



TOXICOLOGICAL REVIEW

OF

**ETHYLENE GLYCOL
MONOBUTYL ETHER (EGBE)**

(CAS No. 111-76-2)

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

October 1999

U.S. Environmental Protection Agency
Washington, DC

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ETHYLENE GLYCOL MONOBUTYL ETHER (CAS No. 111-76-2)

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in the Integrated Risk Information System (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.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. 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.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 202-566-1676.

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In preparing the original draft of the U.S. EPA EGBE IRIS support document, EPA obtained valuable information and research contributions from the Chemical Manufacturers Association (CMA) Ethylene Glycol Ethers Panel, which collaborated with EPA scientists Jeffrey S. Gift, Annie M. Jarabek, and Vicki L. Dellarco in a workshop effort to produce an extensive EGBE health assessment review and support document (U.S. EPA, 1997). The current document and summary information on IRIS have received peer review both by EPA scientists and by independent scientists external to EPA (U.S. EPA, 1994c). Subsequent to external review and incorporation of comments, this assessment has undergone an Agency-wide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects, such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but it provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m³.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. 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 are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m³ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for ethylene glycol monobutyl ether (EGBE) has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1995a), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), and *Proposed Guidelines for Carcinogen Risk Assessment* (1996a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *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); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

EGBE is also known as 2-butoxyethanol. Some relevant physical and chemical properties of EGBE are listed below.

CASRN: 111-76-2

Empirical formula: $C_4H_9O-CH_2CH_2-OH$

Molecular weight: 118.2

Vapor pressure: ≈ 0.88 mm Hg at $25^\circ C$

Water solubility: miscible

Log K_{ow} : 0.81

Henry's Law constant: $2.08 \times 10^{-7} - 2.08 \times 10^{-8}$ atms/m³/mole ($25^\circ C$)

Flash point: $62^\circ C$ (closed cup); $70^\circ C$ (open cup)

Conversion factor: 1 ppm = 4.83 mg/m^3 , $1.0 \text{ mg/m}^3 = 0.207$ ppm

EGBE exists as a colorless liquid at ambient temperature and pressure. Its evaporation rate, relative to butyl acetate, is 0.08, and EGBE is therefore 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, it is unlikely that EGBE bioaccumulates. Based upon the magnitude of Henry's Law constant, it is anticipated that partitioning of EGBE between water and air greatly favors the water phase.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

In laboratory animals, EGBE is absorbed following inhalation, oral (gavage), or percutaneous administration, and it is distributed rapidly to all tissues via the blood stream. The uptake and metabolism of EGBE is essentially linear following a 6-hour inhalation exposure of up to 438 ppm, a concentration that caused mortality (Dill et al., 1998; Sabourin et al., 1992b). 2-Butoxyacetic acid (BAA) is the primary metabolite in rats following drinking water (Medinsky et al., 1990) and inhalation (Dill et al., 1998) exposures to EGBE. 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 either dermally or in the drinking water (Medinsky et al., 1990; Sabourin et al., 1992a; Shyr et al., 1993). Corley et al. (1997) report that 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, sex, age, time of exposure, and exposure concentration (NTP, 1998; Dill et al., 1998).

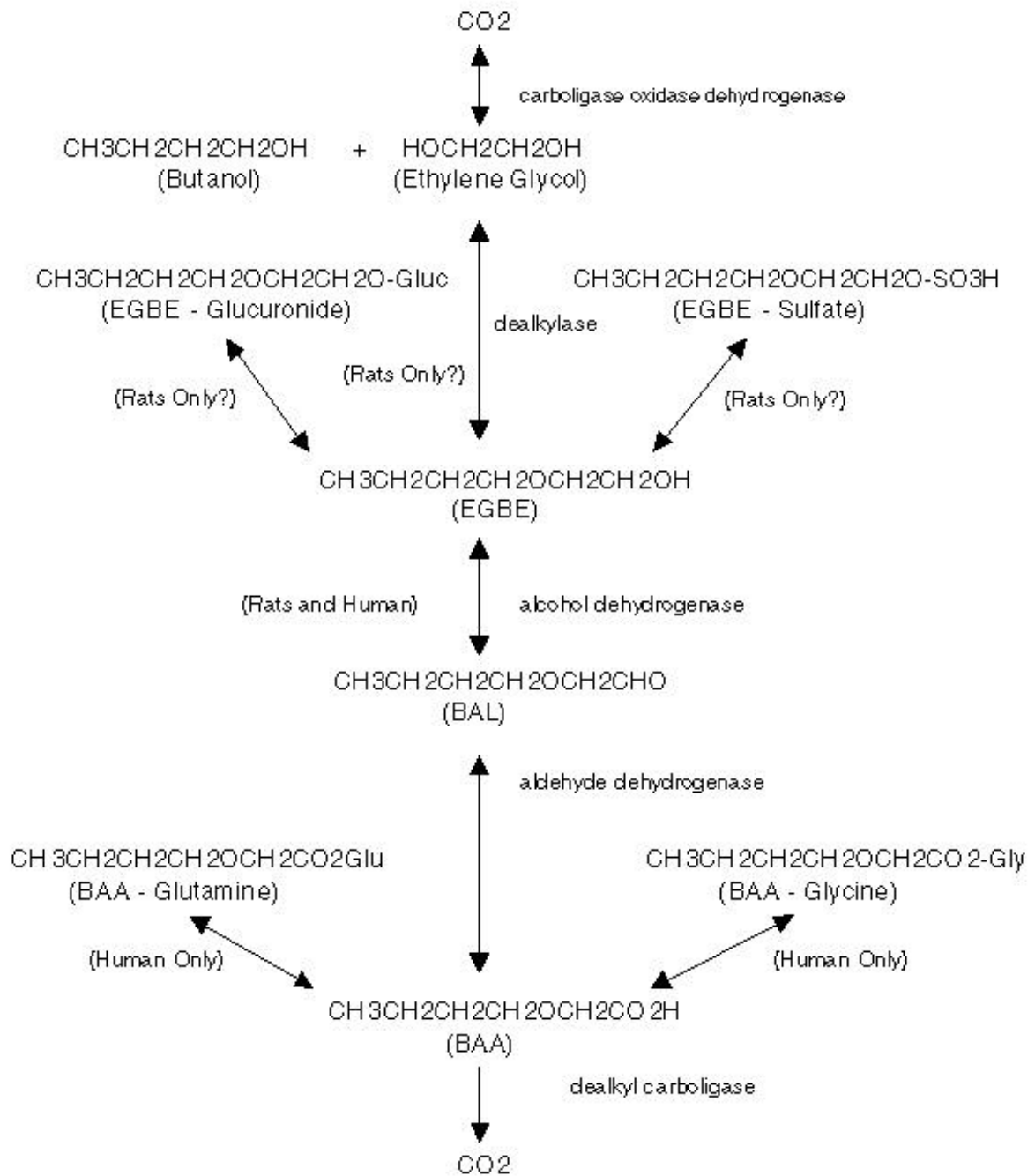
In a human study, Johanson and Boman (1991) attempted to define the relative importance of the skin to the total absorption of EGBE vapors in whole-body exposures. Four volunteers were exposed mouth-only to 50 ppm EGBE for 2 hours, followed by 1 hour of no exposure, followed by 2 hours, of body-only exposure (exposed in a chamber while breathing fresh air via respirator) to 50 ppm. 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 for the concentration of EGBE in the subjects' blood samples following skin-only exposures were threefold to fourfold greater than following mouth-only exposure, Johanson and Boman (1991) 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. Contrary to the conclusions of Johanson and Boman, these simulations 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 is worn that would hinder absorption.

To provide experimental validation of the skin's role in the uptake of EGBE vapors, a study was conducted by Corley et al. (1997) in which human volunteers exposed one arm only to 50 ppm ¹³C-EGBE for 2 hours. Catheters installed in the antecubital vein of the unexposed arm served as the primary site for collecting blood, which was analyzed for both EGBE and BAA. Finger prick blood samples were collected only 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, as 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 effects with the finger prick sampling technique. Corley et al. (1997) reported that the skin permeability coefficients that provided the best simulation of three human data sets (Johanson et al., 1988; Johanson and Boman, 1991; Corley et al., 1997) ranged from 2 to 4 cm/hour, which covers low-high temperatures and relative humidities. Using these permeability coefficients, the relative contribution of the skin to the total uptake of humans exposed to the American Conference of Governmental Industrial Hygienists Threshold Limit Value (ACGIH TLV) concentration of 25 ppm EGBE for 8 hours ranged from (low to high temperature/humidity) 16% to 27.5% under resting conditions (normal ventilation and cardiac output) and 4.6% to 8.7% under working (50 W light exercise) conditions, assuming no clothing is worn that would hinder skin contact with EGBE. If protective clothing is worn, then only that surface area exposed would be available for the absorption of EGBE.

The metabolism of EGBE has been studied extensively, particularly in rats, and the extensive literature on this subject has been thoroughly reviewed (ECETOC, 1994; Commonwealth of Australia, 1996). Carpenter and co-workers (1956) first identified BAA as the metabolite responsible for the hemolytic toxicity of EGBE by incubating the acid with blood from a variety of species. Blood from rats, mice, and rabbits was more rapidly hemolyzed than blood from monkeys, dogs, humans, or guinea pigs when incubated at 37.5°C with a saline solution of 0.1% sodium butoxyacetate. These results correlated well with osmotic fragility studies using blood from these same species following inhalation exposures to EGBE. In contrast, a much higher concentration (2.5%) of EGBE was required to produce hemolysis *in vivo*. Subsequent investigations have shown that hemolytic blood concentrations of the acid may be produced following either oral, inhalation, or dermal administration of EGBE. Proposed pathways for the metabolism of EGBE in rats and humans are presented in Figure 1 (from Medinsky et al., 1990, and Corley et al., 1997).

Figure 1. Proposed Metabolic Scheme of EGBE in Rats and Humans
 [Adapted From Medinsky et al., 1990 and Corley et al., 1997]



The two main oxidative pathways of EGBE metabolism observed in rats are alcohol dehydrogenase (ADH) and O-dealkylation by a cytochrome P450 dealkylase (CYP 2E1) (Medinsky et al., 1990). EGBE may also form conjugates with glucuronide and sulfate to some extent. Primarily because BAA is excreted in the urine of both rats and humans following EGBE exposure, it has been suggested that the former pathway, which involves production of BAA through formation of butoxyacetaldehyde (BAL) by ADH, would be applicable to both rats and humans (Medinsky et al., 1990; Corley et al., 1997). However, the other three proposed metabolic pathways of EGBE may be applicable only to rats, as the metabolites of these pathways—ethylene glycol (EG), EGBE glucuronide, and EGBE sulfate—have been observed only in the urine of rats (Bartnik et al., 1987; Ghanayem et al., 1987a) and not in the urine of humans (Corley et al., 1997). In addition, Corley et al. (1997) confirmed a recent 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. These BAA glutamine and BAA glycine conjugation pathways have not been detected in the rat.

Percutaneous absorption of EGBE in rats is rapid and produces measured blood levels of the acid 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 the acid (0.5 mM) following a single oral dose of 126 mg/kg. 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 inhalation exposure in excess of 200 ppm. A recent report on the NTP (1998) 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 BAA, 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 using pyrazole and cyanamide as metabolic inhibitors of alcohol and aldehyde dehydrogenases, respectively (Ghanayem et al., 1987b). Male F344 rats (9-13 weeks) were pretreated with pyrazole or cyanamide followed by administration of 500 mg/kg EGBE by gavage. 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, but it also resulted in a high mortality rate in rats given cyanamide and EGBE, an effect not observed in animals treated with cyanamide or EGBE alone. Pyrazole completely blocked the increase in spleen weight/body weight ratios seen in EGBE-treated animals. Gavage administration of either BAL or BAA at doses molar equivalent to 125 mg/kg EGBE produced identical increased spleen weight/body weight ratios and identical increases in free hemoglobin (Hgb) 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.

Haufroid et al. (1997) conducted a human study on workers exposed to EGBE to test the possible influence of genetic polymorphism for CYP 2E1 on urinary BAA excretion rate. One exposed individual exhibited a mutant allele with increased cytochrome P450 oxidative activity that coincided with a very low urinary BAA excretion. 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 polymorphism for CYP 2E1 on urinary BAA excretion rate are needed before any firm conclusions can be drawn.

The effect of age, dose, and metabolic inhibitors on the toxicokinetics of EGBE were studied in male F344 rats (Ghanayem et al., 1990). Rats of either 3-4 months of age or 12-13 months of age were dosed by gavage at 31.2, 62.5, or 125 mg/kg. Pretreatments included pyrazole, cyanamide, or probenecid (an inhibitor of renal anion transport). Toxicokinetic parameters for EGBE, including area under the curve (AUC), maximum plasma concentration (C_{max}), and clearance rate (Cl_s) were dose dependent, with AUC and C_{max} increasing and Cl_s decreasing at increasing dose levels. Other measured parameters were unaltered by dose. Age had no effect on half-life ($T_{1/2}$), volume of distribution (V_s), or Cl_s of EGBE, but C_{max} and AUC increased with increasing age. As expected from previous studies, inhibition of EGBE metabolism with either pyrazole or cyanamide resulted in significantly increased $T_{1/2}$ and AUC and decreased Cl_s . BAA toxicokinetics were also altered by dose and age and by administration of metabolic inhibitors. Slight but statistically significant increases in C_{max} , AUC, and $T_{1/2}$ were seen at higher doses and 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 but produced twofold to threefold increases in AUC and twofold to sixfold increases in $T_{1/2}$ for BAA. The results of these studies indicate that renal organic acid transport is vital to the renal elimination mechanism. The increased C_{max} , AUC, and $T_{1/2}$ in older versus younger rats may be due to differences in relative contributions of the two primary metabolic pathways discussed previously, or they may be due to compromised renal clearance.

Several blood and urine samples from the previously discussed human kinetic study by Johanson and Johnsson (1991) were analyzed for BAA. An average peak blood concentration of 44 μ M BAA (range 36-57 μ M) was reached 2-4 hours postexposure. The average $T_{1/2}$ for elimination of BAA from blood was 4.3 hours (range 1.7-9.6 hours), suggesting little chance of accumulation of BAA following repeated occupational exposures to concentrations at or below existing occupational exposure limits of 20-25 ppm. The average renal clearance of BAA was 23-39 mL/minute, which was only about one-third of the glomerular filtration rate. Johanson and Johnsson (1991) suggested that the low clearance of BAA relative to the glomerular filtration rate 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 Johanson and Johnsson) indicates that tubular reabsorption was unlikely since more than 99% of the BAA in normal human urine (pH ~6) is ionized. The volume of distribution (Vd) averaged 15 L (range 6.5-25 L) based on whole blood measurements, which was approximately equal to the volume of extracellular water (13-16 L), a further indication of binding of BAA to blood proteins.

BAA was measured in male workers exposed to low levels of EGBE (average airborne concentration of 2.91 ± 1.30 mg/m³ [0.59 ppm]) in a beverage package production plant (Haufröid et al., 1997). Postshift urine samples showed average BAA concentrations of 10.4 mg/g creatinine in these individuals.

The elimination kinetics of EGBE and BAA following repeated inhalation exposure (NTP, 1998) appear to be dependent on species, sex, age, time of exposure, and exposure concentration (Dill et al., 1998). Postexposure blood samples were collected from rats and mice after 1 day, 2 weeks, and 3, 6, 12, and 18 months of exposure to target EGBE concentrations of 0, 31.2 (rats only), 62.5, 125, or 250 (mice only) ppm by whole-body inhalation for 6 hours/day, 5 days/week. Urine and blood samples were also obtained from a separate set of aged mice (19 months) exposed to EGBE for 3 weeks. While the systemic half-life of EGBE (<10 minutes in rats and <5 minutes in mice after 1-day exposure) was independent of exposure concentration and blood concentrations of EGBE (AUC_{EGBE})

increased proportionally with exposure concentration, the rate of BAA elimination from blood decreased as exposure concentration increased. Female rats were significantly less efficient in clearing BAA from their blood than males, possibly a result of the reduced renal clearance observed in the female rats. EGBE clearance profiles of the 19-month-old mice exposed to 125 ppm EGBE were similar to young mice, but the aged mice eliminated BAA more than 10 times slower than young mice after a 1-day exposure. This difference was not as apparent after 3 weeks of exposure, suggesting that factors other than age may be involved.

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

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, AND CLINICAL CONTROLS

Bauer, P; Weber, M; Mur, JM; et al. (1992) Transient non-cardiogenic pulmonary edema following massive ingestion of ethylene glycol butyl ether. *Inten Care Med* 18:250-251.

The effects of an acute ingestion of 500 mL of window cleaner containing 9.1% EGBE and 2.5% ethanol by a 53-year-old male who was a chronic alcoholic were reported by Bauer et al. (1992). The man was admitted to a hospital comatose with metabolic acidosis, shock, and noncardiogenic pulmonary edema approximately 10 hours after ingestion of the dose. Heart rate was increased, blood pressure was decreased, and there was transient polyuria and hypoxemia. Hypochromic anemia was evident with a Hgb concentration of 9.1 g/100 mL, hematocrit (Hct) was 25%, and thrombocytopenia was noted. The man was discharged from the hospital after 15 days.

Carpenter, CP; Pozzani, UC; Wiel, CS; et al. (1956) The toxicity of butyl cellosolve solvent. *AMA Arch Ind Health* 14:114-131.

Three controlled studies using inhalation exposure were conducted by Carpenter et al. (1956). 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 ft room. Effects observed in humans included nasal and ocular irritation, a metallic taste in the mouth, and belching. Erythrocyte osmotic fragility did not change for the men; however, it rose appreciably for the rats. In a second study, a group of two men, one woman, and three rats was exposed to 195 ppm EGBE for two 4-hour periods, separated by a 30-minute recess, in a 6.5 cubic ft room. There was no change in the blood pressure, erythrocyte fragility, or pulse rate of the human subjects. Irritation of the nose and throat followed by ocular irritation and disturbed taste was noted, as well as one subject reporting a headache. In the rats, an increase in erythrocyte fragility values was noted during exposure. In the third study, a group of two men and two women were exposed for an 8-hour period to an EGBE concentration of 100 ppm. No changes in blood pressure, erythrocyte fragility, or pulse rate were observed. Irritation of the nose and throat followed by ocular irritation and a disturbing metallic taste were mentioned. Two of the subjects reported headaches.

Dean, BS; Krenzelok, EP. (1991) Critical evaluation of pediatric ethylene glycol monobutyl ether poisonings. *Vet Hum Toxicol* 33:362.

Twenty-four children, aged 7 months to 9 years, were observed subsequent to oral ingestion of at least 5 mL (two children drank more than 15 mL) of glass window cleaner containing EGBE in the 0.5%-9.9% range. The two children consuming 15 mL were treated by gastric lavage. No symptoms of EGBE poisoning and no hemolysis were observed in any of the children.

Gijzenbergh, FP; Jenco, M; Veulemans, H; et al. (1989) Acute butylglycol intoxication: a case report. *Hum Toxicol* 8:243-245.

A 23-year-old woman weighing 64 kg ingested approximately 25-30 g of EGBE (~400-500 mg/kg) and ethanol (~ 4 to 1 ratio) as a window cleaner in an apparent suicide attempt. She was admitted comatose to the hospital, exhibiting dilated pupils, obstructive respiration, and metabolic acidosis, including depression of blood Hgb 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. Hgb concentration fell from 11.9 g Hgb/100 mL blood on admission to 8.9 g Hgb/100 mL. The individual was discharged after 8 days.

