# 4 EFFECTS OF EXPOSURE

## 4.1 EFFECTS ON HUMANS

### 4.1.1 Case Studies

A case of severe poisoning from massive ingestion of EGBE has been described by Rambourg-Schepens et al. [1988]. In a suicide attempt, a 50-year-old woman ingested 250 to 500 ml of a window cleaner containing 12% EGBE. When admitted to the hospital 12 hr later, the woman was comatose with no response to painful stimuli; she ventilated poorly and was placed under mechanical ventilation. Metabolic acidosis, hypokalemia (an abnormally small amount of potassium in the blood), a rise in serum creatinine level, hemoglobinuria, and oxaluria were observed. The hemoglobinuria was paralleled by a progressive erythropenia (a deficiency in the number of red blood cells). The clinical status of the woman improved gradually, and she was discharged on the 10th day.

Gijsenbergh et al. [1989] described a suicide attempt by a 23-year-old woman who ingested about 500 ml of a window-cleaning agent that contained EGBE and a small quantity of alcohol (the percentage of each compound was not presented). When admitted to the hospital 1 hr after ingesting the substance, the woman was comatose and suffering from hypotension. She subsequently developed severe metabolic acidosis. Routine laboratory blood examination revealed no abnormalities. The woman recovered after forced diuresis and hemodialysis.

NIOSH conducted a health hazard evaluation in 1983 to evaluate worker exposure to two solvent cleaners—an image remover and a paint remover used in a silk screening process [Boiano 1983]. A silk screener using the image remover was monitored for exposure to ethylene glycol monoethyl ether acetate (EGEEA) and cyclohexanone, and a worker using the paint remover was monitored for a variety of organic solvents, one of which was EGBEA. Although the workers were primarily exposed by inhalation, they also may have been exposed by skin absorption because personal protective equipment was not always worn. The workers complained of headaches, lethargy, sinus problems, nausea, and heartburn. When the workers were away from work, their symptoms improved. Measurement of the silk screener's airborne exposures to EGBEA indicated that the TWA exposures ranged from 0.8 to 3.9 ppm, with a short-term excursion to 5.3 ppm; however, absorption through the skin may have contributed to the workers' overall exposure. Boiano [1983] concluded that a health hazard from exposures to airborne solvent mixtures did not exist at this facility.

### 4.1.2 Experimental Exposure

Two men and six rats were exposed simultaneously in a 1,250-cubic-ft room to 113 ppm EGBE for 4 hr [Carpenter et al. 1956]. Symptoms of the men included nasal and eye irritation, disagreeable metallic taste, occasional belching, and a slight increase in nasal mucous discharge. Although erythrocyte osmotic fragility did not change for the men, it rose appreciably for the rats.

In a second experiment about a year later, the same two men and one woman were exposed to 195 ppm EGBE for two 4-hr periods, separated by a 30-min recess for lunch [Carpenter et al. 1956]. Exposure took place in a 6 1/2-ft cube (7,900 liters). The responses of all three subjects included immediate irritation of the nose and throat, followed by eye irritation and disturbed taste. The woman also developed a headache that lasted about 24 hr. Erythrocyte osmotic fragility did not change. However, increased osmotic fragility was found in vitro with the human erythrocytes. Therefore, increased osmotic fragility would be expected after inhalation of EGBE at concentrations higher than 200 ppm (approximate). Erythrocyte osmotic fragility increased steadily in three female rats concurrently exposed. Concentrations of butoxyacetic acid (BAA), demonstrated as a metabolite of EGBE (see Section 4.2), were determined in urine samples collected during the 24-hr period following exposure. The woman and one of the men excreted considerable amounts of BAA (300 mg and 175 mg, respectively) in the 24-hr period following exposure, but the other man excreted only trace amounts of the metabolite. The three subjects agreed that 195 ppm EGBE caused discomfort when breathed continually.

In a third study, four subjects—two men and two women (including the man who had participated in the second study and had excreted only trace amounts of BAA)—were exposed to 100 ppm EGBE for 8 hr. Following this exposure, all four subjects excreted significant amounts of BAA in their urine (75 to 250 mg). Other effects of exposure included vomiting and headaches; there was no effect on the osmotic fragility of erythrocytes [Carpenter et al. 1956].

## 4.2 METABOLISM, UPTAKE, AND ELIMINATION

### 4.2.1 Research Studies

Carpenter et al. [1956] postulated that EGBE is oxidized to BAA in mammals. The authors verified this postulate with the identification of BAA in the urine of various animal species (dog, rabbit, rat, and guinea pig) exposed to EGBE vapor. The data (excluding that on the dogs) suggested that a correlation existed between the EGBE vapor concentration and urinary BAA [Carpenter et al. 1956]. Although BAA was not identified in the blood of the dogs, rats, or guinea pigs, it was found in the blood of the rabbits 4 hr after i.v. administration of EGBE, and in the urine collected during the 24 hr after injection. The authors [Carpenter et al. 1956] suggested that EGBE was present in the blood at a concentration too low for detection by the analytical method used.

Jonsson and Steen [1978] exposed male albino rats to 414 ppm EGBE for 1 hr, and then collected urine for 20 hr. Gas chromatographic analysis of organic acids in urine after EGBE exposure was conducted. Gas chromatography retention time and mass spectrum of the EGBE metabolite were the same as that of synthetic BAA.

Johanson et al. [1986] exposed seven male volunteers to 20 ppm EGBE for 2 hr during light physical exercise on a bicycle ergometer. Expired air was collected at regular time intervals to estimate respiratory uptake of EGBE. Blood and urine samples were collected and analyzed for EGBE and BAA. The respiratory uptake of EGBE averaged 57% of the inspired amount. The concentration of EGBE in the blood reached a plateau of 7.4 micromoles (μmol)/liter (1 ppm volume/volume) within 1 to 2 hr and could no longer be detected in the blood 2 to 4 hr after exposure. The elimination half-time was 40 min, mean residence time was 42 min, total blood clearance time was 1.2 liters/min, and steady-state volume of distribution was 54 liters. Less than 0.03% of the total uptake of EGBE was excreted in the urine, whereas urinary excretion as BAA ranged from 17% to 55%.

