations for both genders (NTP, 2000). Hematologic evaluation indicated a moderate regenerative anemia (marked by decreased RBC counts, increased reticulocyte counts, and increased MCV) with an increase in platelets at the three highest concentrations in both genders. Histopathological effects consisted of excessive extramedullary splenic hematopoiesis, renal tubular degeneration, hemosiderin deposition in the spleen and kidney and accumulation in Kupffer cells, and testicular degeneration. Forestomach necrosis, ulceration, inflammation, and epithelial hyperplasia were observed at concentrations greater than 31 ppm for females and 62.5 ppm for males. In addition, four females and four males either died or were sacrificed moribund at the highest concentration. The NOAEL for male and female mice was 31 ppm and the LOAEL in mice was 62.5 ppm, based on histopathological changes in the forestomach.

Table 4-3. Hematology and hemosiderin data from a 14-week inhalation study of EGBE in F344 rats.

Endpoint*	Control	31 ppm (150 mg/m^3)	62.5 ppm (302 mg/m^3)	125 ppm (604 mg/m^3)	250 ppm (1,208 mg/m^3)	500 ppm (2,416 mg/m^3)
Hct (%)						
Males	46.8 ± 0.5	45.8 ± 0.6 (98)	47.0 ± 0.4 (100)	44.5 ± 0.5*** (95)	41.1 ± 0.3*** (88)	37.3 ± 0.4*** (80)
Females	48.5 ± 0.5	46.0 ± 0.5*** (95)	45.2 ± 0.5*** (93)	42.9 ± 0.4*** (88)	40.0 ± 0.3*** (82)	36.2 ± 0.6*** (75)
Hb (g/dL)						
Males	15.5 ± 0.1	14.8 ± 0.3 (95)	15.4 ± 0.1 (99)	14.5 ± 0.2*** (94)	13.1 ± 0.1*** (85)	11.7 ± 0.1*** (75)
Females	15.6 ± 0.1	15.0 ± 0.1*** (96)	14.6 ± 0.1*** (94)	13.6 ± 0.1*** (87)	12.5 ± 0.1*** (80)	10.5 ± 0.3*** (67)
Erythrocytes (10^6/µL)						
Males	9.05 ± 0.08	8.71 ± 0.14** (96)	8.91 ± 0.06 (98)	8.01 ± 0.08*** 89)	7.10 ± 0.07*** (78)	5.97 ± 0.05***(66)
Females	8.48 ± 0.05	8.08 ± 0.07*** (95)	7.70 ± 0.08*** (91)	6.91 ± 0.05*** (81)	6.07 ± 0.04*** (72)	4.77 ± 0.15*** (56)
Reticulocytes (10^6/µL)						
Males	0.16 ± 0.02	0.17 ± 0.03	0.15 ± 0.02	0.30 ± 0.04***	0.48 ± 0.06***	0.68 ± 0.07***
Females	0.13 ± 0.02	0.10 ± 0.01	0.16 ± 0.02	0.26 ± 0.04**	0.34 ± 0.04***	0.40 ± 0.11***
Nucleated erythro. (10^3/µL)						
Males	0.04 ± 0.02	0.05 ± 0.01	0.04 ± 0.03	0.11 ± 0.03	0.17 ± 0.04***	0.20 ± 0.06**
Females	0.04 ± 0.02	0.05 ± 0.02	0.12 ± 0.03**	0.18 ± 0.07	0.61 ± 0.24***	0.73 ± 0.27***
MCV (fL)						
Males	50.4 ± 0.3	50.2 ± 0.2 (100)	50.7 ± 0.2 (100)	53.1 ± 0.2*** (105)	53.8 ± 0.3*** (107)	58.5 ± 0.3***(117)
Females	55.1 ± 0.3	55.3 ± 0.2 (100)	56 .4 ± 0.2 (102)	58.7 ± 0.2*** (107)	61.6 ± 0.2*** (112)	66.8 ± 0.9*** (121)
MCH (pg)						
Males	17.1 ± 0.1	17.0 ± 0.1	17.3 ± 0.1	18.1 ± 0.1***	18.4 ± 0.1***	19.5 ± 0.1***
Females	18.4 ± 0.1	18.6 ± 0.2	19.0 ± 0.0***	19.6 ± 0.1***	20.6 ± 0.1***	22.0 ± 0.1***
Hemosiderin (incidence)						
Males	0/10	0/10	0/10	7/10	10/10	10/10
Females	0/10	0/10	10/10	10/10	9/9	5/5

*Values listed are mean ± standard error (percent of control).
**Statistically significant difference, $p \leq 0.05$.
***Statistically significant difference, $p \leq 0.01$.

Source: NTP (1993).

Table 4-4. Hematology and hemosiderin data from a 14-week inhalation study of EGBE in B6C3F1 mice.

Endpoint*	Control	31 ppm (150 mg/m^3)	62.5 ppm (302 mg/m^3)	125 ppm (604 mg/m^3)	250 ppm (1,208 mg/m^3)	500 ppm (2,416 mg/m^3)
Hct (%)						
Males	47.3 ± 1.0	48.3 ± 0.4 (102)	47.6 ± 0.5 (101)	46.6 ± 0.4 (99)	44.2 ± 0.4*** (93)	36.3 ± 1.4*** (77)
Females	46.2 ± 0.3	45.9 ± 0.3 (99)	45.8 ± 0.3 (99)	45.1 ± 0.2*** (98)	42.3 ± 0.4*** (92)	37.8 ± 1.0*** (82)
Hb (g/dL)						
Males	15.7 ± 0.4	16.0 ± 0.1 (102)	15.9 ± 0.1 (101)	15.4 ± 0.1*** (98)	14.4 ± 0.1*** (92)	11.4 ± 0.4*** (73)
Females	15.7 ± 0.1	15.4 ± 0.1** (98)	15.4 ± 0.1** (98)	14.8 ± 0.1*** (94)	13.7 ± 0.1*** (87)	11.6 ± 0.1*** (74)
Erythrocytes (10^6/µL)						
Males	9.71 ± 0.22	10.04 ± 0.08 (103)	9.77 ± 0.1 (101)	9.47 ± 0.06** (98)	8.90 ± 0.07*** (92)	7.21 ± 0.23*** (74)
Females	9.72 ± 0.05	9.55 ± 0.06** (98)	9.51 ± 0.06** (98)	9.18 ± 0.05*** (94)	8.57 ± 0.06*** (88)	7.35 ± 0.07*** (76)
Reticulocytes (10^6/µL)						
Males	0.21 ± 0.03	0.22 ± 0.03	0.21 ± 0.02	0.32 ± 0.03**	0.45 ± 0.04***	0.79 ± 0.20***
Females	0.18 ± 0.02	0.21 ± 0.03	0.19 ± 0.02	0.29 ± 0.02***	0.47 ± 0.04***	1.17 ± 0.28***
MCV (fL)						
Males	49.1 ± 0.4	48.5 ± 0.3 (99)	49.0 ± 0.4 (100)	49.7 ± 0.4 (101)	49.8 ± 0.4 (101)	48.3 ± 0.9 (98)
Females	48.3 ± 0.3	48.8 ± 0.2 (101)	48.8 ± 0.2 (101)	49.5 ± 0.5 (102)	49.0 ± 0.3 (101)	48.8 ± 1.0 (101)
MCH (pg)						
Males	16.2 ± 0.1	16.0 ± 0.1 (99)	16.2 ± 0.1 (100)	16.2 ± 0.0 (100)	16.2 ± 0.1 (100)	15.8 ± 0.2 (98)
Females	16.1 ± 0.1	16.0 ± 0.1 (99)	16.2 ± 0.1 (101)	16.1 ± 0.1 (100)	16.0 ± 0.0 (99)	15.8 ± 0.1 (98)
Hemosiderin (incidence)						
Males	0/10	0/10	0/10	0/10	0/10	6/6
Females	0/10	0/10	0/10	0/10	10/10	6/6

*Values listed are mean ± standard error (percent of control).
**Statistically significant difference, $p \leq 0.05$.
***Statistically significant difference, $p \leq 0.01$.

Source: NTP (1993)

4.2.2. Chronic Studies and Cancer Bioassays

4.2.2.1. Inhalation

NTP (2000) also completed a two-species, 2-year inhalation study on EGBE in both genders of rats and mice. In this chronic study, animals were exposed to EGBE 6 hours/day, 5 days/week at concentrations of 0, 31, 62.5, and 125 ppm (0, 150, 302, and 604 mg/m^3) for groups of 50 F344/N rats and 0, 62.5, 125, and 250 ppm (0, 302, 604, and 1,208 mg/m^3) for groups of 50 B6C3F1 mice. The researchers stated that the highest exposure was selected to produce a 10–15% depression in hematologic indices, and survival was significantly decreased in male mice at 125 and 250 ppm (54.0% and 53.1%, respectively). They reported that no effect on survival was observed in rats, but survival was statistically significantly decreased in male mice exposed to 125 or 250 ppm, compared with chamber controls (NTP, 2000). Although statistics were not reported for mean body weights, the rats exposed to 31 and 62.5 ppm had similar mean body weights to the control rats. Mean body weights of the exposed mice were generally less than for controls, with females experiencing greater and earlier reductions. From week 17 to the end of the study, the mean body weights of 125 ppm female rats were generally less than those of controls. Non-neoplastic effects in rats included hyaline degeneration of the olfactory epithelium in males (13/48, 21/49, 23/49, 40/50) and females (13/50, 18/48, 28/50, 40/49) and Kupffer cell pigmentation in the livers of males (23/50, 30/50, 34/50, 42/50) and females (15/50, 19/50, 36/50, 47/50) (Table 4-5). The severity of the olfactory lesion was not affected by exposure. The Kupffer cell pigmentation is a result of hemosiderin accumulation and is a recognized secondary effect of the hemolytic activity of EGBE (NTP, 2000).

Non-neoplastic, statistically significant effects in mice included forestomach ulcers and epithelial hyperplasia, hematopoietic cell proliferation and hemosiderin pigmentation in the spleen, Kupffer cell pigmentation in the livers, and bone marrow hyperplasia (males only). Hyaline degeneration of the olfactory epithelium (females only) was increased relative to chamber controls, but was not statistically significant. As in the rats, the Kupffer cell pigmentation was considered a secondary effect of the hemolytic activity of EGBE. Bone marrow hyperplasia, hematopoietic cell proliferation and hemosiderin pigmentation in the spleen were also attributed to the primary hemolytic effect; it was followed by regenerative hyperplasia of the hematopoietic tissue. The forestomach lesions did not appear to be related to the hemolytic effect of EGBE. Incidences of ulcer were significantly increased in all exposed female groups , as well as males exposed to 125 ppm. Incidences of epithelial hyperplasia, usually focal, were significantly increased in all exposed groups of males and females. The hyperplasia was often associated with ulceration, particularly in the females, and consisted of thickness of the stratified squamous epithelium and sometimes the keratinized layer of the forestomach.

Table 4-5. Selected Female and Male Rat and Mouse Non-neoplastic effects from the 2-year Chronic EGBE Inhalation Study

	Control	31 ppm	62.5 ppm	125 ppm	250 ppm
Rat					
Kupffer cell pigmentation, hemosiderin in the liver					
Male	23/50	30/50	34/50*	42/50*	NT
Female	15/50	19/50	36/50*	47/50*	NT
Hyaline degeneration of the olfactory epithelium					
Male	13/48	21/49*	23/49*	40/50*	NT
Female	13/50	18/48	28/50*	40/49*	NT
Mouse					
Kupffer cell pigmentation, hemosiderin in the liver					
Male	0/50	NT	0/50	8/49**	30/49**
Female	0/50	NT	5/50*	25/49**	44/50**
Hematopoietic cell proliferation in the spleen					
Male	12/50	NT	11/50	26/48**	42/50**
Female	24/50	NT	29/50	32/49	35/50*
Hemosiderin in the spleen					
Male	0/50	NT	6/50*	45/48**	44/49**
Female	39/50	NT	44/50	46/49**	48/50**
Forestomach ulcers					
Male	1/50	NT	2/50	9/49**	3/48
Female	1/50	NT	7/50*	13/49**	22/50**
Forestomach epithelial hyperplasia					
Male	1/50	NT	7/50*	16/49**	21/48**
Female	6/50	NT	27/50**	42/49**	44/50**
Hyaline degeneration of the olfactory epithelium					
Females (only)	6/50	NT	14/50	11/49	12/50
Bone marrow hyperplasia					
Males (only)	0/50	NT	1/50	9/49*	5/50*

*Statistically significant difference, $p \leq 0.05$.
**Statistically significant difference, $p \leq 0.01$.
NT = not tested

Source: NTP (2000)

Ulceration consisted of a defect in the forestomach wall that penetrated the full thickness of the epithelium and frequently contained accumulations of inflammatory cells and debris.

Using the same exposure levels described above, additional groups of rats (27/gender/exposure group) and mice (30/gender/exposure group) in the 2-year study were examined at 3, 6, and 12 months (8–10 animals/time point) for hematologic effects (NTP, 2000). Nine each male and female rats were exposed to 31 ppm EGBE, specifically to evaluate hematology at 3 months, and to receive a total evaluation at 6 months. Animals were continuously exposed, as described above, until their sacrifice at 3, 6, or 12 months. As in the 14-

week study, inhalation of EGBE by both species resulted in the development of exposure-related hemolytic effects, inducing a responsive anemia. In rats, the anemia was persistent and did not progress or ameliorate in severity from 3 months to the final blood collection at 12 months. Statistically significant ($p < 0.05$) decreases in automated and manual Hct values, Hb concentrations, and RBC counts occurred at 3, 6, anda 12 months in the 125 ppm female mice and the 250 pm male and female mice. Statistically significant decreases in these same endpoints were also observed in 62.5 ppm females at 6 months and in 125 ppm males at 6 and 12 months (decreased in Hct were observed only at 3 and 6 months). MCV was increased in female mice at the highest duration (12 months) and exposure (250 ppm) levels. Reticulocyte counts were increased significantly in the 125 ppm females at 3 and 6 months and in the 125 ppm males at 6 months of exposure. Table 4-6 shows the responses available for a representative measure of the hematologic effects from EGBE exposure. Hct levels for male and female rats and mice measured after 3 months or 12 months are presented.

Table 4-6. Comparison of Female and Male Rat and Mouse Hct (Manual) Values from 3- and 12-month Inhalation Exposures to EGBE.

	Control	31.2 ppm	62.5 ppm	125 ppm	250 ppm
Female rats*					
3 months	46.5 ± 0.5	46.1 ± 0.5 (95)	43.3 ± 0.5*** (93)	42.2 ± 0.5*** (91)	–
12 months	45.4 ± 0.2	–	45.3 ± 0.3 (100)	42.3 ± 0.4*** (93)	–
Male rats*					
3 months	44.9 ± 0.2	46.9 ± 0.5 (104)	44.8 ± 0.4 (100)	42.9 ± 0.5** (95)	–
12 months	47.8 ± 0.4	–	45.9 ± 0.8** (96)	42.9 ± 1.2*** (90)	–
Female mice*					
3 months	49.3 ± 0.5	–	48.9 ± 0.4 (99)	46.2 ± 0.5*** (94)	43.7 ± 0.5*** (89)
12 months	46.9 ± 0.4	–	46.3 ± 0.4 (99)	43.8 ± 0.4*** (93)	41.8 ± 0.3*** (89)
Male mice*					
3 months	47.5 ± 0.3	–	47.3 ± 0.5 (100)	46.0 ± 0.4** (97)	43.7 ± 0.2*** (92)
12 months	47.9 ± 0.4	–	48.7 ± 1.9** (102)	46.4 ± 1.0 (97)	42.1 ± 0.4*** (88)

*These results are from a serial sacrifice conducted as a part of the 2-year chronic inhalation study. Values listed are mean ± standard error (percent of control).
– data were not available.
**Statistically significant difference, $p \leq 0.05$.
***Statistically significant difference, $p \leq 0.01$.

Source: NTP (2000)

In vitro studies by Ghanayem (1989) have shown that the hemolysis caused by the EGBE metabolite BAA is preceded by erythrocyte swelling. If the observed increase in MCV is in response to cell swelling, it could be a preliminary indicator of the hemolytic effect. Other researchers, however, have attributed the increased MCV at all exposures and the increased MCH at higher exposures to the erythropoietic response subsequent to hemolysis and the corresponding increase in the number of larger reticulocytes in circulation (cited in NTP, 2000). Reticulocyte counts were significantly increased in female rats at 62.5 ppm (6 and 12 months)

and in male rats at 125 ppm (3 and 6 months). Since a statistically significant increase in reticulocyte count was not observed at any time point in males or females exposed to 31 ppm or in males exposed to 62.5 ppm, it appears that reticulocyte count alone cannot account for the increase in MCV. The observed increases in MCV may be a combined result of both erythrocyte swelling prior to, and an increased number of reticulocytes subsequent to, hemolysis; the former would be more influential at lower exposure levels, and the latter would have more relative impact at higher levels.

Similar effects indicating anemia were also observed in mice, where females were the more sensitive. As in rats, the anemia response was observed at slightly higher doses, but was persistent and did not progress or ameliorate in severity from 3 months to the final blood collection at 12 months. Table 4-6 shows the manual hematocrit values in male and female rats and mice at 3 months and 12 months. Statistically significant ($p < 0.05$) decreases in automated and manual Hct values and Hb and RBC counts occurred at 3, 6, and 12 months in the 125 ppm females and the 250 ppm males and females. Statistically significant decreases in these endpoints were also observed in 62.5 ppm females exposed for 6 months and in 125 ppm males exposed for 6 and 12 months (decreases in Hct were observed only at 3 and 6 months). No changes were observed in the MCV of mice, except for an increase in female mice at the highest duration (12 months) and exposure (250 ppm) levels. Reticulocyte counts were increased significantly in the 125 ppm females at 3 and 6 months and in 125 ppm males at 6 months of exposure.

At the end of the 2-year chronic bioassay (NTP, 2000), neoplastic effects were observed in female rats and in male and female mice. In female rats, the combined incidence of benign and/or malignant pheochromocytoma of the adrenal medulla was 3/50, 4/50, 1/49, and 8/49. The incidence in the high-dose group (16%) does not represent a statistically significant increase over the chamber control group (6%), but it exceeded the historical control (6.4 ± 3.5%; range 2–13%) for this effect.

The low survival rate in male mice exposed to 125 and 250 ppm EGBE may have been due to carcinogenic effects in the liver. A high rate of hepatocellular carcinomas was found in these exposure groups (10/50 [control], 11/50, 16/50, 21/50); the increase at the high-exposure level was statistically significant ($p < 0.01$). When hepatocellular adenomas and carcinomas are combined, no significant increase was observed in any exposure group. However, the incidence of hemangiosarcomas in males exposed to 250 ppm (8%) was significantly increased ($p = 0.046$) relative to chamber controls (0/50, 1/50, 2/49, 4/49), and exceeded the range of historical controls (14/968; 1.5 ± 1.5%; range 0–4%). No organisms consistent with *Helicobacter hepaticus* were found in the 14 mice evaluated (NTP, 2000). The researchers concluded from this that *H. hepaticus* was not a factor in the development of liver neoplasms. No significant increases in benign or malignant hepatocellular tumors or hemangiosarcomas were noted in the female mice, and the incidence of hepatocellular adenomas actually decreased significantly ($p <$

0.05) in relation to the control chamber group (16/50, 8/50, 7/49, 8/49). However, in light of the high survival rate of the exposed female mice relative to the control animals (29/50, 31/50, 33/50, 36/50), the high exposure of 250 ppm may not have provided the maximum tolerated dose.

Forestomach squamous cell papillomas and carcinomas, combined, were significantly increased (trend test = 0.003) in female mice relative to the chamber controls (0/50, 1/50, 2/50, 6/50). The incidence of these tumor types (12%) at the highest exposure level was statistically significant and exceeded the range for the occurrence of these tumors in historical controls (0.9 ± 1.1%; range 0–3%). The first incidence of these tumors appeared in the group exposed to 250 ppm at 582 days, compared with 731 days at 62.5 and 125 ppm. This indicates a decreased latency period in the highest exposure group. While the incidence of these types of forestomach tumors was not significantly increased over controls in male mice (1/50, 1/50, 2/50, 2/50), the incidence of squamous cell papillomas (4%) in the two highest exposure groups exceeded the range for historical controls (0.5 ± 0.9%; range 0–2%). The increased incidences of forestomach neoplasms in males, as in females, occurred in groups with ulceration and hyperplasia.

Section 4.6 has a discussion of the cancer data from this study. With respect to the noncancer findings, a NOAEL could not be determined, and a LOAEL of 62.5 ppm was determined in mice for hemosiderin deposition. In rats, a NOAEL could not be determined and a LOAEL of 31 ppm was determined for hemosiderin deposition.

4.3. Reproductive and Developmental Studies: Oral and Inhalation

Due to the known reproductive toxicity, such as effects to male testes and sperm, of two other glycol ethers, ethylene glycol methyl ether (EGME; 2-methoxyethanol) and ethylene glycol ethyl ether (EGEE; 2-ethoxyethanol), the reproductive toxicity of EGBE was studied in a variety of oral studies (NTP, 1993; Exon et al., 1991; Heindel et al., 1990; Foster et al., 1987; Grant et al., 1985; Nagano et al., 1984, 1979) and inhalation studies (NTP, 2000; Dodd et al., 1983) using rats, mice, and rabbits. Several developmental studies have addressed EGBE toxicity from conception to sexual maturity, including toxicity to the embryo and fetus, following oral (Sleet et al., 1989; Wier et al., 1987), inhalation (Nelson et al., 1984; Tyl et al., 1984), and dermal (Hardin et al., 1984) exposures in rats, mice, and rabbits. In many instances, LOAELs and NOAELs were reported for both parental and developmental effects; therefore, the developmental studies can also be used to assess systemic toxicity as well as developmental toxicity.

EGBE did not cause adverse effects in the reproductive organs, including testes, in any study. In a two-generation reproductive toxicity study, fertility was reduced in mice only at very high, maternally toxic, doses (>1000 mg/kg). Maternal toxicity, related to the hematologic effects of EGBE, and relatively minor developmental effects, have been reported in developmental

studies and are discussed below. No teratogenic effects were noted in any of the studies. It can be concluded from these studies that EGBE is not significantly toxic to the reproductive organs of adult males or females, or to the developing fetuses of laboratory animals.

As discussed in Section 4.2, Nagano et al. (1979) performed a toxicity study in male JCL/ICR mice (five/group), using gavage doses of 0, 357, 714, or 1,430 mg/kg-day EGBE 5 days/week for 5 weeks. A LOAEL of 357 mg/kg-day based on reduced RBC count was identified, but no changes in testes weight or histology were observed. In another study, Nagano et al. (1984) used the same dosing regimen up to 2,000 mg/kg-day to test EGBE and other glycol ethers. Testicular atrophy was observed for EGEE and EGME, but not for EGBE.

Grant et al. (1985) exposed male F344 rats (six/group) to gavage doses of 0, 500, or 1,000 mg/kg-day EGBE and EGME for 4 days. Severe testicular atrophy was observed in rats fed 500 mg/kg-day EGME, but no significant effect was noted in rats fed up to 1,000 mg/kg-day EGBE.

Krasavage (1986) conducted a toxicity study using groups of 10 COBS CD(SD)BR adult male rats treated by gavage with 222, 443, or 885 mg/kg-day undiluted EGBE 5 days/week for 6 weeks. They found no effects on testicular weight and no histopathological lesions in the testes, seminal vesicles, epididymides, or prostate gland at any exposure level.

Foster et al. (1987) fed Alpk/AP (Wistar-derived) male rats single gavage doses of 0, 174, 434, or 868 mg/kg BAA. Occasional significant decreases in the weight of the prostate gland and seminal vesicles were observed, but the decreases were not time- or dose-related. No treatment-related lesions were noted following histologic examination of the testes, epididymides, or prostate. BAA did not produce any changes in testicular cell populations when introduced in vitro at 5 mM. Simultaneous testing of the acids of EGME and EGEE resulted in significant spermatocyte cell loss and damage in vivo and in vitro.

Subchronic reproductive studies were conducted using male and female Swiss CD-1 mice. Heindel et al. (1990) exposed them to EGBE in drinking water at doses of 0, 700, 1,300, and 2,000 mg/kg-day for 7 days premating; subsequently, they exposed the mice for 98 days while cohoused as breeding pairs. The higher two-dose levels resulted in mortality: 13/20 died during the study in the 2,000 mg/kg-day group and 6/20 died in the 1,300 mg/kg-day dose group, as compared with 1 each in the 700 mg/kg-day group and the control group. Statistically significant toxic effects seen in the 1,300 and 2,000 mg/kg-day dose groups with adult mice included decreased body weight gain, increased kidney and liver weights, and dose-related decreases in water consumption. Statistically significant developmental effects observed in the 1,300 and 2,000 mg/kg-day dose groups included decreased pup weight and fewer and smaller litters produced per pair. A significant reduction (5%) in live pup weight was also observed in the 700 mg/kg-day dose group. No adverse effect on fertility was observed in the 700 mg/kg-day dose group.

At the completion of the 98-day continuous breeding phase, F_0 breeding pairs were separated and housed individually, while exposure to EGBE continued. When the last litter was weaned, a 1-week crossover mating trial was performed to determine effects by gender. F_0 males and females from the 1,300 mg/kg-day dose group were mated with male and female control animals. The exposed mice had significantly lower body weights and increased relative kidney weights, but reproductive organ weights, sperm motility and morphology, and estrous cycle length and frequency did not differ from control mice. In the only histopathological examination carried out on treated females, no kidney lesions were observed. The proportion of successful copulation was equivalent in all groups, and no developmental effects were observed in any offspring. However, the number of fertile females was significantly reduced in the group where treated females were mated with control males, suggesting that fertility effects were primarily due to effects on the female mice.

A final phase of this study assessed the fertility and reproductive effects of EGBE in first-generation (F_1) pups. There were insufficient numbers of offspring to assess the two highest dose groups, and no adverse effect on fertility was noted when offspring of the low, 700 mg/kg-day dose group were mated. Thus, the researchers concluded that the 700 and 1,300 mg/kg-day dose levels are considered to be NOAEL and LOAEL values, respectively, for both maternal and reproductive effects. A minimal LOAEL for developmental effects was 700 mg/kg-day, where a very slight decrease in pup weight was observed.

A study by Exon et al. (1991), also discussed in Section 4.4.5, looked at reproductive parameters in male rats. Groups of six Sprague-Dawley rats were exposed to EGBE in drinking water at doses of 0, 180, or 506 mg/kg-day (males) for 21 days. While testicular atrophy and necrosis and a reduced number of spermatogenic cells were observed in males exposed to EGME, no adverse effects on fertility parameters were seen in males at any of the tested doses of EGBE.

NTP (1993) evaluated the effects of EGBE on the reproductive systems of male and female B6C3F1 mice (five/gender/group) following 2-week drinking water exposure to doses of 93, 148, 210, 370, or 627 mg/kg-day for males and 150, 237, 406, 673, or 1364 mg/kg-day for females. No deaths were reported, and there were no effects on body weight. Thymus weights were decreased in the highest male dose group. There were no treatment-related gross lesions in any of the reproductive organs and histopathological examinations were not performed. NTP (1993) also exposed male and female F344 rats (five/gender/group) to EGBE for 2 weeks in drinking water. Male rats received doses of 73, 108, 174, 242, or 346 mg/kg-day, and females received 77, 102, 152, 203, or 265 mg/kg-day. No treatment-related deaths occurred during the study, and no changes in body weight were observed in male rats that could be related to treatment. However, female rats had lower weight gain in the highest dose group. Water

consumption was lowest in the highest dose group in both genders, and no treatment-related gross lesions of reproductive organs were reported.

Dodd et al. (1983) (also discussed in Section 4.2) performed a 90-day subchronic inhalation study on F344 rats. Male and female rats (16/gender/group) were exposed to EGBE for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, and 77 ppm. They reported no changes in testicular weight or in the pathology of the epididymides or testes of male rats at any exposure level; reproductive organs of the female rats were not examined histologically.

NTP (2000) performed chronic and subchronic inhalation studies of EGBE in F344 rats and B6C3F1 mice in which reproductive organs were examined. In the subchronic portion of the NTP (2000) studies, rats and mice (10/gender/group) were exposed to concentrations of 0, 31, 62.5, 125, 250, and 500 ppm of EGBE 6 hours/day, 5 days/week for 14 weeks. Testicular degeneration was reported in 2/4 mice from the 500 ppm group that died or were killed moribund. No other effects were noted in the reproductive organs of rats or mice. Exposure concentrations were 0, 31, 62.5, and 125 ppm for groups of 50 F344/N rats and 0, 62.5, 125, and 250 ppm for groups of 50 B6C3F1 mice. No effects were noted in the reproductive organs of either species, but the researchers reported that survival was significantly decreased in male mice at 125 and 250 ppm (54.0 and 53.1%, respectively).

Prenatal and postnatal developmental toxicity tests were conducted in CD-1 mice by Wier et al. (1987). Animals received 0, 350, 650, 1,000, 1,500, or 2,000 mg/kg-day via gavage on gestational days (GDs) 8–14. Maternal toxicity included mortality of 3/6 animals in the 1,000 mg/kg-day group and 6/6 in the 2,000 mg/kg-day group. Treatment-related clinical observations were lethargy, abnormal breathing, and green or red vaginal discharge (the latter at ≥1500 mg/kg-day). Based on clinical signs in the prenatal study, the LOAEL for maternal effects was 350 mg/kg-day. The LOAEL for developmental toxicity was determined to be 1,000 mg/kg-day, based on a statistically significant increase in the number of resorptions and a reduced number of viable fetuses. The corresponding NOAEL for prenatal effects was 650 mg/kg-day. In the postnatal study, reproductive effects were evaluated in offspring of CD-1 mice administered EGBE via gavage at 0, 650, or 1,000 mg/kg-day on GDs 8–14. Maternal body weight was lowered at 1,000 mg/kg-day. Survival and body weight gain of offspring were unaffected by treatment. No adverse reproductive or developmental effects were observed. In a simultaneous study with EGEE, developmental toxicity was noted at doses below maternal toxicity levels.

Developmental toxicity was investigated following the administration of EGBE in distilled water by gavage to groups of 28–35 pregnant F344 rats at doses of 0, 30, 100, or 200 mg/kg-day on GDs 9–11, or doses of 0, 30, 100, or 300 mg/kg-day on GDs 11–13 (Sleet et al., 1989). GDs 9–13 were are the most critical periods of fetal cardiovascular development. Food and water measurements, body and organ weights, clinical signs, hematologic analyses of dams, amount of corpora lutea, uterine contents, and number of dead and live fetuses were

monitored. Maternal effects of EGBE given in either dosing sequence included marked, dose-related reductions in body weight and/or weight gain, increases in kidney and spleen weights, severe hematotoxicity as evidenced by a decrease in HCT, Hb, and RBC counts;, and an increase in reticulocytes at doses ≥100 mg/kg-day. No indications of developmental toxicity were observed at the two lower doses. Viability of embryos was reduced by EGBE treatment at the 200 mg/kg-day dose (GDs 9–11), but not at 300 mg/kg-day (GDs 9–13). A decreased platelet count was noted in the fetuses at 300 mg/kg-day (GDs 9–13). Cardiovascular or other types of malformations were not found at any dose. The LOAEL for maternal toxicity was 100 mg/kg-day, based on signs of hematotoxicity, with a NOAEL established at 30 mg/kg-day. The LOAEL for developmental toxicity was 200 mg/kg-day, based on decreased viability of embryos, with a NOAEL for this endpoint at 100 mg/kg-day.

Sprague-Dawley rats (15/group) were exposed to 0, 150, or 200 ppm EGBE via inhalation for 7 hours/day on GDs 7–15 (Nelson et al., 1984). Rats exposed to 200 ppm showed some evidence of hematuria on the first day of exposure; no adverse effects were noted thereafter, not in offspring. The LOAEL was 200 ppm for slight maternal toxicity; a NOAEL was identified at 150 ppm. The NOAEL for developmental toxicity was 200 ppm. Simultaneous testing revealed that 50 ppm exposures to EGME were toxic at all levels of embryonic and fetal development.

Pregnant F344 rats (36/group) and New Zealand white (NZW) rabbits (24/group) were exposed to 0, 25, 50, 100, or 200 ppm EGBE via inhalation for 6 hours/day on GDs 6–15 for rats or days 6–18 for rabbits (Tyl et al., 1984). Fetuses were weighed and evaluated for viability, body weight, and morphological development, including external, visceral, and skeletal malformations. In rats, fetotoxicity was observed at 100 and 200 ppm in the form of retarded skeletal ossification of vertebral arches or centra, sternebrae, or phalanges. Maternal toxicity was also evident at 100 and 200 ppm by increased incidence of hematuria, reduced RBC count, decreased weight gain, and reduced food consumption. For both maternal and developmental toxicity in the rat, the NOAEL and LOAEL values were 50 and 100 ppm, respectively. In rabbits, fetal skeletal ossification of sternebrae and rudimentary ribs was delayed at 200 ppm. Maternal toxicity was also evident at 200 ppm as an increased incidence of clinical signs, reduced gravid uterine weight, and decreased weight gain. For both maternal and developmental effects in the rabbit, the NOAEL and LOAEL values were 100 and 200 ppm, respectively.

Reproductive toxicity tests were performed on female Sprague-Dawley rats (N not specified) via dermal administration during GDs 6–15, four times per day at 1800 and 5400 mg/kg-day (Hardin et al., 1984). In the highest dose group, 10/11 rats died between days 3 and 7 of treatment. Signs associated with treatment included red-stained urine, ataxia, inactivity, rough coats, and necrosis of the tail tip. At the lower dose, body weight was slightly reduced, yet there was no evidence of embryo- or fetotoxicity, gross malformations or variations,.

4.4. Other Studies

4.4.1. Acute and Short-Term Exposure Studies

Ghanayem et al. (1987c) conducted acute toxicity studies designed to assess the effect of age on toxicity by comparing effects in treated young (4–5 weeks old) and adult rats (9–13 weeks, 5–6 months, and/or 16 months old). The researchers exposed male F344 rats (six/group) using single gavage doses of EGBE in water (99% purity) of 0, 32, 63, 125, 250, 250, or 500 mg/kg-day. Evaluations included total RBC and WBC counts, urine Hb concentration, organ weights, and histology of the liver, spleen, bladder, kidney, and testes. Focal necrosis of the liver was observed in adult rats exposed at a dose of 250 or 500 mg/kg. Hematologic effects were found to be dose- and age-dependent; older rats being more sensitive than younger rats. Significant decreases in RBC counts, Hct, and Hb and increases in free plasma Hb occurred at 125 mg/kg-day in both young and adult rats, with the younger rats exhibiting significantly less pronounced responses. The incidence of hemoglobinuria was also dose and age dependent. Concentrations of free Hb in urine also were significantly higher in older rats than in younger rats at all doses. These researchers suggested that the metabolic basis of the age-dependent toxicity of EGBE may be due to a reduced ability by older rats to metabolize the toxic metabolite BAA to CO_2 and a diminished ability to excrete BAA in the urine. Based on increased Hb concentrations in the urine and associated hemolytic effects at higher doses, the LOAEL for this study was determined to be 32 mg/kg-day for both young and adult rats. A NOAEL was not identified.

Ghanayem and Sullivan (1993) performed acute oral toxicity studies in male F344 rats (N not specified), using single gavage doses of 250 mg/kg-day EGBE in tap water. MCV and Hct values increased immediately after treatment and decreased with time following exposure. Hemolysis and decreases in Hb concentrations and RBC counts were reported.

Grant et al. (1985) gavaged groups of 24 male F344 rats with EGBE (purity 99.9%) in water at doses of 0, 500, or 1,000 mg/kg-day for 4 days. Six rats per dose were examined at 1, 4, 8, and 22 days after the last dose. The animals were evaluated for changes in body weight, hematology, organ weight, and histology. Hematology evaluations showed marked dose-related effects on circulating RBCs and WBCs. Changes at 500 and 1,000 mg/kg-day on postdosing day 1 included significant dose-related decreases in Hb concentration and total WBC and lymphocyte counts and increases in MCV, reticulocyte counts, and MCH. Hct was also reduced at 1,000 mg/kg-day. Most of the RBC changes subsequently returned to normal, although MCV and MCH remained increased at day 22. Body weight gain was sufficiently reduced throughout the posttreatment period at 1,000 mg/kg-day. Changes in relative organ weights were evident on posttreatment day 1, including increased liver and spleen weights at 500 and 1,000 mg/kg-day and increased kidney and reduced thymus weights at 1,000 mg/kg-day. These changes returned

to normal by posttreatment day 22, except for liver and spleen weights that were at 1,000 mg/kg-day, somewhat increased levels (~5 and ~20%, respectively). The authors determined that EGBE appears to be relatively inactive as a bone marrow toxicant due to the observed proliferative response and the lack of hemorrhage at any time in the bone marrow of EGBE treated animals. Based on hemolytic anemia with associated reticulocytosis and increased hematopoiesis, a LOAEL was established at 500 mg/kg-day, the lowest dose tested. A NOAEL was not identified.