Gualtieri, JF; Harris, CR; Corley, RA; et al. (1995) Multiple 2-butoxyethanol intoxications in the same patient: clinical findings, pharmacokinetics, and therapy. Rochester, NY: North American Congress of Clinical Toxicology.

A case of an intentional suicide attempt with an industrial-strength window cleaner was reported by Gualtieri et al. (1995). An 18-year-old male weighing 71 kg consumed between 360 and 480 mL of a concentrated glass cleaner containing 22% EGBE (dose 1,131-1,509 mg/kg). The patient was admitted to the hospital within 3 hours postingestion with no abnormalities other than epigastric discomfort. Approximately 10 hours postadmission, the patient was noticeably more lethargic, weak, and hyperventilating, consistent with the onset of metabolic acidosis. BAA and EGBE levels were measured. The patient was transferred to a tertiary care hospital where hemodialysis was initiated (approximately 24 hours postingestion) and ethanol therapy was started 30 minutes later. Treatment also consisted of intravenous 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 following discharge, the patient was readmitted following a second ingestion of 480 mL of the same cleaner (EGBE dose 1,509 mg/kg). Treatment, including ethanol therapy and hemodialysis, was initiated within a few hours of ingestion to control the metabolic acidosis. Since treatment was initiated soon after ingestion, ethanol therapy did have an impact on the disposition of EGBE (higher concentrations were detected than following the first ingestion) and BAA (lower levels were detected). As with the first episode, clinical manifestations of high-dose oral ingestion of nearly 1.1-1.5 g/kg body weight consisted of metabolic acidosis. No evidence of hemolysis or renal abnormalities was detected.

Haufroid, V; Thirion, F; Mertens, P; et al. (1997) Biological monitoring of workers exposed to low levels of 2-butoxyethanol. *Int Arch Occup Environ Health* 70:232-236.

A cross-section of 31 male workers (22-45 years old, employed for 1-6 years) exposed to low levels of EGBE in a beverage packing production plant were monitored by Haufroid et al. (1997). The effect of external EGBE and internal BAA exposure on erythrocyte lineage (red blood cell [RBC] numeration, Hgb, Hct, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], haptoglobin [Hp], reticulocyte numeration [Ret], and osmotic resistance [OR]), as well as hepatic and renal creatinine and urinary retinol binding protein parameters was investigated. The average airborne concentration of EGBE was 2.91 mg/m³ (0.6 ppm) (SD ± 1.30 mg/m³ or 0.27 ppm). Single determinations of BAA in post-shift urine samples were used to assess exposure to low levels of EGBE. No difference between exposed and control workers was observed for RBC count, Hgb, MCV, MCH, Hp, Ret, and OR (a measure of osmotic fragility). 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. No significant differences were observed in hepatic and renal biomarkers.

Rambourg-Schepens, MO; Buffet, M; Bertault, R; et al. (1988) Severe ethylene glycol butyl ether poisoning. Kinetics and metabolic pattern. *Hum Toxicol* 7:187-189.

A 50-year-old woman ingested approximately 250-500 mL of a window cleaner containing 12% EGBE (~30-60 mL of EGBE) in an apparent suicide attempt. The woman was diagnosed with metabolic acidosis, hypokalemia, a rise in serum creatinine level, and a markedly increased urinary excretion of oxalate crystals. Moderate hemoglobinuria appeared on the third day postexposure and a progressive erythropenia was noted. Without more complete hematologic details from this and other similar case studies, it is not possible to determine whether these effects are due to hemolysis or other factors related to the profound changes in blood chemistry observed in these patients. The clinical status improved gradually, and the patient was discharged on the 10th day.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Carpenter, CP; Pozzani, UC; Wiel, CS; et al. (1956) The toxicity of butyl cellosolve solvent. *AMA Arch Ind Health* 14:114-131.

Carpenter et al. (1956) studied the hemolytic effects in various animal species following inhalation of EGBE vapors. An unspecified strain of rats (15 animals/sex) 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 lowest-observed-adverse-effect levels (LOAELs) for these rat and mouse studies were apparently 54 and 100 ppm, respectively. No no-observed-adverse-effect levels (NOAELs) were reported.

Dodd, DE; Snelling, WM; Maronpot, RR; et al. (1983) Ethylene glycol monobutyl ether: acute, 9-day, and 90-day vapor inhalation studies in Fischer 344 rats. *Toxicol Appl Pharmacol* 68:405-414.

A 90-day subchronic inhalation study was performed using F344 rats (16 rats/sex) exposed to EGBE for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, and 77 ppm. During the course of the study, the 77 ppm males exhibited slight (5%) but statistically significant decreases in RBC counts and Hgb levels that were accompanied by increases in MCH. At the end of the study (66 exposures), these effects had either decreased or returned to the range of the control values. The NOAEL was determined to be 25 ppm, and the LOAEL was 77 ppm.

Krasavage, WJ. (1986) Subchronic oral toxicity of ethylene glycol monobutyl ether in male rats. *Fundam Appl Toxicol* 6:349-355.

A toxicity study was conducted 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 following the last treatment. Dose-related changes were observed in the RBC counts of all treatment groups, including significantly decreased RBC count, decreased Hgb concentration, and increased MCH. Hematologic changes occurring at 443 and 885 mg/kg-day were increased MCV and decreased MCHC. The decrease in RBC count at a lower dose (222 mg/kg-day) seems to be inconsistent with the predominant theory that erythrocyte swelling (which is indicated by the increased MCV) precedes lysis of the cell (see discussion in Section 5.1.1). While such swelling has been documented in vitro (Ghanayem, 1989), the associate increases in MCV may not be detectable in vivo, given the sensitivity of the equipment used. Thus, the increased MCV at higher doses is more likely due to an increase in the number of larger reticulocytes (RTCs) in the circulation following this erythropoietic response, as has been suggested recently (NTP, 1998). Based on decreased RBC count and trends in Hgb and other hematologic

endpoints, the LOAEL was determined to be 222 mg/kg-day, the lowest dose tested. A NOAEL was not identified.

Nagano, K; Nakayama, E; Koyano, M.; et al. (1979) Testicular atrophy of mice induced by ethylene glycol mono alkyl ethers. *Jpn J Indust Health* 21:29-35.

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

National Toxicology Program (NTP). (1993) Technical report on toxicity studies of ethylene glycol ethers 2-methoxyethanol, 2-ethoxyethanol, 2-butoxyethanol administered in drinking water to F344/N rats and B6C3F1 mice. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. NTP No. 26. NIH Publ. No. 93-3349.

NTP (1993) performed a 13-week toxicity study in F344 rats and B6C3F1 mice using EGBE. Groups of 10/sex/species received EGBE in drinking water at doses of 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. Complete histologic 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 sexes persisting until or developing by 13 weeks included dose-related indications of mild to moderate anemia. Male rats evaluated at 13 weeks showed significantly reduced RBC counts at ≥ 281 mg/kg-day and reduced Hgb concentration, reduced platelets, and increased bone marrow cellularity at ≥ 367 mg/kg-day. Significant hematologic effects in female rats at week 13 included reduced RBC counts and Hgb concentration at ≥ 82 mg/kg-day and increased RTCs, decreased platelets, and increased bone marrow cellularity at approximately 304 mg/kg-day. There were no histopathologic changes in the testes and epididymis at ≥ 129 mg/kg-day. Liver lesions, including cytoplasmic alterations, hepatocellular degeneration, and pigmentation were observed in the mid- and high-dose groups. 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 low-dose groups (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, 1990). The hematologic (decreased RBC count and Hgb) and hepatic changes were dose related and were associated with more severe blood and liver effects at higher doses; 69-82 mg/kg-day was considered a LOAEL. A NOAEL was not identified. Fewer effects were observed in male and female mice exposed to EGBE. Mean final body weight and body weight gain were essentially the same as control values at the two lower dose levels, but they were slightly reduced at the three highest dose levels.

Werner, HW; Nawrocki, CZ; Mitchell, JL; et al. (1943a) Effects of repeated exposure of rats to vapours of monoalkyl ethers of ethylene glycol. *J Ind Hyg Toxicol* 25:374-379.

Werner, HW; Mitchell, JL; Miller, JW; et al. (1943b) Effects of repeated exposure of dogs to monoalkyl ethylene glycol ether vapors. *J Ind Hyg Toxicol* 25:409-414.

Subchronic inhalation studies were conducted using Wistar-derived rats (23 animals/group) by exposing them to 0, 135, or 320 ppm EGBE for 7 hours/day, 5 days/week for 5 weeks (Werner et al., 1943a). Hematologic endpoints were evaluated (RBC, white blood cell [WBC], differential, and RTC counts, and Hgb estimations). The authors concluded that exposure to 320 ppm EGBE resulted in an increased percentage of circulating immature granulocytes, a decrease in Hgb concentration and RBC count, and an increase in RTC count. These hematologic changes were not severe and reversed 3 weeks after discontinuing exposures. No effect on the WBC count was observed. In another study, the same researchers (Werner et al., 1943b) conducted a subchronic inhalation study using groups of 2 dogs (of unspecified strain) and exposing them to 0 or 415 ppm EGBE for 7 hours/day, 5 days/week for 12 weeks. Necropsies were performed 5 weeks postexposure; 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 Hgb concentration and RBC count, and increased hypochromia, polychromatophilia, and microcytosis. These hematologic effects were not severe and were reversed 5 weeks after the end of exposure.

National Toxicology Program (NTP). (1998) NTP technical report on the toxicology and carcinogenesis studies of 2-butoxyethanol (CAS No. 111-76-2) in F344/N rats and B6C3F1 mice (inhalation studies). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, NIH, Research Triangle Park, NC. NTP TR 484. NIH Draft Publ. No. 98-3974.

In the subchronic portion of this study, both F344 rats and B6C3F1 mice (10/sex) were exposed via inhalation to concentrations of 0, 31, 62.5, 125, 250, and 500 ppm of EGBE 6 hours/day, 5 days/week for 14 weeks (NTP, 1998). Both sexes of rats exhibited clinical signs consistent with hemolytic effects of EGBE at the three highest doses. Hematologic evaluation showed a mild to moderate regenerative anemia at all concentrations in females and at the highest three concentrations in males. Exposure-related trends were noted for RTCs, RBC count, MCV, Hgb, and Hct. Liver-to-body weight ratios were significantly increased in males at the two highest concentrations and in females at the highest concentration. Histopathologic effects 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 hemoglobin and hemosiderin deposition, and bone marrow hyperplasia at concentrations in excess of 62.5 ppm for male rats and 31 ppm for females. Also, five female rats were sacrificed moribund from the highest concentrations and one from the 250 ppm group. The LOAEL for hematologic 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 of exposure exhibited clinical signs consistent with the hemolytic effects of EGBE at the two highest concentrations for both sexes. Hematologic evaluation indicated a moderate regenerative anemia with an increase in platelets at the three higher concentrations in both sexes. Histopathologic effects consisted of excessive extramedullary splenic hematopoiesis and hemosiderosis, hemosiderin accumulation in Kupffer cells, renal tubular degeneration and hemosiderin deposition, 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. Also, 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 histopathologic changes in the forestomach.

NTP also completed a two-species, 2-year inhalation study on EGBE (NTP, 1998). In the chronic study, exposure concentrations of EGBE 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. The highest exposure was selected to produce a 10% to 15% depression in hematologic indices. Survival was significantly decreased in male mice at 125 and 250 ppm (54.0% and 53.1%, respectively), but no effect on survival was observed in rats.

Mean body weights of all groups of male and female rats exposed to 31 and 62.5 ppm were similar to controls. 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. Mean body weights of the exposed male and female mice were generally less than for controls, with females experiencing greater and earlier reductions. Nonneoplastic 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). The severity of the nasal lesion was not affected by exposure and was deemed to be, in general, an adaptive rather than adverse response to exposure (NTP, 1998). The Kupffer cell pigmentation results from hemosiderin accumulation and is a recognized secondary effect of the hemolytic activity of EGBE (NTP, 1998).

Nonneoplastic effects in mice included forestomach ulcers and epithelium hyperplasia, hematopoietic cell proliferation and hemosiderin pigmentation in the spleen, Kupffer cell pigmentation in the livers, hyaline degeneration of the olfactory epithelium (females only), and bone marrow hyperplasia (males only). As in the rats, the nasal lesion is deemed an adaptive rather than adverse response to exposure, and the Kupffer cell pigmentation is considered a secondary effect of the hemolytic activity of EGBE. Bone marrow hyperplasia and hematopoietic cell proliferation and hemosiderin pigmentation in the spleen are also attributed to the primary hemolytic effect, which is followed by regenerative hyperplasia of the hematopoietic tissue. The forestomach lesions do not appear to be related to the hemolytic effect of EGBE. Incidences of ulcer were significantly increased in males exposed to 125 ppm and in all exposed female groups. Ulcer consisted of a defect in the forestomach wall that penetrated the full thickness of the forestomach epithelium, and frequently contained accumulations of inflammatory cells and debris. 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.

Using the same exposure groups described above, additional groups of rats (27/sex/exposure group) and mice (30/sex/exposure group) in the 2-year study were examined at 3, 6, and 12 months (8-10 animals/duration) for hematologic effects. Rats in the 31 ppm exposure group were not examined at 12 months, and only hematology was examined at 3 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 and Hgb and erythrocyte counts, occurred at 3, 6, and 12 months in the 62.5 ppm females and the 125 ppm males and females. Statistically significant decreases in these same endpoints were also observed in 31 ppm females exposed for 3 and 6 months, and in 62.5 ppm males exposed for 12 months. At 3 months, MCV was increased following 31 ppm and higher exposures in both males and females. In vitro studies by Ghanayem (1989) have shown that the hemolysis caused by 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 mean cell hemoglobin at higher exposure levels to the erythropoietic response subsequent to hemolysis and the corresponding increase in the number of larger RTCs in circulation (NTP, 1998). RTC count was increased significantly 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 RTC count was not observed at any duration in males or females exposed to 31 ppm, nor in males exposed to 62.5 ppm, it appears that RTC count alone cannot account for the increase in MCV at these levels of exposure. The observed increases in MCV may be a combined result of both erythrocyte swelling prior to and an increased number of RTCs, subsequent to hemolysis, with the former being more influential at lower exposure levels and the latter having more relative impact at higher exposure levels.

Similar effects indicating anemia were also observed in mice, with females being the more sensitive of the species. However, the anemia response was observed at higher doses and changed somewhat with duration of exposure. Statistically significant ($p < 0.05$) decreases in automated and manual Hct values, Hgb, and erythrocyte 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 females at the highest duration (12 months) and exposure (250 ppm) levels. RTC count was increased significantly in 125 ppm females at 3 and 6 months and in 125 ppm males at 6 months.

At the end of the 2-year chronic bioassay, neoplastic effects were observed in female rats and 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, but exceeded the historical control ($6.4\% \pm 3.5\%$; range 2%-13%) for this type of study.

The low survival rate in male mice exposed to 125 and 250 ppm EGBE may have been due to carcinogenic effects in the liver as a high rate of hepatocellular carcinomas was found in these exposure groups (10/50, 11/50, 16/50, 21/50), the increase at the high exposure level being statistically significant ($p < 0.001$). 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; $1.5\% \pm 1.5\%$; range 0-4%). NTP (1998) noted that no organisms consistent with *Helicobacter hepaticus* were found in any of 14 mice evaluated. It was concluded that *H. hepaticus* was not a factor in the development of liver neoplasms in this study. No significant increase in benign or malignant hepatocellular tumors or hemangiosarcomas was noted in the female mice. In fact, 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 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 = 0.017) in female mice relative to the chamber controls (0/50, 1/50, 2/50, 6/50). The incidence of these tumor types at the highest exposure level (12%) exceeds the range for the occurrence of these tumors in historical controls ($0.9\% \pm 1.1\%$; 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, indicating a decreased latency period in the highest exposure group. While the incidence of these types of forestomach tumors was not significantly increased in male mice over controls (1/50, 1/50, 2/50, 2/50), the incidence of squamous cell papillomas in the two highest exposure groups (4%) exceeded the range for historical controls ($0.5\% \pm 0.9\%$; range 0-2%). Furthermore, the increased incidences of forestomach neoplasms in males, as in females, occurred in groups with ulceration and hyperplasia, suggesting a relation between these nonneoplastic and neoplastic lesions.

A discussion of the cancer data from this study is provided in Section 4.6. With respect to the noncancer findings of this study, a NOAEL could not be determined, and a LOAEL of 62.5 ppm was determined for nonneoplastic lesions in mice. In rats, a NOAEL of 31 ppm and a LOAEL of 62.5 ppm were determined for noncancer effects.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Due to the known reproductive toxicity (i.e., toxicity 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 has been studied in a variety of well-conducted oral (Nagano et al., 1979, 1984; Grant et al., 1985; Foster et al., 1987; Heindel et al., 1990; Exon et al., 1991; NTP, 1993) and inhalation (Dodd et al., 1983; NTP, 1998) studies 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 (Wier et al., 1987; Sleet et al., 1989), inhalation (Nelson et al., 1984; Tyl et al., 1984), and dermal (Hardin et al., 1984) exposures to 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 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 ($> 1,000$ 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 toxicities 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 parents, nor to the developing fetuses of laboratory animals.

As discussed in Section 4.2, Nagano et al. (1979) performed a toxicity study in male mice 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 was identified, but no changes in testes weight or histology were observed. In another study, Nagano et al. (1984) used the same dosing regimen to test EGBE and other glycol ethers to up to 2,000 mg/kg-day. Testicular atrophy was observed for EGEE and EGME, but not for EGBE.

Grant et al. (1985) exposed male F344 rats 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 for rats fed up to 1,000 mg/kg-day EGBE.

As discussed in Section 4.2, 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. The researchers found no effects on testicular weight and no histopathologic lesions in the testes, seminal vesicles, epididymides, or prostate 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 and seminal vessels were observed, but the decreases were not time or dose related. No treatment-related lesions were noted following histologic examination of the testes, epididymides, and 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 by exposing them to EGBE in drinking water at doses of 0, 700, 1,300, and 2,000 mg/kg-day for 7 days pre-mating and 98 days as breeding pairs (Heindel et al., 1990). In the 2,000 mg/kg-day dose group, 13/20 females died, and in the 1,300 mg/kg-day dose group, 6/20 females died during the study. Toxic effects in adult mice in the 1,300 and 2,000 mg/kg-day dose groups included decreased body weight gain, increased kidney and liver weights, and dose-related decreases in water consumption. Decreased pup weight and a decrease in the number of litters produced per pair and in the size of each litter were observed in the 1,300 and 2,000 mg/kg-day dose groups. A significant reduction (5%) of 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 continuous breeding phase, first-generation (F_0) breeding pairs were separated and housed individually and exposure to EGBE continued. When the last litter was weaned, a 1-week crossover mating trial was performed to determine which sex was more affected by treatment. 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 controls. In the only histopathologic examination carried out on treated females, no kidney lesions were observed. The proportion of successful copulation was the same in all groups, and no developmental effects were observed in the offspring of any group. 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 second generation (F₁) pups. There were insufficient numbers of offsprings 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.

In summary, 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 as only a very slight decrease in pup weight was observed at this dose.