Johanson et al. [1988] studied the percutaneous uptake of EGBE in five healthy males (see Section 5.4.5.1). The subjects kept two or four fingers immersed in undiluted EGBE for 2 hr. Blood samples were collected from the unexposed hand before, during, and up to 4 hr after the EGBE-exposure and analyzed for EGBE by gas chromatography. Urine samples were collected for 24 hr and analyzed for BAA by gas chromatography. The authors concluded that detection of EGBE in the blood of all subjects was indicative of systemic uptake of EGBE through the skin in vivo. The percutaneous uptake varied from 127 to 1,891 μmol/subject. The acid metabolite BAA was found in the urine. Urinary excretion peaked 3 hr after exposure and then declined with an average half-life of 3.1 hr. The accumulated excretion of BAA ranged from 8.7 to 313 μmol, corresponding to 2.5% to 39% of the uptake. The authors concluded that their study clearly showed that EGBE is absorbed through human skin in vivo and enters the systemic circulation. A comparison of dermal uptake rate with inhalatory uptake suggested that both skin and respiratory uptake should be considered when workers are exposed to EGBE. Both studies by Johanson et al. [1986, 1988] are assessed and discussed under biological monitoring in Section 5.4.

A lack of data exists concerning the metabolism of EGBEA. However, reports in the literature have identified ethoxyacetic acid (EAA) as the metabolite of both ethylene glycol monoethyl ether (EGEE) and its acetate (EGEEA) [Jonsson et al. 1982; Cheever et al. 1984; Groeseneken et al. 1986c; Groeseneken et al. 1987a]. The findings confirmed that EGEEA is first converted to EGEE by hydrolysis of the ester moiety [Elam 1980] and then passes through the same pathway as EGEE to EAA. By analogy to EGEE and EGEEA, it is reasonable to assume that EGBEA is first hydrolyzed to EGBE and then oxidized to BAA [Carpenter et al. 1956; Jonsson and Steen 1978].

### 4.2.2 Summary of Studies on Metabolism, Uptake, and Elimination

Carpenter et al. [1956] identified BAA in the urine of various animal species exposed to EGBE vapor. Analysis of organic acids in urine after EGBE exposure of rats revealed the presence of BAA [Jonsson and Steen 1978].

Exposure of male volunteers to EGBE vapors during light physical exercise resulted in 57% respiratory uptake of inspired EGBE [Johanson et al. 1986]. Less than 0.03% of the total uptake of EGBE was excreted unchanged in urine, and urinary BAA excretion ranged from 17% to 55% of the EGBE absorbed. Percutaneous exposure of male volunteers to EGBE resulted in systemic uptake of EGBE through the skin in vivo, and BAA was found in the urine [Johanson et al. 1988]. A comparison of dermal and respiratory uptake suggested that both routes of exposure should be considered when workers are exposed to EGBE [Johanson et al. 1988].

The metabolite of EGBEA has not been identified. However, by analogy to EGEEA, which is converted to EGEE and then to EAA [Jonsson et al. 1982; Cheever et al. 1984; Groeseneken et al. 1986c; Groeseneken et al. 1987a], it is logical to assume that EGBEA is metabolized to EGBE and then to BAA.

## 4.3 EFFECTS ON ANIMALS

Kidney, hematologic, and central nervous system (CNS) effects have been observed in experimental animals exposed to EGBE and EGBEA.

### 4.3.1 Acute Toxicity

Many experiments have been performed to investigate the acute toxicity of glycol ethers in animals. These investigations have led to the establishment of a lethal concentration or lethal dose for 50% of the exposed animals ($LC_{50}$ or $LD_{50}$) in a variety of species by a variety of routes (inhalation, oral, dermal, and injection). A summary of the available data by animal species is presented in Table 4-1, and more detailed information is given in Table 4-2.

#### *4.3.1.1 Oral Administration*

When rabbits were dosed by a single gavage with 890 or 1,780 mg EGBE/kg, death occurred within 30 or 22 hr [Gross 1943]. Sluggishness, ruffling of coats, prostration, and narcosis occurred after oral administration of lethal concentrations of EGBE to male and female rats (2,400 and 2,500 mg/kg, respectively) [Carpenter et al. 1956]. Necropsies revealed congested or hemorrhaged lungs, mottled livers, congested kidneys, and hemoglobinuria. When EGBEA was administered by gastric intubation to male and female Wistar rats (3,000 and 2,400 mg/kg, respectively), hypertrophic and bloody kidneys were observed at necropsy [Truhaut et al. 1979].

#### *4.3.1.2 Inhalation Exposure*

Werner et al. [1943c] demonstrated an adverse effect of EGBE on the hematopoietic system. Single 7-hr exposures of White-Swiss mice to EGBE (390 to 1,210 ppm) caused marked follicular phagocytosis in the spleen, congestion of the cavernous veins of the spleen, and hemoglobinuria. The usual sign of toxic action was dyspnea.