Ghanayem et al. (1992) administered EGBE to male F344 rats (six/group) via gavage for 12 days at dose levels of 0 and 125 mg/kg-day. These investigators identified effects of EGBE exposure similar to those identified above. Significant hemolysis occurred, becoming more pronounced up to the third day of dosing. Gradual recovery was observed up to day 12. MCV, ATP concentration, reticulocyte counts, and relative spleen-to-body weight ratios increased up to the sixth day of dosing and declined thereafter. Liver-to-body-weight ratios were slightly lowered on days 3 and 6 and slightly increased on day 12.

Several studies investigated EGBE-induced effects on specific organs and cells. Four male and four female F344 rats were exposed to two, three, or four daily doses of EGBE at 250 mg/kg-day. Ezov et al. (2002) investigated hemolytic anemia and disseminated thrombosis in rats by investigating the organs for hemolysis and histopathologic evidence of disseminated thrombosis. Significant morphological changes in erythrocytes were noted in both genders of rats, while disseminated thrombosis and infarction were seen mainly in females and consisted of tissue necrosis in the brain, liver, bones, eyes, lungs, and heart. Renal tubular necrosis associated with Hb casts was seen in both genders. Koshkaryev et al. (2003) measured changes in adherence, aggregability, and deformability of RBCs. EGBE exposure did not affect RBC aggregability, and its effect on deformability was inconclusive; however, the exposure clearly enhanced RBC adherence to endothelial cells, with adherence highest at day 2, the first day examined, after which it decreased sharply with time. Shabat et al. (2004) studied bone marrow injury and reported extensive vascular thrombosis resulting in necrosis of bone marrow cells, bone-lining cells, and cortical and trabecular osteocytes. The authors concluded that, in EGBE-treated rats, interactions of several factors may generate a thrombotic crisis, such as the release of procoagulant factors from destroyed erythrocytes; they further concluded that disturbed blood flow may result from alterations in the rheology of erythrocytes, including self-aggregation, deformation, and adherence to the endothelium of the blood vessel wall. Redlich et al. (2004) investigated the dental effects from EGBE-induced hemolysis and thrombosis. Odontoblastic necrosis in the dental pulp of incisors and molars and muscle-cell damage in the tongue were observed; the most severe changes occurred in females. These effects were probably the result of ischemic events in the blood vessels supplying these tissues, rather than a direct cytotoxic effect of EGBE.

Corley et al. (1999) conducted a series of studies in B6C3F1 mice investigating aspects of EGBE toxicity, including the occurrence of forestomach lesions in both oral and inhalation exposure routes, the dose-response of forestomach irritation, and the occurrence of forestomach lesions as a consequence of systemic-only exposure. To determine the extent that activities during inhalation exposures (e.g., grooming) could contribute to observed forestomach lesions, groups of female mice were exposed for 6 hours to target concentrations of 250 ppm EGBE via whole-body (n = 20) and nose-only (n = 20) exposures and concentrations on the fur that were available for oral consumption via grooming measured. For whole-body exposures, 9.2 ± 2.9 mg/kg was available compared with 7.5 ± 2.3 mg/kg for the nose-only exposures. Little difference was detected in the internal dose of EGBE from whole-body versus nose-only exposures, as measured by the analysis of EGBE and in BAA detected in blood and urine postexposure. To inform the dose response of toxicity in the forestomach tissues in mice, neat EGBE was administered to male and female mice (five/gender/dose) via gavage (no vehicle) for 1 week at doses of 100, 400, or 800 mg/kg-day. The dose in the 100 mg/kg-day group was increased to 1,200 mg/kg-day after 2 days. Severe hemolysis and mortality were seen, and the 2-week study was terminated after only four doses. Forestomach lesions consisting of focal areas of irritation and epithelial hyperplasia were seen at all exposure levels in this study. Next, the researchers administered saline solutions of EGBE to groups of three mice by either intraperitoneal (i.p.) or subcutaneous (s.c.) injection at 400 or 600 mg/kg-day for 3 consecutive days or 0 and 400 mg/kg-day for 5 consecutive days. Focal irritation in the forestomach, similar to that seen in gavage and inhalation studies, was seen in the three mice administered EGBE by i.p. injection at 600 mg/kg for 3 days, while 1/3 mice at 400 mg/kg i.p. and at 400 and 600 mg/kg s.c. for 3 days also had forestomach lesions, minimal effects. At 400 mg/kg (5-day study), 1/6 mice (i.p.) and 2/6 mice (s.c.) also had minimal lesions. From these results, it can be concluded that: (1) the contribution to forestomach exposure from grooming activities, etc., during whole-body inhalation exposures is incidental; (2) the exposure of forestomach lesions to EGBE is similar from gavage and inhalation exposures; and (3) forestomach tissues show a similar irritative response whether EGBE exposure is systemic or portal of entry.

In another series of studies, male and female B6C3F1 mice (16/gender/group) were exposed by oral gavage to 0, 400, 800, or 1200 mg/kg-day of neat EGBE for 2 days (Poet et al., 2003). A high level of mortality was seen; the dose was reduced by half and the dosing discontinued when survival did not improve after two additional doses. Lesions, including epithelial hyperplasia and inflammation of the forestomach, were seen at the higher dose levels in both males and females, and minimal-to-mild forestomach epithelial hyperplasias were seen in the lower-dose groups, as well as in both genders. In a study similar to that performed by Corley et al. (1999), female mice were exposed, either by whole-body or nose-only inhalation, to a single 6-hour exposure of 250 ppm EGBE; the concentrations on the fur available for oral

consumption via grooming were measured. An average of 205–69 µg of EGBE was detected on the fur of the mice exposed whole-body, while an average of 170–52 µg was detected on the fur of the mice exposed nose-only (Poet et al., 2003).

Green et al. (2002) conducted a series of experiments to examine the effects and distribution of EGBE in vivo. First, female B6C3F1 mice (five/group) were given a single, daily gavage dose of either EGBE or BAA (0, 50, 150, or 500 mg/kg) for 10 days. Eighteen hours after the last dose, animals were sacrificed to look at cell proliferation in the forestomach and glandular stomach. The only dose-dependent finding was a thickening of the keratinized layers or hyperkeratosis of the forestomach (statistics not reported). A NOAEL of 150 mg/kg EGBE and 50 mg/kg BAA was observed. No effects were seen in the glandular stomachs at any dose levels. In the next set of studies, female B6C3F1 mice (n = 12) were exposed whole-body to 250 ppm 2- butoxy[1-^{14}C]ethanol (specific activity 0.365 mCi/mmol) for 6 hours. Following the exposure, animals were given free access to food and water; they were terminated (four per time point) at 5 minutes and 24 and 48 hours after exposure and whole body autoradiography was performed. Female B6C3F1 mice (n = 12) were given a single i.v. injection of 10 mg/kg 2-butoxy[1-^{14}C]ethanol (850 µCi/kg). The animals were then given free access to food and water and terminated (four per time point) at 4, 24, and 48 hours after dosing, and whole body autoradiograms were prepared. These studies showed that whether EGBE was delivered by inhalation or by i.v., radiolabeled EGBE was found in the buccal cavity, esophagus, and stomach. This suggests that EGBE somehow enters the stomach via the buccal cavity and esophagus following inhalation exposure.

Administration of a 2,000 mg/kg oral dose of EGBE to guinea pigs caused complete mortality of females and 60% mortality of males (Shepard, 1994a, b), but a dose of 1,000 mg/kg caused only a 20% mortality of either gender. Clinical signs and gross necropsy indicated toxicity was due to irritation of the stomach. There was no evidence of hemolytic toxicity.

Gingell et al. (1998) performed acute oral and inhalation toxicity studies in the guinea pig. A dose of 2,000 mg/kg EGBE was administered by gavage to five male and five females. After excessive mortality (3/5 males, 5/5 females) was observed at this dose, reduced doses of 500 and 1000 mg/kg were administered. No animals died at 500 mg/kg, and 1/5 males and 1/5 females died at 1,000 mg/kg. The acute oral median lethal dose for both genders was 1,414 mg/kg (95% confidence interval [CI] = 1020–1961 mg/kg). Clinical signs in the guinea pigs included slight-to-severe weakness, salivation and staining of face or abdomen hair, and respiratory difficulties in a few males. No evidence of RBC toxicity or hemolysis was observed. In the inhalation study, male and female guinea pigs were exposed for 1 hour to 633 ppm and 691 ppm, respectively. No mortality, clinical signs of toxicity, or exposure-related pathological signs were noted. Thus, the median lethal concentration for a 1-hour exposure for guinea pigs was >633 ppm in males and >691 ppm in females.

4.4.2. Dermal Exposure Studies

EGBE appears to be readily absorbed after contact with animal skin. Rats and rabbits exhibit varying degrees of hematotoxicity following dermal application of EGBE (Allen, 1993a, b, c, d; Bartnik et al., 1987; Tyler, 1984). Bartnik et al. (1987) performed acute dermal toxicity tests using Wistar rats (six/gender/group). A single application of 200, 260, 320, 375, or 500 mg/kg EGBE was placed on the dorsal shaved skin of rats and covered with a glass capsule. Hemolytic and/or hemoglobinuria effects were observed at 500 mg/kg EGBE within 6 hours of application. No effects were observed at 200 mg/kg.

Repeated occluded application of EGBE either neat or as a dilute aqueous solution to NZW rabbits (five/gender/group) of 18, 90, 180, or 360 mg/kg (6 hours/day, nine consecutive applications) produced hemoglobinuria in males at 360 mg/kg and in females at 180 or 360 mg/kg (Tyler, 1984). Only female rabbits showed decreased RBC counts, decreased Hb and MCHC, and increased MCH concentrations at the highest treatment level. Recovery was noted after 14 days. In a separate 13-week study, occluded dermal administration of EGBE to NZW rabbits (10/gender/group) at exposure levels of 10, 50, or 150 mg/kg 6 hours/day, 5 days/week, produced no observable hematological effects (Tyler, 1984).

Occlusion or semi-occlusion of the site of EGBE administration was also a determining factor. For example, some studies have shown no clinical signs of hematotoxicity in Sprague-Dawley rats (five/gender/group) administered EGBE dermally at 2,000 mg/kg (24-hour exposure) either semi-occluded or occluded (Allen, 1993a, b). However, clinical signs of systemic toxicity were noted following the occluded exposure. In similar studies in NZW rabbits (five/gender/group), red-stained urine was reported at semi-occluded doses of 2,000 mg/kg EGBE, along with other clinical signs of systemic toxicity (Allen, 1993c, d). Similar effects occurred at occluded doses of 500, 707, and 1,000 mg/kg in this species; deaths occurred at the 500 and 1,000 mg/kg exposures. Thus, hematotoxicity varied from nonexistent to severe. In guinea pigs, dermal administration of EGBE at 2,000 mg/kg produced no deaths, clinical signs of toxicity, or treatment-related signs of organ toxicity (Gingell et al., 1998; Shepard, 1994b).

In an assessment of immune parameters, female BALB/c mice (five/group) were topically exposed to EGBE at 100, 500, 1,000, and 1,500 mg/kg-day for 4 consecutive days (Singh et al., 2001). A statistically significant increase in spleen-to-body-weight ratio, and a 29% increase in splenic cellularity was observed at 1,500 mg/kg. Splenic proliferative responses to the T-cell mitogen, concanavalin-A (con-A), were significantly decreased by 32% at 500 mg/kg-day and 35% at 1,000 mg/kg-day. Allogeneic antigen-driven lymphoproliferative responses in the mixed lymphocyte response were significantly reduced by 55% at 500 mg/kg-day and 56% at 1,000 mg/kg-day. However, natural killer (NK) cell activity, cytotoxic T-lymphocyte activity, and the T-dependent plaque-forming cell (PFC) response were not significantly affected by EGBE exposure. A dose of 100 mg/kg-day was a NOAEL.

Singh et al. (2002) exposed female BALB/c mice (five/group) via gavage to 50, 150, or 400 mg/kg EGBE, or topically on the ear to 0.25, 1.0, 4.0, or 16.0 mg EGBE. The researchers measured the oxazolone (OXA)-induced contact hypersensitivity response (CHR). Mice that received the gavage doses of EGBE for 10 consecutive days did not exhibit a significantly altered OXA-induced CHR as measured by ear swelling 24 hours post-challenge. In contrast, topical exposure to EGBE significantly suppressed the OXA-induced CHR at a dose of 4.0 mg EGBE/ear, but not at any other dose.

The studies indicate that while the dermal route of exposure can be expected to contribute to overall exposure, the concentrations at which effects occur in animals are higher than those found following oral and inhalation exposure. In humans, toxicokinetic studies have shown that dermal absorption of EGBE vapors do contribute to the total body burden, showing the importance of the dermal route is of exposure.

4.4.3. Ocular Exposure Studies

EGBE has been found to be an irritant when instilled in rabbit (Jacobs and Marten, 1989; Kennah et al., 1989). Kennah et al. (1989) performed the Draize eye irritation test in rabbits. The percent EGBE concentration and corresponding scores by the Texaco single-digit toxicity classification system were 100%—66, 70%—49, 30%—39, 20%—2, and 10%—1. In an assessment that measured corneal thickness, the highest concentration was classified as severely irritating, the 70% concentration was moderately irritating, and the others were mildly irritating. Jacobs and Marten (1989) conducted ocular tests on NZW rabbits (n = 6) to determine the effects of EGBE (100 µL, 99% pure) on eye irritation. The undiluted chemical was dropped onto the lower lid of one eye; the other eye served as a control. The eyes were examined and graded for ocular reactions at 4, 24, 48, 72, 96, and 168 hours post-instillation. The authors determined that EGBE should be classified as an irritant based on the mean erythema scores and percent corneal thickening.

4.4.4. Genotoxicity

Although weakly genotoxic responses have been obtained in two laboratories (Elias et al., 1996; Hoflack et al., 1995), EGBE is not expected to be mutagenic or clastogenic based on the available data (summarized in Table 4-7). The NTP reported negative responses for mutagenicity when EGBE was tested in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 at up to 10 mg/plate with and without metabolic activation (Zeiger et al., 1992). However, Hoflack et al. (1995) reported that at 38 µmol/plate (4.5 mg/plate), EGBE induced a weak mutagenic response in salmonella tester strain TA97a in the absence of S9 mix (Hoflack et al., 1995). The work of Hoflack and colleagues was repeated by Gollapudi et al. (1996), and EGBE was found to be negative in these tester strains when evaluated at 0.5, 1.0, 2.5, 5.0, 8.5, and 10 mg/plate in the presence and absence of Aroclor-induced rat liver S9 mix. Thus, the weak

positive result reported in salmonella TA97a by Hoflack et al. (1995) is unconfirmed. A plausible explanation put forth by Gollapudi et al. (1996) is that, given the sensitivity of the Ames test, perhaps the weak positive result reported by Hoflack et al. (1995) is attributed to an impurity in their test material.

Elias et al. (1996) reported that EGBE did not induce chromosomal aberrations in Chinese hamster V79 fibroblast cells but that EGBE, at treatment concentrations of ≥8.5 mM, weakly induced SCEs and micronuclei and potentiated the clastogenicity induced by methyl methanesulfonate. Elias et al. (1996) also reported that EGBE weakly induced aneuploidy (numerical chromosomal anomalies) in V79 cells; however, this response was found at very high concentrations (16.8 mM EGBE).

When tested at doses nearing toxicity, EGBE and its metabolite BAL were not mutagenic in an in vitro gene mutation assay using Chinese hamster ovary (CHO) cells (CHO-AS52) (Chiewchanwit and Au, 1995). In contrast, Elias et al. (1996) reported that both EGBE and BAL weakly induced gene mutations in Chinese hamster V79 cells only at high treatment concentrations (≥ 7.5 mg/mL). It should be noted that Chiewchanwit and Au (1995) reported high cytotoxicity at 38.1 mM EGBE (4.5 mg/mL). The gene mutation data presented by Elias et al. (1996) is in graphic form only with mean values and no SDs presented. The presence or absence of cytotoxicity was not reported. BAL was also tested for induction of DNA damage in the mouse endothelial cell line, SVEC4-10, using the comet assay. BAL failed to produce a statistically significant increase in DNA strand breaks at any of the concentrations or time points examined (Klaunig and Kamendulis, 2005, 2004; Reed et al., 2003). Other lines of evidence indicate that direct interaction of BAL with the DNA molecules does not play a significant role in the carcinogenic activity of EGBE. First, BAL causes cytotoxicity at levels associated with chromosome effects, and cytotoxicity itself can have effects that result in chromosome damage, such as reduction in the repair of sister chromatid exchanges (SCEs). Second, acetaldehyde is recognized as “weakly mutagenic” and structural comparisons of the aldehyde metabolites of glycol ethers shows that longer-chain aldehydes such as BAL are less mutagenic (Chiewchanwit and Au, 1995). Third, if BAL were a stable mutagenic metabolite in any of the in vitro assays exposed to EGBE, one would expect them to give positive results; however, the results were generally negative. Elias et al. (1996) suggested that the V79 cells possess neither ALD nor ADH. The relevance of these studies, or of any systems that lack these enzymes, is of limited value in elucidating the MOA of toxicity in biological systems that possess these enzymes.

Table 4-7. Summary of genotoxicity studies on EGBE, BAL, and BAA.

Type of test, test species	Dose*	Result	Reference
In vitro tests: EGBE			
Reverse mutation, *S. typhimurium*, TA97, TA98, TA100, TA1535, TA1537	10 mg/plate	Negative (w/ and w/o metabolic activation)	Zeiger et al. (1992) (work done for NTP)
Reverse mutation, *S. typhimurium* his–TA 98, TA 100, TA 102	115 μmol/plate (14.0 mg/plate)	Negative (w/and w/o metabolic activation)	Hoflack et al. (1995)
Reverse mutation, *S. typhimurium* his-TA97a	38 μmol/plate (4.5 mg/plate)	Weakly positive (w/o metabolic activation)	Hoflack et al. (1995)
Reverse mutation, *S. typhimurium* his-TA97a, TA 100; *Escherichia coli* WP2uvrA	10 mg/plate	Negative (w/and w/o metabolic activation)	Gollapudi et al. (1996)
Sister chromatid exchanges (SCEs), micronuclei (MNs) and aneuploidy (AP) in V79 cells	10–100 mM (SCE) 8.46 mM (MN) 16.8 mM (AP)	Weakly positive (w/o metabolic activation)	Elias et al. (1996)**
Potentiation of clastogenicity induced by methyl methanesulfonate	8.5 mM	Positive (w/o metabolic activation)	Elias et al. (1996)**
Chromosomal aberrations, V79 cells and human lymphocytes	Not available	Negative (w/o metabolic activation)	Elias et al. (1996)**
Gene mutation, Chinese hamster ovary cells	38.1 mM*** (4.5 mg/mL)	Negative (w/o metabolic activation)	Chiewchanwit and Au (1995)
DNA damage, SVEC4-10 mouse endothelial cells	10 mM	Negative	Klaunig and Kamendulis (2005)
In vitro tests: BAL			
Reverse mutation, S. typhimurium his-TA 97a, TA 98, TA 100 and TA 102	43 μmol/plate (5.0 mg/plate)	Negative (w/and w/o metabolic activation)	Hoflack et al. (1995)
Chromosomal aberrations, V79 cells and human lymphocytes	0.1–1 mM;cytotoxicity not reported	Positive (w/o metabolic activation)	Elias et al. (1996)**
DNA damage, SVEC4-10 mouse endothelial cells	1 mM	Negative	Klaunig and Kamendulis (2005)
In vitro tests: BAA			
Reverse mutation, S. typhimurium his-TA 97a, TA 98, TA 100 and TA 102	8 μmol/plate (0.9 mg/plate)	Negative (w/ and w/o metabolic activation); dose limited by toxicity	Hoflack et al. (1995)
SCEs and CAs, V79 cells	0.8 mM	Negative (w/o metabolic activation)	Elias et al. (1996)**
Aneuploidy, V79 cells	0.38 mM	Weakly positive (w/o metabolic activation)	Elias et al. (1996)**
MN assay, V79 cells	10 mM	Positive (w/o metabolic activation	Elias et al. (1996)**
DNA damage, SVEC4-10 mouse endothelial cells	10 mM	Negative	Klaunig and Kamendulis (2005)
In vivo tests: EGBE			
MNs, bone marrow erythrocytes of male mice or rats	550 mg/kg-day, mice 450 mg/kg-day, rats	Negative Negative	NTP (1996)
DNA adducts FVB/N mice Sprague-Dawley rats	120 mg/kg-day; mice and rats	No changes in DNA methylation	Keith et al. (1996)

*Doses are either the lowest effective dose or the highest ineffective dose.
**All in vitro assays were performed without the addition of an exogenous metabolic activation system.
***The authors found that this dose was cytotoxic.

BAA has been found negative for reverse mutations in *S. typhimurium* his^- with and without metabolic activation (Hoflack et al., 1995). Concentrations of up to 8 μmol/plate were tested, and dose was limited by toxicity. BAA (up to 10 mM) was also found negative for induction of DNA damage in SVEC4-10 mouse endothelial cells (Klaunig and Kamendulis, 2005) and in an SCE assay in V79 cells (Elias et al., 1996). BAA was weakly positive for aneuploidy in V79 cells at 0.38 mM and positive for micronuclei induction in the same cell line at 10 mM, as reported by Elias et al. (1996). As noted above, the data means are presented in graphic form without SDs and cannot be critically evaluated; no cytotoxicity data are reported.

EGBE did not increase the incidence of micronuclei in the bone marrow cells of male mice or rats (NTP, 1996). Animals were given three i.p. injections of EGBE 24 hours apart and sacrificed 24 hours after the last injection; rats were dosed at 0, 7, 14, 28, 56, 112.5, 225, or 450 mg/kg and mice were dosed at 0, 17, 34, 69, 137.5, 275, or 550 mg/kg (NTP, 1996). There was high mortality (2/5 mice survived) in mice injected with 1,000 mg/kg doses of EGBE. Keith et al. (1996) treated Sprague-Dawley rats and transgenic FVB/N mice carrying the v-Ha-ras oncogene with a single oral dose of 120 mg/kg EGBE; there was no increase in DNA adducts in the brain, liver, kidney, testes, or spleen of the rats, and no changes in DNA methylation patterns in either species.

In conclusion, EGBE has been tested in conventional genotoxicity tests for its potential to induce gene mutations in systems and cytogenetic damage both in vitro and in vivo. Available data do not support a mutagenic or clastogenic mechanism for EGBE. Two laboratories (Elias et al., 1996; Hoflack et al., 1995) reported weak genotoxicity responses in vitro at toxic doses. These results, however, are questionable given limited published information. Elliott and Ashby (1997) reviewed the results of the available genotoxicity studies on EGBE and concluded that the data indicate that EGBE has no significant genotoxic activity.

4.4.5. Immunotoxicity

Based on the results of the Exon et al. (1991) study, it appears that the immune system is not a sensitive target of EGBE. Groups of six Sprague-Dawley rats were exposed to EGBE in drinking water at doses of 0, 180, or 506 mg/kg-day (males) or 0, 204, or 444 mg/kg-day (females) for 21 days. All rats were injected s.c. with heat-aggregated aqueous keyhole limpet hemocyanin (KLH), a T-cell dependent antigen, on days 7 and 13 following the start of dosing. Endpoints evaluated on day 21 included body weight, absolute and relative organ weights (spleen, thymus, liver, kidney, testis), and histology of thymus, liver, kidney, and testis. Splenic histology was not assessed, because this tissue was used as a source of cells for immune function assays. Immune function assays included NK cell cytotoxicity, antibody response to a T- cell dependent antigen as determined by measurement of KLH-specific serum immunoglobulin G levels, delayed-type hypersensitivity reaction, interleukin-2 and interferon production, and spleen cell counts. Terminal body weights were somewhat lower than controls in all exposed groups,

and the decreases were statistically significant in all groups except the 180 mg/kg-day males. No dose-related changes in organ weights or histology were observed. NK cell cytotoxic responses were significantly enhanced in males at 180 mg/kg-day and females at 204 mg/kg-day. At the highest dose tested in males and females, the NK cell cytotoxic response was increased above control, but this finding was not statistically significant. A decreased NK cell cytotoxic response is an indication of compromised nonspecific immune system integrity. Given that this study showed an increased response and no dose-response relationship, these findings are not considered to be an indication of an adverse response. No significant alterations in other immune parameters were noted.

Smialowicz et al. (1992a) reported on results of experiments that examined the primary antibody response to a T-cell independent antigen (trinitrophenyl-lipopolysaccharide [TNP-LPS]) measured with a PFC assay to determine the immunotoxic potential of a variety of glycol ethers. This author had previously reported that the shorter-chain glycol ether 2-methoxyethanol (ME) and its principal metabolite 2-methoxyacetic acid (MAA) suppressed the antibody response to TNP-LPS as measured by the PFC assay in F344 rats but not CD-1 mice (Smialowicz et al., 1992b). Having established the sensitivity of F344 rats to suppression of the antibody response to the T-cell independent antigen TNP-LPS by ME and MAA, the authors examined other glycol ethers, including 2-butoxyethanol, in the same dose range, for their ability to suppress the antibody response to TNP-LPS by using the same PFC assay. Male F344 rats were immunized with a single i.v. injection of 0.5 mL of 40 μg/mL TNP-LPS, then dosed (six/dose group) by gavage with 50–400 mg/kg-day of various glycol ethers, including EGBE (0, 50, 100, 200, 400 mg/kg-day) for 2 days. All rats exposed to 400 mg /kg-day EGBE died, and the 200 mg/kg-day EGBE dose resulted in one dead and one moribund rat. This finding was not unexpected, as the hematotoxicity of EGBE in older rats has been reported in the literature (Ghanayem et al., 1987c; Tyler, 1984; Carpenter et al., 1956). EGBE did not suppress the primary antibody response to TNP-LPS in the PFC assay.

In an assessment of immune parameters, female BALB/c mice (five/group) were topically exposed to EGBE at 100, 500, 1,000, and 1,500 mg/kg-day for 4 consecutive days (Singh et al., 2001). A statistically significant increase in spleen-to-body-weight ratio was observed at 1,500 mg/kg, and splenic cellularity was increased by 29% at this dose. Splenic proliferative responses to the T-cell mitogen, con-A, were significantly decreased by 32% at 500 mg/kg-day and 35% at 1,000 mg/kg-day. Allogeneic antigen-driven lymphoproliferative responses in the mixed lymphocyte response were significantly reduced by 55% at 500 mg/kg-day and 56% at 1,000 mg/kg-day. However, NK cell activity, cytotoxic T-lymphocyte activity, and the T-dependent PFC response were not significantly affected by EGBE exposure. For those immune parameters measured, 100 mg/kg-day was a NOAEL.

Singh et al. (2002) exposed female BALB/c mice (five/group) via gavage to 50, 150, or 400 mg/kg EGBE or topically to 0.25, 1.0, 4.0, or 16.0 mg EGBE on the ear and measured the OXA-induced CHR. Mice that received the gavage doses of EGBE for 10 consecutive days did not exhibit a significantly altered OXA-induced CHR as measured by ear swelling 24 hours postchallenge. In contrast, topical exposure to EGBE significantly suppressed the OXA-induced CHR at a dose of 4.0 mg EGBE/ear, but not at any other dose.

4.4.6. Other In Vitro Studies

Ghanayem (1989) compared the metabolic and cellular basis of EGBE-induced hemolysis of rat and human erythrocytes in vitro. EGBE is not metabolized when incubated with blood from male F344 rats and causes no hemolysis or metabolic alterations at concentrations of up to 10 mM. A concentration of 20 mM EGBE was required to produce significant hemolysis of rat blood. This may be due to a nonspecific effect occurring at a concentration that is not physiologically relevant. In contrast, incubation of rat blood with BAL or BAA at concentrations of 0.5, 1.0, or 2.0 mM caused a time- and concentration-dependent increase in cell swelling (i.e., increased Hct) followed by hemolysis. This response was more pronounced for BAA, with nearly complete hemolysis observed after 4-hour incubation at 2.0 mM. BAL produced only slight hemolysis under the same conditions. The addition of ADH (with nicotine adenine dinucleotide cofactor) to rat blood followed by BAL produced a potentiation of the hemolytic effects. Addition of cyanamide, an ADH inhibitor, significantly decreased the effects with or without added ADH. Both BAL and BAA caused a time- and concentration-dependent decrease in blood ATP concentrations, although this effect may be secondary to the swelling and lysis observed. Addition of exogenous ATP failed to reverse the hemolytic effects. Neither EGBE nor its metabolites, BAL and BAA, caused any detectable changes in the concentrations of glutathione (GSH) or glucose-6-phosphate dehydrogenase (G6PD) in rat erythrocytes. Blood from male and female human volunteers was unaffected by 4-hour incubations with BAA at concentrations of up to 4.0 mM. At 8 mM, slight but significant hemolysis of human blood was observed: blood from female volunteers showed a slightly greater sensitivity. These studies show that the erythrocyte membrane is the likely target for BAA, that humans of both genders are relatively insensitive to the hemolytic effects of BAA, as compared with rats.

Udden (2000) compared effects on RBC morphology in male F344 rats (five/group) dosed with 125 or 250 mg/kg EGBE via gavage with the effects of incubation of rat erythrocytes in vitro with BAA (1 or 2 mM). In vivo exposure resulted in stomatoacytosis and spherocytosis in blood smears and cup-shaped cells and spherocytosis in fixed samples. In vitro incubation resulted in erythrocytes with cup shapes and spherocytosis in the fixed samples. Since in vivo and in vitro exposure caused similar changes in RBC morphology in rats, this study provides additional evidence for the role of BAA in causing the hemolytic effects from EGBE exposure in rats.

Udden (2002) performed in vitro comparisons of sub-hemolytic and hemolytic effects in rat and human RBCs in response to exposure of high BAA, using samples from hospitalized adults, hospitalized children, and well adults. Erythrocyte parameters measured included deformability, size distribution, density, MCV, count, osmotic fragility, and hemolysis. Exposure for 4 hours resulted in loss of deformability, although at concentrations 150-fold different; the first significant alterations noted in cells exposed was at 0.05 mM BAA in rats and at 7.5 mM BAA in humans. Significant increases in MCV after a 4-hour exposure to BAA were noted at 0.05 mM in rats and at 10.0 mM in humans, a 200-fold difference. A similar differential in concentration range was noted in osmotic fragility. Testing of erythrocytes for changes in MCV and percent hemolysis from in vitro exposure to either 0 or 10 mM BAA showed no differences among the well adults or the hospitalized children. The percent hemolysis seen in the average response of the hospitalized adults to 10 mM BAA was significantly increased, from 0.54–0.69%. Significant increases in MCV were noted for all three of these groups, including the well adults. The mechanisms underlying erythrocyte damage and the resistance of human cells to these effects remain unknown. It should be noted, however, that although the sub-hemolytic responses were similar for human and rat erythrocytes, other aspects of the erythrocytes, including morphology and cell density, were altered in the rats but not in human cells. These differences between species suggest that the mechanisms underlying the observed pre-hemolytic effects may be different between rats and humans.

The possibility exists that certain human subpopulations, including the aged and those predisposed to hemolytic disorders, might be at increased risk from EGBE exposure. Udden (1995, 1994) investigated this possibility using blood from the elderly (mean age 71.9; range 64–79 years; five men and four women), from seven patients with sickle cell disease, and from four subjects with hereditary spherocytosis, three of whom were studied post-splenectomy and one studied pre-splenectomy. Using a sensitive assay for erythrocyte deformability (Udden, 1994; Udden and Patton, 1994), it was shown that blood from all of these potentially sensitive groups was unaffected by incubations of up to 4 hours with 2.0 mM BAA.

Udden and Patton (2005) examined the role of osmolarity and cation composition of the cell suspension buffers in the mechanism of BAA-induced hemolysis of rat RBCs. Adding sucrose to the cell suspension media or replacing external sodium with potassium protected rat RBCs from BAA-induced hemolysis. The authors also observed that adding as little as 0.05 mM $CaCl_2$ to the buffer delayed the time course of the hemolytic response, while adding $MgCl_2$ had no effect. Use of the calcium-activated potassium channel inhibitor charybdotoxin blocked the protective effect of calcium. From this, the authors suggest that BAA causes sodium and calcium to enter the cell. While calcium initially has a protective effect via a loss of potassium through the calcium-activated potassium channel, compensating for the osmotic effect of increased cell

sodium, calcium may subsequently have other deleterious effects through activation of proteases and other calcium-activated processes.

Corthals et al. (2006) tested EGBE, BAL, and BAA in the comet assay to determine their ability to induce DNA damage in SVEC4-10 mouse endothelial cells. EGBE (up to 10 mM), BAL (up to 1 mM), and BAA (up to 10 mM) did not produce significant increases in DNA damage relative to controls at any of the time points examined (2, 4, and 24 hours). The researchers next tested the effect of hemolyzed mouse RBC lysate and ferrous sulfate in the same system and found that the hemolyzed RBCs produced a statistically significant increase in DNA damage at the highest concentration tested (10×10^6 hemolyzed RBCs) for 4 hours. No other time points were significant. Ferrous sulfate produced statistically significant increases in DNA damage at the highest time point and the lowest concentration tested (24 hours, 0.1 μM) and at all time points (2, 4, and 24 hours) in the mid- and high doses that were tested (0.5 μM and 1.0 μM). The next experiment examined the ability of EGBE, BAA, ferrous sulfate, and hemolyzed RBCs to stimulate tumor necrosis factor-alpha (TNFα) release from cultured mouse macrophages (RAW 264.7 cells). Hemolyzed RBCs (10×10^6 cells) resulted in a statistically significant increase ($p < 0.05$) in TNFα release following a 4-hour treatment. Treatment with EGBE, BAA, or ferrous sulfate did not result in increased TNFα release. Finally, the authors report that macrophages activated with hemolyzed RBCs (10×10^6) for 4 hours were able to increase DNA synthesis in mouse endothelial cells through co-culturing for 24 hours. These macrophages, were not, however, able to increase endothelial cell DNA damage (after 4- or 24-hour treatment) as measured by the comet assay. The authors did find that LPS activated macrophages after a 4-hour treatment produced statistically significant increases ($p < 0.05$) in endothelial cell DNA damage, as measured by the comet assay.

4.5. Synthesis and Evaluation of Major Noncancer Effects and Mode of Action: Oral and Inhalation

Intravascular hemolysis is the primary response elicited in sensitive species following inhalation, oral, or dermal administration of EGBE. The carboxylic acid metabolite of EGBE, BAA, has been shown to be the causative agent in this hemolysis. Hemolysis can be induced in vivo following administration of EGBE or in vitro following addition of BAA to either whole blood or washed erythrocytes. In vitro tests have shown that BAA produces a concentration- and time-dependent swelling of rat erythrocytes, and changes in the normal erythrocyte morphology from the typical discocyte form to a spherocyte form prior to lysis. This response appears to be mediated by the erythrocyte membrane and results in an increase in osmotic fragility and a loss of deformability of the erythrocyte, thereby leading to hemolysis. Older erythrocytes are

apparently more sensitive to the hemolytic effects of BAA than are younger cells or newly formed reticulocytes.

The primary response of hemolysis is indicated via dose-related clinical observations of decreases in Hct, Hb concentration, and RBC count in the blood of laboratory animals exposed to EGBE. The hemolysis-related events of macrocytosis and increased MCV were also observed in the rat, considered to be a sensitive species, and are attributed at least partly to the increased number of larger reticulocytes in the circulation following the erythropoietic compensatory response (NTP, 2000). These alterations were persistent throughout the chronic animal exposures but do not appear to progress with extended exposure (from 3 months to 1 year for rats). These changes persist despite functioning, compensatory, homeostatic mechanisms.

Liver effects were noted in the NTP (2000) reports of subchronic and chronic inhalation studies in rats and mice and in the subchronic drinking water study in rats. These included statistically significant increases in the iron-staining hepatic pigmentation attributed to hemosiderin, the storage protein for insoluble iron, believed to be derived from the Hb released during hemolysis. Nyska et al. (2004) examined the possible association between chemically induced hemosiderin deposition and hemangiosarcomas in the liver of mice from 130 NTP bioassays, and found a highly significant association between liver hemangiosarcoma and apparent Kupffer cell pigmentation. The cause for the hemosiderin deposition in all cases was the erythrocyte hemolytic effect of the compounds. However, in an NTP (1993) subchronic drinking water study, hepatocellular cytoplasmic changes were observed in male rats at an exposure level (750 ppm) below the level at which hematological changes were recorded (1,500 ppm). This finding raises the possibility of a direct, primary hepatic toxicity due to either EGBE or an EGBE metabolite. Similar liver effects observed in female rats at the 750 ppm exposure level were accompanied by hematological effects.