In an immunotoxicity study discussed in more detail in section 4.4.5 (Exon et al., 1991), 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. While testicular atrophy and necrosis and reduced number of spermatogenic cells were observed in males exposed to EGBE, no adverse effect on fertility parameters was seen in males exposed to 506 mg/kg-day EGBE.

NTP (1993) evaluated the effects of EGBE on the reproductive systems of male and female B6C3F1 mice by exposing them to doses of 93, 148, 210, 370, or 627 mg/kg-day EGBE for males and 150, 237, 406, 673, or 1,364 mg/kg-day EGBE for females in drinking water for 2 weeks. No deaths were reported, and there were no effects on body weight. Water consumption was decreased at all dosages except the highest in females. Thymus weights were decreased in the highest male dose group. There were no treatment-related gross lesions in any of the reproductive organs, and histopathologic examinations were not performed. NTP (1993) also exposed male and female F344 rats to EGBE in drinking water for 2 weeks. 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 lowered in the highest dose group in both sexes, and there were no treatment-related gross lesions of reproductive organs reported.

As discussed in Section 4.2, Dodd et al. (1983) and NTP (1998) performed 90-day subchronic inhalation studies on F344 rats. NTP (1998) also performed a subchronic study of B6C3F1 mice and chronic inhalation studies of F344 rats and B6C3F1 mice. Dodd et al. (1983) exposed male and female rats (16/sex) to EGBE for 6 hours/day, 5 days/week at concentrations of 0, 5, 25, and 77 ppm. The authors reported no changes in testicular weight or in the pathology of the epididymides and testes of male rats at any exposure level, but reproductive organs of the female rats were not examined histologically. In the subchronic portion of the NTP (1998) studies, no effects were noted in reproductive organs of rats and mice (10/sex) exposed to concentrations of 0, 31, 62.5, 125, 250, and 500 ppm of EGBE 6 hours/day, 5 days/week for 14 weeks, although testicular degeneration was reported in 2 of 4 mice from the 500 ppm group that died or were killed moribund. In the NTP (1998) chronic study, exposure concentrations of EGBE 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; however, 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 days 8-14 of gestation. 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 1,500 mg/kg-day and above). Based on clinical signs in the prenatal study, a LOAEL for maternal effects was 350 mg/kg-day. A LOAEL for developmental toxicity was determined to be 1,000 mg/kg-day based on an increased 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 CD-1 mice administered EGBE via gavage at 0, 650, or 1,000 mg/kg-day on days 8-14 of gestation. 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 gavage to groups of 28-35 pregnant F344 rats at doses of 0, 30, 100, or 200 mg/kg-day on gestation days 9-11, or doses of 0, 30, 100, or 300 mg/kg-day on gestation days 11-13 (Sleet et al., 1989). Gestation days 9-13 were chosen for investigation because they are the most critical periods of fetal cardiovascular development. Food and water measurements, body and organ weights, clinical signs, hematologic analyses (dams) and number of corpora lutea, uterine contents, and dead and live fetuses were monitored. Maternal effects of EGBE given in either dosing sequence included marked reductions in body weight and/or weight gain; increases in kidney and spleen weights; severe hematotoxicity as evidenced by a decrease in HCT, Hgb, and RBC count; and an increase in RTCs at doses greater than or equal to 100 mg/kg-day. These effects were dose related. 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, but not at 300 mg/kg-day. A decreased platelet count was noted in the fetuses at 300 mg/kg-day. No fetal malformations, including cardiovascular malformations, were noted at any dose. The LOAEL for maternal toxicity was 100 mg/kg-day with a NOAEL established at 30 mg/kg-day. The LOAEL for developmental toxicity was 200 mg/kg-day, 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 for days 7-15 of gestation (Nelson et al., 1984). Rats exposed to 200 ppm showed some evidence of hematuria on the first day of exposure; however, no adverse effects were noted thereafter. No adverse effects attributable to EGBE exposure were seen in offspring. The LOAEL was 200 ppm for slight maternal toxicity, and a NOAEL was identified at 100 ppm. The NOAEL for developmental toxicity was 200 ppm. Simultaneous testing revealed that 50 ppm exposures to EGME was toxic at all levels of embryonic and fetal development.

Pregnant F344 rats (36/group) and New Zealand white rabbits (24/group) were exposed to 0, 25, 50, 100, or 200 ppm EGBE via inhalation for 6 hours/day on gestational days 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 in the form of retarded skeletal ossification of vertebral arches or centra, sternbrae, or phalanges at 100 and 200 ppm. Maternal toxicity was also evident at 100 and 200 ppm

as an increased incidence of hematuria, reduced RBC count, decreased weight gain, and reduced food consumption. The NOAEL and LOAEL for maternal and developmental toxicity in the rat were 50 and 100 ppm, respectively. In rabbits, fetal skeletal ossification of sternbrae and rudimentary rib 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. The NOAEL and LOAEL for maternal and developmental effects in the rabbit were 100 and 200 ppm, respectively.

Reproductive toxicity tests were performed on female Sprague-Dawley rats via dermal administration during days 6-15 of gestation, four times per day at 1,800 and 5,400 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 and there was no evidence of embryo- or fetotoxicity, nor were any gross malformations or variations noted.

4.4. OTHER TOXICOLOGICALLY RELEVANT STUDIES

4.4.1. Single Exposure Studies

Ghanayem et al. (1987c) conducted acute toxicity studies in male F344 rats using single gavage doses of 0, 32, 63, 125, 250, or 500 mg/kg-day of EGBE (purity 99%) in water. These studies were designed to assess the effect of age on toxicity by comparing effects in treated young rats (4-5 weeks old) and adult rats (9-13 weeks, 5-6 months, and/or 16 months). Evaluations included hematology (total RBC and WBC counts), urine Hgb concentration, organ weights, and histology (liver, spleen, bladder, kidney, and testes). Focal necrosis of the liver was observed in adult rats exposed at either 250 or 500 mg/kg. Hematologic effects were found to be dose- and age-dependent, with older rats being more sensitive than younger rats. Significant decreases in RBC counts, HCT, and Hgb and increases in free plasma Hgb occurred at 125 mg/kg-day in both adult and young rats, with the younger rats exhibiting significantly less pronounced responses. Incidence of hemoglobinuria was also dose- and age-dependent. Concentrations of free Hgb 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 CO₂ and a diminished ability to excrete BAA in the urine. Based on increased Hgb in the urine and associated hemolytic effects at higher doses, a LOAEL for this study was determined to be 32 mg/kg-day for adult rats. A NOAEL was not identified.

Ghanayem and Sullivan (1993) performed acute oral toxicity studies in rats by administering EGBE in single gavage doses of 250 mg/kg-day in tap water. MCV and HCT values were raised immediately after treatment and decreased with time following exposure. Hemolysis and decreases in Hgb concentrations and RBC counts occurred.

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 weights, and/or histology. Hematology evaluations showed marked dose-related effects on circulating RBCs and WBCs. Changes at 500 and 1,000 mg/kg-day on post-dosing day 1 included

significant dose-related decreases in Hgb, total WBC, and lymphocytes, and increases in MCV, RTC, 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 post-treatment period at 1,000 mg/kg-day. Changes in relative organ weights were evident on post-treatment day 1, including increased liver weight and spleen weight 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 post-treatment day 22 except for liver and spleen weights at 1,000 mg/kg-day, which remained somewhat increased (~5% and ~20%, respectively). 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) also administered EGBE to F344 rats via gavage for a 12-day period 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, which became more pronounced up to the third day of dosing. Gradual recovery was observed up to day 12. MCV, ATP concentration, RTC numbers, and body weight-relative spleen weights increased up to the sixth day of dosing and declined thereafter. Body weight-relative liver weight ratios were slightly lowered on days 3 and 6 and slightly raised on day 12.

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), but only a 20% mortality of either at a dose of 1,000 mg/kg. Clinical signs and gross necropsy indicated toxicity was due to irritation of the stomach. There was no evidence of hemolytic toxicity.

4.4.2. Dermal Exposure Studies

EGBE appears to be readily absorbed after contact with the skin of animals. Rats and rabbits have been shown to 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 rats (unspecified species). 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.

However, in the case of rabbits, repeated application of EGBE either neat or as a dilute aqueous (occluded) to male or female New Zealand rabbits at exposures 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, Hgb concentrations, and MCHC and increased MCH at the highest treatment level. Recovery was noted following a 14-day observation period. In a separate 13-week study, occluded dermal administration of EGBE to male and female New Zealand rabbits (6 hours/day, 5 days/week) at exposure levels of 10, 50, or 150 mg/kg produced no observable hematologic effects (Tyler, 1984).

In addition to differences in effects based on dose and duration, whether the site of EGBE administration was occluded or semioccluded was also a determining factor. For example, some

studies have shown no clinical signs of hematotoxicity in male or female Sprague-Dawley rats administered EGBE dermally at 2,000 mg/kg (24-hour exposure) either semiocluded or occluded (Allen, 1993a,b). However, clinical signs of systemic toxicity were notable in the occluded study. In a similar set of studies in rabbits, red-stained urine was reported at 2,000 mg/kg EGBE (semiocluded) along with other clinical signs of systemic toxicity (Allen, 1993c,d). Similar effects occurred at applied doses of 500, 707, and 1,000 mg/kg and occluded in this species; deaths occurred at the 500 and 1,000 mg/kg dose levels. Thus, hematotoxicity varied from nonexistent to severely affected. In guinea pigs, dermal administration of EGBE at 2,000 mg/kg produced no clinical signs of toxicity or treatment-related signs of organ toxicity (Shepard, 1994b).

4.4.3. Ocular Exposure Studies

EGBE has also been found to be an irritant when instilled in the eyes of rabbits in several studies (Jacobs and Marten, 1989; Kennah et al., 1989). Kennah et al. (1989) performed the Draize test to determine the effects of EGBE on eye irritation in rabbits. Scores for different concentrations tested at 24 hours postinstillation were 100%/66, 70%/49, 30%/39, 20%/2, and 10%/1 by the Texaco single-digit toxicity classification system. In an assessment that measured corneal thickness, the highest concentration was still classified as severely irritating, the 70% concentration was moderately irritating, and the others were mildly irritating. Jacobs and Marten (1989) also conducted ocular tests (no method specified) on rabbits (no concentrations given) to determine the effects of EGBE on eye irritation. Mean erythema scores and percent corneal thickening indicated that the substance should be classified as an irritant.

4.4.4. Genotoxicity

Although weakly genotoxic responses have been obtained in one laboratory, on the basis of the available data, EGBE is not expected to be mutagenic or clastogenic. The NTP has reported negative responses for mutagenicity when EGBE was tested in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 up to 10 mg/plate with or without metabolic activation (Zeiger et al., 1992). However, Hoflack et al. (1995) reported that at a high concentration (38 mmole/plate or 4.5 mg/plate), EGBE induced a weak mutagenic response in *Salmonella* tester strain TA97a with or without S9 mix (Hoflack et al., 1995). The Corning Hazleton Laboratory (Gollapudi et al., 1996) conducted testing in *Salmonella* to confirm the positive result reported by Hoflack et al. (1995). Testing was conducted in *Salmonella* strains TA97a and TA100, as well as *Escherichia coli* WP2uvrA. 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 unexplained. 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.

In an in vitro study, EGBE was reported to induce cell-cycle delay, but neither sister chromatid exchanges (SCEs) nor chromosomal aberrations were observed in Chinese hamster ovary cells with or without liver S9 mix (NTP, 1993); a weak response for the induction of chromosomal aberrations

without S9 mix was observed in a second test, but the response was not reproducible. Elias et al. (1996) reported that EGBE did not induce chromosomal aberrations in Chinese hamster V79 fibroblast cells, but indicated that EGBE at high treatment concentrations (8.5mM and higher) 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, but this response was also found at very high concentrations (8.4 and 16.8 mM EGBE).

When tested at up to toxic doses, EGBE or its metabolite BAL were not found to be mutagenic in an in vitro gene mutation assay using Chinese hamster ovary 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, but at high treatment concentrations (7.5 mg/mL and higher). It should be noted that Chiewchanwit and Au (1995) reported high toxicity at 38.1 mM EGBE (4.5 mg/mL). The gene mutation data presented by Elias et al. (1996) is in graphic form and cannot be critically evaluated given that only mean values are displayed with no standard deviations. Furthermore, survival data are not reported.

It was also determined that EGBE did not increase the incidence of micronuclei in the bone marrow cells of male mice or rats (NTP, 1998). Animals were given three intraperitoneal 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. Furthermore, NTP (1998) reported high mortality (two mice out of five survived) to mice injected with 1,000 mg/kg doses of EGBE. The protocol and results of Elias et al. (1996) appear to be adequate and consistent with the NTP results for this assay.

In conclusion, EGBE has adequately been tested in conventional genotoxicity tests for its potential to induce gene mutations in in vitro systems and cytogenetic damage in both in vitro and in vivo systems. The available data do not support a mutagenic or clastogenic potential for EGBE. One laboratory has reported weak genotoxicity responses at toxic doses (Elias et al., 1996; Hoflack et al., 1995). These data, however, are questionable given the limited information reported on results.

4.4.5. Immunotoxicity

Based upon the results of the Exon et al. (1991) study, it appears that the immune system is not a sensitive target of EGBE. In this immunotoxicity study, 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 subcutaneously with heat-aggregated aqueous keyhole limpet hemocyanin (KLH) 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 natural killer (NK) cell cytotoxicity, serum anti-KLH IgG antibody 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 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 response was significantly enhanced in males at 180 mg/kg-day and females at 204 mg/kg-day, but not at the high dose in either sex. 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 adversity. No significant alterations in other immune parameters were noted.

Smialowicz et al. (1992) reported that EGBE may potentiate the lethality of low-level exposure to lipopolysaccharide (LPS). They immunized F344 rats with a single intravenous injection of 0.5 mL of 40 µg/mL trinitrophenyl-LPS (TNP-LPS), then dosed them (six per dose group) by gavage with 50 to 400 mg/kg-day of various glycol ethers, including EGBE, 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. However, EGBE was not found to be immunosuppressive, as indicated by the fact that it did not suppress the primary plaque-forming cell response to TNP-LPS.

4.4.6. Other In Vitro Studies

Ghanayem (1989) has studied the metabolic and cellular basis of EGBE-induced hemolysis of rat erythrocytes in vitro and has compared this with human erythrocytes. EGBE is not metabolized when incubated with blood from male F344 rats and causes no hemolysis or metabolic alterations at concentrations 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 of no relevance physiologically. 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 (increased HCT) followed by hemolysis. This response was more pronounced for BAA, with nearly complete hemolysis observed following a 4-hour incubation at 2.0 mM. The aldehyde produced only slight hemolysis under the same conditions. The addition of aldehyde dehydrogenase and its cofactors to rat blood followed by BAL produced a potentiation of the hemolytic effects. Addition of cyanamide, an aldehyde dehydrogenase inhibitor, significantly decreased the effects either with or without added aldehyde dehydrogenase. 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, BAL, nor BAA caused any detectable changes in the concentrations of glutathione or glucose-6-phosphate dehydrogenase in rat erythrocytes. Blood from male and female human volunteers was unaffected by 4-hour incubations with BAA at concentrations up to 4.0 mM. At 8 mM, only slight but significant hemolysis of human blood was observed, with blood from female volunteers showing a slightly greater sensitivity. It was concluded from these studies that the erythrocyte membrane is the likely target for the hemolysin BAA. Loss of osmotic homeostasis results in cell swelling and lysis, and humans of both sexes are relatively insensitive to the hemolytic effects of BAA.

The relative insensitivity of human erythrocytes to the hemolytic effects of BAA has been demonstrated *in vitro*. However, the possibility exists that certain human subpopulations, including the aged and those predisposed to hemolytic disorders, might be at an increased risk from exposure to EGBE. Udden (1994, 1995a) has investigated this possibility using blood from the elderly (mean age 71.9 years; range 64-79 years; five men and four women) or from patients with sickle cell disease (seven patients) or hereditary spherocytosis (three subjects; all were studied following splenectomy; one was studied presplenectomy). 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 for up to 4 hours with 2.0 mM concentrations of BAA. In more recent work (Udden, 1995b), the deformability of human erythrocytes incubated at BAA concentrations of 7.5-10 mM displayed a slight but significant decrease that was accompanied by slight increases in osmotic fragility and MCV. These effects were judged prehemolytic and corresponded to similar changes reported in rat erythrocytes but at approximately 15-fold lower concentrations (0.5 mM).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN)—ORAL AND INHALATION

Intravascular hemolysis is the primary response elicited in sensitive species following inhalation, oral, or dermal administration of EGBE. Humans are less sensitive to the hemolytic effects of EGBE than are typical laboratory species. Effects generally thought to be secondary to hemolysis are observed in the liver, kidneys, bone marrow, spleen, and thymus and may result from increased hematopoiesis. The 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 to 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 spherocytic 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. Older erythrocytes are apparently more sensitive to the hemolytic effects of BAA than are younger cells or newly formed RTCs. Macrocytosis and increased MCV have been observed in sensitive species (rat) and are attributed to the increased number of larger RTCs in the circulation following this erythropoietic response (NTP, 1998). The following issues relate to the relevance of this hemolytic effect to humans and to EGBE's mode of action.

The weight of evidence obtained from a variety of studies in animals and humans would suggest that certain species are more susceptible to the hemolytic effects of EGBE. The sensitivities range from that of the guinea pig, which displays no hemolytic effects from EGBE at exposure levels

as high as 1,000 mg/kg when given orally or at 2,000 mg/kg when given dermally, to the rat, 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 has been observed in controlled laboratory acute inhalation exposures of human volunteers to up to 195 ppm EGBE; reversible hemolytic effects have been observed in cases where humans consumed single oral doses of 400 to 1,500 mg/kg EGBE (see Section 4.1). Effects in humans from chronic exposure have not been studied.

With respect to gender sensitivity, it has been consistently noted (Carpenter et al., 1956; Dodd et al., 1983; NTP, 1993, 1998) that female rats are more sensitive to EGBE-induced hemolysis than male rats. This gender difference is consistent with toxicokinetic data for male and female rats reported for the NTP 2-year study (NTP, 1998). Female rats eliminated BAA, the toxic metabolite of EGBE, more slowly from the blood, resulting in a larger area under the blood concentration versus time curve (Appendix K of NTP, 1998). This may be a result of the reduced renal excretion observed in female versus male rats. NTP (1998) 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 anemia found by NTP in female over male mice. However, unlike female rats, female mice excrete slightly more BAA than male mice, and no significant difference between female and male mice has been noted in the overall rate of elimination or the half-life of BAA.

Several studies (Ghanayem et al., 1987c, 1990) 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 retaining more of the EGBE metabolite BAA in their blood and being more sensitive than the younger rats. The increased blood retention (as measured by increased C_{max} , AUC, and $T_{1/2}$) in older versus younger rats may be due to metabolic differences or compromised renal clearance. These 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.