Table 4–1.—Lethal doses or concentrations of glycol ethers*

| Species and sex | $LD_{50}$* oral (mg/kg) | | EGBE $LD_{50}$ i.v. (mg/kg) | $LD_{50}$ dermal (mg/kg) | | $LC_{50}$* inhalation (ppm) | |
|---|---|---|---|---|---|---|---|
| | EGBE | EGBEA | | EGBE | EGBEA | EGBE | EGBEA |
| Rat: | | | | | | | |
| Male | 2,400 | 3,000 | --- | --- | --- | 486 (4 hr) | --- |
| | --- | 7,000 | --- | --- | --- | --- | --- |
| Female | 2,500 | 2,400 | --- | --- | --- | 450 (4 hr) | --- |
| Unspecified | --- | --- | 380 | --- | --- | --- | --- |
| Rabbit: | | | | | | | |
| Male | 320 | --- | --- | 404 to 502 | 1,500 | --- | --- |
| Unspecified | --- | --- | 500 | --- | 1,500 | --- | --- |
| Guinea pig: | | | | | | | |
| Male and female | 1,200 | --- | --- | --- | --- | --- | --- |
| Mouse: | | | | | | | |
| Male | 1,200 | --- | --- | --- | --- | --- | --- |
| Female | --- | --- | --- | --- | --- | --- | --- |
| Unspecified | --- | --- | 1,100 | --- | --- | 700 (7 hr) | --- |

*Abbreviations: $LD_{50}$ = mean lethal dose; $LC_{50}$ = mean lethal concentration.

**Table 4–2.—Acute toxicity of EGBE and EGBEA**

| Species | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| | | | EGBE: |
| Rat | Tail vein injection: $LD_{50}$,* 380 mg/kg | Death | Carpenter et al. 1956 |
| Mouse | Tail vein injection: $LD_{50}$, 1,100 mg/kg | Death | Carpenter et al. 1956 |
| Rabbit | Ear vein injection: $LD_{50}$, 500 mg/kg | Death | Carpenter et al. 1956 |
| Rabbit | Subcutaneous: | | Gross 1943 |
| | 180 mg/kg | Kidney inflammation | |
| | 360 mg/kg | Death | |
| | 2,700 mg/kg | Respiratory paralysis, death | |
| Cat | Subcutaneous: 1,800 mg/kg | Kidney injury, death | Gross 1943 |
| Rabbit | Oral: 900 or 1,800 mg/kg | Death | Gross 1943 |
| Rabbit | Oral: $LD_{50}$, 320 mg/kg | Death | Carpenter et al. 1956 |
| Mouse | Oral: $LD_{50}$, 1,200 mg/kg | Death | Carpenter et al. 1956 |
| Guinea pig | Oral: $LD_{50}$, 1,200 mg/kg | Death | Carpenter et al. 1956 |
| Rat (M) | Oral: $LD_{50}$, 2,400 mg/kg | Congested lungs and kidneys, hemoglobinuria, mottled livers | Carpenter et al. 1956 |
| Rat (F) | Oral: $LD_{50}$, 2,500 mg/kg | Congested lungs and kidneys, hemoglobinuria, mottled livers | Carpenter et al. 1956 |

(Continued)

*Abbreviations: $LC_{50}$ = mean lethal concentration; $LD_{50}$ = mean lethal dose.

Table 4–2 (Continued).—Acute toxicity of EGBE and EGBEA

| Species | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| | | | EGBE: |
| Cat | Inhalation: 518 ppm, 8 hr/day for 8 or 9 days | Death, kidney inflammation | Gross 1943 |
| Guinea pig | Inhalation: 518 ppm, 8 hr/day for 8 or 9 days | Death, kidney inflammation | Gross 1943 |
| Mouse | Inhalation: $LC_{50}$*, 700 ppm for 7 hr | Hemoglobinuria, splenic lesions | Werner et al. 1943c |
| Rat (M) | Inhalation: $LC_{50}$, 486 ppm for 4 hr | Enlarged kidneys, blood in bladders | Dodd et al. 1983 |
| Rat (F) | Inhalation: $LC_{50}$, 450 ppm for 4 hr | Enlarged kidneys, blood in bladders | Dodd et al. 1983 |
| Rabbit | Dermal: $LD_{50}$, 404 to 502 mg/kg | Congested kidneys, hemoglobinuria, pale livers, enlarged spleens | Carpenter et al. 1956 |
| | | | EGBEA: |
| Rat (M) | Oral: $LD_{50}$, 7,000 mg/kg | Death | Smyth et al. 1962 |
| Rat (M) | Oral: $LD_{50}$, 3,000 mg/kg ± 300 mg/kg | Bloody and hypertrophic kidneys | Truhaut et al. 1979 |
| Rat (F) | Oral: $LD_{50}$, 2,400 mg/kg ± 200 mg/kg | Bloody and hypertrophic kidneys | Truhaut et al. 1979 |
| Rabbit (M) | Dermal: $LD_{50}$, 1,500 mg/kg | Death | Smyth et al. 1962 |
| Rabbit | Dermal: $LD_{50}$, 1,500 mg/kg | Bloody and hypertrophic kidneys | Truhaut et al. 1979 |

Inhalation exposures of groups of female rats to 62 ppm EGBE for 4 hr resulted in increased osmotic fragility of rat erythrocytes [Carpenter et al. 1956].

Single 4-hr exposures of male and female Fischer 344 rats to EGBE (486 ppm and 450 ppm, respectively) caused hematuria; enlarged and discolored kidneys were observed at autopsy [Dodd et al. 1983].

#### *4.3.1.3. Dermal Exposure*

The percutaneous toxicity of EGBE and EGBEA has been investigated in the rabbit [Carpenter et al. 1956; Truhaut et al. 1979]. Toxic effects on the kidneys were seen consistently. Male albino New Zealand rabbits were immobilized during 24 hr of skin contact with undiluted EGBE (0.48 to 0.64 ml/kg); they were observed for 14 days thereafter [Carpenter et al. 1956]. Autopsy of the rabbits revealed congestion of the kidneys, hemoglobinuria, pale livers, and engorged spleens. Truhaut et al. [1979] exposed rabbits to 7.5 to 23.5 g EGBEA/kg for 24 hr using an occluded bandage technique. Bloody kidneys were found at necropsy; histologic examination revealed necrotizing, hemorrhagic, atrophic, acute, tubular nephrosis with occasional glomerular lesions.