In the same NTP (1993) report, no liver lesions were reported in mice exposed to drinking water containing up to 6,000 ppm EGBE. The lesions reported in rats consisted of cytoplasmic alterations, hepatocellular degeneration, and pigmentation. The cytoplasmic alterations, the only lesion observed at the 750 ppm exposure level (corresponding to a consumption of roughly 55 mg/kg-day EGBE for adult male rats), were described as hepatocytes staining more eosinophilic and lacking the amphophilic-to-basophilic granularity of the cytoplasm present in hepatocytes from control animals. Greaves (2000) suggested that the lack of cytoplasmic granularity or ground-glass appearance of the hepatocytes is an indication that the response does not involve enzyme induction. The hepatocellular degeneration and pigmentation observed at the higher exposure levels in both genders was centrilobular, which is consistent with the Kupffer cell pigmentation and hemosiderin deposition reported in the NTP (2000) inhalation studies. This information, along with the observation that all other rat and mouse oral and inhalation studies of EGBE report hemolysis at or below exposure levels that result in liver

effects, suggests that at least these cytoplasmic hepatocellular changes in male rats reported in the NTP (1993) drinking water study may reflect adaptation to a subclinical level of hemolysis. However, focal necrosis of the liver observed in male rats following gavage administration of 250 and 500 mg/kg EGBE (Ghanayem et al., 1987b) was judged to be inconsistent with typical anoxic centrilobular necrosis associated with anemia (Edmonson and Peters, 1985). The effects observed in the Ghanayem et al. (1987b) study may be associated with the high bolus exposures employed.

The liver alterations documented throughout these studies suggest a defined progression of pathological events with increasing doses of EGBE with increasing levels of hemolysis. In particular, hepatic hemosiderin deposition in the liver is a dose-related sequela of the hemolytic activity caused by EGBE exposure. This deposition was noted to follow a clear dose-response relationship as well as to increase in severity in the chronic rat and mouse NTP studies; it shows a statistically significantly increase relative to the chamber controls. Although some hemosiderin deposition in the spleen and liver may be expected with increasing age, the extent of hemosiderin deposits noted by NTP in the livers of EGBE exposed animals is not normal. For these reasons, hemosiderin deposition in the liver has been considered a pathological finding (Muller et al., 2006). The following issues relate to the relevance of these hemolytic and hepatic effects to humans and to the MOA of EGBE.

The weight of evidence from a variety of studies in animals and humans suggests that certain species are more susceptible to the hemolytic effects of EGBE. It appears that humans are less sensitive to the hemolytic effects of EGBE than rats and mice. On one end of the sensitivity range is the guinea pig, which displays no hemolytic effects from EGBE at exposure levels as high as an oral dose of 1,000 mg/kg or a dermal dose of 2,000 mg/kg. The rat, conversely which displays increased osmotic fragility of erythrocytes at single-inhalation exposures below 100 ppm and single oral exposures below 100 mg/kg EGBE. No hemolysis was been observed in controlled laboratory acute inhalation exposures of human volunteers at up to 195 ppm EGBE. Some reversible hemolytic effects have been described in addition to more debilitating effects in humans who consumed single oral doses of 400–1500 mg/kg EGBE in cleaning formulations (see Section 4.1). Effects in humans from chronic exposure to EGBE have not been studied.

With respect to gender sensitivity, it has been consistently noted (Ezov et al., 2002; NTP, 2000, 1993; Dodd et al., 1983; Carpenter et al., 1956) that female rats are more sensitive to EGBE-induced hemolysis than males. This gender difference is consistent with toxicokinetic data for male and female rats reported by the NTP (2000) 2-year study. Female rats eliminated BAA, the toxic metabolite of EGBE, more slowly from the blood, resulting in a larger AUC for the blood concentration of BAA versus time. This may be a result of the reduced renal excretion observed in female versus male rats. NTP (2000) also reported that, like female rats, female mice tended to have greater blood concentrations of BAA at any given time than males. This may

explain the slight increase in incidence and severity of the anemic response found in female, as compared to male, mice. However, unlike female rats, female mice excrete slightly more BAA than male mice; no significant difference between female and male mice has been noted in the overall rate of elimination or the $t_{½}$ of BAA.

Some studies (Ghanayem et al., 1990, 1987c) were designed to assess the effect of age on the toxicokinetics and hemolytic effects in young and adult rats treated with single EGBE gavage exposures. Both blood retention (Ghanayem et al., 1990) and hematologic effects (Ghanayem et al., 1987c) were found to be dose- and age-dependent with older rats retained more of the EGBE metabolite BAA in their blood and were more sensitive than younger rats. The increased blood retention of BAA (as measured by increased C_{max}, AUC, and $t_{½}$) in older rats versus younger rats may be due to metabolic differences or compromised renal clearance. The researchers suggested that the pharmacokinetic basis of the age-dependent toxicity of EGBE may be due to a reduced ability by older rats to metabolize the toxic metabolite BAA to CO_2, and a diminished ability to excrete BAA in the urine. No comparable studies exist for an analysis of liver effects.

While older rats appear to be more severely affected by acute doses of EGBE, continuous exposures appear to impart a certain level of tolerance to rats and mice over time. Apparent tolerance to EGBE-induced hemolysis in rats and mice has been seen in subchronic (Krasavage, 1986; Grant et al., 1985) and chronic (NTP, 2000) studies. Ghanayem et al. (1992, 1990) investigated this effect in the male F344 rat. Daily gavage administration of EGBE at 125 mg/kg for 12 days resulted initially in hemolytic anemia, which was more pronounced following the third day, but the animals recovered gradually to near pretreatment levels by day 12. Additionally, rats treated for 3 days at 125 mg/kg followed by a 7-day recovery period were significantly less sensitive to subsequent treatment with EGBE at either 125 or 250 mg/kg, as were rats that were bled and subsequently treated. Ghanayem et al. (1992, 1990) proposed that the tolerance to hemolysis following repetitive dosing is not due to changes in EGBE metabolism, but to the replacement of older and more susceptible erythrocytes with younger, less susceptible cells. However, chronic studies in rats and mice (NTP, 2000) have shown that any increased resistance imparted by these immature erythrocytes diminishes with age. Rats and mice chronically exposed to EGBE experienced anemia that persisted with no apparent progression or amelioration of severity for 9 months, up to the final blood collection at 12 months of age. Apparently, there is a balance in these rodents between the release of reticulocytes to the circulation and the aging process, so that the level of susceptible cells and severity of anemia remains relatively constant.

A number of secondary effects resulting from the hemolytic toxicity of EGBE have been reported in studies with rats, mice, and rabbits. In the rat, the organs generally affected include most prominently the liver (see discussions above) but also the kidneys, spleen, bone marrow,

and, to a lesser extent, the thymus (Shabat et al., 2004; NTP, 1993; Exon et al., 1991; Grant et al., 1985). Typically, increased liver and kidney weights are observed with corresponding decreases in body weights at doses that produce a hematotoxic response. Accompanying this are hepatocellular degeneration, hemosiderin deposition in the liver, and congested spleens. Renal damage is often reported, accompanied by hemosiderin accumulation, renal tubular degeneration, and intracytoplasmic Hb. Often these effects are more pronounced in females. Hematopoiesis in bone marrow and spleen, increased cellularity of bone marrow, and splenic congestion are all secondary to the hematotoxicity of EGBE and develop as a compensatory response to hemolysis. In addition, intact erythrocytes have been observed histopathologically in spleens from EGBE-treated rats, but not in spleens from control animals. This suggests an increased rate of removal of damaged erythrocytes in EGBE-treated rats (Ghanayem et al., 1987c). Mild lymphopenia and neutrophilia were observed at hemolytic doses of EGBE (Ghanayem et al., 1987c) and were reported to be consistent with a "stress" leukogram produced by the release of endogenous corticosteroids (Wintrobe, 1981a). Neutrophilia, commonly associated with acute hemolysis or hemorrhage (Wintrobe, 1981b), was also observed.

In conclusion, humans are significantly less sensitive to the hemolytic toxicity of EGBE than are typical laboratory species such as mice, rats, or rabbits, although human erythrocytes do appear capable of responding similarly to the causative EGBE metabolites, albeit at much higher exposures. This marked species difference in sensitivity has been demonstrated in several laboratory studies and through the use of in vitro studies using either whole blood or washed erythrocytes. Based on the results of in vitro testing, blood concentrations of the hemolytically active metabolite BAA must reach levels in human blood in excess of 7.5 mM for prehemolytic changes to occur. Comparable effects in rat blood occur at in vitro concentrations approximately 150-fold lower. In addition, blood from potentially sensitive individuals, including the elderly or those with congenital hemolytic disorders, does not show an increased hemolytic response when incubated with up to 2 mM BAA for 4 hours. Based on simulations from PBPK modeling, 6-hour whole-body exposure of humans to saturated atmospheres of EGBE will result in maximum blood concentrations of BAA below those needed to produce hemolysis (Corley et al., 2005a).

Most of the liver alterations documented throughout the EGBE exposure database are related directly to hemolysis. Prominent among these alterations is hemosiderin deposition, a pathological finding whose occurrence is related to hemolysis (Muller et al., 2006). Humans also experience hemosiderin deposition in the liver, principally in hepatocytes, as a consequence of excessive hemolysis, such as with thalassemia, a hereditary form of hemolytic anemia (Iancu et al., 1976). Hemosiderin deposition in the liver is a pathological response that follows the precursor hematologic effects, which, as a group, do not appear to progress with changes in duration of exposure from subchronic to chronic.

4.6. Evaluation of Carcinogenicity

4.6.1. Summary of Overall Weight of Evidence

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EGBE is deemed "not likely to be carcinogenic to humans" at expected environmental concentrations, based on laboratory animal evidence, mode-of-action information, and limited human study information. The available data indicate that carcinogenic effects from EGBE are not likely to occur in humans in the absence of the critical noncancer effects, including hepatic hemosiderin staining and irritant effects at the portal of entry and are not likely to be carcinogenic to humans exposed at levels at or below the RfC and RfD values established in this assessment. Carpenter et al. (see Section 4.1) reported that no changes in erythrocyte osmotic fragility were found in human subjects exposed to up to 195 ppm (942 mg/m^3; ~ 600 times the RfC) for two 4-hour periods separated by a 30-minute break. At oral doses of 400-500 mg/kg with a one-time bolus dose (see Section 4.1), hematuria has been noted in two human case reports. This dose is 3000 to 3500 times the RfD and would need to be sustained for a significant period of time to produce hemosiderin deposition. This is unlikely to occur because the primary response of humans to high oral doses of EGBE, as shown in the case studies in Section 4.1, is metabolic acidosis, which, if not treated, can lead to shock and eventually death. No information is available on the carcinogenic effects of EGBE via the oral or inhalation route in humans. A 2-year inhalation bioassay with mice and rats (NTP, 2000) reported tumors of the liver in male mice, forestomach tumors in female mice, and tumors of the adrenal medulla in female rats. Non-neoplastic effects in rats included hyaline degeneration of the olfactory epithelium and Kupffer cell pigmentation. Non-neoplastic effects in mice included forestomach ulcers and epithelial hyperplasia, hematopoietic cell proliferation, Kupffer cell pigmentation, hyaline degeneration of the olfactory epithelium (females only), and bone marrow hyperplasia (males only).

EGBE has been tested in conventional genotoxicity tests for its potential to induce gene mutations in vitro and cytogenetic in both in vitro and in vivo, and the available data do not support a mutagenic or clastogenic mechanism for EGBE. Two laboratories (Elias et al., 1996; Hoflack et al., 1995) did report weak genotoxicity responses in vitro at high treatment concentrations, but results were not replicated in five other labs reporting negative results.

The hypothesized MOA for the tumors observed following EGBE treatment involves exposure to high doses for prolonged periods of time. This MOA is described in the sections that follow. The weight of evidence indicates that EGBE is not likely to be carcinogenic to humans at expected environmental concentrations.

4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence

NTP (2000) conducted a 2-year inhalation study on EGBE in both genders of F344/N rats and B6C3F1 mice. Rats (50/gender/group) were exposed to concentrations of 0, 31, 62.5, and

125 ppm (0, 150, 302, and 604 mg/m^3) and mice (50/gender/group) were exposed to concentrations of 0, 62.5, 125, and 250 pm (0, 302, 604, and 1,208 mg/m^3). The NTP report stated that the highest exposure was selected to produce a 10–15% depression in hematologic indices and survival was significantly decreased in male mice at 125 and 250 ppm (54.0 and 53.1%, respectively). While the NTP researchers report that no effect on survival was observed in rats, the female rats appeared to show a trend toward decreased survival that may have been attributable to the hematological effects. Mean body weights of rats exposed to 31 and 62.5 ppm were similar to those of control animals. Mean body weights of the exposed mice were generally less than for controls, with females experiencing greater and earlier reductions. From week 17 to the end of the study, the mean body weights of 125 ppm female rats were generally less than those of controls.

At the end of the 2-year chronic bioassay (NTP, 2000), neoplastic effects were observed in female rats and in male and female mice. In female rats, the combined incidence of benign and/or malignant pheochromocytoma of the adrenal medulla was 3/50, 4/50, 1/49, and 8/49. The incidence in the high-dose group (16%) did not represent a statistically significant increase over the chamber control group (6%), but it exceeded the historical control (6.4–3.5%; range 2–13%) for this effect.

The low survival rate in male mice exposed to 125 and 250 ppm EGBE may have been due to carcinogenic effects in the liver. A high rate of hepatocellular carcinomas was found in these exposure groups (10/50 [control], 11/50, 16/50, 21/50); the increase at the high-exposure level was statistically significant ($p < 0.01$). However, when hepatocellular adenomas and carcinomas were combined, no significant increase was observed in any exposure group. The incidence of hemangiosarcomas in males exposed to 250 ppm (8%) was also significantly increased ($p = 0.046$) relative to chamber controls (0/50, 1/50, 2/49, 4/49) and exceeded the range of historical controls (14/968; 1.5 ± 1.5%; range 0–4%). No significant increases in benign or malignant hepatocellular tumors or hemangiosarcomas were noted in the female mice, and the incidence of hepatocellular adenomas actually decreased significantly ($p < 0.05$) in relation to the control chamber group (16/50, 8/50, 7/49, 8/49). It should be noted that in light of the high survival rate of the exposed female mice relative to controls (29/50, 31/50, 33/50, 36/50), the high exposure of 250 ppm may not have provided the maximum tolerated dose.

Forestomach squamous cell papillomas and carcinomas, combined), were significantly increased (trend test = 0.003) in female mice relative to the chamber control group (0/50, 1/50, 2/50, 6/50). The incidence of these tumor types (12%) at the highest exposure level was also statistically significant and exceeded the range for the occurrence of these tumors in historical controls (0.9 ± 1.1%; range 0–3%). The first incidence of these tumors appeared in the group exposed to 250 ppm at 582 days, as compared to 731 days at 62.5 and 125 ppm, indicating a decreased latency period in the highest exposure group. While the incidence of these types of

forestomach tumors was not significantly increased over controls in male mice (1/50, 1/50, 2/50, 2/50), the incidence of squamous cell papillomas (4%) in the two highest exposure groups exceeded the range for historical controls (0.5 ± 0.9%; range 0–2%). The increased incidence of forestomach neoplasms in males, as in females, occurred in groups with ulceration and hyperplasia.

The NTP (2000) study concluded that there was no evidence showing carcinogenic activity in male F344/N rats, and equivocal evidence of carcinogenic activity in female F344/N rats, based on increased combined incidences of benign (mainly) and malignant pheochromocytoma of the adrenal medulla. The researchers reported some evidence of carcinogenic activity in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver and an increase in the incidence of hepatocellular carcinoma, as well as some evidence of carcinogenic activity in female B6C3F1 mice based on increased incidence of forestomach squamous cell papilloma (mainly) or carcinoma.

With respect to the pheochromocytomas reported in female rats, while the data showed a positive trend ($p = 0.044$) and the high-dose tumor frequencies (16%) were above the upper range of historical controls (13%), the tumor incidence data were not statistically significant. Further, the NTP (2000) report noted that pheochromocytomas can be difficult to distinguish from non-neoplastic adrenal medullary hyperplasia. The presence of mild-to-moderate compression of the adjacent tissue is a primary criterion used to distinguish pheochromocytomas from medullary hyperplasia; most tumors observed were small and not substantially larger than the more severe grades of adrenal medullary hyperplasia. Interpretation of these tumors should be done cautiously. Given the marginal dose response, lack of tumor evidence in any other organ system of the rats, and reported difficulties in distinguishing pheochromocytomas from non-neoplastic adrenal medullary hyperplasia, this tumor type was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential.

4.6.3. Mode of Action Information

4.6.3.1. Hypothesized Mode of Action for Liver Tumor Development in Male Mice

The hypothesized MOA for EGBE-induced liver tumors in male mice is believed to involve iron accumulation and subsequent oxidative stress due to the hemolytic effects of a metabolite of EGBE. Male mice developed hepatocellular carcinomas and hemangiosarcomas in the liver that appeared to be exposure-related. The incidence of hemangiosarcomas was statistically significant and increased over both concurrent and historical control groups. The hepatocellular carcinomas were within the range of historical controls for male mice, but are also considered in this discussion because the dose-response trend is significant and because a similar MOA has been suggested for this tumor (Klaunig and Kamendulis, 2005). The incidences in the high dose group of these two types of tumors were only slightly higher than the upper end of the

range for historical controls. Furthermore, these two tumor types were not increased in other organs (e.g., bone, bone marrow) and were not noted in either rats or female mice. The hypothesized MOA involves the hemolysis of RBCs, the accumulation of hemosiderin, and subsequent oxidative stress that leads to neoplasia in the two cell types believed to be the origin for these two tumors: hepatocytes for hepatocellular carcinoma and endothelial cells for hemangiosarcomas. Only the male mice developed these tumor types, and this is hypothesized to be due to their susceptibility to oxidative stress. Mice are known to be more susceptible to oxidative stress than rats because of their lower antioxidant capacity (Siesky et al., 2002; Bachowski et al., 1997). The available studies suggest that iron accumulation from the hemolytic effects of EGBE produces liver oxidative damage that is more severe in mice and increased DNA synthesis in both endothelial cells and hepatocytes that may be unique to mice (Corthals et al., 2006; Siesky et al., 2002). From this and reported differences in antioxidant capacity[1] and background rates of these tumors[2] between male and female mice, it can be hypothesized that events leading to oxidative stress contribute to the transformation of male mouse endothelial cells to hemangiosarcomas and hepatocytes to hepatocellular carcinomas. A series of events that may lead to the formation of liver tumors follows.

Step Event

(1) EGBE is metabolized to BAL, which is subsequently oxidized to BAA.
(2) BAA causes RBC swelling and hemolysis and an increase in Hb levels.
(3) Excess Hb from damaged RBCs is taken up by phagocytic (Kupffer) cells of the spleen and liver and stored as hemosiderin.
(4) Oxidative damage and increased synthesis of endothelial and hepatocyte DNA are initiated by one or more of the following events:
    (a) Generation of reactive oxygen species (ROS) from Hb-derived iron within Kupffer cells and perhaps from within hepatocytes and sinusoidal endothelial cells
    (b) Activation of Kupffer cells to produce cytokines/growth factors that suppress apoptosis and promote cell proliferation
(5) ROS results in oxidative DNA damage to hepatocytes and endothelial cells.
(6) ROS modulates hepatocyte and endothelial cell gene expression.

[1]While the reason for the sex difference in liver tumor susceptibility between male and female mice is not clear, it has been shown that estrogens can be protective through their antioxidant capacities and through their modulation of the activities of other antioxidants (Nyska et al., 2004).

[2]NTP has observed liver hemangiosarcomas in 105/4183 (2.51%) male versus just 35/4177 (0.84%) female historical controls (Klaunig and Kamendulis, 2005; NTP, 2000). In addition, other chemicals reported by NTP to cause both early onset hemosiderin buildup and liver tumors have also exhibited this male specificity (U.S. EPA, 2005c).

(7) ROS stimulates hepatocyte and endothelial cell proliferation.
(8) ROS promotes initiation of hepatocyte and endothelial cells.
(9) ROS promotes neoplasm formation.

The first two steps, the metabolism of EGBE to BAA and the association of BAA with hemolytic effects, have been clearly established in many studies, both in vitro and in vivo, with sensitivities ranging from extreme sensitivity as in rats, mice, rabbits and dogs, to moderate to extreme insensitivity seen in monkeys, guinea pigs, and humans (see Section 3). The third step has been seen in both genders of rats and mice exposed to EGBE in multiple studies that observed hemosiderin within Kupffer cells and hepatocytes after RBC breakdown (Siesky et al., 2002; NTP, 2000; Kamendulis et al., 1999; Ghanayem and Sullivan, 1993; Ghanayem et al., 1987a, b; Krasavage, 1986). A number of studies (Siesky et al., 2002; Park et al., 2002; Kamendulis et al., 1999) provide support for Step 4a by showing that in vivo exposure to EGBE increases 8-OHdG levels (an indicator of oxidative damage) in mice, but not rats, and decreases vitamin E levels in rats and mice. Using rat and mouse hepatocytes, Park et al. (2002) showed that $FeSO_4$ produced dose-related changes in these same indicators in mouse hepatocytes, but not in rat hepatocytes, and that treatment with EGBE or BAA did not produce changes in these oxidative stress parameters. Additionally, Nyska et al. (2004) analyzed 130 2-year carcinogenicity studies of B6C3F1 mice from NTP bioassays and concluded that a significantly increased risk of inducing hepatic hemangiosarcomas in male B6C3F1 mice exists in studies with compounds that caused increased tissue burdens of ROS. Klaunig and Kamendulis (2005) and Corthals et al. (2006) provided support for Step 4b, by showing that the activation of Kupffer cells, either through RBC hemolytic components and/or iron accumulation in the Kupffer cells, results in the production of cytokines such as TNFα. The comet assay has been used to assess DNA damage to endothelial cells from ROS (Step 5) (Klaunig and Kamendulis, 2005, 2004; Reed et al., 2003). While Step 6 has not been shown directly for endothelial cells or hepatocytes exposed to EGBE, induction of oxidative damage has been shown to modify gene expression in mammalian cells. In addition, ROS production can stimulate cell proliferation and the inhibition of apoptosis (Klaunig and Kamendulis, 2005; Nyska et al., 2004). Siesky et al. (2002) observed increased DNA synthesis in endothelial cells and hepatocytes in vivo in mice but not in rats at doses that produced hemangiosarcomas in the mouse liver (NTP, 2000) (Step 7). Steps 8 and 9 are consistent with the lack of direct genotoxicity of EGBE (see Section 4.4.4) and the high rate of spontaneous endothelial neoplasms in the male mouse liver relative to the rat (Klaunig and Kamendulis, 2005). The observation of decreased antioxidant capacity and increased 8-OHdG levels in male mice also lends supports to the proposed steps of initiation and promotion of neoplasms by ROS (Klaunig et al., 1998).

4.6.3.1.1. Temporal association and species specificity

Key steps in the proposed MOA (i.e., hemolysis, hepatic hemosiderin buildup, and oxidative damage) have all been observed in subchronic or shorter-duration rat and mouse studies of EGBE (Siesky et al., 2002; NTP, 2000; Kamendulis et al., 1999) well in advance of tumor formation. Differences between rats and mice with respect to these responses may help to explain the relative sensitivity of male mice to the formation of liver tumors following EGBE exposure. In mice, Siesky et al. (2002) observed a dose-dependent increase in levels of liver oxidative stress indicators at exposure days 7 and 90, increased endothelial cell DNA synthesis at exposure days 7 and 14, and increased hepatocyte DNA synthesis at 90 days. No increase in ROS or the DNA synthesis of either cell type was observed in rats at any time point.

4.6.3.1.2. Dose-response relationships

Six chemicals have been determined by the NTP to cause hemosiderin buildup in the livers of mice. As shown in Table 4-8, male mice exposed to chemicals that caused a significant hemosiderin buildup in Kupffer cells within 13 weeks of exposure also showed an increased incidence of hemangiosarcomas and hepatocellular carcinomas following chronic exposure. The dose responses for endpoints describing possible precursor effects, splenic hematopoietic cell proliferation, and liver hemosiderin accumulation appear to be dose-related and coincident to the formation of tumors. Dose-responses for several hemolytic effects were also observed in rats, but liver tumors were not increased in rats at any dose. However, the high dose used in the rat study was only half that of the high dose used in the mouse study, leaving the possibility that similar responses could have been observed in rats if higher doses of EGBE were tolerated by this species.

Of these six chemicals, liver hemangiosarcomas were observed with the only four chemicals that induced hemosiderin buildup by week 13, but not with the other two chemicals for which hemosiderin buildup was not observed until the end of the 2-year study (U.S. EPA, 2005c; Gift, 2005). Two of the four chemicals that induced early liver hemosiderin accumulation and increased hemangiosarcoma incidence, EGBE and p-chloroaniline hydrochloride, also induced an increase in hepatocellular carcinomas. Early buildup of hemosiderin combined with early increases in endothelial cell and hepatocyte DNA synthesis would result in a longer exposure of cells to oxidative damage via iron-generated radicals (Step 4). This would be consistent with a mechanism involving a continuing cycle of damage and repair and accumulation of DNA mutations (Steps 5 and 6). In addition to an earlier onset of hemosiderin buildup, mice also show evidence of a more sustained hemolytic response to EGBE than rats.[3]

[3]Mice experienced an increase in liver and splenic hematopoietic cell proliferation throughout the 2-year NTP (2000) study, while rats tended to compensate for the effects of EGBE after a few months. This increased

Table 4-8. Incidence of liver hemangiosarcomas and hepatocellular carcinomas in studies of NTP chemicals that caused increased hemosiderin in Kupffer cells in male mice.

Chemical (NTP TR #)	Hemosiderin	SC[*]	Hemangiosarcoma	Hepatocarcinoma	Type[***]
2-Butoxyethanol (EGBE) (TR-484)	0/50, 0/50, 8/49[¤], 30/49[¤]	yes	0/50, 1/50, 2/49, 4/49[§]	10/50, 11/50, 16/49, 21/49[¤]	I
p-Chloroaniline hydrochloride (TR-351)	0/50, 0/49, 0/50, 50/50[±]	yes	2/50, 2/49, 1/50, 6/50	3/50, 7/49, 11/50[§], 17/50[¤]	G
p- Nitroaniline (TR-418)	1/50, 1/50, 8/50[§], 50/50[¤]	yes	0/50, 1/50, 2/50, 4/50	10/50, 12/50, 13/50, 6/50	G
Pentachloroanisole (TR-414)	1/50, 50/50[¤], 50/50[**,¤]	yes	2/50, 8/50, 10/50[§]	9/50, 16/50, 12/50	G
C.I. Pigment Red 3 (TR-407)[±]	0/50, 5/50, 30/50, 41/50	no	0/50, 1/50, 1/50, 0/50	5/50, 10/50, 8/50, 4/50	F
o-Nitroanisole (TR-416)	0/50, 0/50, 3/50, 16/50[¤]	no	2/50, 2/50, 1/50, 0/50	7/50, 12/50, 11/50, 7/50	F

[*] Chemicals that caused hemosiderin accumulation in Kupffer cells following subchronic (SC) exposure are identified with a "yes" in this column.
[**] The authors of this study could not identify the source of the Kupffer cell pigmentation. They speculate that it may consist of porphyrins known to be produced from exposure to chlorinated hydrocarbons, but the possibility that it may have consisted of hemosiderin could not be discounted.
[***] I = inhalation, G = gavage, F = feed
[§] $p \leq 0.05$
[¤] $p \leq 0.01$
[±] Statistics not reported

4.6.3.1.3. Biological plausibility and coherence of the database

Oxidative damage plays an important role in the pathogenesis of several diseases, including cancer and cardiovascular disease (Djordjevic, 2004; Lesgards et al., 2002; Klaunig et al., 1998). In support of the proposed hypothesis, increased ROS are known to accompany the release of large amounts of iron from hemolysis (Ziouzenkova et al., 1999). If EGBE causes oxidative stress via hemolysis, then the production of protein and DNA damage would be expected, including the production of 8-OHdG, accompanied by a decrease in antioxidant levels, such as Vitamin E (Houglum et al., 1997; Yamaguchi et al., 1996; Wang et al., 1995). These effects were verified by both Kamendulis et al. (1999) and Siesky et al. (2002), who measured a dose-dependent increase in levels of ROS indicators, including 8-OHdG and malondialdehyde, and a decrease in vitamin E levels in the livers of mice, but not in rats, after acute and subchronic exposure to EGBE. The fact that mice are more susceptible than rats to ROS from EGBE exposure is consistent with the proposed MOA, and is a reasonable explanation for why hemangiosarcomas are not observed in rats following chronic EGBE exposure, despite the fact that rats are at least as sensitive as mice to the hemolytic effects of EGBE.

tolerance in rats is evidenced by a lack of induction of splenic hematopoiesis at the end of the 2-year NTP (2000) study.

Iron, which is known to accumulate in cells of rodent livers following EGBE exposure, can produce hydroxyl radicals in combination with oxidative by-products via the Fenton reaction (Kamendulis et al., 1999). The damaging effects of iron overload to liver sinusoidal cells have been shown in rats following a single i.p. injection of 200 mg iron/kg (Junge et al., 2001). In addition, endothelial cells appear to be relatively sensitive to oxidative stress (Spolarics, 1999; DeLeve, 1998). Liver hemangiosarcomas develop from the endothelial cell component of the vascular sinusoidal cells of the liver (Frith and Ward, 1979).

In vivo studies have indicated that pretreatment of rats with an ALD inhibitor, pyrazole, prior to a single 125 mg EGBE/kg gavage exposure protected against hemolysis (Ghanayem et al., 1987b), presumably by blocking the production of both BAL and BAA. Pretreatment of rats with an ADH inhibitor, cyanamide, prior to a single 125 mg EGBE/kg gavage exposure, reduced hemolytic responses, but increased RBC swelling, increased mortality, decreased BAA formation and excretion in the urine, and increased the urinary excretion of EGBE conjugates with glucuronide and sulfate (Ghanayem et al., 1987b). This hematotoxicity in the presence of cyanamide may be due to BAL, but residual BAA may also be a factor. EGBE + cyanamide decreased BAA concentrations in rats; however, some BAA was formed and the BAA $t_{½}$ was increased Ghanayem et al. (1990), and, when Ghanayem et al. (1987b) administered a gavage dose of 125 mg BAL/kg + cyanamide to rats, the researchers observed almost no hemolytic activity. Furthermore, gavage administration to rats of 125 mg EGBE/kg and the molar equivalent of BAL and BAA resulted in no significant difference between the hemolytic effects of the three chemicals between 2 and 24 hours postexposure (Ghanayem et al., 1987b). These data suggest that EGBE's hemolytic activity (without coexposures) is due to BAA and that the metabolism of EGBE and BAL to BAA takes place rapidly and completely.

4.6.3.1.4. Relevance of the hypothesized MOA to humans

The occurrence of liver tumors in mice exposed to EGBE is hypothesized to occur through an MOA that requires first a dosage of EGBE that is high enough to cause sustained hemolysis of RBCs and, second, leads to sufficient buildup of hemosiderin in the Kupffer cells of the liver to produce ROS and subsequent neoplasm formation.

Several studies have examined the susceptibility of RBCs to BAA-induced hemolysis, and have found a range in sensitivity from the sensitive (rats, mice, rabbits, and dogs) to the less sensitive (monkeys, guinea pigs, and humans). Human volunteers experienced no hemolysis from controlled laboratory acute inhalation exposures (up to 195 ppm), a dosage which caused significant erythrocyte fragility in rats (Carpenter et al., 1956). Only mild hemolytic effects have been observed in humans acutely exposed to oral doses of EGBE (400–1500 mg/kg), doses that have been shown to cause marked hemolytic effects in rats (Ghanayem et al., 1987c; Grant et al., 1985). RBCs from populations that were potentially more sensitive to hemolysis in general—the

elderly, and individuals with sickle cell anemia and hereditary spherocytosis—were tested in vitro and not found to exhibit hemolysis after exposure to concentrations 40-fold higher, the highest tested in the study, as opposed to those shown to induce hemolysis in rat RBCs (Udden, 1994; Udden and Patton, 1994). In an in vitro study of RBCs from hospitalized children and adults, concentrations of up to 150-fold higher than those used in rat studies, the highest tested in the study, did not produce hemolysis (Udden, 2002). The resistance of human RBCs to the initial event of hemolysis makes it unlikely that they would experience the subsequent effects of increased hemosiderin deposition through this pathway, and consequently would not be at increased risk of tumor development through this MOA.

4.6.3.1.5. Other possible MOAs for liver tumor development in male mice

Although certain key events in EGBE's MOA for the development of liver tumors in male mice are fairly well-described and plausible, some alternative considerations (also supported by scientific literature) may be involved. ROS can potentially be derived from two sources: iron overloading in the liver (through Fenton and Haber-Weiss reactions) and Kupffer cell activation. Via either source, oxygen radicals can induce oxidative damage to DNA and lipids as documented in the liver following EGBE treatment (Siesky et al., 2002). The activation of Kupffer cells, through phagocytosis of RBC hemolytic components or iron in the Kupffer cell, results in the production of cytokines, possibly including vascular endothelial growth factor that may elicit a growth response on endothelial cells (Corthals et al., 2006). In addition to the production of oxidative DNA damage, ROS, whether derived from Kupffer cell activation or other biological processes, can alter gene expression (e.g., MAP kinase/AP-1 and NFκB), resulting in stimulation of cell proliferation and/or inhibition of apoptosis (Klaunig and Kamendulis, 2004).

Another recognized mechanism for the development of chemically-induced liver hemangiosarcomas involves direct interaction with DNA. This MOA is recognized for vinyl chloride and thorotrast, two agents that are known to induce hemangiosarcomas in humans. The EGBE metabolite BAL is considered to have the greatest potential to interact with DNA, since it has been shown to cause in vitro SCE at concentrations ranging from 0.2 to 1 mM (Elliot and Ashby, 1997). However, high ADH activity in the liver, as in the forestomach, is expected to result in very short residence time and low C_{max} liver tissue concentrations of BAL. Corley et al. (2005b) extended their 1994 model (Appendix A) to include the metabolism of EGBE to BAL via ALD and the subsequent metabolism of BAL to BAA via ADH in both the liver and forestomach. As shown in Figure 4-2, using rate constants derived from mouse stomach fractions (Green et al., 2002) and making several assumptions about the use of these enzyme activity data, Corley et al. (2005a) estimated that 250 ppm EGBE (the highest concentration used in the NTP

[2000] study) would result in peak C_{max} concentrations of 7 μM EGBE, 0.5 μM BAL, and 3,250 μM BAA in liver tissue of male mice at the end of a 6-hour exposure period.

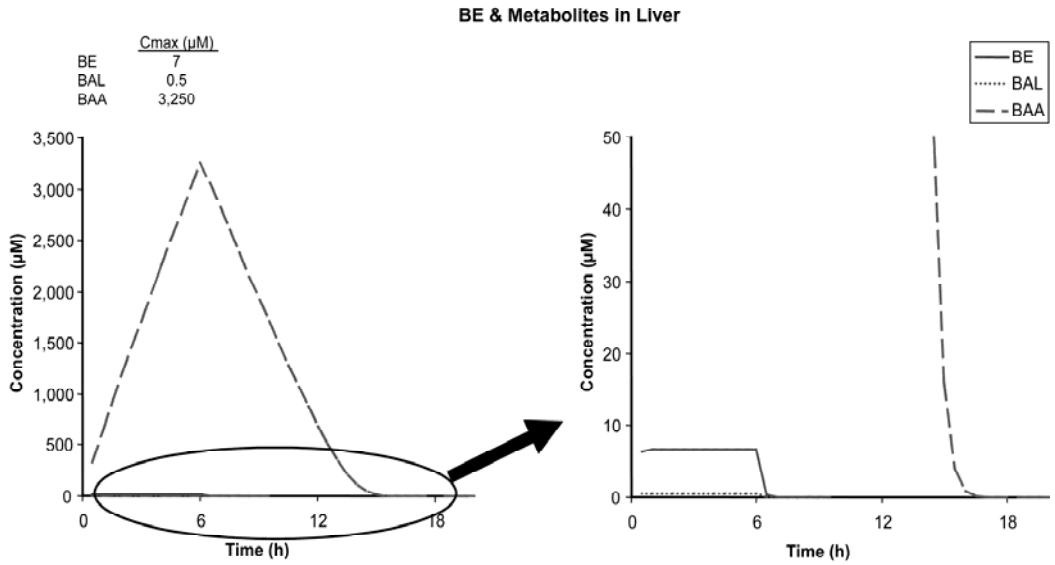

Figure 4-2. Simulated concentrations of EGBE, BAL, and BAA in liver tissues of female mice exposed via inhalation for 6 hours to 250 ppm EGBE.