While older rats appear to be more severely impacted by acute doses of EGBE, chronic 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, 1998) studies. Ghanayem and co-workers (1990, 1992) investigated this adaptive effect in the male F344 rat. Daily gavage administration of EGBE at 125 mg/kg (12 days) resulted initially in hemolytic anemia, which was more pronounced following the third day, but rats 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 and co-workers proposed from the results of these studies that the tolerance to hemolysis following repetitive dosing is not due to changes in EGBE metabolism but is due to the replacement of older and more susceptible erythrocytes with less susceptible, younger cells. However, chronic studies in rats and mice (NTP, 1998) 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 final blood

collection at 12 months of age. Apparently, there is a balance in these rodents between the release of immature erythrocytes (RTCs) to the circulation and the aging process, so that the level of susceptible cells and severity of anemia remain 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 the liver, kidneys, spleen, bone marrow, and, to a lesser extent, the thymus. 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, pigmentation of the liver, and congested spleens. Renal damage is often reported, accompanied by hemosiderin accumulation, renal tubular degeneration, and intracytoplasmic hemoglobin. Often these effects are more pronounced in females. Changes noted in the liver and kidneys generally return to normal following recovery periods of from 2 to 3 weeks. Hematopoiesis in bone marrow and spleen, increased cellularity of bone marrow, and splenic congestion are all secondary to the hematotoxicity of EGBE and result 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, suggesting 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.

All of the liver effects, predominantly hemosiderin pigmentation of Kupffer's cells, noted in the NTP (1998) report of subchronic and chronic inhalation studies in rats and mice are discernible as secondary effects of the hemolytic activity caused by EGBE exposure. 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 hematologic changes were recorded (1,500 ppm), raising possibility of a direct, primary hepatic toxicity of either EGBE or a metabolite. Similar liver effects observed in female rats at the 750 ppm exposure level were accompanied by hematologic 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. 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), consisted of hepatocytes staining more eosinophilic and lacked the amphophilic to basophilic granularity of the cytoplasm present in hepatocytes from control animals. Greaves (1990) has suggested that the lack of cytoplasmic granularity or "ground-glass" appearance of the hepatocytes is an indication that this response was not due to a mechanism involving enzyme induction. The hepatocellular degeneration and pigmentation observed at the higher exposure levels in both sexes was centrilobular, which is consistent with Kupffer's cell pigmentation of the NTP (1998) inhalation studies. These facts, along with the fact that all other rat and mouse oral and inhalation studies of EGBE report hemolysis at or below exposure levels that result in liver effects, suggest that the hepatocellular changes in male rats reported in the NTP (1993) drinking water study may reflect adaptation to a subclinical level of hemolysis. However, other data indicate that there is reason for caution. Focal necrosis of the liver observed in male rats following gavage administration of 250 and 500 mg/kg EGBE (Ghanayem et al., 1987b) has been judged to be inconsistent with typical anoxic centrilobular necrosis associated with anemia (Edmonson and Peters, 1985). The effects observed in

this study may be associated with the high bolus exposures employed. However, these results and the results of the NTP (1993) drinking water study in male rats indicate that an assessment of human risk from EGBE exposure should allow for the possibility of direct liver effects by some as-yet-undetermined mechanism.

In conclusion, humans are significantly less sensitive to the hemolytic toxicity of EGBE than are typical laboratory species such as mice, rats, or rabbits. This 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 minimal prehemolytic changes to occur. Comparable effects in rat blood occur at in vitro concentrations approximately 15-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 BAA. Based on the results of PBPK modeling, 6-hour exposures of humans (whole body) to saturated atmospheres of EGBE will result in maximum blood concentrations of BAA below those needed to produce hemolysis (Corley et al., 1994). Although liver effects observed in rats and mice may be secondary to hemolysis, the possibility of a direct liver effect through a mechanism more relevant to humans has not been ruled out.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION— SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

Under the existing *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), EGBE is deemed to be a *possible human carcinogen* based on limited laboratory animal evidence and a lack of human studies. Under the *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), it is concluded that the human carcinogenic potential of EGBE *cannot be determined* at this time, but suggestive evidence exists from rodent studies. As was discussed in Section 4.2, 2-year inhalation bioassays conducted in mice and rats with EGBE indicate significant increases in several tumor types (NTP, 1998). NTP (1998) 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. They also reported *some evidence of carcinogenic activity* in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver, and *some evidence of carcinogenic activity* in female B6C3F1 mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma). As discussed below, there are questions regarding the relevance of these tumors to an assessment of the carcinogenicity of this compound to humans. No reliable human epidemiologic studies are available that address the potential carcinogenicity of EGBE.

With respect to the pheochromocytomas reported in female rats, the NTP (1998) tables indicate a marginally significant trend ($p=0.044$), and high-dose findings (16%) are only slightly different from the upper range of historical controls (13%). Further, pheochromocytomas can be difficult to distinguish from nonneoplastic adrenal medullary hyperplasia, and according to the NTP

report, most of these tumors were “small and not substantially larger than the more severe grades of adrenal medullary hyperplasia.” Thus, these tumors must be interpreted with caution.

The hemangiosarcomas in livers of male mice appear to be exposure related. However, the fact that the incidence of hemangiosarcomas was only slightly higher than the upper end of the range for historical controls (8% vs. 4%), was not increased in other organs (bone, bone marrow), and was not noted in either rats or female mice raises the question of whether this effect is related to accumulation of hemosiderin from hemolytic effects in the liver and related oxidative stress in male mice. Mice are known to be more susceptible to oxidative stress than are rats because of their lower antioxidant capability (Bachowski et al., 1997). On page 118 of its draft report, NTP (1998) states that a review of past NTP studies found no association between hemosiderin deposition in the liver and liver neoplasms in 79 male mice and 103 female mice from 2-year NTP studies in which liver was a site of chemical-related neoplasms. NTP (1998) goes on to state: “At least for mice, it does not appear that an accumulation of hemosiderin and possible oxidative stress alone were the cause of liver neoplasm in male mice.” However, recent work does suggest that iron accumulation from the hemolytic effects of EGBE occurs in the livers of mice and may lead to oxidative stress (Xue et al., 1999). Humans have been shown to be much less sensitive to the hemolytic effects of EGBE. Thus, if the slight increase in the incidence of hemangiosarcomas in male mice observed in the NTP study is related to the hemolytic effects of EGBE, they are unlikely to be relevant to human risk. Ongoing research on the effects of hemosiderin accumulation in male mice could help to resolve this issue.

The increased incidence of forestomach squamous cell papillomas or carcinomas was another effect observed in mice but not in rats. Increased incidences of forestomach neoplasms in the male and female mice occurred in groups in which ulceration and hyperplasia were also noted. NTP (1998) notes (p. 115): “A direct association of neoplasia with ulceration and hyperplasia was not shown in this study although it is hypothesized that 2-butoxyethanol exposure-induced irritation caused the inflammatory and hyperplastic effects in the forestomach, and that the neoplasia was associated with a continuation of the injury/degeneration process.” The mechanism for forestomach accumulation of EGBE or a metabolite following inhalation exposure is not known. However, Ghanayem et al. (1987a) found that the levels of EGBE in the forestomach of rats 48 hours after gavage exposure were three times the levels in the glandular stomach, suggesting a different reactivity and/or absorption in the two parts of the stomach.

In addition to the 2-year bioassay data, data from short-term tests and subchronic studies were evaluated along with EGBE’s chemical and physical properties to gain some insight into EGBE’s potential carcinogenicity. From what is known of the metabolic pathways of EGBE in animals, metabolic production of a species capable of significant reactivity with DNA is not anticipated. Available data on EGBE derived from conventional genotoxicity tests do not support a mutagenic or clastogenic (chromosomal breaking) potential of the compound. Further details on these genotoxicity tests can be found in Section 4.4. Not all carcinogens, however, are DNA reactive (Ashby and Tennant, 1991). A paucity of information was available on other potential modes of action for EGBE. Some information was available on gap-junctional intercellular communication (GJIC), which is widely believed to play a role in tissue and organ development and in the maintenance of a normal cellular phenotype with tissues. Thus, interference of GJIC may be a contributing factor in tumor development. Elias et al. (1996) reported that EGBE inhibited intercellular communication in Chinese hamster V79 fibroblast cells. They reported negative results for cell transformation in Syrian Chinese

hamster embryo. This cell transformation assay is capable of detecting genotoxic or nongenotoxic carcinogens; however, the gene mutation data presented by Elias et al. (1996) are in graphic form and cannot be critically evaluated given that only mean values are displayed with no standard deviations. Furthermore, survival data are not reported.

Structure-activity relationship (SAR) analyses were conducted to provide some insight into EGBE's potential carcinogenicity. SAR analysis is useful mostly for agents that are believed to initiate carcinogenesis through DNA reactive mechanisms. Based on chemical structure, EGBE does not resemble any known chemical carcinogens and is not expected to have electrophilic or DNA reactive activity. As discussed in Section 4.4.4, this is supported by genotoxicity data on EGBE, which were predominantly negative with the exception of one laboratory reporting weak mutagenic activity in some in vitro tests at toxic concentrations (Elias et al., 1996; Hoflack et al., 1995). Many of the conclusions reached in the Elias et al. (1996) paper cannot be evaluated because of the lack of data reported, and the weak *Salmonella* response in the Hoflack et al. (1995) study could not be repeated (Gollapudi et al., 1996). Thus, considering the weight of evidence on EGBE, it is not expected to be mutagenic or clastogenic.

4.7. SUSCEPTIBLE POPULATIONS

The hemolytic effect of EGBE is caused by its primary metabolite, BAA, presumably on the RBC membrane. Potentially susceptible subpopulations would include individuals with enhanced metabolism or decreased excretion of BAA. As discussed in Section 4.7.1 below, older rats have a reduced ability to metabolize the toxic metabolite BAA to CO₂ and a diminished ability to excrete BAA in the urine (Ghanayem et al., 1987c, 1990). However, the relevance of this finding to the possible susceptibility of elderly humans is uncertain due to the fact that, as discussed in section 3, humans may have conjugation pathways for the excretion of BAA (BAA-glutamine and BAA-glycine) that are not available to the rat.

In addition, it is expected that individuals whose RBC walls are less resistant to the lysis caused by BAA would be more sensitive to EGBE. However, RBCs from normal, aged, sickle-cell anemia, and hereditary spherocytosis patients were all resistant to the hemolytic effects of BAA (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 with rat RBCs may eventually illuminate characteristics in the human population that may indicate increased susceptibility. It is unknown at this time, for instance, whether people with a genetic predisposition to hemolytic anemia from other causes (e.g., glucose-6-phosphate dehydrogenase deficiency) would be more susceptible to EGBE-induced hemolysis. Other human risk factors for anemia include ingestion of certain therapeutic drugs (hydralazine, dilantin, chloramphenicol and others, sulfa, etc.); infections (malaria, parasites, syphilis, herpes, rubella, etc.); family history (e.g., of gallstones, cholecystectomy, jaundice, Rh or ABO isoimmunization); diet (e.g., iron deficiency); and systemic illnesses such as cardiac, gastrointestinal, liver, renal diseases, and hypothyroidism (Berliner et al., 1999).

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), who observed 24 children, age 7 months to 9 years, subsequent to oral ingestion of at least 5 mL of glass window cleaner containing EGBE in the 0.5% to 9.9% range (potentially 25 to 1,500 mg EGBE exposures). The two children who had taken greater than 15 mL amounts of the cleaner 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 a comparison of toxic effects observed to age sensitivity of the human extremely difficult.

As discussed above, numerous risk factors for anemia might predispose an individual to or compound the adverse effects of EGBE-induced hemolysis. It is generally recognized, however, that children have fewer risk factors for anemia than are present for adults due to (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 United States 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 so that valve replacement, malignant hypertension, and the use of certain drugs are not usually a factor (Berliner et al., 1999; Hord and Lukens, 1999).

The primary cause for 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 EGBE's toxic metabolite BAA (Udden, 1994) suggest that certain patients with abnormal hematopoietic systems (sickle-cell anemia and hereditary spherocytosis patients) 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 adults. RBCs of neonates and children (up to 6 months) differ from normal adult RBCs in that they are larger and have higher levels of Hgb F versus adult Hgb A (Lewis, 1970). Frei et al. (1963) showed that the larger calf erythrocytes containing Hgb F were osmotically more resistant than smaller, adult erythrocytes containing Hgb A. Frei et al. (1963) 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 and co-workers (1987c, 1990). These studies also demonstrated the time course for the onset and resolution of hematologic and histopathologic changes accompanying hemolysis. Adult (9-13 weeks) male F344 rats were significantly more sensitive to the hemolytic effects of EGBE than were young (4-5 weeks) male rats following administration of a single gavage dose of EGBE at 32, 63, 125, 250, or 500 mg/kg. In concurrent metabolism studies, it was also found that there was increased blood retention of EGBE metabolite BAA (as measured by increased C_{max} , AUC, and $T_{1/2}$), and that young rats eliminated a significantly greater proportion of the administered EGBE dose as exhaled CO_2 or as urinary metabolites as well as excreting a greater proportion of the EGBE conjugates

(glucuronide and sulfate) in the urine. These 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 CO₂ and a diminished ability to excrete BAA in the urine.

NTP (1998) also found that young mice (6-7 weeks) eliminated BAA 10 times faster than aged mice (19 months) following a 1-day exposure to 125 ppm 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).

Developmental studies, which may also be of possible relevance to this issue, have been conducted using rats, mice, and rabbits dosed orally, by inhalation, or, in one study, dermally (Hardin et al., 1984; Heindel et al., 1990; Nelson et al., 1984; NTP, 1993; Sleet et al., 1989; Tyl et al., 1984; Wier et al., 1987). Maternal toxicity related to the hematologic effects of EGBE and relatively minor developmental effects were reported in most studies. No teratogenic toxicities were noted in any of the studies. It can be concluded from these studies 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, with the female gender being more susceptible. 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 following a 2-week exposure. 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, female rats 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 New Zealand 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 in females at 180 or 360 mg/kg (Tyler, 1984). Only female rabbits showed decreased erythrocyte counts, Hgb concentrations, and MCHC and 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, are more pronounced in females. In drinking water studies conducted by NTP (1993), liver lesions 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 male rats to the hemolytic effects of EGBE. In studies on dogs, 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). Erythrocyte counts and Hgb concentrations were slightly decreased in the female. Erythrocyte permeabilities (determined by radioiodine uptake) were increased in both sexes but were not statistically different from 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 leukocyte count. Autopsy 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 in one adrenal was found. A male dog survived 28 days of inhalation exposure to 385 ppm of EGBE (7 hours/day). Toxic manifestations in the male were similar to the female but developed more slowly. At autopsy, congestion of the kidneys was not observed for the male animal. Also, two monkeys (male and female) were exposed to 100 ppm EGBE by inhalation (exposure period not specified but presumed to be 7 hours) for 90 days. Occasional rises in erythrocyte osmotic fragility were recorded during the exposure period and were more frequent in the female monkey.

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 up to 4.0 mM. At 8 mM, only slight but significant hemolysis of human blood was observed, with blood from female volunteers showing a slightly greater sensitivity.

A recently completed 2-year inhalation bioassay (NTP, 1998; Dill et al., 1998) also reports 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 half-life, and larger area under the blood concentration-versus-time curve. In addition, the C_{max} of BAA were greater for females at each concentration and time point. It has been suggested that higher blood concentrations of BAA accumulate in female rats because a smaller amount is excreted in their urine relative to male rats (Dill et. al., 1998). Mouse data from the NTP (1998) study also suggest a slightly increased hematologic effect among female mice, but while female mice tended to have higher blood concentrations of BAA, they actually excreted more BAA in urine than male mice.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

No chronic oral studies are currently available for EGBE. The results of the only two subchronic 91-day drinking water studies in rats and mice (NTP, 1993) are summarized in Table 1.

Based on a comparison of NOAELs and LOAELs for hematologic and liver effects, rats are clearly more sensitive than mice. As discussed in Section 4.2, hematologic and hepatocellular changes were noted in both sexes of rats. In female rats, 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

Table 1. Subchronic 91-day drinking water studies in rats and mice

Reference	Species (strain)	Sex	# Animals/ dose	Effect levels (mg/kg-day)	
				NOAEL	LOAEL
NTP (1993)	Rat (F344)	M	10	--	54.9 ^a
		F	10	--	58.6 ^a
NTP (1993)	Mouse (B6C3F1)	M	10	223	553 ^b
		F	10	370	676 ^b

^aDoses were calculated using water consumption rates and body weights measured during the last week of exposure, and therefore differ slightly from those presented in Section 4.2.

^bThe LOAEL in mice was based on reduced body weight and body weight gain.

in low-dose male rats (54.9 mg/kg-day using water consumption rates and body weights measured during the last week of exposure). However, as discussed in Section 4.5, these hepatocellular changes probably represent adaptation to a subclinical level of hemolysis produced at this dose. Although a lower LOAEL was reported in male rats, this value gives no indication of the relative slope of the dose-response curve for males and females. Because this is an important factor for benchmark dose(BMD) analyses (U.S. EPA, 1995b, 1996c), a comparison of the MCV and RBC count results for both male and female rats was performed, which demonstrates that female rats are more sensitive to the effects of EGBE than are males. For this reason, dose-response information on the hematologic effects in female rats was selected as the basis for the oral RfD BMD analyses discussed below.

In the female rat study (NTP, 1993), groups of 10 female F344 rats were exposed to 0, 750, 1,500, 3,000, 4,500, and 6,000 ppm EGBE via the drinking water for 13 weeks. Body and organ weights were measured. In addition, clinical, hematologic, 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, Hgb, and Hct, and increased RTC and MCV.

Hematologic effects appear to be the most sensitive of the adverse effects caused by EGBE in laboratory animals. Less clear, however, is the decision as to which of the hematologic endpoints (changes in RBC count, RTC, MCV, Hct, and Hgb) observed in EGBE-exposed animals is the most appropriate basis for an RfC/RfD. The suggested mechanism of action of EGBE is based on the fact that BAA, an oxidative metabolite of EGBE, appears to be the causative agent in hemolysis (Carpenter et al., 1956; Ghanayem et al., 1987b, 1990). The first event in this mechanism of action is the interaction between BAA and cellular molecule(s) in erythrocytes. The second event is erythrocyte swelling. The third event is cell lysis mediated by the increase in osmotic fragility, and a loss of deformability of the erythrocyte (Ghanayem, 1989; Udden, 1994, 1995a; Udden and Patton, 1994), which results in decreased values for RBC count, Hgb, and Hct. The last event is compensatory erythropoiesis; that is, in response to the loss of erythrocytes, the bone marrow responds by increasing the production of young RBCs (RTCs).

Although changes in RTC sometimes represent the largest measurable differences between exposed animals and unexposed controls, this parameter is highly variable (covariance = 30%-60%) and does not always exhibit a clear dose-dependent trend (NTP, 1993, 1998). The use of RTC as the critical endpoint is analogous to the use of cell proliferation versus quantification of cell death (histopathologic). While cell death has a direct relationship to chemical exposure, cell proliferation has multiple feedback control processes that can be both very sensitive and variable. Therefore, changes in RTC are not considered a suitable endpoint for deriving the RfC or RfD. As discussed in Section 4.2, both cell swelling (second event of the proposed mechanism of action) and an increased number of larger RTCs (last event) can result in increased MCV, and decreased RBC count suggests cell lysis (third event). Until more is known about the molecular interaction between BAA and specific cellular molecules, changes in MCV and RBC count must serve as the earliest measurable responses for both oral and inhalation exposures to EGBE. For this reason, dose-response information on MCV and RBC count is considered for derivation of an RfC and an RfD for EGBE.