#### *4.3.1.4 Subcutaneous Administration*

Gross [1943] administered single subcutaneous (s.c.) injections of varying doses of EGBE to 13 rabbits. At 180 mg/kg, a slight, temporary, kidney inflammation was noted. Doses of 360 to 1,800 mg/kg caused death within 20 to 72 hr from kidney inflammation, and a dose of 2,700 mg/kg caused death within 2 hr from respiratory paralysis. A cat injected s.c. with 900 mg EGBE/kg did not show signs of illness; a second cat received 1,800 mg EGBE/kg s.c. and died 3 days later with signs of kidney injury.

#### *4.3.1.5 Summary of Acute Toxicity*

The acute toxicity of EGBE and EGBEA has been investigated in a number of experiments with a variety of species and routes of exposure. Animals exhibited inactivity, weakness, and dyspnea. Autopsies revealed congested lungs and kidneys [Carpenter et al. 1956; Truhaut et al. 1979]. The principal effect exerted by these compounds was damage to the kidneys [Gross 1943; Dodd et al. 1983], which included extreme tubular necrosis and degeneration. Additional adverse effects included increased osmotic fragility of erythrocytes and damaged spleens [Werner et al. 1943c; Carpenter et al. 1956; Truhaut et al. 1979]. The acute toxic effects of EGBE and EGBEA are summarized in Table 4-2.

### 4.3.2 Hematologic Effects

EGBE and EGBEA have been shown to have adverse hematologic effects. These effects include increased osmotic fragility and decreased levels of hemoglobin (Hb), hematocrit (Hct), platelets, red blood cells (RBCs), white blood cells (WBCs), and mean cell volume (MCV).

In an early investigation [von Oettingen and Jirouche 1931], the hemolytic action of EGBE was studied by adding 1 cc of EGBE to 5-cc suspensions of dog or beef blood corpuscles in Ringer solution. The investigators reported that hemolysis occurred in the presence of EGBE.

#### *4.3.2.1 Oral Administration*

In a study by Nagano et al. [1979], male JCL-ICR mice were treated orally with 500 or 1,000 mg EGBE/kg per day, 5 days/wk for 5 wk. Although EGBE exerted no effect on WBC counts, MCV, or Hb levels, it significantly reduced RBC counts at doses of both 500 mg EGBE/kg per day ($P<0.05$) and 1,000 mg EGBE/kg per day ($P<0.01$).

EGBE-induced hematotoxicity in rats is age-dependent, with older rats more susceptible to EGBE treatment than young rats [Ghanayem et al. 1987]. EGBE (0, 125, or 500 mg/kg) was administered orally to young (4- to 5-week-old) and adult (9- to 13-week-old) male F344 rats (≥5 rats/group). No significant hematologic effects were observed in the younger rats at any time intervals (2, 4, 8, 24, and 48 hr) investigated in the group receiving 125 mg EGBE/kg. However, a significant decrease ($P\leq0.05$) in RBCs, Hct, and Hb was detected in the adult rats 8 and 24 hr after oral administration of 125 mg EGBE/kg. Free Hb concentrations in plasma were significantly increased ($P\leq0.05$) in adult rats 8 hr after oral administration of 125 mg EGBE/kg; there was no effect on free Hb concentrations in plasma of young rats. Twenty-four hours after dosing, free Hb concentrations in plasma of older rats were comparable with those in untreated control rats [Ghanayem et al. 1987].

Decreases in RBCs, Hb, and Hct were accompanied by a significant ($P\leq0.05$) dose-dependent increase in the free Hb concentrations of both age groups treated with 500 mg EGBE/kg [Ghanayem et al. 1987]. The authors state that a gradual recovery from hematotoxicity was observed after 48 hr in both of these groups.

Hemoglobinuria secondary to the hemolytic effect of EGBE was also observed. Table 4-3 demonstrates the incidence of hemoglobinuria in rats of various ages treated orally with various doses of EGBE.

**Table 4-3.—Incidence of hemoglobinuria in rats of various ages administered EGBE by gavage***

| | Dose (mg EGBE/kg)† | | | | |
|---|---|---|---|---|---|
| **Age of rats** | **32** | **63** | **125** | **250** | **500** |
| 4-5 wk | 0/6 | 0/6 | 1/11 | 6/6 | 12/12 |
| 9-13 wk | 0/6 | 0/6 | 12/12 | 6/6 | 12/12 |
| 5-6 mo | 0/6 | 6/6 | 6/6 | ND§ | ND |
| 16 mo | 6/6 | 6/6 | 6/6 | ND | ND |

*Adapted from Ghanayem et al. [1987].
†EGBE was administered in water at a dose volume of 5 ml/kg.
§Not done.

A 100% incidence of hemoglobinuria was detected in 16-month-old rats treated with 32 mg EGBE/kg, but no effect was observed in rats younger than 16 months treated with the same dosage. A dose of 125 mg EGBE/kg caused 100% incidence of hemoglobinuria in all rats older than 4 to 5 weeks and a 9% incidence in 4- to 5-wk-old rats.

Histopathologic evaluation of tissues from rats of various ages examined 24 hr after EGBE administration demonstrated that EGBE caused dose- and age-dependent liver and kidney changes. These histopathologic changes exhibited signs of regression when examined 48 hr after EGBE-dosing.

The results presented in this report clearly demonstrate that a direct relationship exists between toxicity of EGBE and the age of the rats [Ghanayem et al. 1987]. Older rats are more susceptible than younger rats to hematotoxicity and liver/kidney damage caused by EGBE. Severe acute hemolytic anemia was evidenced by a decrease in circulating RBCs, an increase in the concentration of free Hb in plasma, and the development of hemoglobinuria. Ghanayem et al. [1987] state that the greater susceptibility of older rats to EGBE-induced toxicity may be caused by (1) a longer half-life of its metabolite butoxyacetic acid (BAA) in older rats compared with younger rats and (2) an enhanced ability of younger rats to degrade BAA to $CO_2$ and/or excrete BAA in the urine.