BE = EGBE.

Source: Adapted from Corley et al. (2005b).

Thus, the Corley et al. (2005b) PBPK model suggests that the high cytotoxic concentrations of BAL that showed some evidence of clastogenicity may not be relevant to the target organ where lower concentrations of BAL would exist in the presence of metabolizing enzymes. A recent gavage study performed by Deisinger and Boatman (2004) provided support for the Corley et al. (2005b) model and the predicted low levels of the BAL metabolite in liver tissue.[4] In addition, as discussed in Section 4.4.4, evidence from in vivo and in vitro genotoxicity assays does not suggest that BAL would have any significant genotoxicity in vivo. [5] Furthermore, the MOA for hemangiosarcoma induction by genotoxins such as vinyl chloride and thorotrast involves the initiation of hepatocellular and sinusoidal cell hyperplasia and sinusoidal compression, leading to the development of fibrous septa, generally in the periportal area, out of which eventually develop multiple areas of angiosarcomas (Foster, 2000). EGBE exposure does not generate this same pattern of effects prior to the development of cancer in mice.

[4]The Corley et al. (2005b) model predicts that the concentrations of BAL in liver tissues of male and female mice would be 17 and 29 μM, respectively, following oral gavage exposure to 600 mg/kg EGBE. The levels of BAL actually observed in the liver tissue of male and female mice, following oral gavage exposure to EGBE at 600 mg/kg, 3 and 4 μM, respectively, were even lower than the predicted values (Deisinger and Boatman, 2004).

[5] The exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC and RfD.

4.6.3.2. Hypothesized Mode of Action for Forestomach Tumor Development in Female Mice

A significant increase over controls (experimental and historical) of papillomas and one carcinoma of the forestomach (6/50; 12%) was reported by NTP (2000) in female mice exposed for 2 years to 250 ppm EGBE by inhalation. An increase was also seen in squamous cell papillomas in male mice, although this did not reach the level of statistical significance. Significant increases in forestomach papillomas and carcinomas were not observed in rats of either gender. The study also showed statistically significant, dose-dependent increases in hyperplasia for both male and female mice, and for ulceration in female mice. Male mice showed significant increases in ulceration at the 125 ppm exposure. The process of irritation, hyperplasia, and ulceration is thought to be a part of the cell injury and regeneration process elicited by irritant chemicals such as EGBE, and the study authors hypothesized that the neoplasia may occur due to an increase in the cell replication associated with regeneration.

A hypothesized series of events that may lead to the formation of forestomach tumors is as follows:

Step Event

(1) Deposition of EGBE/BAA in the stomach and forestomach via consumption or reingestion of EGBE laden mucus, salivary excretions, and fur material
(2) Retention of EGBE/BAA in food particles of the forestomach long after being cleared from other organs
(3) Metabolism of EGBE to BAL, which is rapidly metabolized to BAA systemically and in the forestomach
(4) Irritation of target cells by BAA leading to hyperplasia and ulceration
(5) Continued injury by BAA and degeneration leading to high cell proliferation and turnover
(6) High levels of cell proliferation and turnover leading to clonal growth of spontaneously initiated forestomach cells.

There are a number of studies that have demonstrated Steps 1 and 2 (i.e., the deposition and retention of EGBE and BAA in the forestomach). Studies have shown this occurs following whole-body exposure (Poet et al., 2002; Green et al., 2002), nose-only inhalation (Poet et al., 2002), i.v. exposure (Poet et al., 2002; Green et al., 2002; Bennette, 2001), i.p. exposure (Poet et al., 2002; Corley et al., 1999), s.c. exposure (Corley et al., 1999), and gavage exposures (Poet et al., 2002; Green et al., 2002; Ghanayem et al., 1987a, b). It is of note that following i.v. and inhalation exposures in mice, EGBE metabolites rapidly accumulate in salivary secretions and are swallowed (Green et al., 2002; Bennette, 2001), leading to the collection and retention of the chemical(s) in the forestomach. The same process is likely to occur through other systemic

exposures. The metabolism of EGBE to BAA (Step 3) has been shown in both in vitro and in vivo tests with rats, mice, rabbits, guinea pigs, dogs, monkeys, and humans, (see Section 3) and is further supported by the EGBE PBPK model developed by Corley et al. (2005b). Step 4, the irritation of target cells, has been seen in both genders of B6C3F1 mice, (Poet et al., 2003; Green et al., 2002; NTP, 2000), with irritation and a compensatory proliferative response reported following exposure to EGBE. Female mice were shown to have more extensive and severe forestomach lesions than male mice and were observed in the NTP (2000) study to have statistically significant increases in forestomach tumors. This suggests the importance of the continued damage and high cell proliferation (Step 5) that is associated with tumor formation. Green et al. (2002) found that the number of cells in S-phase (an indication of cell turnover) increased in a dose-dependent fashion after exposure to EGBE and BAA, even though none of the changes were statistically significant due to the high turnover for the control group. Step 6, high levels of cell proliferation and turnover, leads to clonal growth of spontaneously initiated cells and is supported by the continuum of effects observed in the mice (Green et al., 2002; NTP, 2000) and the effects seen with other irritant compounds (Kroes and Wester, 1986).

Green et al. (2002) also provided relevant information regarding Step 3 through examination of the activity and localization of ALD and ADH in the stomach tissues of mice, rats, and a human sample. Whole body autoradiography of mice that had been exposed to radiolabeled EGBE was also performed. Histochemical staining of stomach tissues from the rodent species showed the dehydrogenase enzymes to be heavily concentrated in the stratified squamous epithelium of the forestomach of both rats and mice, whereas their distribution in the glandular stomach was more diffuse. By comparison, histochemical analysis of a human stomach tissue sample showed both enzymes to be present and evenly distributed throughout the epithelial cells of the entire stomach mucosa. A marked species difference in ALD activity in the forestomach was observed between rats (K_m = 0.29 mM; V_{max} = 1.627 nmol/minute per mg protein) and mice (K_m = 46.59 mM; V_{max} = 17.094 nmol/minute per mg protein) with K_m values up to one order of magnitude greater in mice compared to rats. These differences indicate that mice forestomach tissues would have the capacity to metabolize appreciably larger amounts of EGBE to BAL, and subsequently to BAA, than would the rat forestomach. Whole body autoradiography of mice exposed to EGBE clearly demonstrated selective accumulation in the forestomach, which would provide substrate for these enzymes. Collectively, these data demonstrate several points regarding the observed forestomach toxicity in mice following exposure to EGBE, including: (1) the accumulation of EGBE in the target tissue, the forestomach, of mice; (2) a high degree of localization in the forestomach (as compared to the glandular stomach) tissues of both rats and mice of the enzymes that metabolize EGBE to the corresponding carboxylic acid; and (3) kinetic differences in these enzymes consistent with mice being capable of metabolizing more EGBE to BAA than rats. The data also suggest that human

stomach tissues do not have a high localization of the EGBE metabolizing enzymes; the diffuse distribution of these enzymes in the human stomach sample examined is more similar to the distribution seen in the glandular portions of the rodent species examined. These observations suggest that human stomach tissues would be less capable of accumulating and localizing BAA than rat tissues and, thus, would be less likely to be exposed to the irritating effects of BAA.

4.6.3.2.1. Temporal association

All of the steps in the proposed MOA have been observed to occur in female mice prior to tumor formation. NTP (2000) reported that female mice experienced epithelial hyperplasia (1/10, 5/10, 9/10, and 10/10) after just 13 weeks of exposure at the same exposure levels used in the chronic study, 0, 62.5, 125, and 250 ppm. The reported incidence of a forestomach papilloma or carcinoma in female mice was 731, 731, and 582 days in the 62.5, 125, and 250 ppm exposure groups, respectively. This is consistent with the findings of Ghanayem et al. (1994, 1993, 1986), who investigated the temporal relationship between the induction of this type of forestomach lesion by another nonmutagenic irritant, ethyl acrylate (EA), and the development of squamous cell papillomas and carcinomas. These researchers observed cell proliferation/hyperplasia in the forestomach of all rats that received EA by gavage (200 mg/kg, 5 days/week) for 6 or 12 months. All these potentially precancerous forestomach lesions regressed in animals treated with EA for 6 months and allowed 2 or 15 months of recovery, and no forestomach neoplasms were observed. Although EA, an unsaturated aldehyde, is not a metabolite of EGBE, it is an analog of BAL and a much more potent carcinogen than either EGBE or BAL (Gold et al., 1993).

For EGBE, the high incidence of forestomach hyperplasia, the relatively lower incidence of papillomas, and the late occurrence of a single carcinoma in the high, 250-ppm, exposure group, are suggestive of a temporal relationship and tumor progression following EGBE exposure to female mice. Male mice may show the beginnings of tumorigenic effects as the incidence of papillomas increases, but such findings have not been statistically significant compared to concurrent or historical controls. No hyperplasia and no tumors were observed in inhalation studies of rats (NTP, 2000) or in drinking water studies of mice (NTP, 1993), supporting the need for these steps prior to tumor formation.

4.6.3.2.2. Dose-response relationships

The incidence of epithelial hyperplasia (6/50, 27/50, 42/49, 44/50) and ulceration (1/50, 7/50, 13/49, 22/50) in EGBE-exposed female mice were dose-related and significantly increased over both concurrent and historical controls at lower dose levels than the forestomach tumors. The hyperplasia was often associated with ulceration, particularly in the female mice. Forestomach tumors observed by NTP (2000) (incidence 0/50, 1/50, 2/50, 6/50) increased over control animals only at exposure levels above those that caused significant hyperplasia. The

increased incidence of the forestomach neoplasms occurred in groups with ulceration and hyperplasia, suggesting a dose-dependent relationship between the non-neoplastic and the neoplastic lesions.

4.6.3.2.3. Biological plausibility and coherence of the database

Both mutagenic and nonmutagenic chemicals have been shown to induce forestomach tumors in rodents (NTP, 2000; Ghanayem et al., 1994, 1993, 1986; Kroes and Wester, 1986). Some nonmutagenic substances that cause such tumors appear to require long-term contact with the forestomach epithelium, leading to irritation, cell proliferation, and neoplasia. The overstimulation of repair processes and enhancement of growth-promoting factors are believed to be involved (Harrison, 1992). Promotion and other activities associated with the stimulation of cell proliferation have been observed for many of these compounds (Ghanayem et al., 1994; Clayson et al., 1991). High concentrations of EGBE and its BAA metabolite sequestered in the forestomach are assumed to cause chronic irritation and the more serious damage observed in the forestomach lining of female mice. The incidence of ulcers was significantly increased in all exposed groups of females. NTP (2000) suggests that EGBE exposure-induced irritation caused inflammatory and hyperplastic effects in the forestomach and that the neoplasias (papillomas and one carcinoma) were associated with a continuation of the injury/degeneration process.

Other substances that induce forestomach hyperplasia in male and female mice following inhalation exposure include acetonitrile, 1,3-butadiene, and chloroprene (U.S. EPA, 2005c). Both propionic and butyric acid have been shown to induce proliferative responses in forestomach epithelium after only 7 days, and long-term propionic acid exposure has produced papillomas in the rat forestomach (Kroes and Wester, 1986). Since high levels of EGBE and BAA have been observed in the stomachs of mice following i.v., i.p., oral gavage, and inhalation exposures, it is apparent that the chemical partitions to the forestomach via multiple routes, including grooming of fur, systemic blood circulation, ingestion of salivary excretions and respiratory tract mucus, and possibly repartitioning of the stomach contents (Poet et al., 2003; Green et al., 2002). Because the forestomach functions as a storage organ, there is a reduced requirement for vascularization. The planar capillary network within the epithelial layers of the rodent forestomach contrasts strongly with the thick mucosal network of capillaries in the glandular stomach of rodents (Browning et al., 1983). The cells of the forestomach epithelium, especially the more superficial squamous cells, are separated from capillaries by substantial diffusion distances (Bueld and Netter, 1993; Browning et al., 1983). In addition, the glandular stomach contains a complex mucosal protection and buffering system necessary to withstand the high acidity of the digestion process. As a result, irritant substances that concentrate in the forestomach may produce hyperplasia in the forestomach, but not in the glandular stomach or other gastrointestinal tissue (Kroes and Wester, 1986).

4.6.3.2.4. Relevance of the hypothesized MOA to humans

While this proposed MOA is thought to be of qualitative relevance to humans, the EGBE exposure concentrations that would be necessary to cause hyperplastic effects and tumors in humans, if attainable at all, are likely to be much higher than the concentrations necessary to cause forestomach effects in mice for several reasons:

(1) The mouse forestomach serves a food storage function and the slow rate of emptying provides a sink for EGBE where it is metabolized to BAA and remains in contact with squamous epithelium long after EGBE has been cleared from the rest of the body. While the human esophagus is histologically similar to the murine forestomach, the contact time with food and other ingested substances is short, because this organ does not have a storage function. Thus, the risk for esophageal tumors is low (Kroes and Wester, 1986). The human stomach also has a faster rate of emptying than the rodent forestomach, and is further protected from irritant compounds by a mucous layer that is not present in the rodent forestomach.

(2) The localization of the enzymes needed for acid production in the human stomach tissue is not the same as it is in the target, the rodent forestomach (Green et al., 2002).

(3) A margin of exposure analysis (see Appendix C) indicates that the exposure concentrations necessary to cause hyperplastic effects in humans would be much higher than the existing RfD and RfC for EGBE.

4.6.3.2.5. Other possible MOAs for forestomach tumor development in female mice

Though the evidence favors the hypothesis that BAA is the principal toxic metabolite of EGBE, roles for BAL (Ghanayem et al., 1987b) and butyric acid (Harrison et al., 1991) have been suggested. It is not likely that butyric acid plays a significant role in the toxicity of EGBE, particularly at environmentally relevant concentrations. High concentrations of butyric acid have caused ulceration and other preneoplastic lesions in mice (Harrison et al., 1991). However, low concentrations of butyric acid do not appear to be harmful, since it naturally occurs in the diet through the fermentation of fiber and starch and as a significant portion (up to 10 mol%) of total bovine milk fatty acid (Smith and German, 1995).

Another possible alternative MOA could exist if EGBE or one of its metabolites were to have the capability of damaging a cell through direct interaction with its DNA. As has been discussed in Section 4.4.4, there is no clear evidence that EGBE or BAA is genotoxic. BAL, a short-lived metabolite of EGBE, has been found to be clastogenic in in vitro assays without enzyme activation at concentrations ranging from 0.2–1 mM (Elliot and Ashby, 1997). However, as has been discussed, in vivo and in vitro genotoxicity assays do not suggest that BAL would have any significant genotoxicity in vivo. In addition, chemicals for which

mutagenesis/genotoxic effects play a significant role generally induce more tumors at earlier time points than near the end of the conducted bioassays due to their ability to both initiate and promote tumor pathogenesis. The mutagenic compound ethylene dibromide, for instance, was reported to induce forestomach tumors in all dose groups 168–280 days from the start of exposure (U.S. EPA, 2004). EGBE is consistent with other forestomach carcinogens that are not mutagenic, such as EA, in that observed tumors generally did not progress to carcinoma and were not observed until well into the study (i.e., after long periods of forestomach cell damage and repair). The first reported incidence of forestomach papilloma or carcinoma in female mice was 731, 731, and 582 days in the 62.5, 125, and 250 ppm EGBE exposure groups, respectively (NTP, 2000).

It does not appear that EGBE, BAL, or BAA preferentially bind to stomach tissue macromolecules (Poet et al., 2003; Green et al., 2002). Poet et al. (2003) found that high levels of EGBE concentrate in the food content of the forestomach following i.p. exposure (Poet et al., 2003), indicating that the observed sequestering of EGBE in the forestomach is related to its retention in the food that remains there, not to preferential binding to proteins within forestomach tissue.

4.6.3.3. Conclusions About the Hypothesized Modes of Action

Inhalation exposure of B6C3F1 mice to EGBE gave rise to tumors in the liver and forestomach. The liver tumors, hemangiosarcomas and hepatocellular carcinomas occurred in males only and were significantly elevated over controls with a positive trend test for the hemangiosarcomas. The forestomach tumors occurred in females only, had a positive trend test result, and were significantly increased over controls only at the highest dose.

The MOAs that have been developed for these tumors reflect the evidence for the nonmutagenic nature of EGBE and its metabolites. For the liver tumors, the hypothesized key steps of the MOA are metabolism of EGBE to BAA, hemolysis of RBCs with release of Hb and hepatic hemosiderin accumulation, followed by oxidative stress, modulation of gene expression, cell proliferation, promotion and neoplasm, leading to the formation of liver tumors. For the forestomach tumors the hypothesized steps are metabolism to BAA, followed by tissue irritation and subsequent cytotoxicity, compensatory proliferation, and the induction of forestomach tumors. No other viable MOAs have been identified that adequately explain the existing laboratory animal and human observations.

Both of these MOAs have some degree of qualitative significance for humans since the principal biological components supporting them are all present and the processes can occur in humans. Collectively, however, the evidence presented in this assessment for these MOAs suggests that both MOAs have only limited quantitative significance to humans, principally due to kinetic/dynamic differences from the rodents. In the case of the liver tumors, in vitro data suggest there is a 40- to 150-fold difference in the dose that produces hemolytic changes in the

RBCs of humans as compared to rodents. This difference is supported by the Carpenter et al. (1956) study, in which no changes in erythrocyte fragility were measured in humans at the highest tested concentration, 195 ppm, but increased erythrocyte fragility was measured in coexposed rats. Further, PBPK model simulations performed by Corely et al. (2005a) predict that given the vapor pressure of EGBE, the maximum blood level of BAA that can be obtained from inhalation exposure would be lower than the predicted concentrations from bolus exposures that have not resulted in hemolytic effects, and lower than concentrations that have been shown to produce an effect on human RBCs in vitro (Udden, 2002). In the case of the forestomach tumors, the primary difference between mice and humans is in the degree of kinetics in the metabolizing enzymes involved in the production and clearance of BAA. Thus, the hypothesized key events in the MOAs for the animal tumors (liver and forestomach) are not likely to occur in humans, especially at low doses.

Based on the preceding analysis, EGBE is deemed not likely to be carcinogenic to humans at expected environmental concentrations, when examining it on its physical-chemical properties, toxicokinetic and dynamic factors, and MOA information.

4.7. Susceptible Populations

The hemolytic effect of EGBE is presumed to be caused by its primary metabolite, BAA, interacting with the RBC membrane. Potentially susceptible subpopulations would include individuals with enhanced metabolism or decreased excretion of BAA. As discussed in Section 4.7.1, older rats have reduced ability to metabolize the toxic metabolite BAA to CO_2 and a diminished ability to excrete BAA in the urine (Ghanayem et al., 1990, 1987c). However, the relevance of this finding to the possible susceptibility of elderly humans is uncertain; as discussed in Section 3, humans may have conjugation pathways for the excretion of BAA, such as BAA-glutamine and BAA-glycine, that are not present in rats.

It would also be expected that individuals whose RBC membranes are more susceptible to the lysis caused by BAA would be more sensitive to effects from EGBE exposure. However, RBCs from normal, aged, sickle-cell anemia, and hereditary spherocytosis patients were no more sensitive to the hemolytic effects of BAA than RBCs from healthy volunteers when tested in vitro (Udden, 1994). As work in this area continues, further information on the metabolic or structural differences that result in the lower sensitivity of human RBCs compared to rat RBCs may eventually identify characteristics in humans that may indicate increased susceptibility. For instance, it is unknown if a genetic predisposition to hemolytic anemia from other causes, such as G6PD deficiency, would lead to increased susceptibility to EGBE-induced hemolysis. G6PD deficiency appears in approximately 400 variants, thus describing a genetically heterogeneous disorder. The label has been applied to all types of moderate to severe enzyme deficiency with intermittent, induced hemolytic episodes with chronic hemolytic anemia. To date, complete

deficiency of this enzyme has not been identified. The clinical result is the reduced ability to produce nicotinamide adenine dinucleotide phosphate, an enzyme required for reactions of various biosynthetic pathways, as well as for the stability of catalase and the maintenance of GSH levels. Catalase and glutathione peroxidase are the primary enzymes in the detoxification of hydrogen peroxide. Thus, cells are dependent on G6PD for this pathway; without it, they are vulnerable to oxidative damage. RBCs are sensitive to this loss of enzyme: they rely on this system for their antioxidant defenses. Other human risk factors for anemia include ingestion of certain therapeutic drugs, infections, family history, diet, and systemic illnesses (Berliner et al., 1999).

Individuals with hereditary hemochromatosis (HH) represent a population potentially susceptible to increased release of iron from any source. It is reported that 5/1,000 persons of northern European descent are homozygous for the gene or genes that cause hemochromatosis, although it is unknown what proportion of this population will go on to develop the HH phenotype (Pietrangelo, 2004). Individuals with this disorder are not able to reduce their absorption of iron in response to increasing iron levels in the body. Iron stores in the body continue to increase. The iron stores normally start out as ferritin, then eventually as aggregates of a breakdown product of ferritin called hemosiderin. HH is a condition characterized by excessive iron deposition in the form of hemosiderin, found in the liver, heart, skin, joints, pancreas, and other endocrine organs. It is unknown whether individuals with this condition would be susceptible to the effects of EGBE exposure. There is no indication in the literature, however, that RBCs in individuals with HH are more fragile, and is therefore unlikely that HH would increase the risk of hemolysis or additional hemosiderin deposition from EGBE exposure. Studies have shown differences in the localization of iron between HH patients and rodents. In HH patients, iron appears to accumulate preferentially in the parenchymal cells of the liver, early on as ferritin and later as hemosiderin, due to increased iron absorption from the duodenum; late in the disease, iron storage is seen in Kupffer cells and reticular endothelial cells of the bone marrow (Knutson and Wessling-Resnick, 2003; Valberg et al., 1975). In contrast, EGBE-induced toxicity in mice and rats results in initial and preferential accumulation of hemosiderin in Kupffer by phagocytosing senescent RBCs (NTP, 2000). While it is clear that macrophages and other cells can in fact contain hemosiderin, the relative level compared to hepatocytes is much less; staining in these cells is typically seen in late stages of the disease (Kwittken and Tartow, 1966). The human course of developing hepatocellular carcinomas as a consequence of HH is also quite different compared to the development in the mouse model of hemangiosarcoma. Human cases of HH-induced hepatocellular carcinoma are typically observed in the presence of cirrhosis of the liver, another long-term process that reflects the chronic nature of the disease progression in humans (Harrison and Bacon, 2005). Hemangiosarcomas, the tumor type of concern in the male mice, have not been associated with HH in the literature.

4.7.1. Possible Childhood Susceptibility

A number of factors may differentially affect children's responses to toxicants. The only human toxicity information available on the toxicity of EGBE to children is from the case study by Dean and Krenzelok (1991). They observed 24 children, age 7 months to 9 years, after oral ingestion of at least 5 mL of glass window cleaner containing EGBE in the 0.5–9.9% range (potentially 25–1500 mg EGBE exposures). Two children who had taken greater than 15 mL did well after gastric emptying or lavage and observation in the hospital. The remainder were watched at home after receiving diluting oral fluids. No symptoms of EGBE poisoning or hemolysis were observed. While the effects reported in adult poisonings have been more severe than those reported in these children, the adults tended to consume larger volumes and different concentrations of EGBE, making it extremely difficult to assess the correlation of toxic effects with age sensitivity.

As discussed above, there are numerous risk factors for anemia that might predispose an individual to, or compound the adverse effects of, EGBE-induced hemolysis. It is generally recognized, however, that children do not share the same risk factors for anemia as adults for a number of reasons, including: (1) a higher rate of RBC turnover; (2) lower incidence of neoplastic disease in childhood as either a direct or indirect cause of anemia (<7,000 of the 1,000,000 new cases of cancer each year in the U.S. occur in individuals <15 years of age); (3) the fact that iron deficiency is almost always secondary to nutritional factors in children; (4) the relative rarity of alcoholism and its related liver disease; (5) a much lower incidence of anemia associated with thyroid disease; and (6) a rarity of cardiovascular disease other than congenital heart diseases, with the result that valve replacement, malignant hypertension, and the use of certain drugs are not usually a factor (Berliner et al., 1999; Hord and Lukens, 1999).

Anemia in children is usually associated with an abnormality of the hematopoietic system (Berliner et al., 1999; Hord and Lukens, 1999). Studies of the osmotic fragility and deformability of RBCs exposed to BAA, the toxic metabolite of EGBE (Udden, 1994), suggest that certain patients with abnormal hematopoietic systems, such as sickle-cell anemia or hereditary spherocytosis, are not more sensitive to the hemolytic effects of EGBE than normal adults. Other studies suggest that the RBCs of children may be pharmacodynamically less sensitive to hemolysis than those of adults. RBCs of neonates and children up to 6 months of age differ from normal adult RBCs in that they are larger and have higher levels of Hb F versus adult Hb A (Lewis, 1970). Frei et al. (1963) showed that the larger calf erythrocytes containing Hb F were osmotically more resistant than smaller, adult erythrocytes containing Hb A and suggested that, as fetal erythrocytes are replaced by postnatal erythrocytes, the total population of RBCs becomes more susceptible to lysis.

The effect of age on EGBE-induced hematotoxicity was studied in male F344 rats by Ghanayem et al. (1990, 1987c). These studies also demonstrated the time course for the onset

and resolution of the hematological and histopathologic changes accompanying hemolysis. Adult male F344 rats were significantly more sensitive to the hemolytic effects of EGBE than were young (4–5 week) male rats following administration of a single gavage dose of EGBE. Concurrent metabolism studies found increased blood retention of EGBE metabolite BAA (as measured by increased C_{max}, AUC, and $t_{½}$) in young rats and that these rats eliminated a significantly greater proportion of the administered EGBE dose as exhaled CO_2 or as urinary metabolites. The rats also excreted a greater proportion of the EGBE conjugates, glucuronide and sulfate, in the urine. These researchers suggested that a reduced ability by older rats to metabolize the toxic metabolite BAA to CO2 along with a diminished ability to excrete BAA in the urine may explain the age-dependent toxicity of EGBE.

NTP (2000) also found that young mice eliminated BAA 10 times faster than aged mice following a 1-day exposure to EGBE. This difference was not as apparent after 3 weeks of exposure, suggesting that factors other than age may be involved (Dill et al., 1998).

Available in vitro information suggests that children are no more and are possibly less sensitive to the hemolytic effects of BAA than adults. Udden (2002) compared the in vitro responses of erythrocytes (percent hemolysis and MCV alterations) obtained from hospitalized adults (n = 29 to 40) and hospitalized children (n = 25 to 46) to 0 or 10 mM BAA for 4 hours. BAA (10 mM) produced comparable significant increases in MCV in both adults (from 87.1 to 89.2 μm^3; 2%) and children (92.8–95.2 μm^3; 3%). In the case of hemolysis in response to BAA, the response was noted as being significantly increased for hospitalized adults (0.54–0.69%; 28%) but not for hospitalized children (0.68%–0.75%; 10%).

Relatively minor developmental effects due to maternal toxicity related to hematologic effects of EGBE exposure were found in studies using rats, mice, and rabbits dosed orally, by inhalation, or dermally (NTP, 1993; Heindel et al., 1990; Sleet et al., 1989; Wier et al., 1987; Hardin et al., 1984; Tyl et al., 1984). No teratogenicity was noted in any studies. It can be concluded that EGBE is not significantly toxic to developing fetuses of laboratory animals.

4.7.2. Possible Gender Differences

Gender differences have been noted in a number of animal and human studies: females were more susceptible to effects from EGBE exposure. In the NTP (1993) 2-week drinking water studies with EGBE, the absolute and relative thymus weights in female F344 rats at the highest exposure level (265 mg/kg-day) were slightly reduced. In the 13-week studies, male rats in the highest three dose groups and females in all dose groups suffered mild (males) to moderate (females) anemia. In addition, females, but not males, displayed significantly increased urea nitrogen creatine.

Gender differences have also been noted in some studies that observed the hemotoxic effects of dermal administration of EGBE. Repeated application of EGBE either neat or as a dilute aqueous solution (occluded) to male or female NZW rabbits at exposure levels of 18, 90,

180, or 360 mg/kg (6 hours/day, nine applications) produced hemoglobinuria in males at 360 mg/kg and females at 180 or 360 mg/kg (Tyler, 1984). Only female rabbits showed decreased RBC counts, Hb concentrations, and MCHC along with increased MCH at the highest treatment level. Recovery was noted following a 14-day observation period.

A number of secondary effects resulting from the hemolytic toxicity of EGBE, such as effects on the rat liver, kidneys, spleen, bone marrow, and, to a lesser extent, the thymus, were more pronounced in females. In the NTP drinking water studies (1993), liver lesions in females, but not males, included cytoplasmic alterations, hepatocellular degeneration, and pigmentation. These effects were most pronounced in the three highest dose groups.

Carpenter et al. (1956) reported female rats to be more sensitive than males to the hemolytic effects of EGBE. In dogs, slight increases in erythrocyte osmotic fragility in both the male and female basenji hybrids were seen, but RBC counts and Hb concentrations were slightly decreased in the female. Overall, the effects were seen in both genders, but appeared to develop more slowly in the males. In monkeys, occasional rises in erythrocyte osmotic fragility were recorded during the exposure period more frequently in the female than in the male.

In the process of studying and comparing the metabolic and cellular basis of EGBE-induced hemolysis of rat erythrocytes in vitro with human erythrocytes, Ghanayem (1989) observed that the blood from male and female human volunteers was unaffected by 4-hour incubations with BAA at concentrations of up to 4.0 mM. At 8 mM, only slight but significant hemolysis of human blood was observed, with blood from females showing a slightly greater sensitivity.

The NTP 2-year inhalation bioassay (NTP, 2000; Dill et al., 1998) also reported evidence of gender specificity in mice and rats, particularly with respect to the elimination of BAA in rats. Female rats eliminated BAA more slowly from the blood, as indicated by a smaller elimination rate constant, longer elimination $t_{½}$, and larger AUC. In addition, the maximum blood concentrations (C_{max}) of BAA were greater for females at each concentration and time point. It has been suggested because a smaller amount of BAA was excreted in the urine of female rats, higher blood concentrations of BAA accumulated in the females (Dill et al., 1998). Mouse data from the NTP (2000) study also suggest a slightly increased hematologic effect among female mice; however, while female mice tended to have higher blood concentrations of BAA, they excreted more BAA in urine than male mice.

5. Dose-Response Assessments

5.1. Inhalation Reference Concentration (RfC)

In general, the RfC is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population—including susceptible subgroups—that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a lower confidence limit on the benchmark dose (BMDL), a NOAEL, a LOAEL, or another suitable point of departure (POD), with uncertainty/variability factors applied to reflect limitations of the data used. The RfC is expressed in terms of mg/m^3 of exposure to an agent and is derived by a methodology similar to the RfD. Ideally, studies with the greatest duration of exposure and conducted via the inhalation route of exposure give the most confidence for derivation of an RfC.

5.1.1. Choice of Principal Study and Critical Effect, with Rationale and Justification

There are no studies reported in which humans have been exposed subchronically or chronically to EGBE by the inhalation route of exposure. The animal studies considered for selection as principal studies include the 14-week and 2-year inhalation studies by NTP (2000) in rats and mice, the developmental toxicity study by Tyl et al. (1984) in rats and rabbits, the developmental toxicity study by Nelson et al. (1984) in rats, and the subchronic study by Dodd et al. (1983) in rats. The NTP (2000) study was selected as the principal study because it was conducted in two species and provides data for different durations and for more dose groups than the other studies. The developmental toxicity studies identified effects at doses higher than the doses associated with the critical effects identified in the NTP (2000) study and were not used for quantitative purposes. While the subchronic study by Dodd et al. (1983) was a well-conducted one, the NTP (2000) study contained more dose groups, more animals per group, and a longer duration of exposure. Thus, Dodd et al. (1983) was not used for quantitative purposes. Two endpoints from the NTP (2000) study—the hemolytic endpoint from the 14-week inhalation study and the hemosiderin deposition endpoint from the 2-year inhalation study—were used for the critical effect. The hemolytic endpoints in the 1999 EGBE toxicological review were used to derive the reference values (see Section 5.1.5), but were not used to derive the values in this updated assessment. New mode of action information published since the 1999 EGBE toxicological review is included in this document, and this information supports the hemosiderin

deposition endpoint as an important, key event in the proposed MOA. A comparison of the NOAELs and LOAELs for the candidate studies are summarized in Table 5-1.

Table 5-1. Results of candidate studies

Reference	Species (strain)	Gender	No./dose group	Duration/effect	Effect levels (ppm)	
					NOAEL	LOAEL
NTP (2000)	Rat (F344)	M	9–10	14 week, hematologic	31	62.5
			50	2 year, hematologic, hemosiderin	–	31
		F	9–10	14 week, hematologic	–	31
			50	2 year, hematologic, hemosiderin	–	31
NTP (2000)	Mouse (B6C3F1)	M	9–10	2 year, histopathology of the forestomach	–	62.5
			50	2 year, hematologic, hemosiderin	62.5	125
		F	9–10	2 year, histopathology of the forestomach	–	62.5
			50	2 year, hematologic, hemosiderin	–	62.5
Tyl et al. (1984)	Rat (F344)	F	30-31	GD 6-15, hematologic	50 ppm	100 ppm
Nelson et al. (1984)	Rat (Sprague-Dawley)	F	15	GD 7-15, hematologic	150 ppm	200 ppm
Dodd et al. (1983)	Rat (F344)	M, F	10	13 week, hematologic	25 ppm	77 ppm

The primary effects of EGBE exposure, hematological effects, were observed in both species and genders tested. Female rats (NTP, 2000) appeared to be most sensitive among animals studied. A mild-to-moderate regenerative anemia was observed in females exposed to all concentrations, with a LOAEL of 31 ppm identified for hematological effects in male and female rats and no NOAEL. Exposure-related trends were noted for reticulocyte count, RBC count, MCV, Hb concentration, and Hct. The hematological endpoints were considered for the derivation of the RfC; however, they presented a number of difficulties. It was not clear which of the hematological endpoints (changes in RBC count, reticulocyte count, MCV, Hb concentration, and Hct) observed in EGBE-exposed animals should be used to derive an RfC. In the case of benchmark dose (BMD) analysis, the proper benchmark response (BMR) level for the BMD derivation was unclear. In addition, while these hematologic effects were observed in both the

subchronic and chronic studies and persisted with exposure duration, they did not progress in severity in the subchronic-to-chronic study (see Table 4-3, Table 4-6, and Table 5-2). Further, better model fits were obtained from the BMD analysis of the subchronic study, which used two more exposure concentrations than the chronic study. For these reasons, the hematologic responses from the 14-week subchronic study were chosen for use in the BMD analyses of this endpoint (see Section 5.1.2.2.1). Selection of the most appropriate hematologic endpoints for use in the BMD analysis also required consideration of EGBE's MOA for hemolysis.

Table 5-2. Female and male rat and mouse liver hemosiderin staining incidence and RBC from subchronic and chronic EGBE inhalation studies.