While the toxicokinetic mechanism proposed above may 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 gavage studies of Ghanayem et al. (1987c) and NTP (1998) inhalation studies, Hct, a measure of erythrocyte volume relative to blood volume, tended to decrease along with RBC count and Hgb at all exposure levels for which a hematologic effect was observed. However, Hct did not change as RBC count and Hgb decreased following drinking water exposures (NTP, 1993). Thus, the loss of erythrocytes (reduced RBC count) was apparently offset by a concurrent increase in the size of the individual cells (increased MCV) in the drinking water studies. This was not the case in the gavage and inhalation studies. Until the reason for this difference is known, EPA has chosen to make use of the empirically more sensitive endpoint (the endpoint that results in the steepest dose response curve) in the following RfD/RfC derivations.

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

The human equivalent doses (HEDs) have been calculated via four methods summarized below.

5.1.2.1. *Standard Default Methods*

Because animals were exposed continuously to EGBE in the critical study (NTP, 1993), adjustments to the male rat LOAEL of 55 mg/kg-day were not required to obtain an HED. The male rat LOAEL was chosen for this method because it provides the more conservative RfD. The preceding paragraphs discuss why female rat data were used for the BMD analysis used in two of the other three RfD derivation methods discussed below. The female rat data were also used for the following PBPK method because this method was not applicable to the male rat endpoint.

5.1.2.2. *PBPK Methods*

Several PBPK models have been developed for EGBE, all of which are capable of estimating internal doses. These models are summarized briefly in Table 2.

Table 2. 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. (1994, 1997)	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

Of these models, the Corley et al. (1994, 1997) model is considered the most complete and appropriate for use in the derivation of the oral RfD because it has been experimentally validated, covers all routes of exposure, and addresses both the distribution and excretion of the toxic metabolite, BAA, via the oral route of exposure. This model is summarized in Appendix B. In addition to selecting a PBPK model, it is also important to determine what estimate of internal dose (i.e., dose metric) can serve as the most appropriate for adverse health effects.

The PBPK model of Corley et al. (1994, 1997) is capable of calculating several measures of dose for both EGBE and BAA, including the following:

- C_{\max} —this represents the peak concentration of EGBE or BAA in the blood during the exposure period.
- AUC—On the other hand, this represents the cumulative product of concentration and time for EGBE and BAA in the blood.

Two important pieces of information were used to select C_{\max} for BAA in the blood as the more appropriate dose metric. 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, Hgb and Hct, and kidney pathology (Hgb casts and intracytoplasmic Hgb). However, hemolytic effects were not reported at a similar acute drinking water dose of 140 mg/kg (Medinsky et al., 1990). While a slight drop in RBC count and Hgb (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 to up to 452 mg/kg-day EGBE (NTP, 1993). Finally, Corley et al. (1994) have also suggested that C_{\max} may be a better dose metric than AUC.

Four steps were involved in using the Corley et al. (1994, 1997) PBPK model as modified by Corley et al. (1997) to calculate the HED corresponding to the LOAEL identified in the animal study ($LOAEL_{HED}$): (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 liters of water during a 12-hour awake cycle; and (4) calculate the HED (mg/kg-day) for the amount of EGBE consumed in 2 liters of water that resulted in the same internal dose (C_{\max} BAA) simulated for the animal in Step 1 as shown below.

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 hematologic determination)

$$C_{\max} \text{ BAA} = 103 \mu\text{M}$$

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 hours/day, 7 days/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.

Water concentration (ppm)	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 $\text{LOAEL}_{\text{HED}}$ for a 70 kg human consuming EGBE in 2 liters 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

$\text{LOAEL}_{\text{HED}}$ continuous exposure = 7.6 mg/kg-day (calculated by regression of the internal dose vs. the dose of EGBE from Step 3).

The $\text{LOAEL}_{\text{HED}}$ calculated using the PBPK model is likely a conservative 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 ($\text{LOAEL}_{\text{HED}}$) 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 upon 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 peak 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 hours/day dosing period.

5.1.2.3. BMD Method

All BMD assessments in this review were performed using EPA Benchmark Dose Software (BMDS). A copy of the latest version of BMDS can be obtained from the Internet at www.epa.gov/ncea/bmds.htm. For the purposes of deriving an RfD for EGBE, hematologic endpoints (see discussion in Section 5.1.1) were evaluated as continuous data. For this reason, the BMD₀₅ was considered a more appropriate basis than the BMD₁₀ (U.S. EPA, 1995b, 1996c). MCV was the most sensitive hematologic endpoint in the NTP (1993) study. Observed versus predicted MCV responses using the BMDS Power model (version 1.1.1b) are provided in Figure 2 for EGBE-exposed female rats. A textual description of these results is provided in Appendix C. The BMD₀₅ was determined to be 49 mg/kg-day, using the 95% lower confidence limit of the dose-response curve expressed in terms of administered dose. This value is considered to be an HED under this method of analysis because animals were exposed continuously, negating the need for a duration adjustment.

5.1.2.4. PBPK and BMD Methods Combined

C_{max} for BAA in arterial blood was determined using the PBPK model of Corley et al. (1994) as modified by Corley et al. (1997). The results of this modeling effort are summarized in Table 3.

Graphic results of the BMDS Power model (version 1.1.1b) assessment of MCV responses in female rats (NTP, 1998) versus corresponding PBPK estimates of C_{max} for BAA in female rat blood are provided in Figure 3. A textual description of these results is provided in Appendix C. The BMD₀₅ was determined to be 64 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. (1994, 1997) PBPK model was used to “back-calculate” an HED of 5.1 mg/kg-day, assuming that rats and humans receive their entire dose of EGBE from drinking water over a 12-hour period each day.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors and Modifying Factors

Uncertainty factors (UFs) are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). Historically, UFs are applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF of 10^{1/2} (3.162; U.S. EPA, 1994b) on the assumption that the actual values for the UFs are log-normally distributed. Application of these factors in the assessments is that, when a single partial UF is applied, the factor is rounded to 3; the total factor for a UF of 3 and 10, for example, would be 30 (3 × 10). When two partial UFs are evoked, however, they are not rounded, so UFs of 3, 3, and 10 would result in a total uncertainty of 100 (actually 10^{1/2} × 10^{1/2} × 10¹). Uncertainty factors applied for this RfD assessment and the justification for their use are as follows.

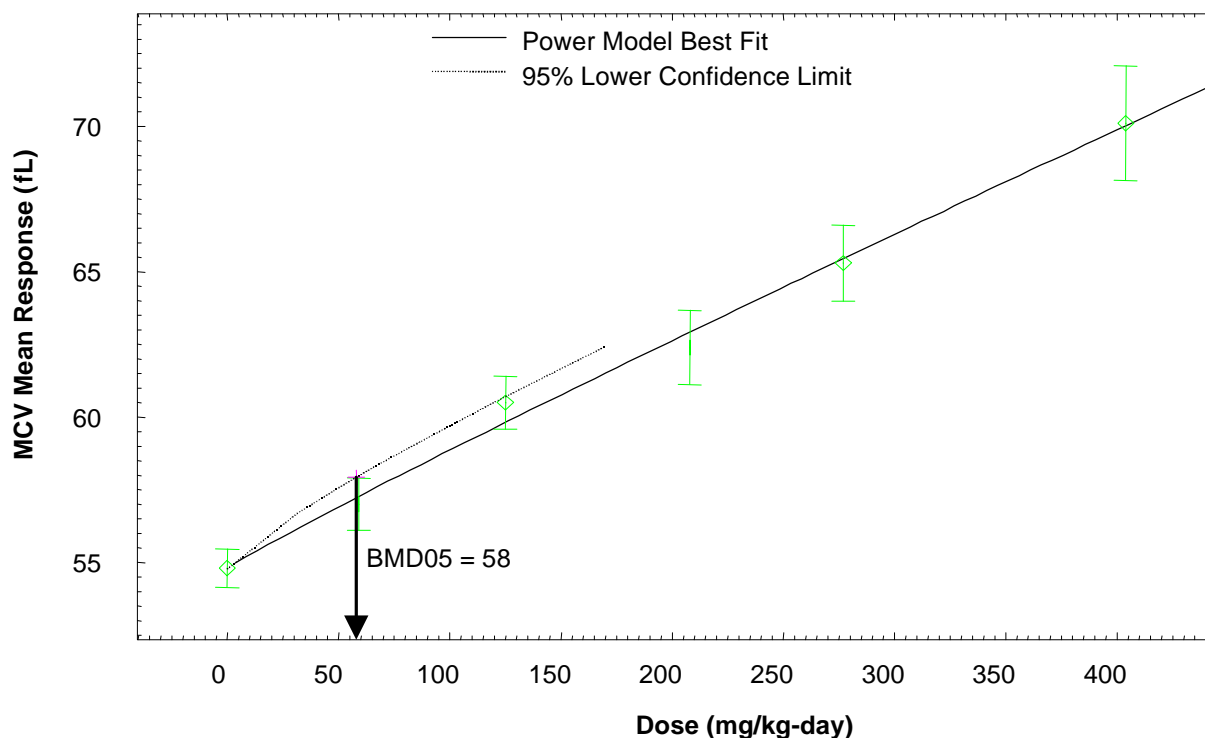


Figure 2. BMD plot of MCV data (expressed in terms of femtoliters, fL) in female rats following oral exposure to EGBE (NTP, 1993) using external dose (mg/kg-day).

Table 3. Corley et al. (1994, 1997) model estimates of BAA blood levels in female rats following oral exposures

Water conc. (ppm)	Water intake (L/day)	Female Body weight (g)	BAA in Blood	
			Dose (mg/kg-day)	C _{max} (uM)
750	0.0147	188	59	103
1,500	0.0155	185	125	253
3,000	0.0125	180	208	495
4,500	0.0101	164	277	738
6,000	0.0101	150	404	1,355

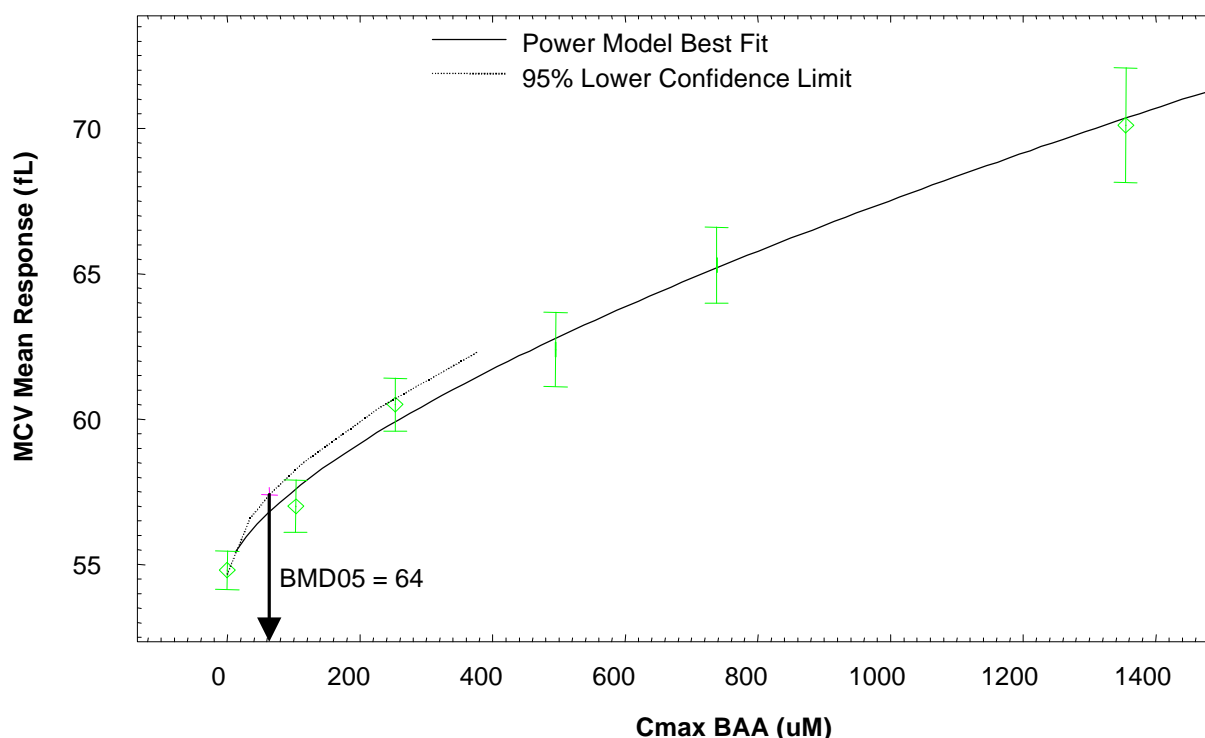


Figure 3. BMD plot of MCV data (expressed in terms of fL) in female rats following oral exposure to EGBE (NTP, 1993) using internal dosimetry (BAA C_{max} , μM).

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 walls are less resistant 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 *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 other details in 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.

The UF for interspecies variation (UF_A) accounts for pharmacodynamic and pharmacokinetic differences between animals and humans. There is *in vivo* (Carpenter et al., 1956) and *in vitro* (Ghanayem and Sullivan, 1993; Udden and Patton, 1994; Udden, 1995b) information indicating that, pharmacodynamically, humans are less sensitive than rats to the hematologic effects of EGBE. For this reason, a fractional component of the UF_A was considered. However, the *in vivo* relative insensitivity of humans cannot be quantified at this time. Thus, for all RfD derivation approaches discussed above, a value of 1 was used to account for pharmacodynamic differences between rats and humans. Under the standard default and BMD approaches described above, an overall UF_A of 3 (1 for

pharmacodynamics \times 3 for pharmacokinetics) was used. For the PBPK and the combined PBPK/BMC (benchmark concentration) approaches, an overall UF_A of 1 (1 for pharmacodynamics \times 1 for pharmacokinetics) was used because pharmacokinetic differences between rats and humans are adequately accounted for by a PBPK model.

For all RfD calculation approaches, a value of 1 was selected for extrapolating the results from a subchronic study to chronic exposures (UF_S). Although no chronic oral studies are currently available for EGBE, there does not appear to be a significant increase in the severity of hemolytic effects beyond 1-3 weeks of oral (NTP, 1993) or inhalation (NTP, 1998) EGBE exposures.

For the standard default and PBPK methods, a value of 3 was selected for extrapolating a LOAEL to a NOAEL (UF_L). A value of less than 10 is justifiable because there is information that indicates the LOAEL is very near the threshold level for the hematologic effects of concern. For example, the effects observed in the critical study at the lowest drinking water doses were fairly mild. All hematologic endpoints except for RTCs were within 5% of the control value. For BMD analyses, a value of 1 was used because of the minimal and precursive nature of the critical lesion (cell swelling as measured by increased MCV) and the fact that a BMD_{05} for a minimally adverse effect is typically deemed to be equivalent to a NOAEL for continuous data sets (U.S. EPA, 1995b, 1996c).

A value of 1 was used for the database UF_D for all methods of analyses. While no chronic oral studies or adequate human data are available for EGBE, oral and inhalation dose-response data indicate that there would be little if any increase in severity of hemolytic effects beyond subchronic exposure durations (NTP, 1993, 1998). There are chronic and subchronic studies available in two species (rats and mice) and adequate reproductive and developmental studies, as well as limited studies in humans following short-term inhalation exposure.

A modifying factor (MF) of 1 was used for all approaches. A summary of how the five UF s and one MF were applied for the four RfD calculation approaches discussed is provided in Table 4.

The combined PBPK and BMD method was used to derive the RfD, since this approach incorporates much of the mechanistic information available for EGBE, best characterizes the dose-response relationships for EGBE-induced hematologic effects, and reduces the potential uncertainties to the greatest extent. Thus, the total UF is 10 and the MF is 1. The $RfD = 5.1 \text{ mg/kg-day} \div 10 = 0.5 \text{ mg/kg-day}$.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

Short-term studies by Dodd et al. (1983) in rats and Tyl et al. (1984) in rats and rabbits, although well conducted, were not considered long enough to be useful for predicting toxicity following chronic inhalation exposures. In addition, the blood samples evaluated by Tyl et al. (1984)

were collected 6 days following the last exposure. For this reason, these studies are not considered further. The results of several candidate subchronic and chronic studies are summarized in Table 5.

Hematologic effects from EGBE exposure are the only effects consistently observed across both sexes and species that have been studied. NTP (1998) observed forestomach ulcers in female mice at all exposure levels, but this effect has not been observed in any other species, nor in mice exposed orally to EGBE (NTP, 1993), and although incidence of this lesion increased with exposure, severity of the lesion did not increase with increasing dose. For these reasons, and because hematologic effects have been observed in humans acutely exposed to EGBE, hematologic effects are considered the critical effects of concern for the purpose of the RfC derivation. Based on a comparison of effect levels, female rats (NTP, 1998) appear to be more sensitive to the hematologic

Table 4. Summary of application of uncertainty factors and modifying factor for RfD calculation

Factor	Approach			
	Standard	PBPK	BMD	PBPK & BMD
UF _H	10	10	10	10
UF _A	3	1	3	1
UF _S	1	1	1	1
UF _L	3	3	1	1
UF _D	1	1	1	1
UF _(Total)	100	30	30	10
RfD (mg/kg-day)	55/100 = 0.6	7.6/30 = 0.3	49/30 = 2	5.1/10 = 0.5

Table 5. Results of candidate studies

Reference	Species (strain)	Sex	#/Dose group	Duration (months)	Hematologic effect levels (ppm)	
					NOAEL	LOAEL
NTP (1998)	Rat (Fischer)	M	9-10	3, 9, 12; 14 weeks	31	62.5
		F	9-10	3, 9, 12; 14 weeks	--	31
NTP (1998)	Mouse (B6C3F1)	M	9-10	3, 9, 12; 14 weeks	31	62.5
		F	9-10	3, 9, 12; 14 weeks	31	62.5
Dodd et al. (1983)	Rat (F344)	MF	16	9	25	77

effects of EGBE than the other animals. In the female rat study, NTP (1998) exposed groups of 9-10 female F344 rats to 0, 31, 62.5, 125, 250, and 500 ppm EGBE in air for 3 to 12 months (6 hours/day, 5 days/week). Body and organ weights were measured, and clinical, hematologic, gross, and histopathologic examinations were conducted. Female rats exposed to the three highest concentrations at all exposure durations developed clinical signs consistent with the hemolytic effects associated with EGBE exposures. Mild to moderate regenerative anemia was observed in females exposed to all concentrations. Exposure-related trends were noted for RTC, RBC count, MCV, Hgb, and Hct. Liver-to-body weight ratios were significantly increased in females exposed to the highest concentration. Histopathologic effects observed in rats included excessive extramedullary splenic hematopoiesis, hemosiderosis, and hemosiderin accumulation in Kupffer cells of the liver (secondary to hemolysis). A LOAEL of 31 ppm was identified in this study for hematologic and histopathologic effects in female rats. A NOAEL for these effects was not identified. Therefore, the female rat data from this study were used as the basis for an RfC.

It is recognized that the NOAEL/LOAEL designations listed above for each study do not necessarily indicate the slope of the concentration-response curve, an important factor in BMC (used to assess inhalation studies in the same manner as BMDs are used to assess oral studies) analysis (U.S. EPA, 1995b, 1996c). For this reason, BMC analyses were also performed on the other subchronic and chronic studies (Dodd et al., 1983; NTP, 1998 in mice). The rationale for choice of the critical effect for use in the BMC analyses, changes in MCV and RBC count, is the same as for oral exposure to EGBE and is summarized in Section 5.1.1.

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

The human equivalent concentrations (HECs) have been calculated via four methods.