Grant et al. [1985] exposed male F344 rats (24 per group) orally to 500 or 1,000 mg EGBE/kg per day for 4 consecutive days. Six animals from each group were bled from a lateral caudal vein and then sacrificed and necropsied 1, 4, 8, and 22 days after the last EGBE treatment. EGBE caused pronounced dose-dependent effects on circulating RBCs and WBCs. Reduced erythrocyte counts, reduced Hct and Hb levels, and elevated MCV, reticulocyte counts, and mean cell hemoglobin concentration (MCHC) ($P<0.001$) were noted at the end of treatment in animals dosed with 1,000 mg EGBE/kg per day. Most of these alterations in the RBCs disappeared over the 22-day recovery period, although the MCV and MCHC values were slightly elevated at day 22. In the high-dose EGBE group (1,000 mg/kg per day), leukocyte counts were also depressed on day 1 ($P<0.001$); this was principally because of reduced numbers of circulating lymphocytes. Although the leukocyte numbers gradually increased, they did not reach control levels by the end of the recovery period. These effects on RBCs and WBCs were also observed in the group receiving 500 mg EGBE/kg per day, although the severity of the changes was less marked.

EGBE was administered by gavage to groups of 10 male rats at doses of 0, 222, 443, or 885 mg/kg per day, 5 days/wk for 6 wk [Krasavage 1986]. At termination, the animals were sacrificed and blood was collected for hematologic and serum chemistry determinations. During the treatment period, two rats in the high-dose group and one rat in the intermediate dose group died. All the other animals survived to termination. The principal effect of EGBE was on the RBC. Hb and RBC levels were significantly reduced at all doses ($P\leq0.05$), and MCHC was statistically lower ($P<0.05$) than the control at the high and intermediate doses. Statistically significant increases were noted for mean cell hemoglobin (MCHb) at all doses and for MCV at the high and intermediate doses. Hct and WBC counts were unaffected.

#### 4.3.2.2 Inhalation

In two separate inhalation studies, Wistar-derived rats [Werner et al. 1943a] and dogs of an unspecified strain [Werner et al. 1943b] were exposed to EGBE, and the effects on hematologic parameters were examined. In the rat study, 23 animals per group were exposed to 0, 135, or 320 ppm EGBE for 7 hr/day, 5 days per wk over a 5-wk period; the animals were sacrificed 3 wk after termination of exposure. Hematologic examinations were made before, during, and after the 5 wk of exposure; they consisted of RBC and WBC counts, differential counts, reticulocyte counts, and Hb estimations. No statistical analysis was presented. The authors [Werner et al. 1943a] concluded that exposure of rats to 320 ppm EGBE resulted in an increased percentage of circulating immature granulocytes, a decrease in Hb concentration and RBC count, and an increase in the reticulocyte count. These hematologic changes were not severe and were reversed 3 wk after discontinuing exposure. There was no effect on the WBC population.

In the second inhalation study [Werner et al. 1943b], groups of 2 dogs were exposed to 0 or 415 ppm EGBE for 7 hr/day, 5 days/wk during a 12-wk period. Animals were sacrificed 5 wk after discontinuing exposure. Hematologic determinations were again made before, during, and after the exposure period. No statistical analysis was presented. The authors [Werner et al. 1943b] concluded that exposure of dogs to EGBE vapors resulted in (1) decreased Hb concentration and RBC count, and (2) increased hypochromia, polychromatophilia, and microcystosis, as shown by the RBC. These hematologic changes were not severe and were reversed 5 wk after exposure ceased [Werner et al. 1943b].

Carpenter et al. [1956] also studied the hemolytic effects exerted by the inhalation of EGBE vapors on various animal species. No statistical evaluations were presented. Exposure to 62 ppm EGBE for 4 hr caused significant osmotic fragility of erythrocytes in six female Carworth-Wistar rats. Groups of 15 rats of both sexes and 10 male guinea pigs (strains not specified) inhaled various concentrations of EGBE, 7 hr/day, 5 days/wk for 30 days. Erythrocyte osmotic fragility was found in rats immediately after a single 7-hr exposure to 107 ppm or higher (203, 314, or 432 ppm EGBE) and after 30 daily 7-hr exposures to 54 ppm EGBE. Osmotic fragility values for females usually exceeded those for the males. In almost all cases, these high fragility values returned to normal after the rats rested overnight. No effect was exerted on the osmotic fragility of guinea pig erythrocytes at the concentrations tested (54, 107, 203, 376, or 494 ppm EGBE). The authors [Carpenter et al. 1956] also exposed groups of 10 male $C_3H$ mice to 100, 200, or 400 ppm EGBE for 7 hr/day over 30-, 60-, or 90-day periods. Increased erythrocyte osmotic fragility occurred at all concentrations. The increase was as great after the first exposure as it was after the 89th exposure. In all instances, erythrocyte osmotic fragility was normal after a 17-hr rest.

Repeated exposure of one male and one female basenji dog to 385 ppm EGBE caused increased RBC osmotic fragility in both dogs, but a significant reduction in RBC count and Hb level occurred only in the male [Carpenter et al. 1956]. The female dog died after eight exposures. When one male and one female basenji from the same litter were exposed to 200 ppm EGBE for 7 hr/day over a 31-day period, RBC osmotic fragility increased slightly in both dogs. WBC counts doubled in the male, and the RBC count and Hb level fell slightly

in the female. When one male and one female wire-haired terrier from the same litter were exposed to 100 ppm EGBE for 7 hr/day over a 90-day period, a transitory doubling of WBC counts occurred in both dogs midway in the 90-day period. At the end of the exposure period, the female's WBC count returned to the preexposure level, but the male's remained 50% higher. The Hct level of the male also dropped by 8.5%.