Effect/time*	Control	31 ppm	62.5 ppm	125 ppm	250 ppm	500 ppm
Female rat						
Hemosiderin 14 week 2 year	0/10 15/50	0/10 19/50	10/10§ 36/50¤	10/10*§ 47/50¤	9/9§ NT	5/5§ NT
RBC count# 14 week 1 year	8.48±0.05 7.81±0.05	8.08±0.07§ (95) NT	7.70±0.08§ (91) 7.42±0.06§ (95)	6.91±0.05§ (82) 6.75±0.05§ (86)	6.07±0.04§ (72) NT	4.77±0.15§ (56) NT
Male rat						
Hemosiderin 14 week 2 year	0/10 23/50	0/10 30/50	0/10 34/50¤	7/10§ 42/50¤	10/10§ NT	10/10§ NT
RBC count# 14 week 1 year	9.05±0.08 8.88±0.08	8.71±0.14¤ (96) NT	8.91±0.06 (94) 8.39±0.15§ (94)	8.01±0.08§ (89) 7.43±0.20§ (84)	7.10±0.07§ (78) NT	5.97±0.05§ (66) NT
Female mouse						
Hemosiderin 14 week 2 year	0/10 0/50	0/10 NT	0/10 5/50¤	0/10 25/49§	10/10§ 44/50§	6/6§ NT
RBC count# 14 week 1 year	9.72±0.05 9.32±0.09	9.55±0.06¤ (98) NT	9.51±0.06¤ (98) 9.14±0.08 (98)	9.18±0.05§ (94) 8.50±0.12§ (91)	8.57±0.06§ (88) 8.08±0.09§ (87)	7.35±0.07§ (76) NT
Male mouse						
Hemosiderin 14 week 2 year	0/10 0/50	0/10 NT	0/10 0/50	0/10 8/49§	0/10 30/49§	6/6§ NT
RBC count# 14 week 1 year	9.71±0.22 9.58±0.07	10.04±0.08 (103) NT	9.77±0.10 (101) 9.73±0.49 (102)	9.47±0.06¤ (98) 9.36±0.32¤ (98)	8.90±0.07§ (92) 8.33±0.10§ (87)	7.21±0.23§ (74) NT

*Mean ± standard error; percent of control in parentheses.
#RBC count as 106/µL.
¤Statistically significant difference, $p \leq 0.05$.
§Statistically significant difference, $p \leq 0.01$; NT = not tested.

Source**:** NTP (2000).

The suggested MOA of EGBE hemolysis is based on data indicating that BAA, an oxidative metabolite of EGBE, the first hypothesized event in the MOA, is likely to be the causative agent in hemolysis (Ghanayem et al., 1990, 1987b; Carpenter et al., 1956). The second event in the MOA is erythrocyte swelling, and cell lysis, which is believed to be preceded by an increase in the osmotic fragility and a loss of deformability of the erythrocyte (Udden, 1995b, 1994; Udden and Patton, 1994; Ghanayem, 1989). This results in decreased values for RBC count, Hb, and Hct and in response, an increase in the production of immature RBCs (reticulocytes) by the bone marrow.

Although changes in reticulocyte and nucleated erythrocyte counts sometimes represent the largest measurable differences between exposed animals and unexposed control animals, this parameter is highly variable and does not always exhibit a clear dose-dependent trend (NTP, 2000, 1993). While these endpoints can be indirect markers of RBC lysis, they are governed by multiple feedback control processes that can be both very sensitive and variable. Therefore, a change in reticulocyte or nucleated erythrocyte count is not considered a suitable endpoint for deriving the RfC or RfD.

Until more is known about the molecular interaction between BAA and specific cellular molecules, it is reasonable to assume that changes in MCV and RBC count are measurements of precursor events in response to both oral and inhalation EGBE exposure. Therefore, dose-response information on MCV and RBC count are key endpoints used in the BMD analyses, and were considered for derivation of the RfC and RfD for EGBE.

While the toxicokinetic data suggest that MCV should theoretically be the earlier indicator of hemolytic effects from EGBE exposure, recent studies suggest that the relationship between the rate of MCV increase and RBC count decrease may not be consistent across exposure protocols. In the gavage studies of Ghanayem et al. (1987c) and the inhalation studies of NTP (2000), Hct, a measure of RBC volume relative to blood volume, tended to decrease along with RBC count and Hb at all exposure levels for which a hematologic effect was observed. However, Hct did not change as RBC count and Hb decreased, following drinking water exposures (NTP, 1993). Thus, the loss of erythrocytes in the drinking water studies (reduced RBC count) was apparently offset by a concurrent increase in the size of the individual cells (increased MCV). This was not the case in the gavage and inhalation studies. For these reasons, greater weight is given to reduced RBC count, as opposed to increased MCV.

While the hemolytic effects appeared to be among the earliest effects from EGBE exposure, the hemosiderin deposition endpoint was selected as the critical effect. This effect was found to occur in both species and genders of animals tested, with rats being the more sensitive species; the effect also occurred in the 14-week subchronic NTP inhalation study. The suggested MOA of EGBE-induced liver effects is based on the observation that the hemolytic effects led to

compensatory erythropoiesis and significant increases in blood degradation products, including an increased accumulation of hemosiderin in the liver Kupffer cells of EGBE-exposed animals. The hemosiderin accumulation seen in the Kupffer cells was found to increase in severity with increasing dose and exposure duration (Table 5-2), unlike the hemolytic endpoints, such as decreased Hct, which did not progress from 3 to 12 months (Table 4-6). Thus, hemosiderin deposition in Kupffer cells in the rat liver is believed to be a sequela to the hematologic effects. Because of the progression of this effect with chronic exposure, hemosiderin is deemed to be the most sensitive effect. A NOAEL was not identified, while a LOAEL of 31 ppm was identified in both male and female rats.

The 2-year chronic inhalation study by the NTP (2000) observed forestomach ulcers in female mice at all exposure levels, but this effect has not been observed in any other species, including mice exposed orally to EGBE (NTP, 1993). Though the incidence of this lesion increased with exposure, severity of the lesion did not increase with increasing dose. While this effect was not considered a critical effect for the derivation of an RfC, Appendix C contains the BMD analysis for this endpoint for comparison purposes.

5.1.2. Methods of Analysis, Including Models (PBPK, BMD, etc.)

PODs for the RfC derivation in terms of the human equivalent concentrations (HECs) have been calculated via the application of PBPK modeling and the use of internal dose metrics published by Dill et al. (1988) to obtain NOAEL and BMCL estimates.

5.1.2.1. Derivation of the POD Using PBPK Modeling and the NOAEL/LOAEL Method

The lowest NOAEL/LOAEL observed in any subchronic or chronic study of EGBE is the 31 ppm LOAEL for hematologic effects observed in the NTP study (2000) in rats. In order to estimate a corresponding human equivalent exposure, an internal dose metric associated with this exposure level is estimated and a PBPK model is used to estimate the human exposure level that would result in that internal dose.

Initially, it was important to determine what estimate of internal dose (i.e., dose metric) could serve as the most appropriate metric for the effects under consideration. PBPK models of Lee et al. (1998) and Corley et al. (1994, 1997) are capable of calculating several measures of dose for both EGBE and BAA, including:

C_{max}C The peak concentration of EGBE or BAA in the blood during the exposure period;

AUCC Area under the curve (AUC), represents the cumulative product of concentration and time for EGBE and BAA in the blood.

Dill et al. (1998) published measured AUC, but not C_{max}, blood concentrations of EGBE and its principal metabolite BAA at various exposure durations in both genders of B6C3F1 mice

and F344 rats exposed to the same concentrations used in the NTP (2000) chronic studies. C_{max} values would need to be derived from a PBPK model. Two pieces of information were used to select C_{max} for BAA in the blood as the more appropriate dose metric for the main hemolytic endpoint associated with this LOAEL. First, as discussed in Section 4.5, there is convincing evidence to indicate that an oxidative metabolite, BAA, is the causative agent for EGBE-induced hemolysis (Carpenter et al., 1956; Ghanayem et al., 1987b, 1990). With this in mind, dose metrics for BAA in blood appear to be more appropriate than those for EGBE in blood, since they are more closely linked mechanistically to the toxic response. Second, EGBE-induced hemolysis appears to be dependent upon the dose rate. Ghanayem et al. (1987c) found that gavage doses to F344 male rats of 125 mg/kg EGBE resulted in hemolytic effects including reduced RBC count, Hb, and Hct, as well as kidney pathology (Hb casts and intracytoplasmic Hb). However, it should be noted that hemolytic effects were not reported at a similar acute drinking water dose of 140 mg/kg (Medinsky et al., 1990). While a drop in RBC count and Hb (9% and 7%, respectively) was noted in F344 male rats after 1 week of drinking water exposure to 129 mg/kg-day EGBE, dose-related kidney pathology was not observed in these rats, even after 13 weeks of drinking water exposure up to 452 mg/kg-day EGBE (NTP, 1993). Finally, as is discussed in Section 5.1.1, hematological endpoints indicative of hemolysis do not progress with increased inhalation duration. Corley et al. (1994) also suggested that C_{max} is a more appropriate dose metric for the hemolytic effects of EGBE than AUC.

The PBPK models developed for EGBE are briefly summarized in Table 5-3. Johanson (1986) and Shyr et al. (1993) do not address BAA distribution, and are only parameterized for humans and rats, respectively. The model described by Lee et al. (1998) is the most appropriate model for the estimation of rat and mouse internal doses following inhalation exposure. That model is used here to estimate the C_{max} of BAA in blood following inhalation exposure to female rats, the more sensitive gender.[6] The human PBPK model of Corley et al. (1994, 1997) was then used to obtain estimates of human inhalation exposure concentrations associated with the female rat BAA blood concentrations.[7] Established EPA (2006c) methods and procedures were used to review, select and apply these chosen PBPK models. [8]

[6] The Lee et al. (1998) model was chosen in this case because it is an extension of the Corley et al. (1994, 1997) model that includes added parameters for female rats.

[7] The basic components of the Corley model are summarized in Appendix A.

[8] EPA notes that the review of the PBPK models was conducted prior to their use in the 1999 EGBE toxicological review.

Table 5-3. Summary of PBPK models.

Model	Species	Routes of exposure	Comments
Johanson (1986)	Human	Inhalation	BAA not addressed
Shyr et al. (1993)	Rat	Inhalation, oral, dermal	BAA excretion
Corley et al. (1997, 1994)	Rat and human	Inhalation, oral, dermal	BAA distribution and excretion; male rats only
Lee et al. (1998)	Rat and mouse	Inhalation	BAA distribution and excretion; males and females

A five-step procedure was used to calculate the LOAEL HEC:

Step 1: Calculate the internal dose surrogate (C_{max} for BAA in blood) corresponding to female rat LOAEL (Lee et al., 1998) by using the actual experimental exposure regimen (6 hours/day, 5 days/week) in model simulations.

Female rat LOAEL = 31 ppm

C_{max} BAA = 41 μM

Step 2: Verify that steady state was achieved (e.g., no change in BAA C_{max} as a result of prolonging the exposure regimen).

There were no changes in the C_{max} of BAA in blood during any 24-hour simulation period, using a 6 hour/day, 5 days/week exposure regimen at the female rat LOAEL, indicating that steady state was achieved.

Step 3: Simulate the internal dose surrogate by calculating the Cmax for BAA in blood for humans continuously exposed (24 hours/day, 7 days/week) to varying concentrations of EGBE (Corley et al., 1997, 1994).

Concentration of EGBE in air (ppm)	C_{max} BAA in blood (μM)
1	2.6
5	13.0
10	26.1
20	52.9
50	137.1
100	295.0

Step 4: Calculate the $LOAEL_{HEC}$ of EGBE for continuous human exposure in air that resulted in the same internal dose (C_{max} of BAA) in blood calculated for the animal study in step 1.

Female rat C_{max} BAA = 41 μM

HEC continuous exposure = 18 ppm (calculated by regression of internal dose versus the concentration of EGBE in air from step 3).

Step 5: Convert the EGBE exposure units from ppm to mg/m3

$$LOAEL_{HEC}\ (mg/m^3) = \text{conversion factor} \times LOAEL_{HEC}\ (ppm)$$
$$= 4.84\ (mg/m^3) \div (ppm) \times 18\ ppm$$
$$= 88\ mg/m^3$$

5.1.2.2. Derivation of the POD Using PBPK Modeling and BMD Modeling Methods

It is recognized that the NOAEL/LOAEL designations listed in Table 5-1 do not necessarily indicate the slope of the concentration-response curve, an important factor in benchmark concentration (BMC) analysis, used to assess inhalation studies in the same manner as BMDs are used to assess oral studies (U.S. EPA, 2000, 1995b). For this reason, BMC analyses were performed on selected hemolytic endpoints from the subchronic study and on the hemosiderin staining endpoint from the chronic study in male and female rats (NTP, 2000).

5.1.2.2.1. BMD approach applied to hematological data.

For the purposes of deriving an RfC for EGBE from hemolytic endpoints, both MCV and RBC count response data were evaluated in female rats from the 14-week subchronic NTP (2000) study (see Section 5.1.1). The current BMD technical guidelines (U.S. EPA, 2000) suggest the use of 1 SD from the control mean for the BMR level for continuous data in the absence of additional information, such as a minimal level of change in the endpoint that is generally considered to be biologically significant. Because the hemolytic endpoints are continuous measurements that have a relatively small historical variance in rats, and because low-dose responses for these endpoints were generally near or within 5% of the control mean, the $BMCL_{05}$ was considered to be an appropriate POD for derivation of the RfC (U.S. EPA, 2000, 1995b). The steepest concentration-response curves (and the lowest $BMCL_{05}$ estimate) were obtained for decreased RBC count in female rats, and a 5% change was found to be statistically significant. Higher levels of response (e.g., ≥ 10% reductions) are in the exposure range where other more severe responses related to anemia occur, such as MCV increases and increased reticulocyte counts. Lower levels of response, for instance, 1 SD from the control mean, an approximate 2% reduction for these data, are relatively distant from the observable data and other responses related to anemia (see Appendix B for the 1 SD data). C_{max} for BAA in arterial blood of rats was determined by using the PBPK model of Lee et al. (1998). Dermal exposure to EGBE vapor was not considered in the predicted blood levels, because the estimated relative contribution of the skin to the total uptake of unclothed humans exposed to 25 ppm

EGBE for 8 hours ranged from only 4.6 to 27.5%, depending on temperature, humidity, and exercise level (Corley et al., 1997). Thus, dermal uptake is predicted to contribute less than 10%, even if 50% of an individual's skin is exposed. The results of this modeling effort are summarized in Table 5-4.

Table 5-4. Model estimates of BAA blood levels in female rats following inhalation exposures.

Exposure concentration (ppm)	Female rat body weight (g)	BAA in arterial blood C_{max} in female rats (µM)
31	216	40.4
61.5	211	85.9
125	214	189.5
250	210	451.3
500	201	1143.3

Source: Lee et al. (1998).

All BMD analyses were performed using models in EPA BMD software (BMDS), version 1.4.1 (U.S. EPA, 2000). Graphical figures and text output files for selected BMC analyses are provided in Appendix B. The fit statistics and BMC information derived from the continuous models available in the BMD software as applied to the female rat RBC count data versus C_{max} BAA are shown in Table 5-5. All models were fit using restrictions and option settings suggested in the EPA BMD technical guidance document (U.S. EPA, 2000). The best model fit to these data, from visual inspection and comparison of Akaike Information Criterion (AIC) values, was obtained using the Hill model. The $BMCL_{05}$ was determined to be 37.2 µM, using the 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} for BAA in blood. The Corley et al. (1997) PBPK model was used to back-calculate an HEC of 17 ppm (81.4 mg/m^3), assuming continuous exposure (24 hours/day).

Table 5-5. Comparison of BMC/BMCL values for female rat RBC count data from a 14-week subchronic inhalation study, using modeled blood Cmax (3 months) of the EGBE metabolite BAA as a common dose metric.

Model	BMC_{05} (µM)	$BMCL_{05}$ (µM)	*p* Value	AIC*	Scaled residual**
2nd degree polynomial	60.8414	54.5902	<0.0001	–90.947514	0.15
Power	74.5253	68.2041	<0.0001	–52.341720	0.218
Hill***	42.2297	37.1792	0.1669	–109.272293	0.168

*AIC = −2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters and P is the number of model degrees of freedom (usually the number of parameters estimated).
**Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMC scaled by an estimate of its SD. Provides a comparative measure of model fit near the BMC. Residuals that exceed 2.0 in absolute value should cause questioning model fit in this region.
***Model choice based on adequate p value (>0.1), visual inspection, low AIC and low (absolute) scaled residual. .

5.1.2.2.2. BMD approach applied to hemosiderin staining data.

For the purposes of deriving an RfC for EGBE, hemosiderin staining data were evaluated in male and female rats from the 2-year chronic study by NTP (2000). The current BMD technical guidelines (U.S. EPA, 2000) suggest the use of 10% extra risk as a BMR level for quantal data as this is at or near the limit of sensitivity in most cancer bioassays and in some noncancer bioassays as well. Because the hemosiderin staining endpoint was observed in control animals and a 10% increase in incidence was within the observable range of the data, 10% extra risk was considered an appropriate BMR and a $BMCL_{10}$ an appropriate POD for derivation of the RfC (U.S. EPA, 2000, 1995b). All BMD assessments in this review were performed using EPA BMDS version 1.4.1. Graphical figures and text output files for selected BMC analyses are provided in Appendix B.

The AUC was selected as the appropriate dose metric due to the nature of the endpoint, hemosiderin deposition. This endpoint increased in severity with increased duration (subchronic to chronic) and is believed to be the result of the cumulative exposure to EGBE as opposed to a peak event. Table 5-6 reports AUC BAA blood concentrations measured at 12 months[9] published by Dill et al. (1998) in both genders of B6C3F1 mice and F344 rats exposed to the same concentrations used in the NTP (2000) chronic studies of these test animals.

Table 5-6. AUC BAA blood concentrations measured at 12 months in both sexesgenders of B6C3F1 mice and F344 rats.

Exposure Concentration (ppm)	Gender		AUC_{BAA} (μmol-h/L)*	
		n	mean	SE
Rats				
31.2	Male	7	358.3	16.6
	Female	5	638.8	18.7
62.5	Male	6	973.0	86.2
	Female	9	1128.9	50.9
125	Male	9	2225.6	71.1
	Female	12	3461.8	154.8
Mice				
62.5	Male	10	1206.6	205.6
	Female	12	1863.6	112.4
125	Male	9	2819.8	685.1
	Female	6	5451.6	508.9
250	Male	10	17951.5	1770.4
	Female	11	18297.1	609.7

*Authors reported AUC values in terms of μg-min/g, which were converted to units consistent with the PBPK model of μmol-h/L by dividing by 60 min/h and 132.16 g/mol and multiplying by 1060 g/L.

Source: Dill et al. (1998).

[9] Dill et al. (1998) also reported 18 month data, but due to the smaller number of animals and higher variability in this data the 12 month data were used for the purposes of this analysis.

The fit statistics and BMC information derived from the dichotomous models available in the BMD software as applied to the male and female rat hemosiderin staining data versus AUC BAA are shown in Table 5-7. All models were fit using restrictions and option settings suggested in the EPA BMD technical guidance document (U.S. EPA, 2000). The best model fit to these data, as determined by visual inspection, examination of low dose model fit (i.e., scaled residual for the dose group closest to the BMD) and comparison of overall fit (i.e., AIC values), was obtained using a multistage model (1st degree) for the male response data and a Log-Logistic model for the female response data. The male rat BMC10 was 196 μmol-hour/L and the BMCL10 was determined to be 133 μmol-hour/L, using the 95% lower confidence limit of the dose-response curve expressed in terms of the AUC for BAA in blood. The BMC_{10} and $BMCL_{10}$ values for the female rat were determined to be 425 and 244 μmol-h/L, respectively. Assuming continuous exposure (24 h/day), the Corley et al. (1997) PBPK model was used to back-calculate human equivalent concentrations of 3.4 ppm (16 mg/m^3) from the male rat data and 4.9 ppm (24 mg/m^3) from the female rat data,

Likewise, the fit statistics and BMC information for male and female mouse hemosiderin staining data versus AUC BAA are shown in Table 5-8. All models were fit using restrictions and option settings suggested in the EPA BMD technical guidance document (EPA, 2000). The best model fit to these data, as determined by visual inspection, examination of low dose model fit (i.e., scaled residual for the dose group closest to the BMD) and comparison of overall fit (i.e., AIC values), was obtained using a log-probit model for both the male and female response data. The male mouse BMC_{10} was 3077 μmol-h/L and the $BMCL_{10}$ was determined to be 2448 μmol-h/L using the 95% lower confidence limit of the dose-response curve expressed in terms of the AUC for BAA in blood. The BMC_{10} and $BMCL_{10}$ values for the female mouse were determined to be 1735 and 1322 μmol-h/L, respectively. Assuming continuous exposure (24 h/day), the Corley et al. (1997) PBPK model was used to back-calculate human equivalent concentrations of 36 ppm (174 mg/m^3) from the male mouse data and 20 ppm (97mg/m^3) from the female mouse data.

Table 5-7. Comparison of BMC/BMCL values for male and female rat liver hemosiderin staining data from inhalation chronic study using measured blood AUC (12 months) of the EGBE metabolite BAA as a common dose metric.

Model	BMC_{10} (µmol-h/L)	$BMCL_{10}$ (µmol-h/L)	p value	AIC*	Scaled Residual**
Male Rats					
Multistage-1st degree***	196.252	133.141	0.8680	247.234	0.441
Gamma***	196.253	133.141	0.8680	247.234	0.441
Logistic	259.296	192.773	0.7692	247.476	0.526
Log-Logistic	166.376	69.3279	0.5623	249.283	0.313
Probit	271.525	205.882	0.7450	247.54	0.517
Log-Probit	368.336	241.992	0.6309	247.876	0.765
Weibull***	196.253	133.141	0.8680	247.234	0.441
Female Rats					
Multistage-1st degree	122.166	214.555	0.0698	218.868	-1.945
Gamma	316.635	134.02	0.0554	219.229	-1.238
Logistic	273.693	221.689	0.0993	218.188	-1.294
Log-Logistic* **	**424.527**	**243.69**	**0.1533**	**217.526**	**-0.896**
Probit	291.017	241.206	0.0683	218.985	-1.260
Log-Probit	427.728	248.683	0.1238	217.884	-0.965
Weibull	266.515	130.801	0.0454	219.58	-1.377

* AIC = Akaike Information Criteria = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and p is the number of model degrees of freedom (usually the number of parameters estimated)
** Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMC scaled by an estimate of its standard deviation. Provides a comparative measure of model fit near the BMC. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.
*** Model choice based on adequate p value (> 0.1), visual inspection, low AIC and low (absolute) scaled residual. The Multistage (1st degree) is referred to as the chosen model for male rats, though equivalent fit was obtained by the restricted Gamma and Weibull models.

Table 5-8. Comparison of BMC/BMCL values for male and female mouse liver hemosiderin staining data from inhalation chronic study using measured blood AUC (12 months) of the EGBE metabolite BAA as a common dose metric

Model	BMC_{10} (µmol-h/L)	$BMCL_{10}$ (µmol-h/L)	p value	AIC*	Scaled Residual**
Male Mice					
Multistage-1st degree	2100.07	1613.9	0.3067	117.571	-1.766
Gamma	2725.35	1702.27	0.1452	118.559	1.358
Logistic	6605.45	5333.72	0.0022	127.326	2.789
Log-Logistic	2616.51	1628.48	0.1882	118.02	1.193
Probit	5917.06	4825.09	0.0031	126.405	2.734
Log-Probit* **	**3076.8**	**2448.3**	**0.1290**	**116.614**	**1.946**
Weibull	2689.76	1687.09	0.1445	118.712	-1.448
Female Mice					
Multistage-1st degree	946.491	769.879	0.3680	142.669	-1.583
Gamma	1402.92	818.367	0.3420	143.288	-0.817
Logistic	2897.15	2341.03	0.0002	162.338	-0.942
Log-Logistic	1705.75	1121.43	0.8223	141.501	-0.343
Probit	2860.03	2364.52	0.0002	161.681	-0.829
Log-Probit* **	**1734.53**	**1322.06**	**0.8237**	**141.498**	**-0.315**
Weibull	1282.82	804.234	0.2958	143.631	-0.988

* AIC = Akaike Information Criteria = -2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters, and p is the number of model degrees of freedom (usually the number of parameters estimated)
** Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMC scaled by an estimate of its standard deviation. Provides a comparative measure of model fit near the BMC. Residuals that exceed 2.0 in absolute value should cause one to question model fit in this region.
*** Model choice based on adequate p value (> 0.1), visual inspection, low AIC and low (absolute) scaled residual. The Log-Probit model provides a slightly better fitthan other models for both genders.

5.1.2.3. Selection of the POD

Consideration of the available data has led to the selection of the two-year inhalation study (NTP, 2000) and increased hemosiderin staining in the liver of male F344 rats as the principal study and critical effect for deriving the chronic RfC for EGBE. This is a high-quality study and when coupled with information on the mode of action, EPA concluded this is a precursor to an adverse effect and is appropriate for use in deriving the RfC. A BMCL10 of 133 µmol-h/L for hemosiderin staining in liver of male rats chronically exposed to EGBE (NTP, 2000) was used as the point of departure to calculate the RfC. A human PBPK model (Corley et al., 1997) was used to back-calculate to a human equivalent concentration of 16 mg/m3 (3.4 ppm) for the BMCLHEC. A total uncertainty factor (UF) of 10 was applied to the BMCLHEC:10 for consideration of intraspecies variation (UF_H: human variability) to obtain an RfC of 1.6 mg/m^3. The rationale for the application of these UFs is provided in Section 5.1.3. The final

calculation of an RfC derived from the NOAEL/LOAEL and BMC modeling approaches are presented in Table 5-9.

Table 5-9. Summary of the application of UFs for RfC derivations using NOAEL/LOAEL and BMC modeling approaches for male rat liver hemosiderin staining.

Factor**	Approach*	
	LOAEL	$BMCL_{10}$
UF_H	10	10
UF_A	1	1
UF_S	1	1
UF_L	3	1
UF_D	1	1
UF (total)	30	10
POD/UFs = RfC (mg/m^3)	88/30 = 2.9	16/10 = 1.6

*PODs—$LOAEL_{HEC}$ = 88 mg/m^3 based on hematological effects in male and female rats; $BMCL_{10HEC}$ = 16 mg/m^3 based on hemosiderin staining in male rats.
**The rationale for the selection of these UFs is discussed in Section 5.1.3. UF_H—intrahuman variability, UF_A—interspecies variation, UF_S—subchronic to chronic extrapolation, UF_L—LOAEL to NOAEL extrapolation, UF_D—database insufficiencies.

Source: NTP (2000).

5.1.3. RfC Derivation, Including Application of Uncertainty Factors (UFs)

UFs are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (e.g., chronic exposure over a lifetime when subchronic studies are used for deriving a toxicity value). The application of UFs may include the use of a partial UF of $10^{1/2}$ (3.2) based on the assumption that the actual values for the UFs are log-normally distributed. Application of these factors in the assessments is such that, when a single partial UF is applied, the factor is rounded to 3—for instance, the total factor for individual UFs of 3 and 10 would be 30 (3 × 10). When two partial UFs are evoked, however, they are not rounded, such that a UF of 3, 3, and 10 would result in a total uncertainty of 100 (actually $10^{1/2} \times 10^{1/2} \times 10^{1}$) (U.S. EPA, 2002). UFs applied for this RfC assessment and the justification for their use are as follows.

A value of 10 was selected to account for variation in sensitivity within the human population (UF_H). Potentially susceptible subpopulations include individuals with enhanced metabolism or decreased excretion of BAA and individuals whose RBC membranes are more susceptible to the lysis caused by BAA, the precursor step to developing hemosiderin staining in the liver. A UF of 10 was used to account for the uncertainty associated with the variability of the human response to the effects of EGBE. Human in vitro studies suggest that the elderly and patients with fragile RBCs would not be more sensitive to the hemolytic effects of EGBE than

normal adults. Laboratory animal studies suggest that older animals are more sensitive than neonates, and that females are more sensitive than males (see Section 4.7). However, actual human responses to EGBE have not been observed under a broad enough range of exposure conditions, such as repeat/long-term exposures, and potentially sensitive subjects, such as individuals predisposed to hemolytic anemia or infants, to warrant the reduction of the UF_H below the default value of 10. While developmental studies do not reveal increased susceptibility in infants, none of the developmental studies examined fetal or infant blood for signs of effects from prenatal exposure to EGBE.

A UF of 1 was used to account for interspecies variation (UF_A) for toxicodynamic and toxicokinetic differences between animals and humans. Traditionally, these components are individually represented by partial UFs of 3 for a total UF of 10 in the absence of chemical-specific information; thus, application of a full UF of 10 would depend on two areas of uncertainty (i.e., toxicokinetic and toxicodynamic uncertainties). In this assessment, the toxicokinetic uncertainty is addressed by the determination of an HEC, using a combination of measured internal blood levels in the test animals and PBPK modeling. In vivo (Carpenter et al., 1956) and in vitro (Udden, 2002; Udden and Patton, 1994; Ghanayem and Sullivan, 1993) results indicate that, toxicodynamically, humans may be less sensitive than rats to the hematological effects of EGBE.

A UF to account for extrapolation from subchronic to chronic exposure (UF_S) was not needed because the RfC was derived from a chronic study.

A UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in hemosiderin staining was selected under an assumption that it represents a biologically significant change.

A value of 1 was used for the database UF (UF_D). Chronic and subchronic studies are available for two species (rats and mice), and several reproductive and developmental studies, including a two-generation reproductive toxicity study. There are limited studies available looking at humans following short-term inhalation exposure

The combined PBPK and BMC modeling method using hemosiderin as an endpoint was used to derive the RfC. In addition, MOA information was used to inform the choice of the critical effect. The total UF is 10. Thus the RfC, is 16 $mg/m^3 \div 10 = 1.6$ mg/m^3.

5.1.4. RfC Comparison Information

For comparison purposes, Figure 5-3 presents the POD, applied UFs, and derived reference values, including the RfC, for the effect endpoints discussed. BMC modeling was done using EPA BMDS version 1.4.1 (U.S. EPA, 2000), and results are provided in Appendices B and

C. This comparison is intended to provide information on alternative endpoints associated with EGBE exposure. The selected RfC value is circled; BMC analyses are provided in Appendices C and D.

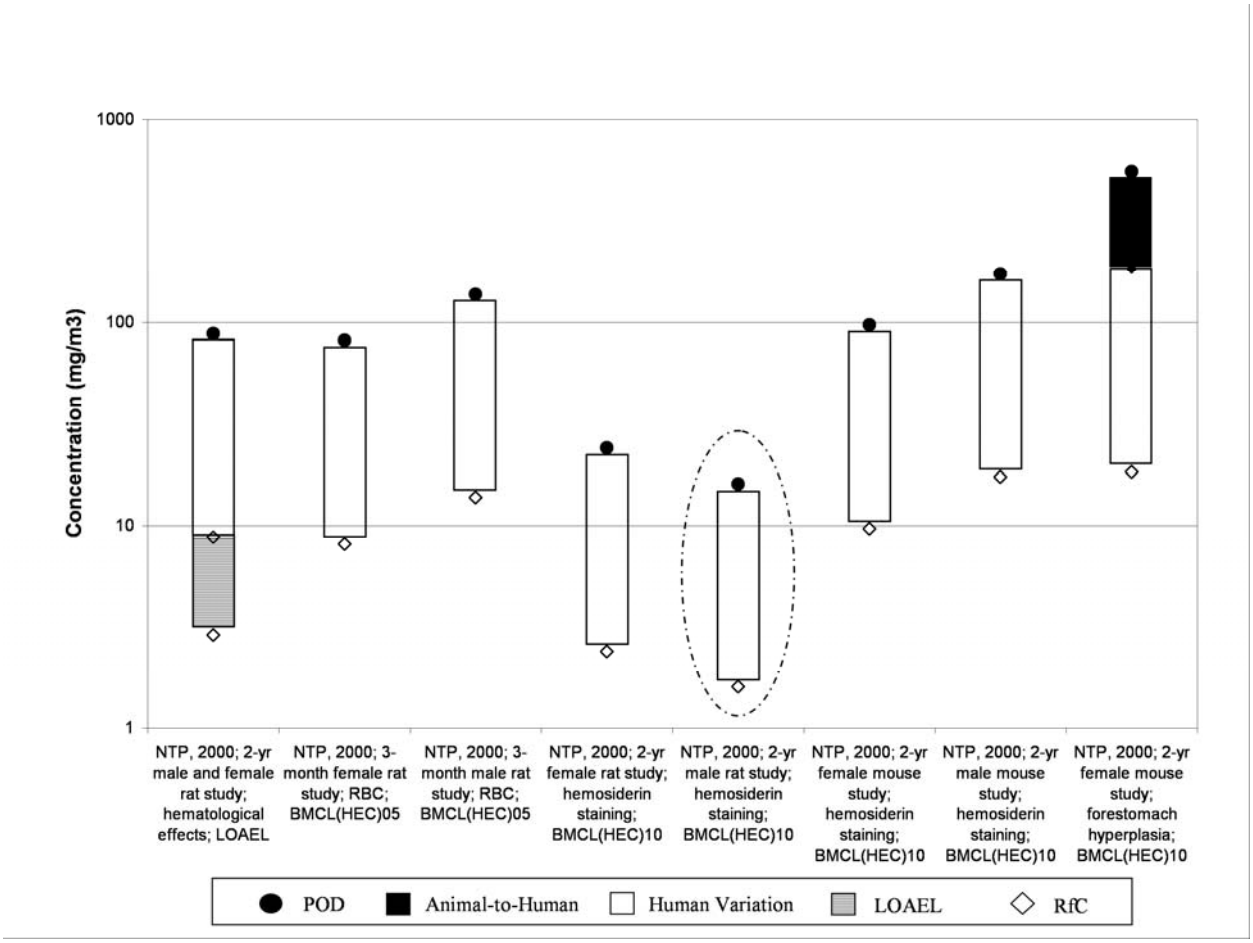

Figure 5-3. Points of departure for selected endpoints with corresponding applied UFs and derived RfC.

Figure 5-3 shows PODs and comparison reference values (including the RfC) that could be derived from the various endpoints, to allow a comparison with the chosen critical effect and the resultant RfC for the critical effect. Hemolytic effects and effects related to hemolysis (i.e., hemosiderin deposition) are the most sensitive endpoints for identification of a NOAEL or a BMCL in the subchronic and chronic studies available; these endpoints have been considered as the critical endpoint for derivation of the RfC. The BMCL05 for RBC count changes in female rats was 37.2 µM, using Cmax at 3 months, and was converted to an inhalation HEC (BMCLHEC) of 81.4 mg/m3 using the EPA model. Though adequate model fit per EPA BMD technical guidance (U.S. EPA, 2000) could not be obtained for the NTP (2000) 3-month male rat RBC response (see Appendix B), this BMCL result ($BMCL_{05}$ of 37.2 µM converted to a

$BMCL_{HEC}$ of 81.4 mg/m^3) is provided for comparison purposes. For the hemosiderin endpoint, both male and female data from the chronic study were considered. The $BMCL_{10}$ for hemosiderin staining in male rats was 133 μM-h/L using the AUC for BAA in arterial blood at 12 months and was converted to a $BMCL_{HEC}$ of 16 mg/m^3 using the Corley et al (1994; 1997) human PBPK model. The $BMCL_{10}$ for hemosiderin staining in female rats was 244 μM-h/L using the AUC for BAA in arterial blood at 12 months and was converted to a $BMCL_{HEC}$ of 24 mg/m^3 using the Corley et al (1994; 1997) human PBPK model.

5.1.5. Previous Inhalation Assessment

The previous IRIS assessment for EGBE was entered into the database on December 31, 1999; it contains an inhalation RfC of 13 mg/m^3. The RfC was based on the BMC_{05} HEC of 380 mg/m^3 for changes in RBC count in female F344 rats following a 14-week inhalation exposure (NTP, 1998). A total UF of 30 was used to account for human variability and extrapolation from an adverse effect level.

5.2. Oral Reference Dose (RfD)

In general, the RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population—including susceptible subgroups—that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a statistical BMDL, a NOAEL, a LOAEL, or another suitable POD, with uncertainty/variability factors applied to reflect limitations of the data used. The RfD is expressed in terms of mg/kg-day of exposure to an agent and is derived by a similar methodology to the RfC. Ideally, studies with the greatest duration of exposure and conducted via the oral route of exposure give the most confidence for derivation of an RfD. The database of oral studies for EGBE is more limited than the database of inhalation studies. For this reason, a PBPK model for EGBE has been applied to the inhalation data for derivation of an RfD.

5.2.1. Choice of Principal Study and Critical Effect, with Rationale and Justification

No studies have been reported in which humans have been exposed subchronically or chronically to EGBE by the oral route of exposure, and thus would be suitable for derivation of an oral RfD. No chronic oral laboratory animal studies are currently available for EGBE. The results of the only two available subchronic 91-day drinking water studies in rats and mice (NTP, 1993) are summarized in Table 5-10.

Table 5-10. Subchronic 91-day drinking water studies in rats and mice

Reference	Species (strain)	Gender	Animals/dose	Effect	Effect levels (mg/kg-day)	
					NOAEL	LOAEL
NTP (1993)	Rat (F344)	M	10	Hepatocellular changes	–	54.9*
		F	10	Hematological	–	58.6*
NTP (1993)	Mouse (B6C3F1)	M	10	Body weight	223	553**
		F	10	Body weight	370	676**

*Doses were calculated using water consumption rates and body weights measured during the last week of exposure and, therefore, differ slightly from those reported by the authors and presented in Section 4.2.
**The LOAEL in mice was based on reduced body weight and body weight gain.