5.2.2.1. Standard Default Methods

There are two steps to calculating an HEC ($LOAEL_{HEC}$) from a LOAEL identified in an animal study: (1) convert units to mg/m^3 ($31 \text{ ppm} \times 4.84 [mg/m^3]/ppm = 150 \text{ mg/m}^3$), and (2) account for the ratio of blood:air partitioning of the chemical for laboratory animals to humans ($150 \text{ mg/m}^3 \times 1 [\text{default ratio}] = 150 \text{ mg/m}^3$). This value is considered an HED and is not adjusted for less than continuous exposure because, as discussed in Section 5.1.2, dose rate is considered a more important determinant of effects from EGBE than total dose. Thus, a $LOAEL_{HEC}$ of 150 mg/m^3 was calculated for EGBE by this standard default method.

5.2.2.2. PBPK Methods

The model of Lee et al. (1998) was used to estimate BAA blood concentrations in female rats following inhalation exposure to EGBE because it is a recent extension of the Corley et al. (1994, 1997) model for inhalation exposures and includes added parameters for female rats. As in the case of the RfD (see Section 5.1.2.2), C_{max} is considered a more appropriate dose metric than AUC, and the PBPK model of Corley et al. (1994, 1997) was used to obtain estimates of human C_{max} concentrations from the female rat data. The same procedure was used to calculate the HEC corresponding to the LOAEL identified in the animal study ($LOAEL_{HEC}$): (1) calculate the internal dose surrogate (C_{max}

BAA in blood) corresponding to the female rat LOAEL using the actual experimental exposure regimen (6 hours/day, 5 days/week) 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 continuously exposed (24 hours/day, 7 days/week) to varying concentrations of EGBE; (4) calculate the continuous HEC for EGBE in air that resulted in the same internal dose (C_{\max} BAA) simulated for the animal in Step 1; and (5) convert the EGBE exposure units from ppm to mg/m^3 as shown below.

Step 1: Calculate C_{\max} for BAA in blood corresponding to female rat LOAEL (Lee et al., 1998).

Female rat LOAEL = 31 ppm

C_{\max} BAA = 285 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 6 hours/day, 5 days/week 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 (Corley et al., 1994, 1997).

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 $\text{LOAEL}_{\text{HEC}}$ of EGBE for continuous human exposures producing the same C_{\max} of BAA in blood calculated for the animal study in Step 1.

Female rat C_{\max} BAA = 285 μM

HEC continuous exposure = 98 ppm (calculated by regression of internal dose versus the concentration of EGBE in air from Step 3).

Step 5: Unit conversion.

$$\begin{aligned} \text{LOAEL}_{\text{HEC}} (\text{mg}/\text{m}^3) &= \text{Conversion factor} \times \text{LOAEL}_{\text{HEC}} (\text{ppm}) \\ &= 4.84 (\text{mg}/\text{m}^3)/(\text{ppm}) \times 98 \text{ ppm} \\ &= 474 \text{ mg}/\text{m}^3 \end{aligned}$$

5.2.2.3. BMC Method

For the purposes of deriving an RfC for EGBE, both MCV and RBC count response data were evaluated in female rats (see discussion in Section 5.1.1) for all exposure durations studied by

NTP (1998). Because these endpoints were evaluated as continuous data, the BMC_{05} was considered a more appropriate basis than the BMC_{10} (U.S. EPA, 1995b, 1996c). The steepest concentration-response curves (and the lowest BMC_{05} estimate) were obtained for decreased RBC count in female rats. Since severity of the effect did not change with increasing exposure duration, all exposure durations (14 weeks, 3, 6, and 12 months) were considered. The best model fit ($p=0.8652$) was obtained from response data from the 14-week subchronic study, using the BMDS polynomial model and excluding the two highest exposure levels (250 and 500 ppm). Observed versus predicted RBC count responses using a polynomial model are provided in Figure 4. A textual description of these results is provided in Appendix C. As discussed in Section 5.1.2.3, all BMD assessments in this review were performed using EPA BMDS version 1.1b. The BMC_{05} was determined to be 27 ppm (130 mg/m^3) using the 95% lower confidence limit of the dose-response curve expressed in terms of administered concentration. Use of response information from just the lowest four exposure groups (0, 31, 62.5, and 125 ppm) is justified based on both a significantly improved model fit and increased relevancy to any potential human exposure and effect scenario. Although poorer model fits were obtained, assessments using other model and duration combinations were supportive of these results (BMC_{05} estimates ranging from 15 to 58 ppm). The estimated BMC_{05} of 130 mg/m^3 is considered an HED and is not adjusted for less than continuous exposure because, as discussed in Section 5.1.2.3, dose rate is considered a more important determinant of effects from EGBE than total dose.

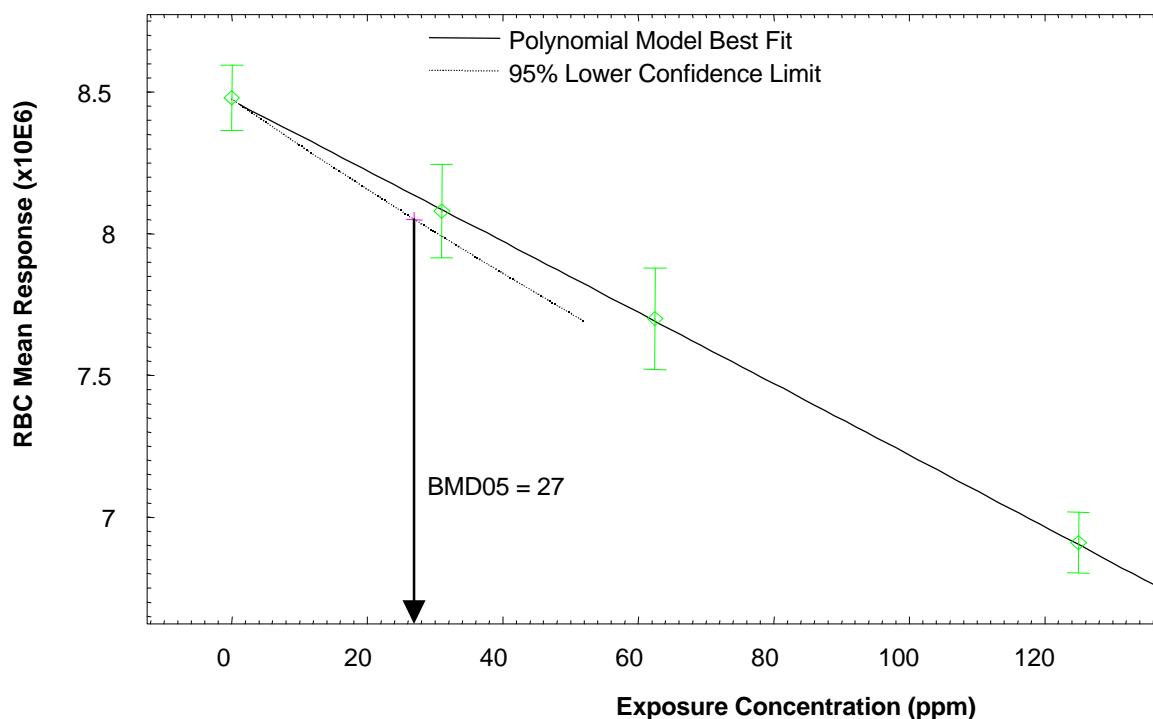


Figure 4. BMC plot of RBC count in female rats following 14-week inhalation exposure to EGBE (NTP, 1998) using external dose (ppm).

5.2.2.4. PBPK and BMC Methods Combined

C_{\max} for BAA in arterial blood of rats was determined using the PBPK model of Lee et al. (1998). Dermal exposures to the EGBE vapor were not considered in the predicted blood levels. This is because the estimated relative contribution of the skin to the total uptake of unclothed humans exposed to 25 ppm EGBE for 8 hours ranged only from 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 6.

Graphic results of a Power model assessment of RBC count responses in female rats (NTP, 1998) versus corresponding PBPK estimates of C_{\max} for BAA in female rat blood are provided in Figure 5. A textual description of these results is provided in Appendix C. As discussed in Section 5.1.2.3, all BMD assessments in this review were performed using EPA BMDS version 1.1b. The BMD_{05} was determined to be 225 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" HEC of 78 ppm (380 mg/m^3) assuming continuous exposure (24 hours/day).

5.2.3. RfC Derivation—Including Application of Uncertainty Factors and Modifying Factors

UFs are applied to account for recognized uncertainties in extrapolation from experimental conditions to the assumed human scenario (i.e., chronic exposure over a lifetime). Historically, UFs are applied as values of 10 in a multiplicative fashion (Dourson and Stara, 1983). Recent EPA practice, however, also includes use of a partial UF of $10^{1/2}$ (3.162; U.S. EPA, 1994b) on the assumption that the actual values for the UFs are log-normally distributed. Application of these factors in the assessments is that, when a single partial UF is applied, the factor is rounded to 3, such that the total factor for a UF of 3 and 10, for example, 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 $10^{1/2} \times 10^{1/2} \times 10^1$). Uncertainty factors applied for this RfC assessment and the justification for their use are as follows.

Table 6. Lee et al. (1998) 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} (μM) in female rats
31	216	285
61.5	211	603
125	214	1243
250	210	1959
500	201	4227

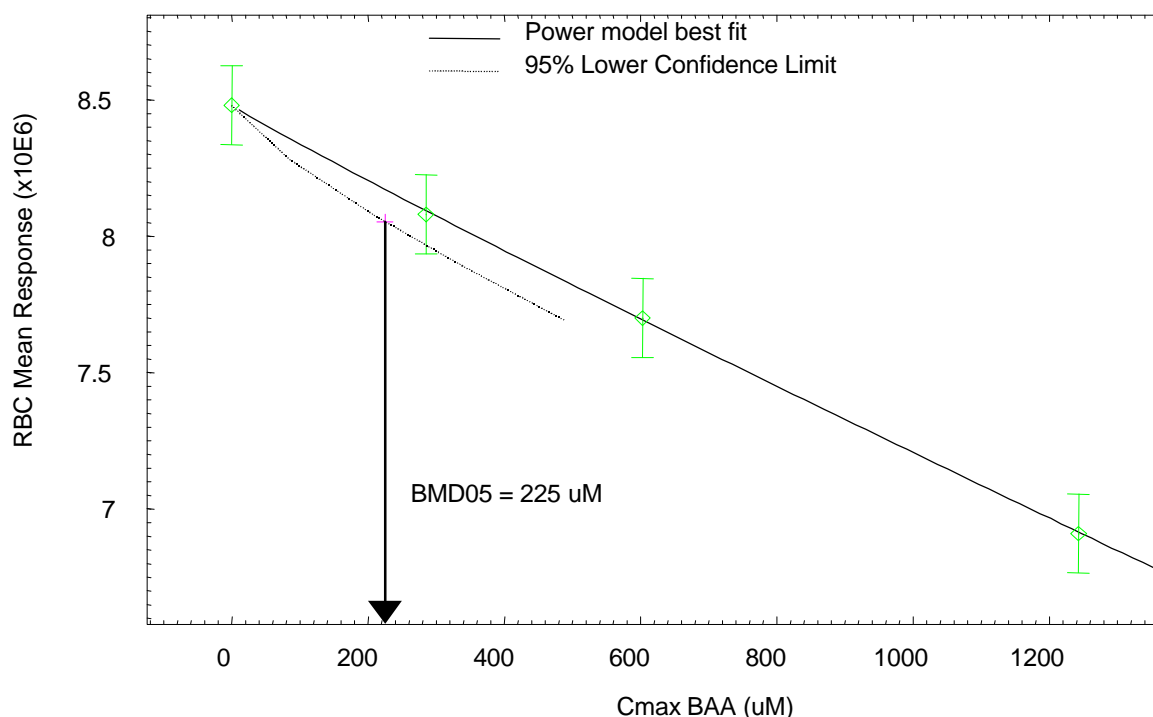


Figure 5. BMC plot of RBC count in female rats following 14-week inhalation exposure to EGBE (NTP, 1998) using internal dosimetry (BAA, C_{max} , μM).

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 walls are less resistant 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 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 other details in 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.

The UF for interspecies variation (UF_A) accounts for pharmacodynamic and pharmacokinetic differences between animals and humans. There is in vivo (Carpenter et al., 1956) and in vitro (Ghanayem and Sullivan, 1993; Udden and Patton, 1994; Udden, 1995b) information indicating that, pharmacodynamically, humans are less sensitive than rats to the hematologic effects of EGBE. For this reason, a fractional component of the UF_A was considered. However, the in vivo relative insensitivity of humans cannot be quantified at this time. Thus, for all RfC derivation approaches discussed above, a value of 1 was used to account for pharmacodynamic differences between rats and humans. Further, each approach accounts for pharmacokinetic differences between rats and humans by either PBPK

models or EPA default methods. Thus, an overall UF_A of 1 (1 for pharmacodynamics \times 1 for pharmacokinetics) was used for all of the RfC derivation approaches.

For all RfC calculation approaches, a value of 1 was selected for extrapolating the results from a subchronic study to chronic exposures (UF_S). Recent chronic studies indicate that a significant increase in the severity of hemolytic effects beyond 1-3 weeks of inhalation exposure time to EGBE (NTP, 1998) would not be expected.

For RfC derivation methods, a value of 3 was selected for extrapolating a LOAEL or BMC estimate to a NOAEL (UF_L). A UF_L value of less than 10 is justifiable because there is information that indicates that both the chosen LOAEL and estimated BMC_{05} values are near the threshold level for the hematologic effects of concern. The measured hematologic effects that formed the basis for these values were mildly adverse and within 5% of the control value. In addition, the female rat LOAEL (150 mg/m^3) and BMC_{05} (130 mg/m^3) values derived from the NTP (1998) subchronic/chronic inhalation study are very close to the 121 mg/m^3 (25 ppm) NOAEL identified for male and female rats in the Dodd et al. (1983) subchronic inhalation study. In the case of the RfD (Section 5.1.3), a UF_L value of 1 was used for the BMD analyses because the RfD BMDs were based on a minimal and precursive lesion (cell swelling as measured by increased MCV). A threefold UF_L is retained for the RfC BMC analyses because the RfC BMCs are based on a more serious hematologic endpoint (RBC lysis as measured by a decrease in RBC count).

A value of 1 was used for the database UF_D for all methods of analyses. Subchronic and chronic inhalation studies suggest that there is little, if any, increase in severity of hemolytic effects beyond subchronic exposure durations (NTP, 1993, 1998). There are chronic and subchronic studies available in two species (rats and mice) and adequate reproductive and developmental studies, as well as limited studies in humans following short-term inhalation exposure.

An MF of 1 was used for all approaches. A summary of how the five UFs and one MF were applied for the four RfC calculation approaches discussed is provided in Table 7.

The combined PBPK and BMD/C method was used to derive the RfC, since this approach incorporates much of the mechanistic information available for EGBE, best characterizes the dose-response relationships for EGBE-induced hematologic effects, and reduces the potential uncertainties to the greatest extent. Thus, the total UF is 30 and the MF is 1; $RfC = 380 \text{ mg/m}^3 \div 30 = 13 \text{ mg/m}^3$.

5.3. CANCER ASSESSMENT

As discussed above (Sections 4.2 and 4.6), there are currently no human epidemiologic, occupational studies addressing the potential carcinogenicity of EGBE. A 2-year inhalation bioassay using mice and rats has recently been completed (NTP, 1998) and reports significant increases in certain types of tumors in exposed mice compared with controls, but not in rats. The relevancy of these tumors to humans is not clear at this time, as is discussed in Section 4.6, and a quantitative assessment was not performed.

Table 7. Summary of application of UFs and MF for RFC calculation

Factor	Approach			
	Standard	PBPK	BMC	PBPK & BMC
UF _H	10	10	10	10
UF _A	1	1	1	1
UF _S	1	1	1	1
UF _L	3	3	3	3
UF _D	1	1	1	1
UF _(Total)	30	30	30	30
RfC mg/m ³	150/30 = 5	474/30 = 16	130/30 = 4	380/30 = 13

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 BAA, the proximate toxicant, in both humans and animals. This acid and its conjugates are readily excreted in the urine.

Hemolysis has been identified as the critical endpoint of concern in toxicological studies on EGBE. Humans are significantly less sensitive to the hemolytic toxicity of EGBE than are typical laboratory species such as mice, rats, or rabbits. This has been demonstrated in numerous laboratory studies and through the use of in vitro studies using either whole blood or washed erythrocytes. In addition to hemolytic effects of EGBE, other effects (e.g., liver, spleen, and kidney) have been observed in laboratory animals with exposure to EGBE. While male rats in one study (NTP, 1993) experienced mild liver effects at a drinking water dose lower than that which caused observable hemolytic effects, human case report and controlled study data and most laboratory animal evidence suggest that these other effects are secondary to hemolysis. Available human toxicity data show that after acute oral ingestion of large doses of EGBE combined with other solvents, hematologic changes and metabolic acidosis are the primary effects. 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 (2-methoxyethanol) and EGEE (2-ethoxyethanol), the reproductive toxicity of EGBE has been studied in a variety of well-conducted oral (Nagano et al., 1979, 1984; Grant et al., 1985; Foster et al., 1987; Heindel et al., 1990; Exon, 1991; NTP, 1993) and inhalation (Dodd et al., 1983; Doe, 1984; Nachreiner, 1994; NTP, 1998) studies 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 (Wier et al., 1987; Sleet et al., 1989), inhalation (Nelson et al., 1984; Tyl et al., 1984), and dermal (Hardin et al., 1984) exposures 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 (> 1,000 mg/kg). Maternal toxicity related to the hematologic effects of EGBE and relatively minor developmental effects have been reported in developmental studies. No teratogenic toxicities 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 parents, nor to the developing fetuses of laboratory animals.

No reliable human epidemiologic studies are available that address the potential carcinogenicity of EGBE. A draft report of the results of a 2-year inhalation bioassay performed using rats and mice has recently become available (NTP, 1998). NTP (1998) 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. They also reported *some evidence of carcinogenic activity* in male B6C3F1 mice based on increased incidences of hemangiosarcoma of the liver, and *some evidence of carcinogenic activity* in female B6C3F1 mice based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma). As discussed in more detail in Section 4.6, because of the uncertain relevance of these tumor increases to humans, the fact that EGBE is generally negative in genotoxic tests, and the lack of human data to support the findings in rodents, the human carcinogenic potential of EGBE, in accordance with the recent *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1996a), *cannot be determined* at this time, but suggestive evidence exists from rodent studies. Under existing EPA guidelines (U.S. EPA, 1986a), EGBE is judged to be a *possible human carcinogen*. 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, because no relevant human data exist.

The human oral dose that is likely to be without an appreciable risk of deleterious noncancer effect during a lifetime (the RfD) is 0.5 mg/kg-day. This value was obtained by dividing the estimated human equivalent BMC_{05} of 5 mg/kg-day by a UF of 10 (see summary of UF below). The human equivalent BMC_{05} was estimated using C_{max} for BAA in blood as the dose metric, calculating a BMD_{05} of 64 μ M, and then using the BMD approach described in the previous section and a PBPK model to “back-calculate” an HED, assuming that rats and humans receive 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 the combined PBPK/BMD method. A higher confidence is placed in the RfD values derived from internal dose measures, since pharmacokinetic differences between rats and humans were accounted for using a validated PBPK model (Corley et al., 1994, 1997). Medium confidence is placed on the NTP (1993) study because it was not a chronic study; however, the study employed both male and female rats and mice, provided a wide range of exposure levels (0-6,000 ppm EGBE in drinking water), and observed animals 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 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 walls are less resistant 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. An MF was not employed ($MF = 1$). For a more detailed discussion of the RfD UF, see Section 5.1.3.