In another part of the study by Carpenter et al. [1956], two monkeys were exposed to 100 ppm EGBE for 7 hr/day, 5 days/wk over a 90-day period. RBC osmotic fragility rose on several occasions, higher in the female than in the male, but returned to normal at the end of the exposure period. RBC counts also fell briefly but returned to normal. In addition, a rhesus monkey inhaled 210 ppm EGBE for 7 hr/day, 5 days/wk over a 30-day period. RBC osmotic fragility rose after the fourth exposure but returned to normal overnight. At the end of the exposure period, the RBC count and Hb level had been reduced to one-half the initial values.

Dodd et al. [1983] conducted inhalation studies with Fischer 344 rats of both sexes. In these studies, 8 animals per group were exposed to 0, 20, 86, or 245 ppm EGBE during a 9-day period, or 16 animals per group were exposed to 0, 5, 25, or 77 ppm EGBE during a 90-day period. For the 9-day study, the rats were exposed for 6 hr/day during 5 consecutive days, followed by 2 days of nonexposure and 4 consecutive days of exposure. For the 90-day study, rats were exposed for 6 hr/day, 5 days/wk over a 13-wk period. All blood samples for hematologic measurements were obtained on the day before sacrifice.

In the 9-day study, both sexes of the group exposed to 245 ppm EGBE had significantly depressed RBC counts ($P<0.001$), Hb levels ($P<0.001$), and MCHC ($P<0.01$). These rats also had a significant increase ($P<0.001$ in all cases) in MCV, nucleated RBC, reticulocytes, and (in males only) lymphocytes ($P<0.001$). Following a 14-day postexposure period, a substantial recovery of the affected erythroid parameters was observed; however, statistically significant differences from controls were still present for the males (i.e., RBC count [$P<0.01$], MCV [$P<0.001$], and MCHb [$P<0.001$]). During the 14-day postexposure recovery period, the WBC count, which had been elevated ($P<0.001$) in males, returned to control values. There was a significant but less profound effect on erythroid parameters in both sexes of the group exposed to 86 ppm EGBE. In male rats, Hb concentration was reduced relative to that of the controls ($P<0.01$); in female rats, Hb concentration ($P<0.001$) and MCHC ($P<0.01$) were reduced, and Hct ($P<0.01$) and MCV ($P<0.05$) were increased.

In the 90-day study, after 6 wk of the exposure regimen, the authors concluded that the female rats exposed to 77 ppm EGBE had slight but statistically significant decreases in RBC counts ($P<0.01$) and Hb levels (statistics not reported) accompanied by an increase in MCHb 11% above the values for controls ($P<0.001$). At the end of the study, these effects had either decreased or returned to the ranges of the control values. The only significant hematologic finding for male rats in the group exposed to 77 ppm EGBE was a 5% decrease in RBC count after 66 EGBE exposures (statistics not given).

In a later study by Tyl et al. [1984], Fischer 344 rats and New Zealand white rabbits were exposed to EGBE vapors (25 to 200 ppm) on g.d. 6 through 15 (rats) or 6 through 18 (rabbits).

Blood samples were collected before sacrifice. Hematologic determinations in rats exposed to EGBE indicated no alterations in RBC osmotic fragility, but there were significant reductions in RBC count, and significant increases in Hb and Hct at 200 ppm ($P<0.001$). RBC count was also reduced at 100 ppm ($P<0.001$). In rats exposed to 100 and 200 ppm EGBE, MCV and MCHb were significantly increased relative to those of the controls ($P<0.001$). In addition, the MCHC was reduced significantly at 100 ppm EGBE ($P<0.01$) and 200 ppm EGBE ($P<0.001$) relative to that of the controls. Hematologic determinations in rabbits exposed to EGBE revealed no apparent exposure-related effects. Statistically significant increases in Hb concentration and Hct were observed at 100 ppm ($P<0.01$) but not at 200 ppm EGBE.

Truhaut et al. [1979] carried out inhalation studies of EGBEA using groups of 10 Wistar rats of both sexes and 4 New Zealand rabbits, two of each sex. Exposure of rats and rabbits to 400 ppm EGBEA for 4 hr resulted in slight and transient hemoglobinuria and/or hematuria only in rabbits, but this effect did not last more than 24 to 48 hr. Exposure of rats (10 male and 10 female) and rabbits (2 male and 2 female) to 400 ppm EGBEA for 4 hr/day, 5 days/wk over a 1-month period resulted in hemoglobinuria and/or hematuria (slight in rats, more pronounced in rabbits) from the second week of exposure onward. RBC counts and Hb concentrations were normal during the first 3 weeks, then decreased slightly in two of four rabbits, and severely in the two others. These latter two rabbits died during the fourth week. Administration of 100 ppm EGBEA for 4 hr/day, 5 days/wk over a 10-month period to rats and rabbits of both sexes had no effect on hematologic parameters.

#### *4.3.2.3 Dermal Exposure*

Percutaneous treatment by Truhaut et al. [1979] of New Zealand rabbits of both sexes with a single application of 1,500 mg/kg EGBEA resulted in RBC counts and Hb levels that were 20% to 25% of the control value (statistics not given). These values returned to normal after 8 to 14 days.