Based on a comparison of NOAELs and LOAELs for hematological and liver effects, rats are clearly more sensitive to the noncancer effects of EGBE than mice. As discussed in Section 4.2, hematologic and hepatocellular changes were noted in both rat genders. In females, both hematologic and hepatocellular changes were noted at the low-dose level (58.6 mg/kg-day, using water consumption rates and body weights measured during the last week of exposure). Only hepatocellular cytoplasmic changes were observed in low-dose male rats (54.9 mg/kg-day, using water consumption rates and body weights measured during the last week of exposure).

In the female rat study (NTP, 1993), groups of 10 F344 rats were exposed to 0, 82, 151, 304, 363, and 470 mg/kg-day EGBE via drinking water for 13 weeks. Body and organ weights were measured, and clinical, hematological, gross, and histopathologic examinations were conducted. Decreases in body weight were observed in female rats exposed to the two highest dose levels. Hematologic changes were observed at all dose levels after 13 weeks and were indicative of mild-to-moderate anemia. These changes included reduced RBC count, Hb, and Hct, as well as increased reticulocyte count and MCV. Liver hemosiderin pigmentation was noted in the cytoplasm of Kupffer cells in both genders. In females it was noted in 0/10 controls in 82 mg/kg-day treated animals, 2/10 with a severity grade of 1 (minimal) at 151 mg/kg-day, and 10/10 in the three highest dose levels, with the severities noted as increasing from a numerical grade of 1.2 in the 304 mg/kg-day group to 1.9 in both of the upper two dose groups. In males the pigmentation was noted in animals exposed to the highest dose only (452 mg/kg-day) at an incidence of 7/10 and a severity rating of 1 (minimal). No hepatic pigmentation was reported in the mice exposed for 13 weeks.

Hematological effects leading to RBC lysis—organ accumulation of iron in the form of hemosiderin accumulation—appear to be the most sensitive of the effects caused by EGBE in laboratory animals. Less clear, however, is whether one of the hematological endpoints (changes in RBC count, reticulocyte count, MCV, HCT, and Hb) or incidence of hemosiderin pigmentation

observed in EGBE-exposed animals is the most appropriate basis for an RfC/RfD (see Section 5.1.1).

A BMD analysis has also been performed on the hemosiderin pigmentation endpoint observed in the NTP (2000) chronic EGBE inhalation study (Section 5.1.2.2.2), and PBPK models have been applied to extrapolate this BMD to a human equivalent oral exposure (Section 5.2.2.2.2). As discussed, hematological endpoints indicative of hemolysis do not progress with increasing duration of inhalation exposure, whereas the incidence of hemosiderin pigmentation did progress considerably with chronic exposure (Table 5-2). Furthermore, not enough is known about the mechanism of action of EGBE to make a biologically-informed determination regarding which hematological endpoints—changes in RBC count, reticulocyte count, MCV, Hct, and Hb—observed in EGBE-exposed animals should be used to derive an RfC and, in the case of BMD analysis, what a proper BMR level should be for the BMD derivation. Finally, hematologic effects signified by changes in RBC count, reticulocyte count, MCV, HCT, and Hb are considered precursor effects to the pathological finding of hemosiderin deposition (Section 4.5). For these reasons, and because the hemolytic effects of EGBE appear to be consistent between oral and inhalation routes of exposure, an RfD has been derived via the application of a PBPK model to perform a route-to-route extrapolation from the incidence of hemosiderin pigmentation observed in the NTP (2000) chronic inhalation study of EGBE. BMD/NOAEL analyses of hematologic endpoints and hemosiderin pigmentation observed in the oral NTP (1993) subchronic study are provided below for comparison purposes.

Another issue that needs to be addressed with respect to the NTP (1993) study is the lack of reported forestomach lesions at even the highest drinking water doses administered in this study relative to the considerable incidence of forestomach hyperplasia and ulceration observed at all exposure levels in the NTP (2000) subchronic and chronic inhalation studies of mice. This is difficult to explain, considering the lowest dose in the subchronic drinking water study is predicted to result in similar, if not higher, C_{max} blood levels of the EGBE metabolite BAA (the presumed irritant), compared to the lowest exposure concentration in the NTP (2000) subchronic inhalation study. There is no clear explanation. It has been suggested that oral non-bolus dosing of EGBE does not result in high enough local concentrations of EGBE and BAA (Poet et al., 2003). Studies with other forestomach carcinogens that are not mutagenic have demonstrated that forestomach effects are dependent not only on the dose but also on the chemical concentration in the dosing solution (Ghanayem et al., 1985), and other effects of EGBE appear to be highly dependent on the concentration attained (Ghanayem et al., 2001, 2000; Long et al., 2000; Nyska et al., 1999). In addition, first-pass liver metabolism of orally administered EGBE may affect the extent to which EGBE reaches the forestomach via the route that has been proposed following i.v. injection, distribution to salivary glands followed by the swallowing of EGBE-laden saliva

(Poet et al., 2003; Green et al., 2002). In any case, since forestomach irritation was not reported in rats or mice in the NTP (1993) drinking water study, this is not considered a sensitive endpoint, and route-to-route extrapolation of this endpoint from inhalation data is not considered appropriate for use in the RfD derivation.

5.2.2. Methods of Analysis, Including Models (PBPK, BMD, etc.)

PODs for the RfD derivation in terms of the human equivalent doses (HEDs) have been calculated via the application of PBPK modeling to NOAEL and BMDL estimates.

Of the available PBPK models (Table 5-3), the Corley et al. (1997, 1994) model is considered the most complete and appropriate for use in the derivation of the oral RfD, because it has been experimentally validated for the most sensitive species (rats) and humans, covers both oral and inhalation routes of exposure, and addresses both the distribution and excretion of the toxic metabolite, BAA, following oral EGBE exposure. This model is summarized in Appendix A. As in the case of the RfC (see Section 5.1.2.1), C_{max} is considered a more appropriate dose metric than AUC for the hematological effects. The PBPK model of Corley et al. (1997, 1994) was used to obtain estimates of human C_{max} concentrations from the female rat drinking water study data.

The four steps involved in using the Corley et al. (1997, 1994) PBPK model as modified by Corley et al. (1997) to calculate the HED corresponding to the LOAEL identified in the animal study ($LOAEL_{HED}$) were to (1) calculate the internal dose surrogate (C_{max} BAA in blood) corresponding to the female rat LOAEL, assuming that the drinking water was consumed only during a 12-hour awake cycle on a 7 day/week schedule in model simulations; (2) verify that steady state was achieved (e.g., no change in BAA C_{max} as a result of prolonging the exposure regimen); (3) simulate the internal dose surrogate (C_{max} BAA in blood) for humans consuming EGBE in drinking water, assuming that a 70-kg human consumes an average of 2 L of water during a 12-hour awake cycle; and (4) calculate the HED (mg/kg-day) for the amount of EGBE consumed in 2 L of water that resulted in the same internal dose (C_{max} BAA) simulated for the animal in step 1 as shown below.

5.2.2.1. Derivation of POD Using PBPK Model and NOAEL/LOAEL Method

Step 1: Calculate the C_{max} for BAA in blood corresponding to female rat LOAEL.

Female rat LOAEL = 59 mg/kg-day (calculated for final week of 13-week study to correspond with the final hematological determination) C_{max} BAA = 103 μM

Step 2: Verify steady state.

There were no changes in the C_{max} of BAA in blood during any 24-hour simulation period using a 12 hour/day, 7 day/week drinking water exposure regimen at the female rat LOAEL, indicating that steady state was achieved.

Step 3: Calculate the C_{max} for BAA in blood for humans continuously exposed to varying concentrations of EGBE.

Table 5-11 shows modeled estimates of BAA in blood of humans exposed continuously to varying concentrations of EGBE in water (Corley et al., 1997, 1994). Drinking water volume is 2 L consumed over 12 hours in a day.

Table 5-11. Modeled estimates of BAA in human blood exposed to EGBE in water.

EGBE concentration (ppm) in water	Calculated dose of EGBE from drinking water (mg/kg-day)	C_{max} BAA in blood (µM)
24	0.7	9
48	1.4	18
94	2.7	36
188	5.4	73
375	10.7	147
750	21.4	299

Step 4: Calculate the LOAEL HED for a 70-kg human consuming EGBE in 2 L of drinking water/day that results in the same internal dose of EGBE (C_{max} of BAA in blood) calculated for the animal study in step 1.

Female rat C_{max} for BAA in blood at LOAEL = 103 µM

$LOAEL_{HED}$ continuous exposure = 7.6 mg/kg-day (calculated by regression of the internal dose versus the dose of EGBE from step 3)

The $LOAEL_{HED}$ calculated using the PBPK model is likely a high estimate of the HED, since the model is based on male rat kinetic data, and female rats have been observed to have slightly higher concentrations of BAA in blood than male rats at similar exposure levels. In other words, use of male rat kinetic data results in estimates of the BAA concentrations in human blood associated with an effect ($LOAEL_{HED}$) that are lower than if female rat kinetic data had been used. In addition, the internal dose surrogate, C_{max} for BAA in blood, is highly dependent on the rate of water ingestion. Since drinking-water exposures are highly complex and variable, a simplifying assumption was used in all simulations that the entire dose of EGBE in drinking water was consumed over a 12-hour period each day corresponding to the awake cycle for both rats and humans. This assumption resulted in higher C_{max} blood concentrations of BAA in both rats and humans than would have been calculated using the original Corley et al. (1994) structure that assumed that drinking-water uptake occurred over a 24 hour/day dosing period.

5.2.2.2. Derivation of POD Using PBPK and BMD Methods

5.2.2.2.1. BMD approach applied to hematological data.

Although a lower LOAEL was reported in male rats, this value gives no indication of the relative slopes of the dose-response curves for males and females. Because this is an important factor for BMD analyses (U.S. EPA, 2000, 1995b), a comparison of the MCV and RBC count results for both male and female rats was performed and demonstrated that female rats are more sensitive to the effects of EGBE than are males. Therefore, dose-response information on these hematological effects in female rats was selected as the basis for the oral RfD BMD analyses discussed below.

As was discussed in Section 5.2.1, MCV and RBC count are continuous response measurements of precursor events associated with EGBE exposure and are considered the most appropriate hematologic endpoints for use in a BMD analysis. C_{max} is considered the more appropriate dose metric for use in evaluating the chosen hemolytic endpoints. C_{max} for BAA in arterial blood, as was determined using the PBPK model of Corley et al. (1994) as modified by Corley et al. (1997) (see Section 5.2.2.1). The results of this modeling effort are summarized in Table 5-12.

Table 5-12. Model estimates of BAA blood levels in female rats following oral exposures.

EGBE concentration in water (ppm)	Water EGBE intake (L/day)	Female body weight (g)	BAA in blood	
			Dose (mg/kg-day)	C_{max} (µM)
750	0.0147	188	59	103
1500	0.0155	185	125	253
3000	0.0125	180	208	495
4500	0.0101	164	277	738
6000	0.0101	150	404	1355

Source: Corley et al. (1997, 1994).

A BMD analysis was performed using EPA BMDS version 1.4.1. As can be seen from the results in Table 5-13, RBC count was the more sensitive of the two hematological endpoints assessed. All models were fit using restrictions and option settings suggested in the EPA BMD technical guidance document (U.S. EPA, 2000) except for the choice of BMR.

Table 5-13. Comparison of female rat RBC count and MCV BMD/BMDL values from an oral subchronic study using modeled blood Cmax (3 months) of the EGBE metabolite BAA as a common dose metric.

Model	BMD_{05} (μM)	$BMDL_{05}$ (μM)	*p* Value	AIC*	Scaled residual**
***RBC* count**					
1st degree polynomial	393.105	325.835	<0.0001	–26.323	–2.35
Power	393.105	325.835	<0.0001	–26.323	–2.35
Hill***	62.3999	36.2595	0.7038	–48.884	0.204
MCV					
1st degree polynomial	229.179	200.063	<0.0001	141.85	3.12
Power	229.179	200.063	<0.0001	141.85	3.12
Hill***	126.844	104.244	0.1757	120.96	–0.315

*AIC = −2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters and P is the number of model degrees of freedom (number of parameters estimated).
**Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMD scaled by an estimate of its SD provides a comparative measure of model fit near the BMD. Residuals that exceed 2.0 in absolute value should cause questioning of model fit in this region.
***Model choice based on adequate *p* value (>0.1), visual inspection, low AIC, and low (absolute) scaled residual.

Source: NTP (1993).

For continuous response data, the current BMD technical guidelines (U.S. EPA, 2000) suggest the use of 1 SD for the BMR level in the absence of additional information, such as a minimal level of change in the endpoint that is generally considered to be biologically significant. Because the chosen hemolytic endpoints are continuous measurements that have a relatively small historical variance in rats and because low-dose responses for these endpoints were generally near or within 5% of the control mean, the $BMDL_{05}$ was considered to be a more appropriate POD for derivation of the RfD (U.S. EPA, 2000, 1995b). Higher levels of response (e.g., ≥10% reduction) are in the exposure range where other more severe responses related to anemia occur (e.g., RBC decreases, increased reticulocyte counts). Lower levels of response (e.g., 1 SD, approximately a 3% reduction for these data) are relatively distant from the observable data and other responses related to anemia (1 SD data have been provided in Appendix B).

The best model fit to the RBC count and MCV data (from visual inspection and comparison of AIC values and scaled residuals near the BMD) was obtained using a Hill model (see Table 5-13). A graphical plot and textual description of the results of the Hill model assessment of RBC count responses in female rats (NTP, 2000) versus corresponding PBPK estimates of C_{max} for BAA in female rat blood are provided in Appendix B.

The BMD_{05} was 62 μM and the $BMDL_{05}$ was determined to be 36 μM using the 95% lower confidence limit of the dose-response curve expressed in terms of the C_{max} for BAA in blood. The Corley et al. (1997, 1994) PBPK model was used to back-calculate an HED

($BMDL_{HED}$) of 3 mg/kg-day, assuming that rats and humans receive their entire dose of EGBE from drinking water over a 12-hour period each day.

5.2.2.2.2. BMD approach applied to hemosiderin endpoint.

Due to the limited oral database, EPA believes that a route-to-route extrapolation will increase the confidence in the determination of the RfD POD. Inhalation studies considered for derivation of the RfC are used to supplement the oral database using the route-to-route extrapolation as described below.

5.2.2.3. Route-to-Route Extrapolation from Inhalation Data

A route-to-route extrapolation was performed on the data used in the derivation of the RfC from the NTP (2000) chronic inhalation study because of the lack of a chronic oral study for EGBE. As with the species-to-species extrapolation used in the development of the RfC, the dose metric used for species-to-species (rat to human) and route-to-route (inhalation to oral) is the AUC of BAA at 12 months in arterial blood. This dose metric was used for dose-response modeling of chronic inhalation data (Section 5.1.2.2.2) to derive the POD of 133 μmol-h/L, expressed as a BMDL. The BMDL was then back-calculated using the human PBPK model (Corley et al. 1997; 1994) to obtain an equivalent human oral drinking water dose ($BMDL_{HED}$) of 1.4 mg/kg-day. As for the HED estimations in Sections 5.2.2.1 and 5.2.2.2.1, a simplifying assumption was used that the entire dose of drinking water EGBE was consumed over a 12-hour period each day.

5.2.2.4. Selection of the POD

The BMCL chosen for the RfC is used to determine the POD for the RfD. This value is based on a more comprehensive chronic data set and is below the range of estimates from available oral data of shorter duration of exposure. Hemosiderin deposition in male rat liver is the critical effect chosen for derivation of the RfC. New mode of action information (see Section 4.6.3.1) supports the hemosiderin deposition endpoint as an important key event in the proposed MOA. The BMCL for the RfC (AUC of 133 μM-H/L BAA in arterial blood at 12 months) is converted using the Corley et al. (1994, 1997) model to an oral human equivalent dose ($BMDL_{HED}$) of 1.4 mg/kg-day. This extrapolated oral value is consistent with and slightly lower than the $LOAEL_{HED}$ of 7.6 mg/kg-day and the $BMDL_{HED}$ of 3 mg/kg-day estimated from the subchronic oral (NTP, 1993) study. The final calculation of an RfD derived from the NOAEL/LOAEL and BMC modeling approaches is presented in Table 5-14.

Table 5-14. Summary of the application of UFs for RfD derivations using NOAEL/LOAEL and BMD modeling approaches.

Factor**	Approach*	
	LOAEL (NTP, 1993)	$BMDL_{10}$ male rat liver hemosiderin staining (NTP, 2000)
UF_H	10	10
UF_A	1	1
UF_S	1	1
UF_L	3	1
UF_D	1	1
UF (total)	30	10
POD/UFs = RfD (mg/kg-day)	7.6/30 = 0.3	1.4/10 = 0.14

*The rationale for the selection of these UFs is discussed in Section 5.1.3 below.
**PODs—$LOAEL_{HED}$ = 7.6 mg/kg-day based on hematological effects from the oral study; the $BMDL_{10HED}$ = 1.4 mg/kg-day, and the approach is based on a route-to-route extrapolation from inhalation data (see Section 5.2.2.3).

5.2.3. RfD Derivation, Including Application of Uncertainty Factors (UFs)

UFs are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (e.g., chronic exposure over a lifetime when subchronic studies are used for deriving a toxicity value). The application of UFs may include the use of a partial UF of $10^{1/2}$ (3.2) based on the assumption that the actual values for the UFs are log-normally distributed. Application of these factors in the assessment is such that, when a single partial UF is applied, the factor is rounded to 3 (e.g., the total factor for individual UFs of 3 and 10 would be 30 [3 × 10]). When two partial UFs are evoked, however, they are not rounded, such that a UF of 3, 3, and 10 would result in a total uncertainty of 100 (actually $10^{1/2} \times 10^{1/2} \times 10^{1}$). UFs applied for this RfD assessment and the justification for their use follow.

A value of 10 was selected to account for variation in sensitivity within the human population (UF_H). Potentially susceptible subpopulations include individuals with enhanced metabolism or decreased excretion of BAA, and individuals whose RBC membranes are more susceptible to the lysis caused by BAA. A UF of 10 was used to account for the uncertainty associated with the variability of the human response to the effects of EGBE. Human in vitro studies suggest that the elderly and patients with fragile RBCs would not be more sensitive to the hemolytic effects of EGBE than normal adults, and laboratory animal (rats, calves, and mice) studies suggest that older animals are more sensitive than neonates, and that females are more sensitive than males (see Section 4.7). However, actual human responses to EGBE have not been observed in a broad enough range of exposure conditions (e.g., repeat/long-term exposures) and potentially sensitive subjects (e.g., individuals predisposed to hemolytic anemia, infants) to warrant the reduction of the UF_H below the default value of 10. While developmental studies do not reveal increased susceptibility in infants, none of the developmental studies examined fetal or infant blood for signs of effects from prenatal exposure to EGBE.

A UF of 1 was used to account for interspecies variation (UF_A) for toxicodynamic and toxicokinetic differences between animals and humans. Traditionally, these components are individually represented by partial UFs of 3 for a total UF of 10 in the absence of chemical-specific information; thus, application of a full UF of 10 would depend on two areas of uncertainty: toxicokinetic and toxicodynamic. In this assessment, the toxicokinetic uncertainty is addressed by the determination of an HEC using PBPK modeling. There is in vivo (Carpenter et al., 1956) and in vitro (Udden, 2002; Udden and Patton, 1994; Ghanayem and Sullivan, 1993) information indicating that, toxicodynamically, humans may be less sensitive than rats to the hematological effects of EGBE. Thus, a value of 1 was used to account for toxicodynamic differences between rats and humans.

A UF to account for extrapolating from subchronic to chronic exposure (UF_S) was not needed because the RfC was derived from a chronic study.

A UF for LOAEL-to-NOAEL extrapolation was not used because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of a 10% change in hemosiderin staining was selected under an assumption that it represents a biologically significant change.

A value of 1 was used for the database UF_D. While no chronic oral studies or adequate human data are available for EGBE, PBPK models allow for deriving a BMDL from the chronic inhalation study using measured internal dose metrics and then extrapolating it back to an equivalent human oral dose. A comparison of NOAELs and BMDLs from the subchronic oral and chronic inhalation studies using a common internal dose metric (AUC BAA) shows that effects occur at approximately the same internal dose following inhalation and oral exposures adding confidence to the application of the chosen PBPK model (Corley et al., 1997, 1994) to derive an oral RfD from the NTP (2000) chronic inhalation study. Thus, the total UF is 10. The RfD is 1.4 mg/kg-day ÷ 10 = 0.1 mg/kg-day.

5.2.4. Previous Oral Assessment

The previous IRIS assessment for EGBE was entered into the database on December 30, 1999 and contains an oral RfD of 0.5 mg/kg-day. The RfD was based on the BMD_{05} HED of 5.1 mg/kg-day for changes in MCV in female F344 rats following a 91-day drinking-water exposure (NTP, 1993). A total UF of 10 was used to account for human variability. This assessment was conducted prior to the adoption of the current BMD technical guidance document (U.S. EPA, 2000c).

5.3. Uncertainties in the Derivation of the Inhalation Reference Concentration (RfC) and Oral Reference Dose (RfD)

The following is a more extensive discussion of uncertainties associated with the RfC and RfD for EGBE beyond the quantitative discussion in Sections 5.1.2, 5.1.3, 5.2.2, and 5.2.3. A summary of these uncertainties, along with uncertainties specific to the Section 5.4 cancer analysis, is presented in Table 5-15.

5.3.1. Choice of endpoint

The impact of endpoint selection on the derivation of the RfC and RfD was discussed in Sections 5.1.2.4 and 5.2.2.4. Comparison RfC values were also calculated (see Section 5.1.4) and are intended to provide information on alternative health effects associated with EGBE exposure. The comparison RfCs ranged from 1−18 mg/m^3, depending on whether irritation (forestomach), hematologic effects, or hemosiderin deposition data were used to derive the POD, with the latter endpoint representing the lower end of the RfC range.

5.3.2. Choice of dose metric

The AUC for BAA in arterial blood was selected as the appropriate measure of dose due to evidence for a causal association between BAA and hemolysis, between hemolysis and the accumulation of hemosiderin in the liver, and between hemosiderin accumulation in the liver and increased incidence of liver hemangiosarcoma. AUC is considered to be a more appropriate response measure because hemosiderin pigmentation increases in incidence and severity with increased duration (subchronic to chronic), and is believed to be the result of the cumulative exposure to EGBE/BAA as opposed to a peak exposure. The corresponding aldehyde of BAA, BAL, was also considered as a choice for an internal dose measure. As discussed in Section 4.6, BAL is the EGBE metabolite considered to have the greatest potential to interact directly with DNA. However, high ADH activity in the liver and forestomach is expected to result in very short residence time and in very low tissue concentrations of BAL; this scenario has been demonstrated in simulations using the Corley et al. (2005b) PBPK model. Also, the Corley et al. (2005b) PBPK model along with the gavage study of Deisinger and Boatman (2004) suggest that the conditions of in vitro assays showing BAL to be clastogenic (e.g., no metabolic activation; high cytotoxic concentrations of BAL) are considered to be of little relevance to the expected target organ (liver) environment (e.g., high metabolic activity; low concentrations of BAL). Use of an alternate measure of internal dose, for instance, a parent compound or C_{max}, would be more difficult to justify based on available empirical information. However, for comparative purposes, a BMD analysis was done on the critical endpoint to determine the impact that choosing C_{max} of BAA in blood rather than AUC of BAA in blood would have had on the $BMCL_{10}$(HEC) derivation. If C_{max} had been used as the dose measure, the $BMCL_{10}$(HEC) value would have been

39 mg/m^3, approximately 2.4-fold higher than the 16 mg/m^3 $BMCL_{10}$(HEC) value derived using AUC as the dose measure.

5.3.3. Use of BMC approach

Utilization of the BMC approach has advantages over other approaches to dose-response analysis, such as, the NOAEL/LOAEL approach. These advantages include the capacity of the BMC approach to accommodate study sample size and reflect this in providing confidence bounds to the lower limit on dose. As shown in Tables 5-9 and 5-14, use of the BMC approach on the incidence of chronic hemosiderin deposition resulted in RfC and RfD values about threefold lower than RfC and RfD values that would have been derived via the NOAEL/LOAEL approach.

5.3.4. Choice of model for BMCL derivations

$BMCL_{10}$ estimates used in the derivation of the RfC, which formed the basis for both the noncancer and cancer quantitative assessments, spanned a twofold range (60–124 μmol-hour/L) for female rats and threefold range (40–126 μmol-hour/L) for male rats. All of the models fit reasonably well (*p* values above 0.1). Hence, this range of results can be considered a reflection, in part, of model uncertainty.

5.3.5. Choice of animal to human extrapolation method

A PBPK model (Corley et al., 1997) was used to extrapolate animal to human concentration. An AUC blood level of BAA associated with a 10% increase in male mice with hemosiderin pigmentation of 69.6 μmol-hour/L was estimated using the mouse PBPK model; the human PBPK model was used to convert back to a human equivalent exposure concentration, or a $BMCL_{10}$(HEC), of 12 mg/m^3. If no PBPK models were available, the $BMCL_{10}$(HEC) would have been derived by dividing the $BMCL_{10}$ for external exposure concentration of 75 mg/m^3 by the threefold pharmacokinetic portion of the animal to human default adjustment factor (U.S. EPA, 1994), resulting in a $BMCL_{10}$(HEC) of 25 mg/m^3. This default value would have been twofold higher than the value derived using the PBPK model.

5.3.6. Route-to-route extrapolation

To estimate an oral dose POD for chronic hemosiderin deposition, a route-to-route extrapolation was performed on the inhalation exposure POD used to derive the RfC using a PBPK model and assuming, as is discussed above, that the metric most closely associated with the effects seen is the AUC measure in blood of BAA. One way of characterizing the uncertainty associated with this approach is to compare dose levels (BMCL values) using this metric for hemolytic effects—assumed to be associated with chronic hemosiderin deposition—derived from (1) an existing oral subchronic NTP (1993) study; or (2) from a model estimating this metric from an existing inhalation subchronic NTP (2000, 1998) study. This analysis was performed

(see Section 5.2.2.3) and shows that the values derived using the second procedure were consistently lower than using the former (i.e., were more potent by a factor of up to fourfold). Thus, estimates using this procedure for route-to-route extrapolation would uniformly overestimate the toxicity value and would result in lower RfD estimates.

5.3.7. Statistical uncertainty at the POD

Parameter uncertainty can be assessed through CIs. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the linear multistage model applied to the male mouse hemosiderin data, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the POD for derivation of the RfC), with the 95%, single-sided lower confidence limit (BMCL) being about 30% below the maximum likelihood estimate of the BMC.

5.3.8. Choice of bioassay

The NTP (2000) inhalation study was used for development of the RfC and RfD. This was a well-designed study, conducted in both genders in two species with an adequate number of animals per dose group and with examination of appropriate toxicological endpoints in both genders of rats and mice. Alternative comparable bioassays were unavailable.

5.3.9. Choice of species/gender

The RfC was based on the incidence of liver hemosiderin pigmentation in male rats, the species and gender most sensitive to this effect (NTP, 2000). This event also occurs in female rats and in mice, and is thought to be a precursor to the observed increase in liver tumors in male mice.

[10] If the RfC had been based on increased incidence of liver hemosiderin in another species/gender, such as male mice, a higher RfC value would have been derived. Similarly, the RfC would also have been higher had it been based on forestomach irritation, an effect less sensitive than hemosiderin deposition but considered to be a precursor event leading to the increase in incidence of forestomach squamous cell papillomas and one high-dose carcinoma observed by NTP (2000) in female mice.

5.3.10. Human relevance of noncancer responses observed in mice

The effects of hemosiderin deposition and forestomach irritation may both have qualitative relevance to humans. However, for reasons discussed in Section 4.5, the exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC/RfD and well above concentrations necessary to cause these effects in mice.

[10] Increased liver hemangiosarcomas were not observed in the NTP (2000) chronic study of rats or female mice. Possible reasons for this species and gender specificity are discussed in Section 4.6 and 4.7.2, respectively.

5.3.11. Human population variability

The extent of interindividual variability associated with EGBE metabolism is not well characterized in humans. As is discussed in Section 4.7, the hemolytic effect of EGBE is presumed to be caused by the interaction of its primary metabolite, BAA, with the RBC membrane. Potentially susceptible subpopulations or life stages would include individuals with enhanced metabolism or decreased excretion of BAA. In addition, individuals whose RBC membranes are more susceptible to the lysis caused by BAA could be more sensitive to EGBE. However, RBCs from normal, aged, sickle-cell anemia, and hereditary spherocytosis patients were no more sensitive to the hemolytic effects of BAA than RBCs from healthy volunteers (Udden, 1994). As is discussed further in Section 4.7, some potentially susceptible subpopulations or life stages have not been tested, and, when combined with the lack of understanding about EGBE's mechanism of hemolytic action, this represents a considerable source of uncertainty and forms the principal basis for the 10-fold UF applied to derive the RfD and RfC values.

5.4. Cancer Assessment

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the method used to characterize and quantify cancer risk from a chemical depends on what is known about the MOA of carcinogenicity and the shape of the cancer dose-response curve for that chemical. An assumption of linearity is appropriate when evidence supports an MOA of gene mutation due to DNA reactivity or supports an MOA that is anticipated to be linear. The linear approach is used as a default option if the MOA of carcinogenicity is not understood. The nonlinear approach "can be used for cases with sufficient data to ascertain the mode of action and to conclude that it is not linear at low doses…" (U.S. EPA, 2005). Alternatively, the MOA may theoretically have a threshold, that is, the carcinogenicity may be a secondary effect of toxicity that is itself a threshold phenomenon.

In the case of EGBE, the MOA of carcinogenicity for hepatic hemangiosarcoma, hepatocellular adenoma and carcinoma, and forestomach tumor formation in animals is reasonably well understood. A reference concentration and reference dose approach has been used for EGBE because "When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action for the general population and/or any subpopulations of concern, a different approach — a reference dose/reference concentration that assumes that nonlinearity – is used." (U.S. EPA, 2005). It is recognized, however, that while this approach fits this case, other nonlinear approaches may be appropriate in other settings. Available data indicate that EGBE is not likely to be mutagenic and that it is not expected to produce rodent tumors via a mutagenic MOA. Rather, there is evidence that carcinogenic responses observed in animals are

associated with erythrocyte hemolysis, leading to oxidative damage and increased hepatocyte and endothelial cell proliferation and initiation for the liver tumors, and associated with the formation of BAA, an acidic metabolite, that leads to regenerative hyperplasia in response to irritation for the forestomach tumors. Because cytolethality occurs only at exposure levels above some critical dose, a nonlinear (threshold) approach is considered to be the most appropriate method for characterizing the cancer risk from EGBE.

As discussed in Sections 4.2 and 4.6, there are currently no human studies addressing the potential carcinogenicity of EGBE. A 2-year inhalation bioassay in mice and rats (NTP, 2000) reported some evidence of carcinogenic activity in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver and an increase in the incidence of hepatocellular carcinoma, as well as some evidence of carcinogenic activity in female B6C3F1 mice, based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma). The study also reported no evidence of carcinogenic activity in male F344/N rats and equivocal evidence of carcinogenic activity in female F344/N rats based on increased combined incidences of benign and malignant pheochromocytoma (mainly benign) of the adrenal medulla. The hypothesized MOA for the induction of hepatic hemangiosarcomas and hepatocellular carcinomas reported in male mice exposed to EGBE may comprise a sequence of events that are dose-dependent. First, EGBE is metabolized to the carboxylic acid, which then hemolyses RBCs. This event leads to the release of excess iron that in turn could result in iron-induced formation of ROS and subsequent oxidative damage to target tissues within the liver. Induction of cell proliferation and neoplasm formation follows. This sequence of events is considered necessary for the formation of the observed neoplasms. Strategies intended to control or omit any of these key events, including the initial hemolytic event, would interrupt the process and prevent formation of neoplasms. For these reasons, formation of neoplasms in humans would likely be prevented by establishing levels of EGBE exposure in the dose range that does not result in the initial hemolytic events and subsequent ROS-mediated cellular damage. Thus, for the assessment of human cancer risk associated with the formation of hemangiosarcomas and hepatocellular adenomas and carcinomas in animals, the RfD and RfC derived in Sections 5.1 and 5.2 should be considered protective, as the carcinogenicity may be a secondary effect of toxicity that is itself a threshold phenomenon.

Available data suggest that the MOA for the induction of forestomach tumors reported in female mice exposed to EGBE is dependent on the initial formation of the irritating metabolite BAA. This acidic metabolite produces chronic cytotoxicity, which results in compensatory epithelial cell regeneration. Chronic cell proliferation in preneoplastic cells is in turn associated with the formation of forestomach papillomas and carcinomas. In addition, the available data indicate that BAA may be preferentially formed in the forestomach due to the presence of levels of ADH that are higher than those found in the human stomach or esophagus. BMD modeling of

the dose response for epithelial hyperplasia in female mice forestomachs was performed as a comparison to reference values derived for precursor effects in the liver as discussed above and is presented in Appendix C. The analysis in Appendix C shows that, had hyperplastic effects in female mice been used as a POD, the resultant RfD and RfC values would have been much higher than the RfD and RfC values derived in Sections 5.1 and 5.2 using hemosiderin deposition as the critical effect for EGBE exposure. Thus, the analysis indicates that the RfD and RfC based on liver effects would also be protective of forestomach toxicity. The exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC and RfD.

Female rats reported a marginally significant trend for pheochromocytomas, and the high dose frequencies reported in the rats were only slightly different from the upper range of historical controls (see Section 4.6). In addition, the histopathologic data indicated there was difficulty distinguishing pheochromocytomas from non-neoplastic adrenal medullary hyperplasia. Thus, these lesions are interpreted with caution as tumors. Given the marginal dose response, lack of tumor evidence in any other organ system of the rats, and reported difficulties in distinguishing pheochromocytomas from non-neoplastic adrenal medullary hyperplasia, this tumor was not given significant weight in the qualitative or quantitative assessment of EGBE cancer potential.

5.4.1. Uncertainties in Cancer Risk Assessment

The cancer assessment of EGBE is based largely on the premise that key events in the MOAs proposed for mice (forestomach irritation and hemolysis leading to increased hemosiderin deposition) are not likely to occur in humans at concentrations at or below the RfC and RfD values. Uncertainties in the RfC and RfD derivations are addressed in part in Sections 5.1.2, 5.1.3, 5.2.2, 5.2.3, and 5.3. This section will discuss additional uncertainties relative to the human relevance of cancer responses observed in mice and the extrapolation method employed for the estimation of low-dose cancer risk. All of these uncertainties are summarized in Table 5-15.

Table 5-15. Summary of uncertainty in the EGBE noncancer and cancer risk assessments.

Consideration	Potential impact*	Decision	Justification*
Choice of low-dose extrapolation method	A linear low-dose extrapolation would likely drive the risk estimation when combined with site-specific exposure data..	Nonlinear approach; RfC and RfD are considered protective of the key events leading to cancer.	Proposed key events in the two MOAs (forestomach irritation and hemolysis leading to ↑ liver hemosiderin) are not likely to occur in humans at the RfC or RfD.
Choice of endpoint	Use of forestomach endpoint could ↑ RfC by up to 18-fold (see Section 5.3).	RfC is based on the most sensitive endpoint, increased liver hemosiderin.	Chosen endpoint is considered most relevant due to forestomach toxicokinetic and exposure issues.
Choice of dose metric	Alternatives could ↑ or ↓ RfC/RfD (e.g., use of C_{max} BAA increases RfC by two to threefold).	AUC for BAA in arterial blood.	Evidence for a causal association between the proposed key steps of BAA and hemolysis leading to ↑ liver hemosiderin and ↑ liver tumors. AUC chosen because hemosiderin ↑ with cumulative exposure to EGBE/BAA.
POD derivation method	RfC/RfD threefold lower than for NOAEL.	BMD method used.	Advantages include capacity to account for sample size and to provide confidence bounds on dose..
Choice of model for BMCL derivation	Alternative models could ↑ RfC up to threefold (see Section 5.3).	Multistage (1st degree) model chosen.	The best-fitting model was chosen based on EPA (2000) BMD technical guidance.
Choice of animal to human extrapolation method	Alternatives could ↑ or ↓ RfC/RfD (e.g., default would ↑ RfC by twofold) (see Section 5.3).	A PBPK model was used to extrapolate animal to human concentrations.	Use of a PBPK model reduces uncertainty associated with the animal to human extrapolation. AUC blood levels of BAA are an appropriate dose metric, and a peer-reviewed and verified PBPK model exists that estimates this metric.
Statistical uncertainty at POD	POD would be ~40% higher if BMD were used.	BMDL used per EPA BMD guidance (U.S. EPA, 2000).	Limited size of bioassay results in sampling variability; lower bound is 95% CI on administered exposure.
Choice of bioassay	Alternatives could ↑ or ↓ RfC/RfD.	NTP (2000) study.	Alternative bioassays were inadequate.
Choice of species/ gender	RfC would be ↑ if based on another species/gender.	RfC is based on the most sensitive endpoint, increased liver hemosiderin, in the most sensitive species and gender, male rats.	Male mice are the only species/gender to experience an increase in liver tumors from EGBE exposure. If one relies on the MOA, however, liver hemosiderin increase in male rats is the appropriate key event and species to utilize for the RfC. Female mice exhibit forestomach papilloma but at higher doses.
Human relevance of rodent tumor data	If MOAs for tumors from EGBE were deemed not relevant, cancer descriptor would be "suggestive of human carcinogenic potential."	MOAs for liver and forestomach tumors are used.	Although EGBE has some evidence for liver tumors in male mice and forestomach tumors in female mice, the MOAs describe rationales as to why humans are unlikely to experience appreciable risk at environmental concentrations.
Human population variability	Low-dose toxicity ↑ to an unknown extent.	10-fold UF applied to derive the RfD and RfC values.	10-fold UF is applied principally because of limited data on human variability or potential susceptible subpopulations.