The daily inhalation exposure to the human population that is likely to be without an appreciable risk of deleterious noncancer effect during a lifetime (the RfC) is 13 mg/m^3 . This amount is $1/30$ the human equivalent BMC_{05} of 380 mg/m^3 , which was “back-calculated” from rat data using the BMD and PBPK approach described in the previous section.

The overall confidence in the RfC assessment is medium to high. A 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., 1994, 1997). High confidence is placed on the NTP (1998) 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.

In the derivation of the RfC, a 30-fold UF was applied, which is intended to account for intrahuman variability and extrapolation from an adverse effect level. 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 walls are less resistant to the lysis caused by BAA. An uncertainty factor 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. In the case of the RfC, a partial threefold LOAEL to NOAEL UF (UF_L) is retained because the BMC used in the derivation of the RfC was based on a more serious hematologic endpoint (RBC lysis as measured by a decrease in RBC count) than the effect that formed the basis for the RfD BMD (cell swelling as measured by an increase in MCV). An MF was not employed ($MF = 1$). For a more detailed discussion of the RfC UF, see Section 5.2.3.

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APPENDIX A. EXTERNAL PEER REVIEW— SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for ethylene glycol butyl ether (EGBE) have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists chosen by EPA's contractor in accordance with guidance on peer review (U.S. EPA, 1994c). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. Public comments also were read and carefully considered. The four external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of comments made by the external reviewers and EPA's response to these comments follows.

(1) General Comments

Reviewers felt that the IRIS file for EGBE was an acceptable basis for the derivation of an RfD and RfC, calling it “an excellent synthesis of the current state of knowledge,” “a well written, concise review,” and “a very thorough, credible, and lucid scientific presentation.” However, two reviewers felt that the cancer assessment was premature and should be postponed pending publication of a final report on the NTP chronic inhalation bioassay. This key issue is addressed briefly in the general comment review below and more extensively in Section 6, “Comments on Chemical-Specific Questions.”

A. Comment: One reviewer commented extensively regarding his belief that “the NTP 2-year studies must be addressed more appropriately.” This and one other reviewer suggested that EPA should wait for the final report on the NTP 2-year cancer bioassay.

Response to Comment: The NTP 2-year studies could not be addressed fully in the external peer review EGBE file because the report on its findings was not available. The only materials available at the time were pathology tables from the NTP Internet Web site, which did not include any descriptive text, nor did it include any hematologic information or blood chemistry data. The external peer review draft made appropriate conclusions based on the preliminary nature of the information available. Subsequent to the external peer review, a draft of the NTP 2-year study has been forwarded to EPA and considered in the preparation of the consensus review draft. The final NTP report will also be considered when it becomes available.

B. Comment: One reviewer suggested that EPA consider the following additional studies:

1. The 14-week studies performed in conjunction with the NTP 2-year study.
2. Lee, K; et al. (in press) Physiologically-based pharmacokinetics model for chronic inhalation of 2-butoxyethanol. *Toxicol Appl Pharmacol*.
3. Dill, J; et al. (in press) Toxicokinetics of 2-butoxyethanol and its major metabolites, 2-butoxyacetic acid, in F344 rats and B6C3F1 mice. *Toxicol Appl Pharmacol*.
4. Ghanayem, BI. (1996) An overview of the hematotoxicity of ethylene glycol ethers. *Occup Hyg* 2:253-268.

Response to Comment: Like the NTP 2-year study, the draft report of the 14-week NTP study was not available at the time of the EGBE IRIS file's external peer review. Much more information was available on this study in tabular form, however, and EPA was able to derive an RfC from the available preliminary information on the 14-week study. As with the 2-year study, the draft 14-week study has since been made available to EPA and has been considered in the current EGBE IRIS file version. Studies 2, 3 and 4 above are now available for consideration in the consensus review draft as well, and have been retrieved, reviewed, and cited in the current EGBE IRIS file version.

(2) Study Descriptions

A. Comment: One reviewer suggested inclusion of a “developmental/repro” studies discussion in Section 6.1.

Response to Comment: A paragraph has been added to Section 6.1 to summarize the developmental and reproductive system effect findings of the document.

B. Comment: One reviewer suggested that the lesions observed in rats and mice in the NTP 14-week inhalation exposure study were not discussed.

Response to Comment: This comment is incorrect. These lesions are discussed in Section 4.2 in the discussion of the NTP (1998) study.

C. Comment: One reviewer did not understand the statement in section 4.2 in the discussion of the Krasavage (1986) study, “hematologic changes occurring at 443 and 885 mg/kg-day were increased MCV and decreased MCHC. This seems to be inconsistent with the predominant theory that erythrocyte swelling precedes lysis of the cell.”

Response to Comment: The sentence before this statement indicates that *all* doses of EGBE caused decreased RBC counts and decreased Hgb concentrations. The fact that RBC counts were decreased at doses lower than doses that caused increased MCV is what was intended to be proffered as inconsistent with “swelling precedes lysis.” Thus, the latter sentence above has been replaced with “The decrease in RBC count at a lower dose (222 mg/kg-day) seems to be inconsistent with the predominant theory that erythrocyte swelling (which is indicated by the increased MCV) precedes...”

D. Comment: This same reviewer stated that “the report failed to address the effects of EGBE on the morphology of erythrocytes and how these changes resemble morphological changes reported in certain human blood disorders,” and provided a citation.

Response to Comment: The effects of EGBE on the morphology of erythrocytes, particularly in rats, are discussed, to the extent that they are currently known with any degree of certainty, in several places in Sections 4.2 and 4.5 of the IRIS support document. Additional language has been added to Section 4.5 regarding erythrocyte morphological changes in rats from EGBE exposure that are similar to morphological changes observed in certain human blood disorders. However, no evidence exists that suggests that humans with these blood disorders represent sensitive subpopulations. In fact, experiments by Udden et al. (1994) have shown that blood from persons with

sickle cell anemia is no more sensitive to the hemolytic effects of EGBE than blood from normal persons.

E. Comment: One reviewer pointed out some additional reservations concerning the Haufroid et al. (1997) occupational EGBE exposure study, including the fact that the authors did not account for an important metabolic detoxification pathway (BAA conjugation with glutamine), the higher alcohol consumption among exposed workers, and the lack of any relation between hematologic results and parameters of internal exposure.

Response to Comment: The additional caveats have been added to the EGBE IRIS file.

F. Comment: One reviewer commented that “in the absence of a complete hematologic investigation, a role for hemolysis [in cases of anemia observed after human ingestion during suicide attempts] cannot be substantiated.”

Response to Comment: EPA agrees that the anemia reported in human case reports, particularly Rambourg-Schepens et al. (1988), may have been related to more than just the hemolytic effect of EGBE. A statement regarding the speculative nature of any conclusions in the absence of more detailed hemolysis data has been added to the support document’s discussion of this case report and to Section 4.5, Synthesis and Evaluation of Major Noncancer Effects and Mode of Action—Oral and Inhalation.

G. Comment: A reviewer suggested that the eosinophilic inclusions in rat hepatocytes observed after subchronic exposure to EGBE are reminiscent of Mallory bodies seen in cases of alcoholic hepatitis. The reviewer pointed out that “there is some evidence that Vitamin A depletion accentuates this finding [Mallory bodies],” and that a comparison of the rat diet and liver lesions from the subchronic versus chronic studies would help to resolve whether diet was a contributing factor.

Response to Comment: An association between diet and the eosinophilic inclusions would be difficult, if not impossible, to determine even if the suggested dietary comparison could be performed. Further, since the eosinophilic inclusions were observed in a high percentage (40%-100%) in male and female rats of EGBE exposure groups, and not in the control rats of either sex, it is not likely that they would be due to a dietary deficiency. A comparison was not attempted.

H. Comment: One reviewer did not agree that the bone marrow hyperplasia observed in rats in the NTP subchronic oral and inhalation studies was an indication of “bone marrow toxicity” or “leukemogenicity.” This reviewer argued that this hyperplasia was a secondary adaptive response to hemolysis and that changes in white cell and platelet counts “can be attributed to splenomegaly or splenic congestion.”

Response to Comment: The section of text this reviewer was referring to has been edited. To account for the reviewer’s comment, the sentence that refers to bone marrow and liver toxicity has been supplemented with language indicating that they are possible secondary effects of hemolysis. New information relevant to the association of liver toxicity and hemolysis has been added to Section 4.5 as well.

I. Comment: According to one reviewer, the table in Section 5.1.1 falsely implies that hematologic effects were observed in mice from the NTP subchronic drinking water study.

Response to Comment: A column header for the table has been changed and a footnote was added to the LOAEL for mice indicating that it was based on reduced body weight and reduced body weight gain.

(3) RfD/RfC Calculation

A. Comment: The use of a male rat LOAEL for the standard default method of deriving an oral RfD is “contradictory to the basis of selection of LOAEL provided in preceding paras.”

Response to Comment: The basis for the LOAEL used in the standard default RfD derivation method was not the focus of the paragraphs that precede this derivation. The preceding paragraphs focused on the assumptions, including the use of female rats, for the benchmark dose analysis used in two of the other three RfD derivation methods discussed.

B. Comment: “A sentence to justify the choice of C_{max} as the appropriate dose surrogate may be added” to the PBPK method discussions in Sections 5.1.2 and 5.2.2.

Response to Comment: The PBPK methods discussion in Section 5.1.2 has been rewritten to include an additional page of discussion on this issue. Section 5.2.2 refers to the discussion in Section 5.1.2.

C. Comment: There is no need to adjust for less-than-continuous exposures for the BMC RfC derivation method if, as is stated earlier, “BAA blood concentrations attain steady state during any 24-hr period.” The reviewer contends that “the fact that steady state is attained implies that regardless of the duration of further exposure, there will be no further change in blood concentration of the dose surrogate.”

Response to Comment: The Agency agrees with this comment, and both the standard and BMC methods discussed in Section 5.2.2 have been revised to reflect that these values are not adjusted for less than continuous exposure because, as is now discussed in section 5.1.2, dose rate is considered a more important determinant of effects from EGBE than total dose.

D. Comment: A reviewer argued that the selection of “mean cell volume (MCV) as the earlier endpoint and development of the HEC without further explanation may create conflicting and confusing scientific discussions,” that “using the MCV as an endpoint may have overestimated [the oral HED] by approximately 8 to 20 fold,” and that RBC count data should have been used to determine the NOAEL for hematologic parameters.

Response to Comment: The reviewer is correct in that better dose-response curves are available from RBC count data in the NTP (1998) inhalation study. This was realized originally, but the MCV data were used because it was felt that there were mechanistic reasons to use MCV as the endpoint that measured an early event in the RBC hemolysis process. This thinking has changed after review of the recently released draft NTP (1998) report (see response to Question C, section 6, below,

and current discussions of the 1998 NTP study in Toxicological Review Section 4.2 and Section 5.1.2). Both MCV and RBC count data were considered for the current assessment, with RBC count data being chosen for derivation of the current RfC value.

(4) Uncertainty Factors

Intrahuman Uncertainty Factor (UF_H)—The proposed UF_H for both the RfD and RfC was 3, based on the fact that red blood cells from the elderly and from patients with hemolytic disorders do not show an increased sensitivity to the hemolytic effects of EGBE (Udden, 1994; Udden and Patton, 1994). A UF of 1 was not considered justifiable given that not all potentially sensitive subpopulations have been tested in this manner.

A. Comments on UF_H : One reviewer commented that the threefold UF_H should be retained based on known toxicodynamic differences (factor of 1) and unresolved toxicokinetic differences (factor of 3) between animals and humans.

A second reviewer suggested that the UF_H should be increased to 10-fold because (a) “some humans have exhibited signs of hemolytic anemia after ingestion of EGBE” and “some humans, for reasons not characterized at this time, may be more sensitive than the average population”; (b) some lesions observed in laboratory animals exposed to EGBE resemble lesions observed in humans with certain diseases such as hereditary spherocytosis and sickle cell anemia; and (c) the yet to be published NTP report of a 2-year bioassay “suggests that EGBE is a multisite carcinogen in mice.”

A third reviewer supports an intrahuman UF of 1 because he felt confident in the “decrease in sensitivity of human red cells for hemolysis” and was “not certain at all that the liver toxicity described is independent of hemolysis.” This reviewer felt that it was more reasonable to attribute the hepatotoxicity of EGBE to the secondary changes related to hemolysis and iron deposition. He cited evidence from the NTP drinking water study that rats experienced both liver damage and hemolysis at 55 mg/kg-day, whereas mice experienced hemolysis at much higher doses (550-670 mg/kg-day) and showed no signs of liver damage.

Like the first reviewer, the fourth reviewer commented that “since it was clearly established that humans are more resistant (less sensitive) to potential hemolytic effects of EGBE than the female rat, it is scientifically justifiable to assign an uncertainty factor of 1, not 3, for this part of the determination.” This reviewer felt that the Agency should apply UF to account for “potential chronic hepatic effects in humans *and* for subchronic to chronic extrapolation by applying factors ranging from 1 to 10.”

Response to Comments: In their comments on this issue, all four reviewers have confused the UF_H with the interspecies UF_A to some extent. The purpose of the UF_H is to ensure protection of sensitive subpopulations. Differences between species are more appropriately accounted for by the UF_A . Nevertheless, it has been determined that, as suggested by the second reviewer, a UF_H of 10 is required to fully account for possible human sensitive subpopulations, including children (see expanded discussion in Section 4.7.1). Potentially susceptible subpopulations include individuals with enhanced metabolism or decreased excretion of BAA and individuals whose RBC walls are less resistant to the lysis caused by BAA. 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. 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. The concern over undiagnosed human liver effects expressed by the fourth reviewer is also a valid argument for retention of the 10-fold UF_H default.

Subchronic to Chronic Uncertainty Factor (UF_S)—For all RfC and RfD calculation approaches proposed in the EGBE IRIS Support Document, a partial UF_S of 3 was selected for extrapolating from a subchronic study to chronic exposures. This was based on the fact that, although no chronic studies are currently available (results of a 2-year bioassay have not been reported completely), there does not appear to be a significant increase in the severity of hemolytic effects beyond 1-3 weeks of exposure to EGBE (NTP, 1993).

B. Comment on UF_S : One reviewer commented that “if C_{max} is chosen as the dose surrogate and steady state is attained during subchronic exposures, then the use of a factor of 3 [for the UF_S] is not defensible.” He supported a UF_S of 1 based on this and earlier statements in the document that suggested “no increase in severity of hemolytic effects beyond subchronic exposure durations.”

Response to Comment: The Agency basically agrees with this comment. While the day-to-day attainment of steady state with respect to the C_{max} does not necessarily ensure that the health effects observed at the end of a subchronic study would not progress as a result of chronic exposures, recent dose-response information obtained from the NTP (1998) chronic inhalation study does suggest that the hemolytic effects of EGBE do not progress significantly with chronic exposure. Information from the NTP (1993) drinking water study also suggests a lack of progression in severity beyond the first 1-3 weeks of exposure. For this reason, the UF_S used in the derivation of the oral RfD and the inhalation RfC were reduced to 1.

(5) Weight-of-Evidence/Confidence Levels

No comments were received regarding the cancer weight-of-evidence classification or the noncancer confidence levels; however, two reviewers felt that the cancer assessment was premature and should be postponed, pending publication of a final report on the NTP chronic inhalation bioassay. This was done.

(6) Comments on Chemical-Specific Questions

A. Question: Have we gone too far in analyzing the NTP (1998) chronic inhalation study given that it exists only in the form of data tables on the Internet that have not been peer reviewed at this time?

Comments: Two reviewers felt strongly that EPA should wait for the complete report of the chronic NTP bioassay before finalizing the IRIS support document’s cancer assessment. One reviewer did not address the question directly. Another reviewer had some suggestions on how to interpret the mouse liver and forestomach tumors, but did not feel that it was necessarily inappropriate to use the data tables to support a cancer assessment at this time.

Response to Comments: The draft NTP (1998) report on the chronic inhalation study became available after this peer review and is incorporated into the IRIS file for EGBE.

B. Question: Is adequate justification provided for basing the RfC and RfD for hematologic effects in rats, despite in vitro indications of human insensitivity? Are the liver effects observed by NTP (1993) in rats adequately addressed as likely secondary effects of hemolysis (e.g., in Section 4.5 and as they would impact the RfD/RfC derivations in Section 5)?

Comments: One reviewer did not address this question. All reviewers generally agreed with the use of hematologic data as the basis for the RfD and RfC. One reviewer suggested “modifying factors” be used (or applied to the UF_A) to take into account the difference in sensitivity to hematologic effects of EGBE between humans and rats, and the reviewer did not feel that concern over a possible direct liver effect of EGBE warranted maintaining a threefold intrahuman UF_H . Another reviewer suggested that any conclusion regarding the potential for EGBE to cause liver effects that are not secondary to hemolysis “can only be determined after chronic oral exposure to EGBE or comparison with other similar chemicals that have linked findings in subchronic to observations in chronic studies.”

Response to Comments: The use of a fractional component of the interspecies UF_A or “modifying factors” as suggested by the reviewer, was considered. However, due primarily to limitations in the database of human response information, the in vivo relative insensitivity of humans cannot be quantified at this time. Thus, a value of 1 was used to account for pharmacodynamic differences between rats and humans, and an overall UF_A of 1 (1 for pharmacodynamics \times 1 for pharmacokinetics) was used for derivation of the RfD and RfC.

With respect to the possible direct liver effect of EGBE, the Agency concurs with the second reviewer in believing that this issue requires further oral animal and oral/inhalation human studies before it can be conclusively resolved. Thus, a threefold UF_D has been retained for both the RfD and the RfC.

C. Question: Is the rationale in Section 5 for the selection of MCV as the hematologic endpoint convincing?

Comments: One reviewer did not address this question. Two reviewers agreed with the rationale and choice of MCV as the basis for the RfD and RfC. However, one indicated that he would have measured the MCV and RTC counts by different, more sensitive and reproducible methods. One commenter disagreed with the use of MCV, pointing out that MCV is not a “bench-level measured clinical determination.”

Response to Comments: Data from the recently released NTP (1998) chronic inhalation bioassays in rats and mice caused the Agency to rethink its position on the strict use of MCV as the basis for both the RfD and RfC. In vitro studies by Ghanayem (1989) show that the hemolysis caused by EGBE metabolite BAA is preceded by erythrocyte swelling. However, increased MCV from EGBE exposures can also be attributed to the erythropoietic response subsequent to hemolysis and the corresponding increase in the number of larger RTCs in circulation (NTP, 1998). RTC count was increased significantly in females at 62.5 ppm (6 and 12 months) and in males at 125 ppm (3 and 6 months) of the NTP (1998) chronic rat bioassay. On the other hand, since a statistically significant

increase in RTC count was not observed in this study at any duration in males or females exposed to 31 ppm, nor in males exposed to 62.5 ppm, it appears that RTC count alone cannot account for the increase in MCV at these levels of exposure. The observed increases in MCV may be a combined result of both erythrocyte swelling prior to and an increased number of RTCs subsequent to hemolysis, with the former being more influential at lower exposure levels and the latter having more relative impact at higher exposure levels. Thus, other endpoints, including RBC count changes, were considered for use in the derivation of the RfD and RfC. One reviewer commented that the Agency rationale for use of MCV was reasonable, but that more sensitive and reproducible methods should have been used to measure both MCV and RTC counts. The Agency agrees with this reviewer and with the reviewer who commented that the MCV as measured was not a bench-level measured clinical determination. Nevertheless, the Agency concurs with the former reviewer's contention that MCV should be considered (along with other endpoints) because of the potential for MCV to provide an indication of primary events in the pathology of EGBE-induced hemolysis.