Bartnik et al. [1987] applied a single dose of 260, 320, 375, or 500 mg EGBE/kg to the shaved dorsal skin of groups of three female rats; they also applied 200 mg/kg EGBE to the shaved dorsal skin of six female rats. Blood was collected retroorbitally six hr after dosing. Test animals were then sacrificed by carbon dioxide asphyxiation, and blood samples were taken immediately by cardiac puncture. Preliminary test results indicated that 500 mg EGBE/kg caused adverse effects such as an increase in mean cell volume, a lowered erythrocyte count and Hb level, and hemoglobinuria within 6 hr of dosing. No adverse effects were caused by 200 mg EGBE/kg. EGBE at doses of 260, 320, 375, and 500 mg/kg produced the effects described above in at least some animals in each group, but there was no discernible dose-response relationship. Bartnik et al. [1987] attributed this result to inherent biologic variation in percutaneous absorption and hemolytic susceptibility of erythrocytes, and to the small number of animals per group.

#### *4.3.2.4 In Vitro Exposure*

Bartnik et al. [1987] also examined the effects of EGBE and BAA on human and rat erythrocytes. Human erythrocytes were isolated from the blood of healthy adult male donors

(number unspecified) and rat erythrocytes were collected from four adult male Wistar rats. Under in vitro conditions, 175, 200, 225, and 250 millimoles (mmol) EGBE/liter induced complete lysis of rat erythrocytes, and 200, 225, and 250 mmol EGBE/liter induced complete lysis of human erythrocytes. At a concentration of 3.75 to 7.5 mmol/liter, BAA caused complete lysis of rat erythrocytes but failed to cause lysis of human erythrocytes. These results indicate that the rat may be more susceptible than humans to the effects of EGBE [Bartnik et al. 1987] (see Tables 4-4 and 4-5).

Ghanayem [1989] examined the effect of EGBE and its metabolite BAA on whole blood collected by cardiac puncture from male F344 rats. The addition of 5 or 10 mM EGBE to whole blood exerted no effect on Hct levels and rat erythrocytes, whereas 20 mM EGBE caused a significant reduction in Hct along with significant hemolysis ($P \leq 0.05$). The addition of 0.5 or 1 mM BAA to rat erythrocytes caused a time- and concentration-dependent increase in Hct followed by hemolysis, while adding 2 mM BAA caused a faster time-dependent increase in Hct. The Hct level reached its maximum after 2 hr followed by nearly complete hemolysis after 4 hr. Ghanayem [1989] also examined the effect of BAA (0.5, 1, 2, 4, 8 mM) on human blood obtained from healthy young male and female volunteers. No significant changes in Hct or hemolysis occurred at BAA concentrations of 4 mM and below. However, at 8 mM BAA there was a slight but significant increase in Hct ($P \leq 0.05$), followed by slight but significant hemolysis ($P < 0.05$) of human erythrocytes.

#### *4.3.2.5 Summary of Hematologic Effects*

Early investigators [von Oettingen and Jirouche 1931] demonstrated the hemolytic activity of EGBE in vitro. In later studies, adverse hematologic effects of EGBE were shown in a variety of species by various exposure routes (i.e., oral, inhalation, dermal). These effects included decreased RBC, Hb, and Hct levels [Werner et al. 1943a,b; Carpenter et al. 1956; Nagano et al. 1979; Dodd et al. 1983; Grant et al. 1985; Tyl et al. 1984; Bartnik et al. 1987; Ghanayem et al. 1987]; the responses were transitory and their severity was dose-dependent. In addition Ghanayem et al. [1987] demonstrated that EGBE-induced hematotoxicity in rats is age-dependent, with older rats more susceptible than younger rats. In the study by Carpenter et al. [1956], exposure to EGBE vapors induced a transitory increase in RBC osmotic fragility in mice, rats, and monkeys. Although inhalation of EGBE vapors caused an increase in WBC levels [Dodd et al. 1983], oral EGBE exposure reduced WBC levels in a dose-dependent manner [Grant et al. 1985]. Exposure of rats and rabbits to EGBEA vapors caused slight hematuria and hemoglobinuria [Truhaut et al. 1979]. Under in vitro conditions, 200, 225, and 250 mmol EGBE/liter induced complete lysis of human erythrocytes [Bartnik et al. 1987]. At a concentration of 3.75 to 7.5 mmol/liter, BAA caused complete lysis of rat erythrocytes [Bartnik et al. 1987]; 8 mM BAA caused a slight but statistically significant lysis of human erythrocytes [Ghanayem 1989]. Studies of the hematologic effects of EGBE and EGBEA are summarized in Table 4-6.

### 4.3.3 Reproductive Effects in Males

A number of experimental animal studies have demonstrated that EGBE does not exert adverse effects on the male reproductive system.

Table 4–4.—Percentage hemolysis of human and rat erythrocytes by EGBE[*, †, §]

| BE (mmol/liter) | 15 min | | 30 min | | 45 min | | 60 min | | 120 min | | 180 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R[**] | H[††] | R | H | R | H | R | H | R | H | R | H |
| 100 | [§§] | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 125 | --- | --- | --- | --- | --- | --- | --- | --- | 10.8 | --- | 64.4 | --- |
| 150 | --- | --- | --- | --- | --- | --- | 8.2 | --- | 62.8 | 3.4 | 92.2 | 11 |
| 175 | --- | --- | 6.2 | --- | 41.4 | --- | 91.1 | 2.1 | 100 | 23.4 | 100 | 89.5 |
| 200 | 5.4 | --- | 100 | --- | 100 | 2.8 | 100 | 8.8 | 100 | 100 | 100 | 100 |
| 225 | 96.4 | --- | 100 | 14.8 | 100 | 89.5 | 100 | 100 | 100 | 100 | 100 | 100 |
| 250 | 200 | 8.6 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

[*]Source: Bartnik et al. [1987].
[†]Data are mean values of up to three measurements, as percentage of total hemolysis (100%) induced by Saponion.
[§]Final erythrocyte concentration = 1%.
[**]R = rat erythrocytes.
[††]H = human erythrocytes.
[§§]No hemolysis.