*↑ = increase; ↓ = decrease.

5.1.1.1. Choice of low-dose extrapolation method

The MOA is a consideration in estimating risks. A linear low-dose extrapolation approach was not considered to be optimal for the estimation of human carcinogenic risk associated with EGBE exposure because of the physicochemical properties of EGBE, toxicokinetic limitations, specific MOAs articulated earlier, and limitations in data to parameterize appropriate models. It should be noted that the demonstration that a chemical is not mutagenic is insufficient, alone, to postulate a nonlinear dose response. Key events in the MOAs proposed for mice (forestomach irritation and hemolysis leading to increased hemosiderin deposition) are not likely to occur in humans at concentrations at or below the RfC and RfD values. This assumes that the proposed MOAs in mice are reasonably correct. If, for instance, hemosiderin accumulation in the mouse liver is not the result of increased hemolysis but of EGBE interaction with other cell types, a more direct, linear MOA for the observed increase in male mouse hemangiosarcomas might be hypothesized. In order to illustrate the predicted cancer risk under such a scenario, the cancer risks associated with the tumor types that were increased following EGBE exposure were calculated using the default approach of low-dose linear extrapolation outlined in the cancer guidelines (U.S. EPA, 2005). The results of this linear analysis are presented in Table 5-16.

For illustrative purposes, an estimate of the increased cancer risk at the RfC (1.6 mg/m^3) was calculated using the inhalation unit risk, for hepatocellular tumors in male mice, that was derived using a default linear low-dose extrapolation approach. The estimated increased cancer risk at the RfC in this comparison exercise would be 1.6 mg/m^3 x 4.8 x 10^{-4} = 7.6×10^{-4}. This value is only for illustrative purposes and indicates the differences in the two approaches. It should not be misconstrued as an estimate of the cancer risk at the RfC since the default linear approach is not recommended.

Table 5-16. Illustrative potency estimates for tumors in mice, using a linear analysis approach.

.	$BMDL_{10HEC}$ (mg/m^3)*	Slope factor $0.1/BMDL_{10HEC}$ (risk/mg-m^3)
Hepatocellular carcinoma (males)	208	4.8×10^{-4}
Hemangiosarcomas (males)	575	1.7×10^{-4}
Papilloma or carcinoma of the forestomach (females)	544	1.8×10^{-4}

*$BMDL_{10HEC}$ values were calculated using AUC as the dose metric.

5.4.1.2. Human relevance of cancer responses observed in mice

The hypothesized MOAs for EGBE-induced liver tumors observed in male mice and EGBE-induced forestomach tumors observed in female mice may both have qualitative

relevance to humans. However, for reasons discussed in Section 4.6, the exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC and RfD.

5.5. Potential Impact of Select Uncertainties on the RfC

In this assessment, the RfC forms the basis for the RfD. The range of possible results associated with some of the areas of uncertainty in the RfC derivation can be estimated (see Sections 5.3 and 5.4). Figure 5-4 graphically illustrates the change in the RfC that would result had particular choices, other than those presented in this assessment, been made (see summary in Table 5-15). These specific areas were presented in this illustration because there are data available that could be used to quantify their contribution to the uncertainty in the noncancer and cancer assessments.

The area of uncertainty that has the potential to have the greatest impact on the EGBE assessment is that which is associated with the approach used to extrapolate human cancer risk from the available rodent studies, that is, if human cancer risk had been estimated from a linear extrapolation of responses observed in rodent studies. Figure 5-4 provides a graphic of the areas of uncertainty, described in Table 5-15, for which there is quantitative information and impact on the RfC can be estimated. The "Cancer Approach" value in Figure 5-4 represents the difference in the dose that was selected for the RfC and the dose that would represent a 10^{-5} increased cancer risk if a default linear low-dose extrapolation approach were to be used. In this instance, the comparison indicates that the dose associated with a 10^{-6} cancer risk, estimated via the linear dose-response method, using the inhalation unit risk for hepatocellular tumors in male mice as indicated in Table 5-16, is 56-fold lower than the RfC. Note that the default linear approach is not recommended.

These discussions and illustrative tools should not be viewed as a comprehensive analysis of all possible uncertainty considerations; rather, they exemplify what are believed to be important areas of uncertainties for which data are available. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

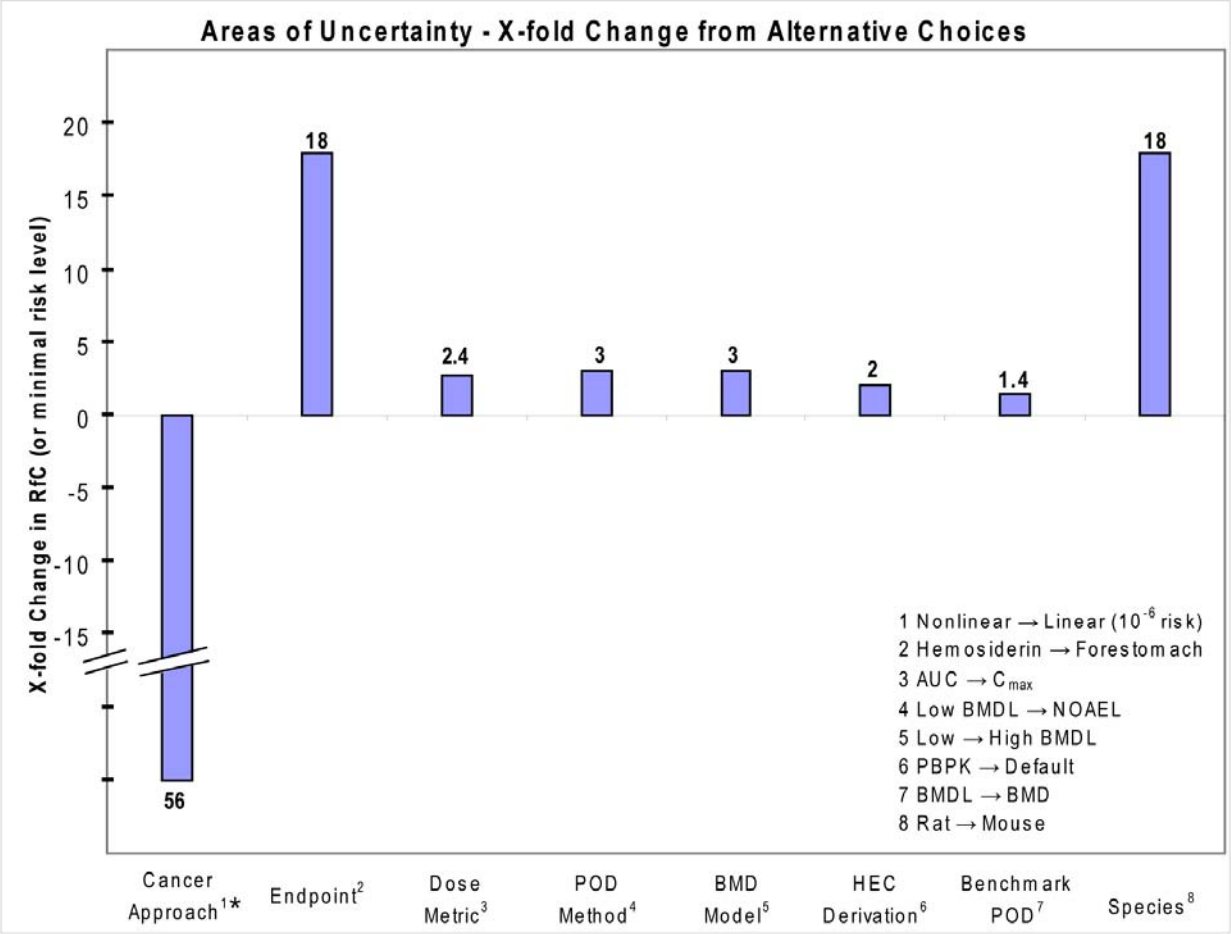

Figure 5-4. Potential impact of select uncertainties on the RfC for EGBE.

*Illustrates the dose that would represent a 10^{-6} increased cancer risk if a default linear low-dose extrapolation approach were used. This dose is expressed as X-fold change in the RfC.

6. Major Conclusions in the Characterization of Hazard and Dose Response

6.1. Human Hazard Potential

EGBE is a clear, miscible solvent used in formulating cleaning products and protective coatings. It is metabolized primarily to the carboxylic acid, BAA, the proximate toxicant, in both humans and animals. BAA and its conjugates are readily excreted in the urine.

Hemosiderin deposition in the liver of male rats, as a consequence of hemolysis, is identified as the critical endpoint of concern in toxicological studies on EGBE. Toxicologically, this effect increases with both exposure concentration and duration, is not compensated for (as with hemolysis), and is considered to be a clearly pathological finding. Mechanistically, this effect is at the interface between noncancer and cancer effects from exposure to EGBE.

Observations regarding the potential relevance of EGBE toxicity to humans include the insensitivity of human RBCs to the hemolytic effects of EGBE and its metabolite, BAA. While it is established that humans can experience hemosiderin deposition in the liver as a consequence of excessive hemolysis (Iancu et al., 1976), the relative insensitivity of human blood to the effects of EGBE have been demonstrated in numerous in vitro studies through the use of either whole blood or washed erythrocytes (e.g., Udden, 2002; Ghanayem and Sullivan, 1993). Humans appear significantly less sensitive to the hemolytic toxicity of EGBE than are typical laboratory species, such as mice, rats, or rabbits, with reports from analyses of isolated RBCs demonstrating that human RBCs are 40- to 150-fold less sensitive than rat RBCs (Udden, 2002). These observations are inclusive of human RBCs from individuals with hereditary spherocytosis and sickle cell anemia, conditions characterized by RBC sensitivity to hemolysis. Available in vivo information with human exposure supports this species disparity in sensitivity to the hemolytic effects of EGBE. Male rats in one study (NTP, 1993) experienced mild liver effects at a drinking-water dose lower than that which caused observable hemolytic effects. Available human toxicity data show that the primary effects after acute oral ingestion of large doses of EGBE (most often combined with other solvents) are reversible metabolic acidosis from the production of BAA and some hematological changes. Occupational exposure to low levels of EGBE did not cause adverse changes in hepatic, renal, or hematologic parameters (Haufroid et al., 1997).

Due to the known reproductive toxicity (i.e., toxicity to male testes and sperm) of two other glycol ethers, EGME and EGEE, the reproductive toxicity of EGBE has been studied in a variety of well-conducted oral studies (NTP, 1993; Exon et al., 1991; Heindel et al., 1990; Foster et al., 1987; Grant et al., 1985; Nagano et al., 1984, 1979) and inhalation studies (NTP, 2000;

Nachreiner, 1994; Doe, 1984; Dodd et al., 1983) using rats, mice, and rabbits. In addition, several developmental studies have addressed EGBE's toxicity from conception to sexual maturity, including toxicity to the embryo and fetus, following oral exposures (Sleet et al., 1989; Wier et al., 1987), inhalation exposures (Nelson et al., 1984; Tyl et al., 1984), and dermal exposures (Hardin et al., 1984) to rats, mice, and rabbits. EGBE did not cause adverse effects in any reproductive organ, including testes, in any study. In a two-generation reproductive toxicity study, fertility was reduced in mice only at very high maternally toxic doses (>1000 mg/kg). Maternal toxicity related to the hematologic effects of EGBE and relatively minor developmental effects have been reported in developmental studies. No teratogenic effects were noted in any of the studies. It can be concluded from these studies that EGBE is not significantly toxic to the reproductive organs (male or female) of either parents or to the developing fetuses of laboratory animals.

No reliable human epidemiological studies are available that address the potential carcinogenicity of EGBE. The NTP (2000) performed a 2-year inhalation bioassay with rats and mice and found no evidence of carcinogenic activity in male F344/N rats and equivocal evidence of carcinogenic activity in female F344/N rats, based on increased combined incidences of benign and malignant pheochromocytoma (mainly benign) of the adrenal medulla. The researchers reported some evidence of carcinogenic activity in male B6C3F1 mice, based on an increased incidence of hemangiosarcoma of the liver and an increase in the incidence of hepatocellular carcinoma that may have been exposure related. They also reported some evidence of carcinogenic activity in female B6C3F1 mice, based on an increased incidence of forestomach squamous cell papilloma or carcinoma (mainly papilloma). Based on its physical-chemical properties, toxicokinetic and dynamic factors, and MOA information, under existing EPA guidelines (U.S. EPA, 2005), EGBE is judged not likely to be carcinogenic to humans at expected environmental concentrations (see Section 4.6). The MOAs presented for the animal tumors indicate that both high doses and sustained periods of exposure are necessary for the carcinogenic response. The available human exposure/response information indicates that these conditions are unlikely to occur because the primary response of humans to high oral doses of EGBE, as shown in the case studies in Section 4.1 is metabolic acidosis, which, if not treated, can lead to shock and eventually death. Further, based on simulations from PBPK modeling, the maximum blood concentrations of BAA that could be produced in humans following exposure to a saturated atmosphere of EGBE would be below those needed to produce hemolysis (Corley et al., 2005a). Evidence from the only human inhalation exposure study available showed that, while nasal and ocular irritation were reported in research subjects exposed to up to 195 ppm, no changes in erythrocyte fragility were observed (Carpenter et al., 1956). For a more complete discussion of the carcinogenic potential of EGBE, see Section 4.6.

6.2. Dose Response

The quantitative estimates of human risk from lifetime exposure to EGBE are based on animal experiments, as no relevant human data exist.

For derivation of the RfC, the most sensitive endpoint for dose-response assessment is the effect of inhalation exposure on hemosiderin staining in Kupffer cells, as reported in the study by NTP (2000). The RfC based on this endpoint is 1.6 mg/m^3. This concentration is based on the human equivalent $BMCL_{10}$ of 16 mg/m^3, which was back-calculated from rat data using the BMD and PBPK approach and the application of a tenfold UF.

The overall confidence in the RfC assessment is medium to high. Higher confidence is placed in the RfC values derived from internal dose measures (PBPK method and combined PBPK/BMC method) because pharmacokinetic differences between rats and humans were accounted for using PBPK models (Lee et al., 1998; Corley et al., 1997, 1994) and actual measurements of internal blood concentrations in test aniumals of interest (Dill et al., 1998). Higher confidence is placed on the NTP (2000) study; because it was a chronic study, it employed both male and female rats and mice, it had a wide range of exposure levels, and animals were observed twice daily. Medium-to-high confidence is placed on the database, because data are available for a variety of animal species, including humans. While the database lacks long-term human studies, the available short-term human controlled studies and case reports as well as laboratory animal and in vitro studies provide evidence to suggest that, with respect to the hemolytic effects of EGBE, long-term human exposures would be no more adverse than long-term rat exposures. Confidence is not high, because the potential for effects in humans from repeat, long-term exposures has not been investigated.

In the derivation of the RfC, a tenfold UF was applied, which was intended to account for intrahuman variability. A value of 10 was selected to account for variation in sensitivity within the human population (UF_H). Potentially susceptible subpopulations include individuals with enhanced metabolism or decreased excretion of BAA and individuals whose RBC membranes are more susceptible to the lysis caused by BAA. A UF of 10 was retained to account for the uncertainty associated with the variability of the human response to the effects of EGBE. Human responses to EGBE have not been observed in a broad enough range of exposure conditions (e.g., repeat/long-term exposures) and potentially sensitive subjects (e.g., individuals predisposed to hemolytic anemia, infants) to warrant the reduction of the UF_H below the default value of 10.

For derivation of the RfD, the BMD PBPK approach, along with the same internal dose metric used in the derivation of the RfC, was used. As with the RfC, the RfD was based on hepatic hemosiderin deposition data. The RfD based on this endpoint is 0.1 mg/kg-day. This value was obtained by using a route-route extrapolation from the RfC, and dividing the estimated human equivalent by a UF of 10. The HED was estimated using the AUC values for BAA in blood as the dose metric, and calculating a $BMDL_{10}$ of 133 μM-hour/L BAA in arterial blood at

12 months. The PBPK model was then used to back-calculate an HED, assuming that rats and humans received their entire dose of EGBE from drinking water over a 12-hour period each day.

The overall confidence in the RfD assessment is medium to high. The RfD value has been calculated for EGBE using a route-to-route extrapolation from the inhalation PBPK/BMC method used to derive the RfC. A higher confidence is placed in the RfD values derived from this combined method, since pharmacokinetic differences between rats and humans were accounted for using a validated PBPK model (Corley et al., 1997 1994). High confidence is placed on the NTP (2000) study because it was a chronic study, it employed both male and female rats and mice, it had a wide range of exposure levels, and animals were observed twice daily. Medium-to-high confidence is placed on the database, because data are available for a variety of animal species, including humans. While the database lacks long-term human studies, the available short-term human controlled studies and case reports and laboratory animal and in vitro studies provide ample evidence to suggest that, with respect to the hemolytic effects of EGBE, long-term human exposures would be no more adverse than long-term rat exposures. Confidence is not high, because the potential for effects in humans from repeat, long-term exposures has not been investigated.

A value of 10 was selected to account for variation in sensitivity within the human population (UF_H). Potentially susceptible subpopulations include individuals with enhanced metabolism or decreased excretion of BAA and individuals whose RBC membranes are more susceptible to the BAA-induced hemolysis, the principal precursor event to the critical effect of hemosiderin deposition in the liver. A UF of 10 was retained to account for the uncertainty associated with the variability of the human response to the effects of EGBE. Human responses to in vivo EGBE exposure have not been observed in a broad enough range of exposure conditions (e.g., repeat or long-term exposures) and potentially sensitive subjects (e.g., individuals predisposed to hemolytic anemia, infants) to warrant the reduction of the UF_H below the default value of 10. For a more detailed discussion of the RfD UF, see Section 5.2.3.

Information regarding the reported liver and forestomach tumors observed in laboratory animals exposed to EGBE indicates that the modes of action underlying these lesions are nonmutagenic and include intermediate processes that have nonlinear dose-response characteristics. Control or omission of these intermediate events would likely be sufficient to prevent the occurrence of such tumors in humans, including potentially sensitive subpopulations such as children.

[11] Application of nonlinear quantitative assessment methods indicate that the noncancer RfD (0.1 mg/kg/day) and RfC (1.6 mg/m^3) values developed for EGBE are considered protective

[11]These analyses are consistent with the nonlinear assessment approach described in the 2005 cancer guidelines (U.S. EPA, 2005).

of these key events and would serve to prevent the occurrence of carcinogenic effects in humans. The exposure concentrations that would be necessary to cause these effects in humans, if attainable at all, are likely to be much higher than the RfC and RfD.

The estimation of uncertainty in this analysis is based on the alternative approaches for estimating the dose response that are discussed and shown in Sections 5.1.3 and 5.2.3 for the RfC and RfD, respectively. These alternatives include using the NOAEL approach combined with the measure of the internal dose estimated with the PBPK model and the BMC approach combined with the same PBPK model. Table 5-9 and Table 5-14 show the uncertainty associated with each of these approaches for the RfC and RfD, respectively. Other uncertainties associated with the noncancer and cancer assessments presented in this toxicological review are summarized in Table 5-15 and in Sections 5.4.1 and 5.5.

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Appendix A
Corley et al. (1997, 1994) PBPK Model

Corley et al. (1994) developed physiologically based pharmacokinetic (PBPK) models for rats and humans with the primary objective of describing the concentration of 2-butoxyacetic acid (BAA) in the target tissue (blood) of rats and humans for use in risk assessment (Figure B-1). The models incorporate allometrically scalable physiological and biochemical parameters (e.g., blood flows, tissue volumes, and metabolic capacity) in place of the standard values for a 70 kg human. These parameters normalize standard values to the actual body weights of the subjects in several human kinetic studies. The physiology of humans under exercise conditions was maintained in the model. The rat was included to expand the database for model validation and to assist in interspecies comparisons of target tissue doses (BAA in blood).

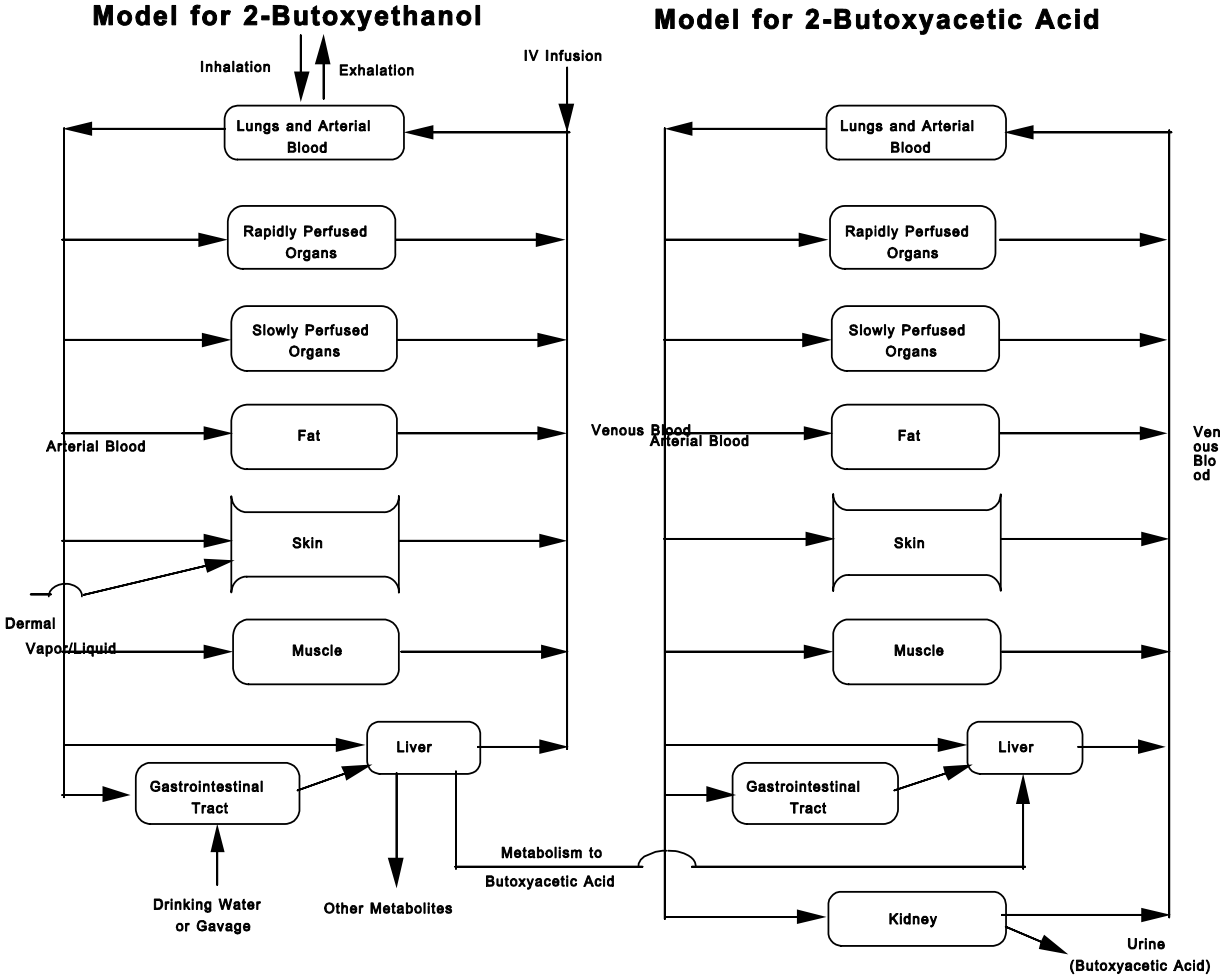


Figure A-1. PBPK model of Corley et al. (1994). The formation of BAA from ethylene glycol monobutyl ether was assumed to occur only in the liver and was simulated in a second model linked via the formation of BAA.

Source: Corley et al. (1994).

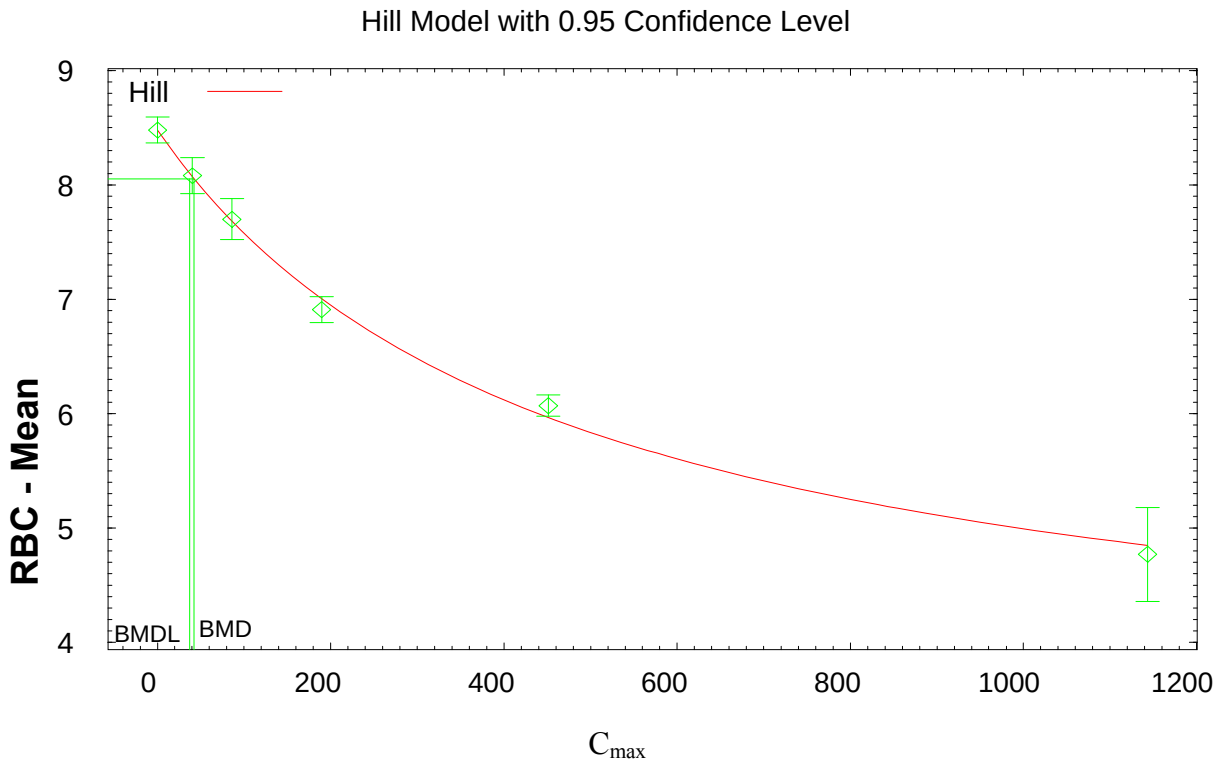
The Corley et al. (1994) model included additional routes of exposure such as oral (gavage), drinking water, intravenous (i.v.) infusion, and dermal (liquids and vapor). The formation of BAA was assumed to occur only in the liver, using the rat liver perfusion data of Johanson et al. (1986) scaled to the human. A second model was linked to the ethylene glycol monobutyl ether (EGBE) model specifically to track the disposition of BAA following its formation in the liver. The kidney was added to the BAA model, because it is the organ of elimination for BAA. All other metabolic routes for EGBE (formation of ethylene glycol [EG] and glucuronide conjugate) were combined, as they were used only to account for the total disposition of EGBE in the rat metabolism studies and not for cross-species extrapolations. Contrary to observations in rats, Corley et al. (1997) found no evidence of metabolites in urine that would indicate that humans form conjugates of EGBE or EG. Thus, these pathways, which were lumped together in the model of Corley et al. (1994) to simulate rat kinetic data, were eliminated for human simulations. The human blood:air partition coefficient of 7,965, from Johanson and Dynesius (1988), was also used in the Corley et al. (1994) model. In addition, the partition coefficients for both EGBE and BAA were measured in human blood, rat blood, and rat tissues by using a modification of the Jepson et al. (1994) technique for ultrafiltration. Human tissue:blood partition coefficients were assumed to be equal to those of the rat. The skin:air partition coefficient, used to calculate the dermal uptake of vapors, was assumed to be the same as the blood:air partition coefficient. With the exception of the lung:blood partition coefficient for EGBE (11.3), the tissue:blood partition coefficients ranged from 0.64–4.33 for EGBE and 0.77–1.58 for BAA. Protein binding of BAA in blood and saturable elimination of BAA by the kidneys were necessary components to describe the BAA kinetic data in rats and humans as discussed above. Since no direct measurements of protein binding were available, these parameters were arbitrarily set to the molar equivalent values reported for phenolsulfonphthalein as described by Russel et al. (1987). Constants for the saturable elimination of BAA by the kidneys were then estimated by optimization from the data of Ghanayem et al. (1990), where rats were administered EGBE i.v. and the concentrations of BAA in blood were determined, following three different dose levels. These parameters were then held constant (protein binding) or scaled by (body weight) $0.74 \times$ (renal elimination) for all simulations. Significant increases in the concentrations of EGBE were observed by Corley et al. (1997) in the first postexposure blood samples. Because the subjects were able to move their arms freely after the exposure, Corley et al. (1997) hypothesized that the local blood flow to the exposed arm increased for a few minutes postexposure. By adjusting the blood flow to the skin by fourfold for 5 minutes postexposure, the model is able to simulate this change in the concentration of EGBE in blood.

Table A-1. Selected parameters used in the PBPK model for EGBE developed by Corley et al. (1994)

Parameter	Human	Rat
Weights		
Body weight (kg)	70	0.23
Liver	3.14%	2.53
Rapidly perfused (BAA model)	3.71% 3.27%	5.1 4.39%
Slowly perfused	9.4	24.8
Flows (L/hour)		
Alveolar ventilation	347.9	5.06
Cardiac output (COP)	347.9	5.06
Liver (% COP)	25.0	25.0
Rapidly perfused (% COP) (BAA model)	50.0 25.0	51.0 26.0
Slowly perfused (% COP)	2	2
Partition coefficients		
Blood/air	7,965	7,965
Liver/blood (BAA model)	1.46 1.30	1.46 1.30
Rapidly perfused/blood (BAA model)	1.46 1.30	1.46 1.30
Slowly perfused/blood (BAA model)	0.64 1.31	0.64 1.31
Metabolic constants		
EGBE to BAA	375	375
K_{m1} (mg/L)	26.9	26.9

Appendix B

Text Output from Benchmark Dose Software Runs Used in the Derivation of RfC and RfD Values




```
==========================================================================

        Hill Model, Version Number: 1.4.1
        Input Data File: C:\BMDS\DATA\EGBE\REDBLOODCELL_F.(D)
       Tue Mar 06 09:07:25 2007
==========================================================================
BMD Method for RfC: Red blood cell count in female rats versus Cmax BAA, 14 week inhalation
study (NTP, 2000)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the response function is:
 Y[dose] = intercept + v*dose^n/(k^n + dose^n)

 Dependent variable = MEAN
 Independent variable = CmaxInternalD
 Power parameter restricted to be greater than 1
 The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))
```

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
lalpha = -3.17322
rho = 0
intercept = 8.48
v = -3.71
n = 1.07589
k = 278.325

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

lalpha rho intercept v k

lalpha 1 -1 0.11 0.2 -0.21

rho -1 1 -0.11 -0.2 0.21

intercept 0.11 -0.11 1 0.27 -0.61

v 0.2 -0.2 0.27 1 -0.88

k -0.21 0.21 -0.61 -0.88 1

Parameter Estimates

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
lalpha -1.10102 2.15389 -5.32256 3.12053
rho -1.07311 1.09214 -3.21366 1.06743
intercept 8.47695 0.0493243 8.38028 8.57363
v -5.12159 0.285841 -5.68183 -4.56135
n 1 NA
k 468.055 60.889 348.715 587.395

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N Obs	Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	8.48	8.48	0.16	0.183	0.0526
40.4	10	8.08	8.07	0.22	0.188	0.168
85.9	10	7.7	7.68	0.25	0.193	0.282
189.5	10	6.91	7	0.16	0.203	-1.42
451.3	9	6.07	5.96	0.12	0.221	1.45
1143	5	4.77	4.84	0.33	0.247	-0.66

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2
Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	61.856962	7	-109.713924
A2	66.476628	12	-108.953256
A3	62.170236	8	-108.340472
fitted	59.636147	5	-109.272293
R	-34.183663	2	72.367326

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test -2*log(Likelihood Ratio) Test df p-value

Test 1 201.321 10 <.0001
Test 2 9.23933 5 0.09989
Test 3 8.61278 4 0.07154
Test 4 5.06818 3 0.1669

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is greater than .1. The model chosen seems to adequately describe the data

Benchmark Dose Computation

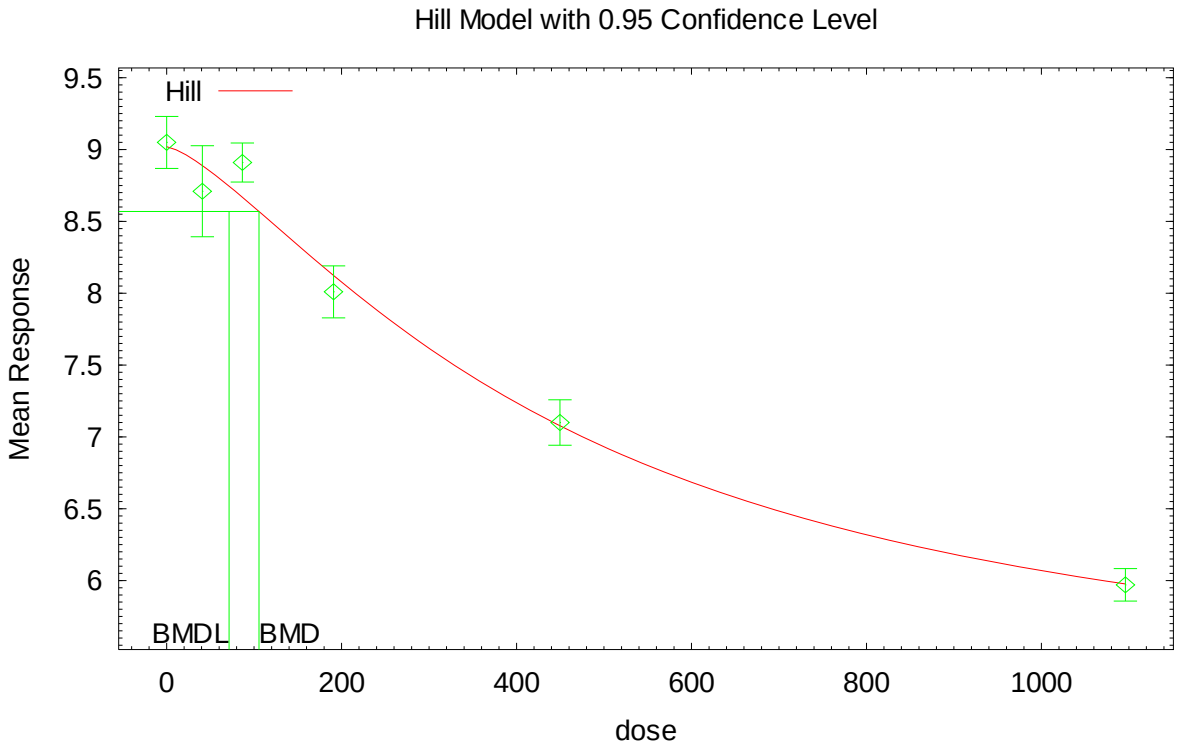
Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