APPENDIX B. CORLEY ET AL. (1994, 1997) PBPK MODEL

Corley et al. (1994) developed PBPK models for rats and humans with the primary objective of describing the concentration of BAA in the target tissue (blood) of rats and humans for use in risk assessment (Figure A-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).

The Corley et al. (1994) model included additional routes of exposure such as oral (gavage), drinking water, intravenous 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 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 EG and glucuronide conjugate) were combined since 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 ethylene glycol. 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 7965, 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 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 to 4.33 for EGBE and 0.77 to 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 intravenously 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 $(\text{body weight})^{0.74} \times (\text{renal elimination})$ for all simulations. Significant increases in the concentrations of EGBE were observed by Corley et al. (1997) in the first postexposure blood samples. Since the subjects were able to freely move their arms after the exposure, Corley et al. 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.

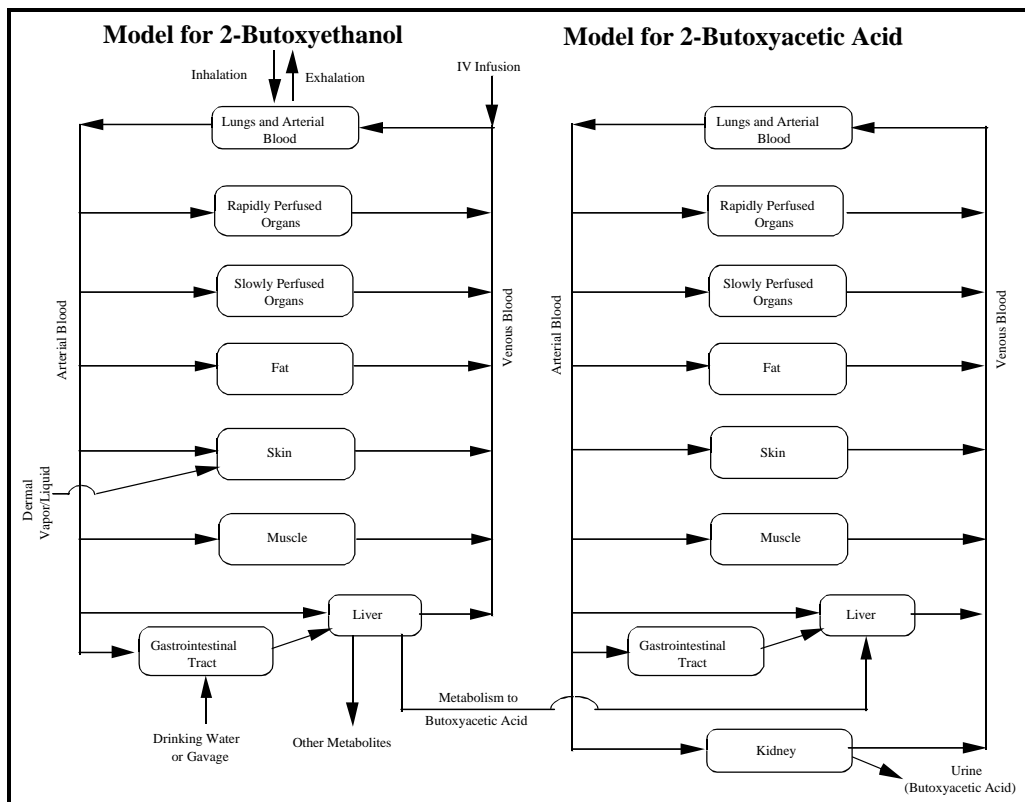


Figure A-1. PBPK model of Corley et al. (1994). The formation of BAA from EGBE was assumed to occur only in the liver and was simulated in a second model linked via the formation of BAA.

**APPENDIX C. TEXT OUTPUT FROM BENCHMARK DOSE SOFTWARE
RUNS USED IN THE DERIVATION OF RfD AND RfC VALUES**

=====
Power Model, Version Number: 1.1.1b
Input Data File: C:\BMDS4ME\DATA\EGBE\EGBEORAL.(D)

Fri Jan 08 13:18:25 1999
=====

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

The form of the response function is:

$$Y[\text{dose}] = \text{control} + \text{slope} * \text{dose}^{\text{power}}$$

Dependent variable = MEAN

Independent variable = DOSE

The power is not restricted

The variance is to be modeled as  $\text{Var}(i) = \alpha * \text{mean}(i)^{\rho}$

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: 2.22045e-016

Parameter convergence has been set to: 1.49012e-008

Default Initial Parameter Values

alpha = 3.02612

rho = 0

control = 54.8

slope = 0.0438974

power = 0.976715

Parameter Estimates

| Variable | Estimate  | Standard Error |
|----------|-----------|----------------|
| alpha    | 0.0008464 | 0.00159172     |
| rho      | 1.95327   | 0.449801       |
| control  | 54.7753   | 0.330267       |
| slope    | 0.0520655 | 0.00218389     |
| power    | 0.946046  | 0.069827       |

Asymptotic Correlation Matrix of Parameter Estimates

|         | alpha    | rho      | control | slope  | power    |
|---------|----------|----------|---------|--------|----------|
| alpha   | -1       | 1        | -0.0078 | 0.012  | 9.52-005 |
| rho     | 1        | -1       | 0.0057  | -0.012 | -0.00011 |
| control | -0.0078  | 0.0057   | 1       | -0.77  | -0.02    |
| slope   | 0.012    | -0.012   | -0.77   | 1      | -0.029   |
| power   | 9.5e-005 | -0.00011 | -0.02   | -0.029 | 1        |

Table of Data and Estimated Values of Interest

| Dose | N  | Obs Mean | Obs Std Dev | Est Mean | Est Std Dev |
|------|----|----------|-------------|----------|-------------|
| 0    | 10 | 54.8     | 0.92        | 54.8     | 1.45        |
| 59   | 10 | 57       | 1.25        | 57.2     | 1.52        |
| 125  | 10 | 60.5     | 1.27        | 59.8     | 1.58        |
| 208  | 10 | 62.4     | 1.78        | 62.9     | 1.66        |
| 277  | 10 | 65.3     | 1.83        | 65.4     | 1.73        |
| 404  | 10 | 70.1     | 2.76        | 70       | 184         |

Model Descriptions for Likelihoods Calculated

Model A1:  $Y_{ij} = \mu(i) + e_{ij}$   
 $\text{Var}\{e_{ij}\} = \sigma^2$

Model A2:  $Y_{ij} = \mu(i) + e_{ij}$   
 $\text{Var}\{e_{ij}\} = \sigma(i)^2$

Model A3:  $Y_{ij} = \mu(i) + e_{ij}$   
 $\text{Var}\{e_{ij}\} = \alpha * (\mu(i))^\rho$

Model R:  $Y_i = \mu + e(i)$   
 $\text{Var}\{e(i)\} = \sigma^2$

#### Likelihoods of Interest

| Model  | Log(likelihood) | DF | AIC         |
|--------|-----------------|----|-------------|
| A1     | -60.057590      | 7  | -67.057590  |
| A2     | -52.588573      | 12 | -64.588573  |
| A3     | -57.206449      | 8  | -65.206449  |
| fitted | -58.818246      | 5  | -63.818246  |
| R      | -131.090823     | 2  | -133.090823 |

#### Explanation of Tests

Test 1: Do response 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)

#### Tests of Interest

| Test   | $-2 * \log(\text{likelihood ratio})$ | DF | $p$ -value |
|--------|--------------------------------------|----|------------|
| Test 1 | 157.005                              | 10 | <0.00001   |
| Test 2 | 14.938                               | 5  | 0.01063    |
| Test 3 | 9.23575                              | 4  | 0.05547    |
| Test4  | 3.22359                              | 3  | 0.3584     |

The  $p$ -value for Test 1 is less than 0.05. 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 0.05. A nonhomogeneous variance model appears to be appropriate.

The  $p$ -value for Test 3 is greater than 0.05. The modeled variance appears to be appropriate here.

The  $p$ -value for Test 4 is greater than 0.05. The model chosen seems to adequately describe the data.

#### Benchmark Dose Computation

Specified effect = 2.7388 (5% of background estimate of 54.7753)

Risk type = Added response

Confidence level = 0.950000

BMD = 65.941824

BMDL = 48.792442

=====  
Power Model, Version Number: 1.1.1b  
Input Data File: C:\BMDS4ME\DATA\EGBE\EGBEORAL.(D)  
=====

Fri Jan 08 15:26:07 1999  
=====

**BMD + PBPK Method for RfD: MCV in Orally Exposed Female Rats (NTP, 1993)**  
~~~~~

The form of the response function is:

$$Y[\text{dose}] = \text{control} + \text{slope} * \text{dose}^{\text{power}}$$

Dependent variable = MEAN

Independent variable = CMAX

The power is not restricted

The variance is to be modeled as $\text{Var}(i) = \alpha * \text{mean}(i)^{\rho}$

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: 2.22045e-016

Parameter convergence has been set to: 1.49012e-008

Default Initial Parameter Values

alpha = 3.02612

rho = 0

control = 54.8

slope = 0.0828926

power = 0.731677

Parameter Estimates

Variable	Estimate	Standard Error
alpha	0.0008464	0.00161642
rho	1.95428	0.456969
control	54.6662	0.335163
slope	0.136613	0.00575923
power	0.656823	0.0430356

Asymptotic Correlation Matrix of Parameter Estimates

	Alpha	Rho	Control	Slope	Power
alpha	-1	1	-0.015	0.02	-0.00069
rho	1	-1	0.013	-0.021	0.00077
control	-0.015	0.013	1	-0.78	-0.023
slope	0.02	-0.021	-0.78	1	-0.039
power	-0.00069	0.00077	-0.023	-0.039	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev
0	10	54.8	0.92	54.7	1.45
103	10	57	1.25	57.5	1.53
253	10	60.5	1.27	59.8	1.59
495	10	62.4	1.78	62.7	1.66
738	10	65.3	1.83	65.1	1.72
1355	10	70.1	2.76	70.2	1.85

Model Descriptions for Likelihoods Calculated

$$\text{Model A1: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma^2$$

$$\text{Model A2: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma(i)^2$$

$$\text{Model A3: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \alpha * (\mu(i))^\rho$$

$$\text{Model R: } Y_i = \mu + e(i) \\ \text{Var}\{e(i)\} = \sigma^2$$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-60.057590	7	-67.057590
A2	-52.588573	12	-64.588573
A3	-57.206449	8	-65.206449
fitted	-58.950987	5	-63.950987
R	-131.090823	2	-133.090823

Explanation of Tests

Test 1: Do response 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)

Tests of Interest

Test	-2*log(likelihood ratio)	DF	<i>p</i> -value
Test 1	157.005	10	<0.00001
Test 2	14.938	5	0.01063
Test 3	9.23575	4	0.05547
Test4	3.48907	3	0.3222

The *p*-value for Test 1 is less than 0.05. 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 0.05. A nonhomogeneous variance model appears to be appropriate.

The *p*-value for Test 3 is greater than 0.05. The modeled variance appears to be appropriate here.

The *p*-value for Test 4 is greater than 0.05. The model chosen seems to adequately describe the data.

Benchmark Dose Computation

Specified effect = 2.733000 (5% of background estimate of 54.66)

Risk type = Added response

Confidence level = 0.950000

BMD = 95.712853

BMDL = 63.695782

=====

Polynomial Model, Version Number: 1.1.0b
Input Data File: C:\BMDS4ME\DATA\EGBE\EGBE_F.(D)

Tue Jan 12 08:56:59 1999

=====

BMD Method for RfC: RBC Count for Female Rats Exposed 14 Weeks (NTP, 1998)

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The form of the response function is:

$$Y[\text{dose}] = \text{beta}_0 + \text{beta}_1 * \text{dose} + \text{beta}_2 * \text{dose}^2 + \dots$$

Dependent variable = MEAN

Independent variable = DOSE

Signs of the polynomial coefficients are not restricted

The variance is to be modeled as  $\text{Var}(i) = \text{alpha} * \text{mean}(i)^\rho$

Total number of dose groups = 4

Total number of records with missing values = 0

Maximum number of iterations = 250

Relative function convergence has been set to: 2.22045e-016

Parameter convergence has been set to: 1.49012e-008

Default Initial Parameter Values

alpha = 0.040875

rho = 0

beta\_0 = 8.47683

beta\_1 = -0.0125321

beta\_2 = 4.71226e-008

Parameter Estimates

| Variable | Estimate      | Standard Error |
|----------|---------------|----------------|
| alpha    | 0.000870086   | 0.0064509      |
| rho      | 1.82359       | 3.61441        |
| beta_0   | 8.47429       | 0.0626104      |
| beta_1   | -0.0123721    | 0.0024627      |
| beta_2   | -1.16375e-006 | 1.78152e-005   |

Asymptotic Correlation Matrix of Parameter Estimates

|        | alpha | rho   | beta_0 | beta_1 | beta_2 |
|--------|-------|-------|--------|--------|--------|
| alpha  | 1     | -1    | 0.1    | -0.14  | 0.13   |
| rho    | -1    | 1     | -0.1   | 0.14   | -0.13  |
| beta_0 | 0.1   | -0.1  | 1      | -0.76  | 0.62   |
| beta_1 | -0.14 | 0.14  | -0.76  | 1      | -0.97  |
| beta_2 | 0.13  | -0.13 | 0.61   | -0.97  | 1      |

Table of Data and Estimated Values of Interest

| Dose    | N  | Obs Mean | Obs Std Dev | Est Mean | Est Std Dev |
|---------|----|----------|-------------|----------|-------------|
| 0.0000  | 10 | 8.480    | 0.160       | 8.474    | 0.043       |
| 31.0000 | 10 | 8.080    | 0.230       | 8.090    | 0.039       |
| 62.5000 | 10 | 7.700    | 0.250       | 7.696    | 0.036       |
| 125.000 | 10 | 6.910    | 0.150       | 6.909    | 0.030       |

## Model Descriptions for Likelihoods Calculated

$$\text{Model A1: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma^2$$

$$\text{Model A2: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma(i)^2$$

$$\text{Model A3: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \alpha * (\mu(i))^\rho$$

$$\text{Model R: } Y_i = \mu + e(i) \\ \text{Var}\{e(i)\} = \sigma^2$$

Warning: Likelihood for model A1 larger than or equal to that one for model A2.

Warning: Likelihood for model A3 larger than or equal to that one for model A2.

Warning: Likelihood for model R larger than or equal to that one for model A2.

## Likelihoods of Interest

| Model  | Log(likelihood) | DF | AIC       |
|--------|-----------------|----|-----------|
| A1     | 46.051943       | 5  | 41.051943 |
| A2     | -0.501998       | 8  | -8.501998 |
| A3     | 46.173884       | 6  | 40.173884 |
| fitted | 46.159466       | 5  | 41.159466 |
| R      | 1.343542        | 2  | -0.656458 |

## Explanation of Tests

Test 1: Do response 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)



## Tests of Interest

| Test   | $-2*\log(\text{likelihood ratio})$ | DF | $p$ -value |
|--------|------------------------------------|----|------------|
| Test 1 | 0                                  | 6  | <0.00001   |
| Test 2 | 0                                  | 3  | <0.00001   |
| Test 3 | 0                                  | 2  | <0.00001   |
| Test4  | 0.0288374                          | 1  | 0.8652     |

The  $p$ -value for Test 1 is less than 0.05. 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 0.05. A nonhomogeneous variance model appears to be appropriate.

The  $p$ -value for Test 3 is less than 0.05. You may want to consider a different variance model.

The  $p$ -value for Test 4 is greater than 0.05. The model chosen seems to adequately describe the data.

## Benchmark Dose Computation

Specified effect = 0.423700 (5% of background estimate of 8.474)

Risk type = Added response

Confidence level = 0.950000

BMD = 34.136806

BMDL = 26.942074

=====  
Power Model, Version Number: 1.1.1b  
Input Data File: C:\BMDS4ME\DATA\EGBE\EGBE\_F.(D)  
=====

Mon Jan 11 13:20:21 1999  
=====

**BMD + PBPK Method for RfC: RBC Count for Female Rats Exposed 14 Wks (NTP, 1998)**  
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The form of the response function is:

$$Y[\text{dose}] = \text{control} + \text{slope} * \text{dose}^{\text{power}}$$

Dependent variable = MEAN

Independent variable = CMAX3

rho is set to 0

The power is not restricted

A constant variance model is fit

Total number of dose groups = 4

Total number of records with missing values = 0

Maximum number of iterations = 250

Relative function convergence has been set to: 2.22045e-016

Parameter convergence has been set to: 1.49012e-008

Default Initial Parameter Values

alpha = 0.040875

control = 8.48

slope = -0.00208697

power = 0.928193

Parameter Estimates

Variable	Estimate	Standard Error
alpha	0.0368453	0.00823886
control	8.47733	0.0469956
slope	-0.00181442	9.50703e-005
power	0.948686	0.132835

Asymptotic Correlation Matrix of Parameter Estimates

	Alpha	Control	Slope	Power
alpha	1	-1.6e-006	-2.7e-006	-0.00016
control	-1.6e-006	1	-0.76	0.01
slope	-2.7e-006	-0.76	1	0.017
power	-0.00016	0.01	0.017	1

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev
0	10	8.48	0.16	8.48	0.192
285	10	8.08	0.23	8.09	0.192
603	10	7.7	0.25	7.69	0.192
1243	10	6.91	0.15	6.91	0.192

Model Descriptions for Likelihoods Calculated

$$\text{Model A1: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma^2$$

$$\text{Model A2: } Y_{ij} = \mu(i) + e(ij) \\ \text{Var}\{e(ij)\} = \sigma(i)^2$$

$$\text{Model R: } Y_i = \mu + e(i) \\ \text{Var}\{e(i)\} = \sigma^2$$

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	46.051943	5	41.051943
A2	47.963928	8	39.963928
fitted	46.020539	4	42.020539
R	1.323169	2	-0.676831

Explanation of Tests

Test 1: Do response and/or variances differ among dose levels? (A2 vs. R)

Test 2: Are variances homogeneous? (A1 vs. A2)

Test 3: Does the model for the mean fit? (A1 vs. fitted)

Tests of Interest

Test	$-2 \cdot \log(\text{likelihood ratio})$	DF	p -value
Test 1	89.4575	6	<0.00001
Test 2	3.82397	3	0.2811
Test 3	0.0628093	1	0.8021

The p -value for Test 1 is less than 0.05. 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 0.05. A homogeneous variance model appears to be appropriate here.

The p -value for Test 3 is greater than 0.05. The model chosen appears to adequately describe the data.

Benchmark Dose Computation

Specified effect = 0.424000 (5% of background estimate of 8.48)

Risk type = Added response

Confidence level = 0.950000

BMD = 313.866166

BMDL = 224.956831