BMD = 42.2297

BMDL = 37.1792



16:39 07/05 2007

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Hill Model. (Version: 2.12; Date: 02/20/2007)
Input Data File: U:\BMDS\DATA\EGBE\EGBE_M_14WK.(d)
Gnuplot Plotting File: U:\BMDS\DATA\EGBE\EGBE_M_14WK.plt
Thu Jul 05 16:39:03 2007

==========

BMD Method for RfC: Red blood cell count in male rats versus C_{max} BAA, 14 week inhalation study (NTP, 2000)

~~~~~~

The form of the response function is:

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = C_{max}-90d
Power parameter restricted to be greater than 1
The variance is to be modeled as Var(i) = exp(lalpha + rho * ln(mean(i)))

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
~~~~~~

Default Initial Parameter Values
lalpha = -2.62607
rho = 0
intercept = 9.05
v = -3.08
n = 1.56979
k = 333.008

Asymptotic Correlation Matrix of Parameter Estimates

lalpha rho intercept v n k

lalpha 1 -1 0.0097 0.064 0.075 -0.09

rho -1 1 -0.014 -0.064 -0.075 0.091

intercept 0.0097 -0.014 1 -0.63 -0.64 0.25

v 0.064 -0.064 -0.63 1 0.96 -0.9

n 0.075 -0.075 -0.64 0.96 1 -0.82

k -0.09 0.091 0.25 -0.9 -0.82 1

Parameter Estimates

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
lalpha -11.3409 2.55713 -16.3527 -6.32896
rho 4.21018 1.23612 1.78742 6.63294
intercept 9.02037 0.0937743 8.83657 9.20416
v -3.96474 0.470028 -4.88597 -3.0435
n 1.39015 0.229376 0.940583 1.83972
k 462.11 80.7255 303.891 620.329

Table of Data and Estimated Values of Interest

Dose	N Obs	Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	9.05	9.02	0.253	0.353	0.265
40.8	10	8.71	8.89	0.443	0.343	-1.65
86.6	10	8.91	8.67	0.19	0.325	2.35
190.7	10	8.01	8.12	0.253	0.283	-1.27
449.7	10	7.1	7.08	0.221	0.212	0.366
1096	10	5.97	5.97	0.158	0.148	-0.0614

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	51.943035	7	-89.886070
A2	58.944827	12	-93.889653
A3	54.680821	8	-93.361643
fitted	49.559802	6	-87.119604
R	-37.662661	2	79.325322

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	193.215	10	<.0001
Test 2	14.0036	5	0.01559
Test 3	8.52801	4	0.07404
Test 4	10.242	2	0.00597

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .1. You may want to consider a different variance model

The p-value for Test 4 is less than .1. You may want to try a different model

Benchmark Dose Computation

Specified effect = 0.05

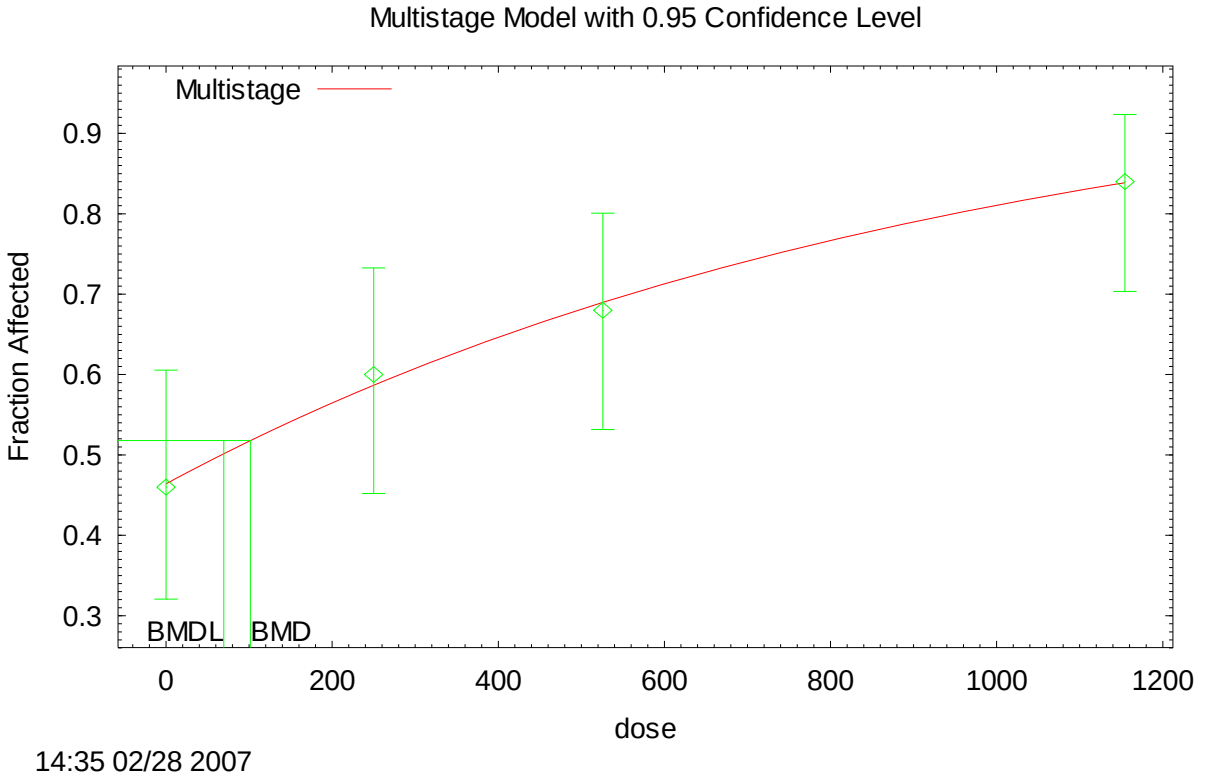
Risk Type = Relative risk

Confidence level = 0.95

BMD = 105.535

BMDL = 71.4622

==========



```
Multistage Model, Version Number: 1.4.1
          Input Data File: C:\BMDS4ME\DATA\\EGBE\HEMOSIDERIN_M.(D)
          Wed Feb 28 01:46:52 2007
================================================================================
 BMD Method for RfC: Hemosiderin deposition in male rats versus AUC BAA, 2 year inhalation
study (NTP, 2000)
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~


 The form of the probability function is:

 P[response] = background + (1-background)*[1-EXP(
 -beta1*dose^1)]

 The parameter betas are restricted to be positive


Dependent variable = Hemosiderin_M
Independent variable = MAUCDose3mo

 Total number of observations = 4
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



 Default Initial Parameter Values
 Background = 0.464249
 Beta(1) = 0.00104028


 Asymptotic Correlation Matrix of Parameter Estimates

 Background Beta(1)

Background 1 -0.67

 Beta(1) -0.67 1


 Parameter Estimates
```

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.464473 0.0851405 0.297601 0.631346
Beta(1) 0.00103871 0.000331559 0.000388869 0.00168856

Analysis of Deviance Table

Model Log(likelihood) # Param's Deviance Test d.f. P-value
Full model -121.475 4
Fitted model -121.506 2 0.0626604 2 0.9692
Reduced model -130.097 1 17.2453 3 0.0006292

AIC: 247.012

Goodness of Fit
Scaled
Dose Est._Prob. Expected Observed Size Residual

0.0000 0.4645 23.224 23 50 -0.063
250.0000 0.5869 29.347 30 50 0.187
525.9000 0.6899 34.494 34 50 -0.151
1154.5000 0.8386 41.929 42 50 0.027

Chi^2 = 0.06 d.f. = 2 P-value = 0.9691

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

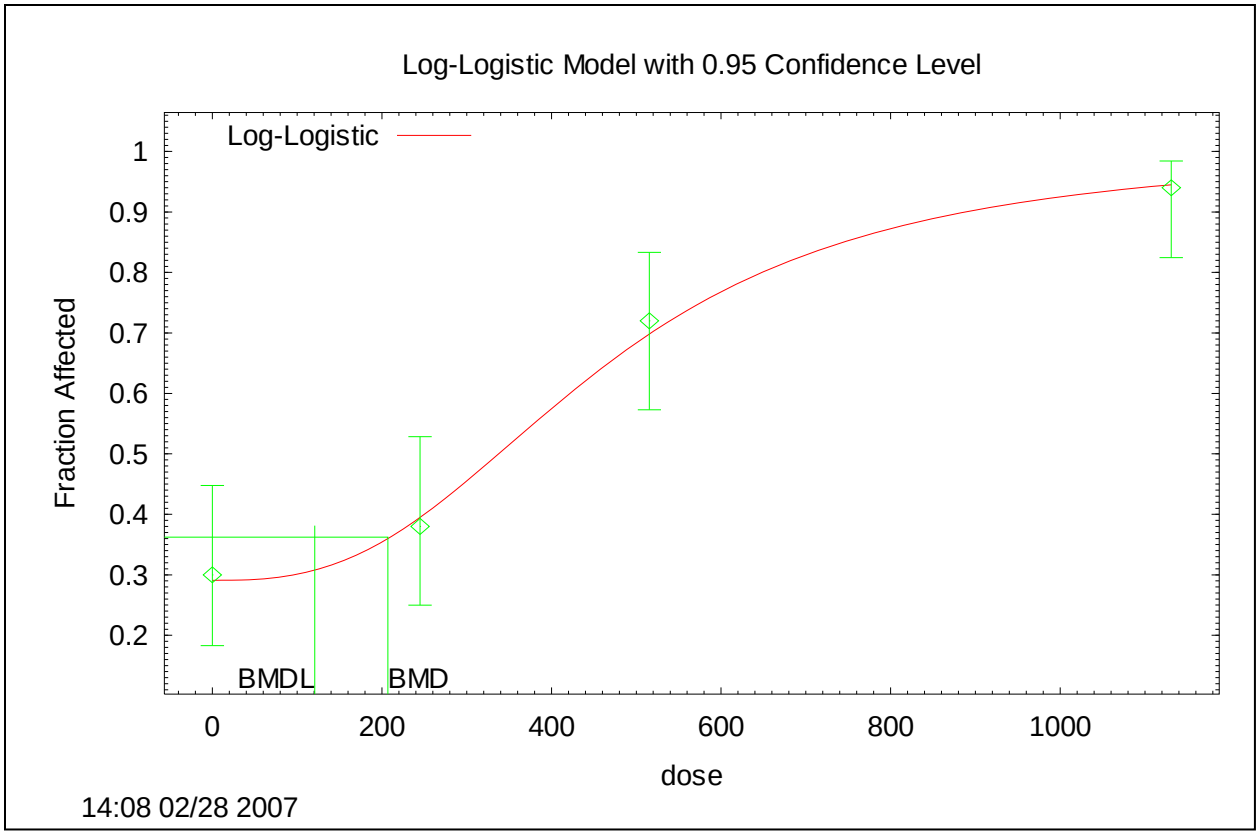
BMD = 101.434

BMDL = 69.6269

BMDU = 174.647

Taken together, (69.6269, 174.647) is a 90% two-sided confidence
interval for the BMD

==========



Log-Logistic Model, Version Number: 1.4.1
Input Data File: C:\BMDS4ME\DATA\\EGBE\HEMOSIDERIN_F.(D)
Wed Feb 28 02:03:32 2007

BMD Method for RfC: Hemosiderin deposition in female rats versus AUC BAA, 2 year inhalation study (NTP, 2000)

~~~~~~

The form of the probability function is:

P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = Hemosiderin_F
Independent variable = FAUCDose3mo
Slope parameter is restricted as slope >= 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model
~~~~~~

Default Initial Parameter Values
background = 0.3
intercept = -17.8009
slope = 2.88261

Asymptotic Correlation Matrix of Parameter Estimates

background intercept slope

background 1 -0.39 0.35

intercept -0.39 1 -1

slope 0.35 -1 1

Parameter Estimates

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
background 0.291372 0.0594025 0.174945 0.407799
intercept -16.9704 4.29214 -25.3829 -8.55798
slope 2.77044 0.669882 1.45749 4.08338

Analysis of Deviance Table

Model Log(likelihood) # Param's Deviance Test d.f. P-value
Full model -104.742 4
Fitted model -104.839 3 0.192188 1 0.6611
Reduced model -135.725 1 61.9658 3 <.0001

AIC: 215.677

Goodness of Fit
Scaled
Dose Est._Prob. Expected Observed Size Residual

0.0000 0.2914 14.569 15 50 0.134
245.0000 0.3981 19.906 19 50 -0.262
515.5000 0.7039 35.193 36 50 0.250
1131.0000 0.9466 47.332 47 50 -0.209

Chi^2 = 0.19 d.f. = 1 P-value = 0.6606

Benchmark Dose Computation

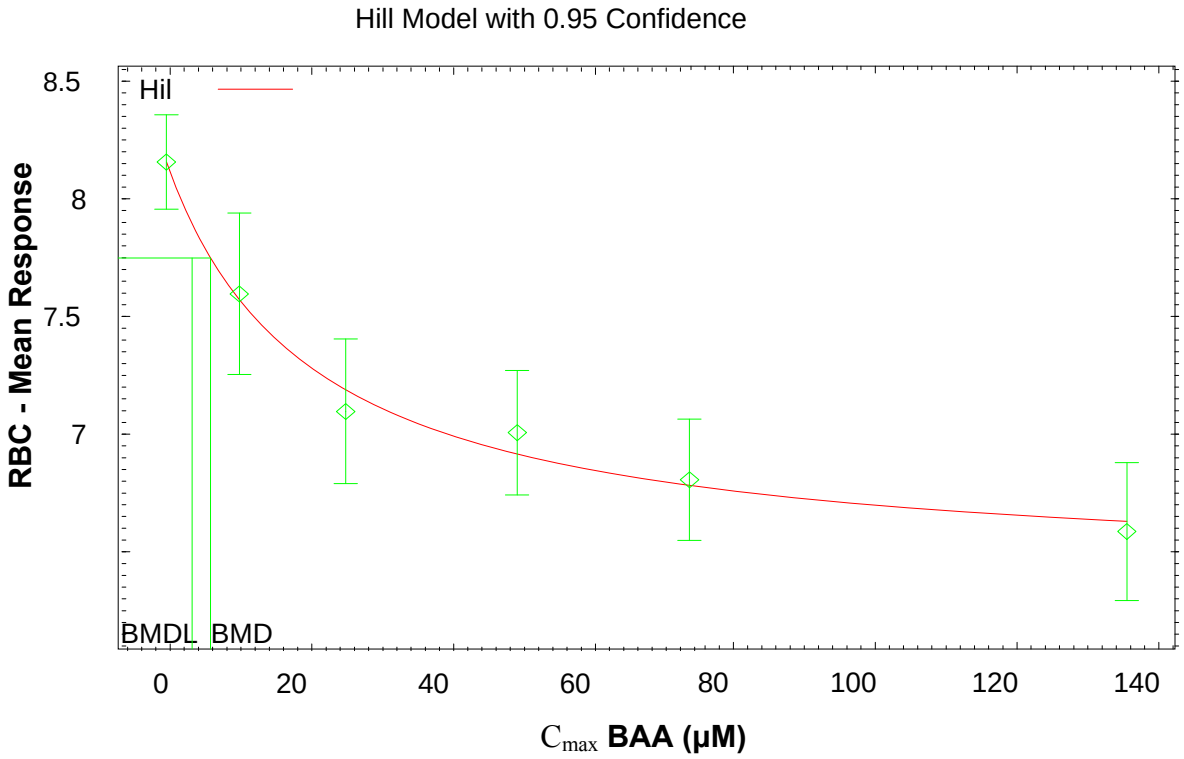
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 206.942

BMDL = 120.82



==========

Hill Model. (Version: 2.12; Date: 02/20/2007)
Input Data File: U:\BMDS\DATA\EGBE\EGBEORAL.(d)
Gnuplot Plotting File: U:\BMDS\DATA\EGBE\EGBEORAL.plt
Wed Mar 07 08:21:49 2007

==========

BMD Method for RfD: RBC Response in Orally Exposed Female Rats (NTP, 1993)

~~~~~~

The form of the response function is:
~~~~~~

Modeling Results Using 1SD as the BMR for Blood Effects

Table 5-5 and Table 5-13 present the modeling results for the potential blood effect endpoints considered for deriving a reference concentration (RfC) and reference dose (RfD) by using a benchmark response (BMR) of 5% relative change in the mean. The following tables present the same effects using a BMR of 1 standard deviation (SD). A discussion for why this BMR was not considered the best choice can be found in Section 5.1.2.2.1 for the RfC and in Section 5.2.2.2.1 for the RfD.

Table B-1. Comparison of BMC/BMCL values for female rat RBC count data from inhalation subchronic study (14 week) using modeled blood C_{max} (3 months) of the EGBE metabolite BAA as a common dose metric

Model	BMC_{1SD} (µM)	$BMCL_{1SD}$ (µM)	*p* Value	AIC*	Scaled residual**
2nd degree polynomial	31.5363	22.6222	<0.0001	–90.947514	0.15
Power	41.9568	33.8953	<0.0001	–52.341720	0.218
Hill***	17.361	13.476	0.1669	–109.272293	0.168

*AIC = Akaike Information Criterion = –2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters and P is the number of model degrees of freedom (usually the number of parameters estimated).
**Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMC scaled by an estimate of its SD. Provides a comparative measure of model fit near the BMC. Residuals that exceed 2.0 in absolute value should cause questioning of model fit in this region.
**Model choice based on adequate p value (>0.1), visual inspection, low AIC, and low (absolute) scaled residual.

Table B-2. Comparison of BMC/BMCL values for female rat RBC count and MCV from an oral subchronic study using modeled blood C_{max} (3 months) of the EGBE metabolite AA as a common dose metric

Model	BMC_{1SD} (µM)	$BMCL_{1SD}$ (µM)	*p* Value	AIC*	Scaled residual**
RBC count					
1st degree polynomial	474.233	374.108	<0.0001	–26.323	−1.35
Power	474.233	374.108	<0.0001	–26.323	−1.35
Hill***	56.5593	32.0476	0.7038	–48.884	0.204
MCV					
1st degree polynomial	108.691	76.203	<0.0001	141.85	−0.223
Power	108.691	76.203	<0.0001	141.85	−0.223
Hill	40.516	failed	0.1768	120.94	–0.317

*AIC = –2L + 2P, where L is the log-likelihood at the maximum likelihood estimates for the parameters and P is the number of model degrees of freedom (number of parameters estimated).
**Chi-square residual (measure of how model predicted response deviates from the actual data) for the dose group closest to the BMC scaled by an estimate of its SD. Provides a comparative measure of model fit near the BMC. Residuals that exceed 2.0 in absolute value should cause questioning of model fit in this region.
***Model choice based on adequate *p* value (>0.1), visual inspection, low AIC, and low (absolute) scaled residual.

Source: NTP (1993).

==========

Multistage Cancer Model. (Version: 1.5; Date: 02/20/2007)

Y[dose] = intercept + v*dose^n/(k^n + dose^n)

Dependent variable = MEAN
Independent variable = C_{max}
rho is set to 0
Power parameter restricted to be greater than 1
A constant variance model is fit

Total number of dose groups = 6
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
alpha = 0.154717
rho = 0 Specified
intercept = 8.15
v = -1.57
n = 1.30181
k = 170.5

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -rho -n
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

alpha intercept v k

alpha 1 1.4e-009 1.2e-007 -7.3e-008

intercept 1.4e-009 1 -0.41 -0.51
v 1.2e-007 -0.41 1 -0.48

k -7.3e-008 -0.51 -0.48 1

Parameter Estimates

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
alpha 0.142549 0.0260259 0.0915397 0.193559
intercept 8.15021 0.117707 7.91951 8.38092

v -1.75852 0.187534 -2.12608 -1.39096
n 1 NA
k 206.872 79.7468 50.5715 363.173

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Table of Data and Estimated Values of Interest

Dose	N Obs	Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	8.15	8.15	0.28	0.378	-0.0018
103	10	7.59	7.57	0.48	0.378	0.204
253	10	7.09	7.18	0.43	0.378	-0.777
495	10	7	6.91	0.37	0.378	0.754
738	10	6.8	6.78	0.36	0.378	0.195
1355	10	6.58	6.62	0.41	0.378	-0.374

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	29.145609	7	-44.291218
A2	30.744881	12	-37.489762
A3	29.145609	7	-44.291218
fitted	28.441981	4	-48.883963
R	-3.574142	2	11.148285

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test -2*log(Likelihood Ratio) Test df p-value

Test 1 68.638 10 <.0001
Test 2 3.19854 5 0.6694
Test 3 3.19854 5 0.6694
Test 4 1.40726 3 0.7038

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Relative risk

Confidence level = 0.95

BMD = 62.3999

BMDL = 36.2595

```
        Input Data File: C:\BMDS\Data\EGBE_M_HC.(d)
        Gnuplot Plotting File: C:\BMDS\Data\EGBE_M_HC.(d)
       Thu Feb 21 10:55:46 2008
================================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

The parameter betas are restricted to be positive


Dependent variable = Hep_Car_ADJ_M
Independent variable = AUC_Mouse_M

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 4
Total number of specified parameters = 0
Degree of polynomial = 3


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008



Default Initial Parameter Values
Background = 0.209552
Beta(1) = 0.000187331
Beta(2) = 0
Beta(3) = 0


Asymptotic Correlation Matrix of Parameter Estimates

( *** The model parameter(s) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix )

Background Beta(1)

Background 1 -0.71

```

Beta(1) -0.71 1

Parameter Estimates

95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.195776 * * *
Beta(1) 0.000198015 * * *
Beta(2) 0 * * *
Beta(3) 0 * * *

* - Indicates that this value is not calculated.

Warning: Likelihood for the fitted model larger than the Likelihood for the full model.
Error in computing chi-square; returning 2

Analysis of Deviance Table

Model Log(likelihood) # Param's Deviance Test d.f. P-value
Full model -119.553 4
Fitted model -118.389 2 -2.32685 2 2
Reduced model -124.691 1 10.2756 3 0.01636

AIC: 240.779

Goodness of Fit
Scaled
Dose Est._Prob. Expected Observed Size Residual

0.0000 0.1958 9.867 10 50 0.189
402.0000 0.2573 12.981 11 50 -0.493
925.0000 0.3304 16.209 19 49 0.866
2120.0000 0.4715 23.334 22 49 -0.240

Chi^2 = 1.09 d.f. = 2 P-value = 0.5809

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 532.083

BMDL = 333.797

BMDU = 1475.14

Taken together, (333.797, 1475.14) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000299583

Appendix C

Benchmark Dose Assessment of Forestomach Lesions in Female Mice Using PBPK Models to Estimate Human Equivalent Exposures

Several PBPK models have been developed for EGBE, all of which are capable of estimating internal doses. These models are summarized briefly in Section 5 of this assessment. Consistent with the assessment approach outlined in Section 5, the Lee et al. (1998) model was used to estimate internal dose levels from inhalation exposures to the experimental animal, in this case the female mouse.

Peak concentration in the blood during the exposure period (C_{max}) of BAA was used in the derivation of the RfC when using hematological endpoints because of (1) convincing evidence that BAA is the causative agent for EGBE-induced hemolysis and (2) EGBE-induced hemolysis appears to be highly dependent on the BAA concentration attained. Area under the curve was used in the derivation of the RfC during use of the hemosiderin deposition as the critical endpoint, because this endpoint increases in severity with increasing duration of exposure and because it is representative of a continuous process and not a one-time event.

C_{max} of blood BAA is considered a reasonable measure of internal dose for the forestomach lesions reported from EGBE exposure. BAA is believed to be the toxic moiety responsible for the forestomach effects observed following EGBE exposure, with concentration (rather than AUC) appearing to be determinative in the development of these effects as well (Corley et al., 2005b). Other information supportive of the C_{max} as an appropriate metric include the findings of NTP (1993), where no signs of forestomach irritation were observed in mice at very high dose levels of 1,400 mg/kg-day in 2-week and 13-week drinking-water studies conducted by NTP (1993). It is likely that such oral non-bolus dosing of EGBE does not result in high enough local concentrations of EGBE and BAA (Poet et al., 2003) to produce irritation. Consistent with this observation are results with other forestomach carcinogens that are not mutagenic, demonstrating that forestomach effects are dependent not only on the dose but also on the chemical concentration in the dosing solution (Ghanayem et al., 1985).

The blood level of BAA is apparently a valid surrogate for the local (i.e., forestomach) BAA concentration as blood levels of BAA follow the severity of the irritant and irritant-associated hyperplastic responses in the forestomach. Further, this relationship between blood BAA levels and irritant response holds true for routes of EGBE administration other than oral, (e.g., inhalation, intraperitoneal, and i.v.) (Green et al., 2002; Corley et al., 1999). The basis for this route-independent response may be related to the Green et al. (2002) results from whole-

body autoradiography of mice exposed via inhalation, showing appreciable amounts of EGBE-associated radioactivity being present in salivary glands and ducts.

Plausible evidence exists for considering the incidence of forestomach tumors in female mice, following chronic inhalation exposure to EGBE to occur via a nonlinear, nongenotoxic mode of action. EGBE appears to be one of a group of compounds that are not mutagenic but can indirectly cause forestomach tumors through the sustained cytotoxicity and cell regeneration brought about by irritant and irritant-associated hyperplastic effects and breakdown of the forestomach's gastric mucosal barrier. This sequence of events is considered obligate for the formation of the observed neoplasms. Strategies intended to control or omit any of these key mode-of-action events, including the initial hyperplastic event, would interrupt the process and prevent formation of neoplasms. While this mode of action may be of qualitative relevance to humans, the exposure concentrations that would be necessary to cause these hyperplastic effects and resultant tumors in humans, if attainable, are likely to be much higher than the concentrations necessary to cause forestomach effects in mice, primarily because humans lack a comparable organ for storage and long-term retention of EGBE.

Another line of reasoning that may be used to address issues relating to the occurrence of these irritant/hyperplastic lesions (and potential for progression) in humans is to order the dose-response among those effects noted for EGBE, most prominently the hematologic effects that underlie the hepatic tumors and that are the basis of the oral RfD and inhalation RfC. A benchmark dose (BMD) analysis of the irritant/hyperplastic response observed in the NTP (2000) follows.

The endpoint used in this analysis was epithelial hyperplasia of the female mouse forestomach, since it was the most sensitive forestomach effect observed in the NTP (2000) study. Consistent with the 1999 IRIS assessment, four steps were employed to estimate human equivalent oral and inhalation benchmark exposures from this endpoint: (1) a $BMDL_{10}$ value was estimated using modeled "end-of-the-week" internal dose (C_{max} BAA in blood) levels, (2) verify that steady state was achieved (e.g., no change in BAA C_{max} as a result of prolonging the exposure regimen), (3) simulate the internal dose surrogate (C_{max} BAA in blood) for humans (continuous air exposure; drinking water assumption was that a 70 kg human consumes an average of 2 L of water during a 12-hour awake cycle), and (4) calculate the human equivalent dose/concentration that resulted in the same internal dose (C_{max} BAA) simulated for the animal in Step 1.

Step 1: Estimation of $BMDL_{10}$ (C_{max}) dose.

C_{max} for BAA in arterial blood was determined using the PBPK model of Lee et al. (1998). The model results and incidence data for the endpoint of concern are summarized in Table C-1.

Table C-1. PBPK model estimates of BAA C_{max} blood levels and incidence of forestomach epithelial hyperplasia in female mice

Air concentration (ppm)	C_{max} BAA (µM)	Incidence of forestomach hyperplasia
0	0	0/50
62.5	529	6/50
125	1,200	27/50
250	2,620	42/49

BMD and $BMDL_{10}$ estimates were derived using the available models in version 1.3.2 of the EPA benchmark dose software (BMDS). The estimates for each model, along with statistical goodness-of-fit information, are provided in Table C-2.

Table C-2. BMDS model estimates of C_{max} BMD_{10} and $BMDL_{10}$ values for forestomach epithelial hyperplasia in female mice

BMDS model	BMD (µM)	BMDL (µM)	AIC (lowest = best fit)	*p* Value (>0.1 = adequate fit)
Gamma	420.56	266.87	151.16	0.5287
Logistic	544.757	444.896	162.191	0.0067
Log-logistic	462.513	329.04	150.153	0.8717
Multistage (1st degree)	177.442	145.713	156.244	0.0648
Multistage (2nd degree)	338.483	202.437	152.681	0.0976
Multistage (3rd degree)	338.485	197.436	152.681	0.2535
Probit	525.521	430.612	161.304	0.0086
Log-probit	470.876	344.412	150.163	0.8673
Weibull	376.085	238.952	151.855	0.3807

Step 2: Verification of steady state.

As can be seen from Table C-3, C_{max} levels are relatively constant through 6 months then increase at and beyond 12 months, presumably due to clearance problems in aging animals. However, the earlier steady-state levels are appropriate for use in this assessment, because that is the more conservative approach and because similar effects were observed during the subchronic portion of the NTP (2000) study at the same dose levels, indicating that the higher internal doses at and beyond 12 months were not required for the effects to appear.

Table C-3. Female mouse C_{max} values for various time points of the NTP (2000) study estimated by the Lee et al. (1998) model

Months on study	62.5 ppm		125 ppm		250 ppm	
	Male	Female	Male	Female	Male	Female
1	403	529	921	1,200	2,080	2,620
3	402	527	925	1,202	2,120	2,652
6	399	523	914	1,184	2,071	2,582
12	484	639	1,079	1,414	2,349	2,951
16	643	849	1,443	1,839	2,798	3,501
18	756	995	1,625	2,102	3,067	3,803

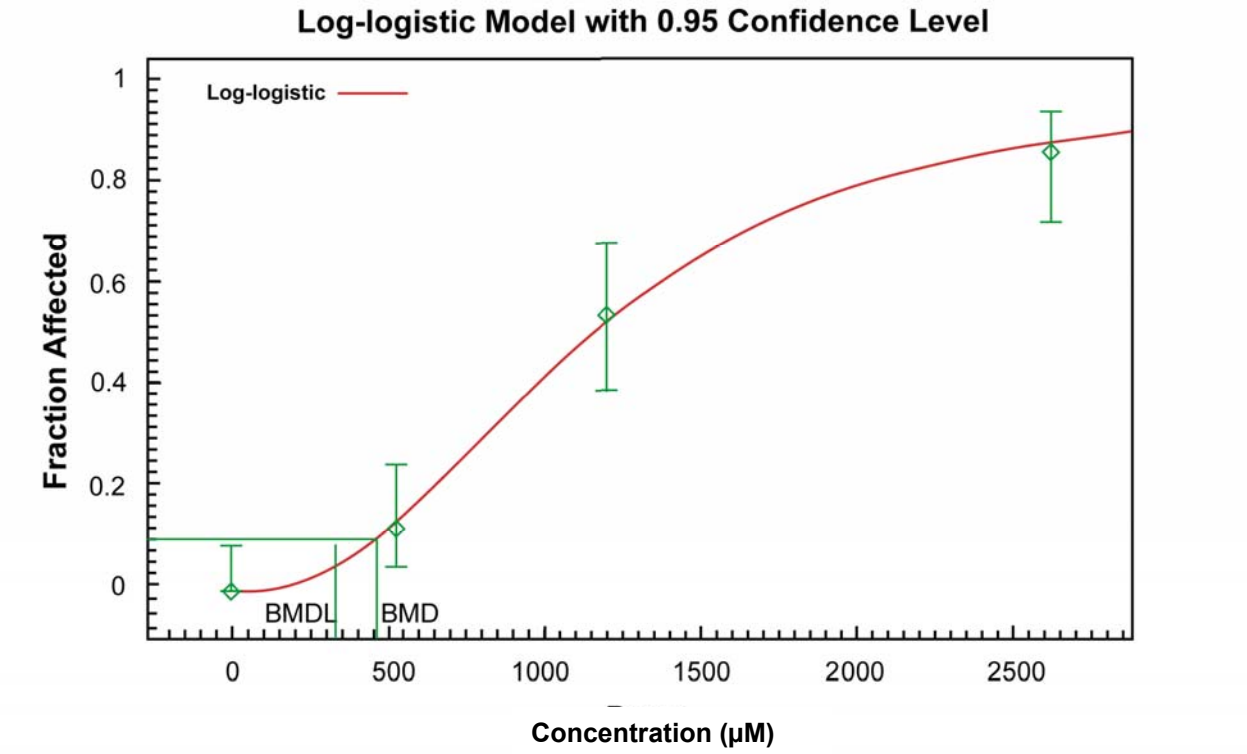


Figure C-1. BMD plot of fraction of female mice with forestomach epithelial hyperplasia following inhalation exposure (NTP, 2000) versus internal dose metric (BAA C_{max}, µM).

Step 3: Simulation of internal human doses.

The tables below summarize the results of model simulations of the internal dose surrogate (C_{max} BAA in blood) for a 70 kg human who consumes an average of 2 L of drinking water during a 12-hour awake cycle (Table C-4) or is continuously exposed to air concentrations (Table C-5) of EGBE.

Table C-4. Estimated C_{max} for BAA in blood for humans continuously exposed to varying drinking water concentrations of EGBE

EGBE concentration in water (ppm)	Calculated dose of EGBE from drinking water (mg/kg-day)	Cmax BAA in blood (µM)
24	0.7	9
48	1.4	18
94	2.7	36
188	5.4	73
375	10.7	147
750	21.4	299

Source: Corley et al. (1997, 1994).

Table C-5. Estimated C_{max} for BAA in blood for humans continuously exposed to varying concentrations of EGBE

Concentration of EGBE in air (ppm)	C_{max} BAA in blood (µM)
1	2.6
5	13.0
10	26.1
20	52.9
50	137.1
100	295.0
200	733.7

Source: Corley et al. (1997, 1994).

Step 4: Calculate the human equivalent dose/concentration

The Corley et al. (1997, 1994) PBPK model was used to back-calculate a human equivalent oral dose of 23.6 mg/kg-day from the C_{max} $BMDL_{10}$ of 320 µM estimated in step 1, assuming that mice and humans receive their entire dose of EGBE from drinking water over a 12-hour period each day. The Corley et al. (1997) PBPK model was used to back-calculate human equivalent air concentration of 551 mg/m^3 (113 ppm) from the C_{max} $BMDL_{10}$ of 320 µM estimated in step 1, assuming continuous exposure (24 hours/day).

Conclusion

The PODs calculated above are significantly higher than the PODs of 1 mg/kg-day and 12 mg/m^3 used to derive the RfD and RfC, respectively. Thus, these results indicate that the RfD and RfC values for EGBE, which were based on hemosiderin accumulation due to hemolytic effects in rats, should be adequate for the prevention of gastrointestinal hyperplastic effects as well.

Log-Logistic Model Results for Forestomach Epithelial Hyperplasia in Female Mice (NTP, 2000)

```
1
2   ================================================================
3            Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
4            Input Data File: F:\BMDS\DATA\EGBE\F_MOUSE_HYP_LOG-LOGIST.(d)
5            Gnuplot Plotting File: F:\BMDS\DATA\EGBE\F_MOUSE_HYP_LOG-LOGIST.plt
6           Fri Jul 11 19:53:31 2003
7   ================================================================
8
9   BMDS MODEL RUN
10  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
11
12   The form of the probability function is:
13
14   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]
15
16   Dependent variable = F_Hyperplasia
17   Independent variable = Dose
18   Slope parameter is restricted as slope >= 1
19
20   Total number of observations = 4
21   Total number of records with missing values = 0
22   Maximum number of iterations = 250
23   Relative Function Convergence has been set to: 1e-008
24   Parameter Convergence has been set to: 1e-008
25
26   User has chosen the log transformed model
27
28
29   Default Initial Parameter Values
30   background = 0
31   intercept = -16.768
32   slope = 2.36735
33
34   Asymptotic Correlation Matrix of Parameter Estimates
35
36   ( *** The model parameter(s) -background
37   have been estimated at a boundary point, or have been specified by the user,
38   and do not appear in the correlation matrix )
39
40   intercept slope
41
42   intercept 1 -1
43   slope -1 1
44
45   Parameter Estimates
```

Variable Estimate Std. Err.
background 0 NA
intercept -16.7132 2.64108
slope 2.36545 0.372243

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model Log(likelihood) Deviance Test DF P-value
Full model -72.9391
Fitted model -73.0765 0.274637 2 0.8717
Reduced model -131.841 117.804 3 <.0001

AIC: 150.153

Goodness of Fit

Scaled
Dose Est._Prob. Expected Observed Size Residual

0.0000 0.0000 0.000 0 50 0
529.0000 0.1324 6.622 6 50 -0.2596
1200.0000 0.5145 25.725 27 50 0.3608
2620.0000 0.8705 42.653 42 49 -0.2777

Chi-square = 0.27 DF = 2 P-value = 0.8717

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 462.513

BMDL = 329.04