Table 4–5.—Percentage hemolysis of human and rat erythrocytes by BAA[*, †, §]

| BAA (mmol/liter) | 15 min | | 30 min | | 45 min | | 60 min | | 120 min | | 180 min | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R[**] | H[††] | R | H | R | H | R | H | R | H | R | H |
| 1.25 | §§ | --- | --- | --- | --- | --- | --- | --- | 6.9 | --- | 25.0 | --- |
| 2.50 | --- | --- | --- | --- | 4.0 | --- | 20.0 | --- | 79.3 | --- | 95.0 | --- |
| 3.75 | --- | --- | --- | --- | 17.9 | --- | 49.7 | --- | 86.2 | --- | 100 | --- |
| 5.0 | --- | --- | 13.0 | --- | 59.7 | --- | 88.3 | --- | 100 | --- | 100 | --- |
| 6.25 | --- | --- | 17.4 | --- | 59.9 | --- | 89.0 | --- | 100 | --- | 100 | --- |
| 7.5 | --- | --- | 25.0 | --- | 69.2 | --- | 100 | --- | 100 | --- | 100 | --- |
| 10 | n.m.[***] | --- | n.m. | --- | n.m. | --- | n.m. | --- | n.m. | --- | n.m. | --- |
| 15 | n.m. | --- | n.m. | --- | n.m. | --- | n.m. | --- | n.m. | --- | n.m. | --- |

[*]Source: Bartnik et al. [1987].
[†]Data are mean values of up to three measurements, as percentage of total hemolysis (100%) induced by Saponion.
[§]Final erythrocyte concentration = 1%.
[**]R = rat erythrocytes.
[††]H = human erythrocytes.
[§§]No hemolysis.
[***]Not measured.

**Table 4–6.—Hematologic effects of EGBE and EGBEA**

| Species and sex | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| | | | EGBE: |
| | In vitro RBC (beef blood) | Hemolysis | von Oettingen and Jirouche 1931 |
| Mouse (M)* | Oral: 500 or 1,000 mg/kg per day, 5 days/wk for 5 wk | Reduced RBC counts (500 and 1,000 mg/kg per day) | Nagano et al. 1979 |
| Rat (M) 4–5 wk old | Oral: 125 or 500 mg/kg (single dose) | No effect (125 mg/kg); reduced RBCs, Hb, Hct (500 mg/kg) | Ghanayem et al. 1987 |
| Rat (M) 9–13 wk old | | Reduced RBCs, Hb, and Hct (125 and 500 mg/kg) | |
| Rat (M) | Oral: 500 or 1,000 mg/kg per day for 4 consecutive days | Reduce RBC and WBC counts, Hct and Hb levels; increased MCV, reticulocyte counts and MCHC (1,000 mg/kg per day; same effects but less severe in 500 mg/kg per day group | Grant et al. 1985 |
| Rat (M) | Oral: 222, 443, or 885 mg/kg per day, 5 days/wk for 6 wk | Reduced Hb and RBC count (all doses); decreased MCHC (443 or 885 mg/kg per day); increased MCHb (all doses) | Krasavage 1986 |
| Rat | Inhalation: 135 or 320 ppm, 7 hr/day, 5 days/wk for 5 wk | Increased circulating, immature granulocytes; decreased Hb and RBC count; increased reticulocyte count (320 ppm); effects for 5 wk are reversible | Werner et al. 1943a |
| Dog | Inhalation: 415 ppm, 7 hr/day, 5 days/wk for 12 wk | Decreased Hb and RBC count; increased hypochromia, polychromatophilia and microcystosis; changes are reversible | Werner et al. 1943b |
| Rat (F)† | Inhalation: 62 ppm for 4 hr | Increased osmotic fragility | Carpenter et al. 1956 |
| Rat (M, F) | Inhalation: 107, 203, 314, or 432 ppm for 7 hr | Increased osmotic fragility | Carpenter et al. 1956 |
| | Inhalation: 54 ppm, 7 hr/day, 5 days/wk for 30 days | Increased osmotic fragility | Carpenter et al. 1956 |

(Continued)

*Male.
†Female.

Table 4–6 (Continued).—Hematologic effects of EGBE and EGBEA

| Species and sex | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| Guinea Pig (M, F) | Inhalation: 54, 107, 203, 376, or 494 ppm, 7 hr/day, 5 days/wk for 30 days | No effect on osmotic fragility | Carpenter et al. 1956 |
| Mouse (M) | Inhalation: 100, 200, or 400 ppm, 7 hr/day for 30, 60, or 90 days | Increased osmotic fragility | Carpenter et al. 1956 |
| Dog (F) | Inhalation: 385 ppm (8 exposures) | Death; increased osmotic fragility | Carpenter et al. 1956 |
| Dog (M) | Inhalation: 385 ppm (28 exposures) | Death; increased osmotic fragility, decreased Hb and RBC counts | Carpenter et al. 1956 |
| Dog (M) | Inhalation: 200 ppm, 7 hr/day for 31 days | Slightly increased osmotic fragility, increased WBC count | Carpenter et al. 1956 |
| Dog (F) | Inhalation: 200 ppm, 7 hr/day for 31 days | Slightly increased osmotic fragility, decreased Hb and RBC counts | Carpenter et al. 1956 |
| Dog (M, F) | Inhalation: 100 ppm, 7 hr/day for 90 days | Transitory increase in WBC; female's WBC returned to pre-exposure level, but males remained 50% higher; decrease Hct in male | Carpenter et al. 1956 |
| Monkey (M, F) | Inhalation: 100 ppm, 7 hr/day for 90 days | Transitory increase in osmotic fragility and decrease in RBC counts | Carpenter et al. 1956 |
| Monkey | Inhalation: 210 ppm, 7 hr/day, for 30 days | Reduced Hb and RBC count; transitory increase in osmotic fragility | Carpenter et al. 1956 |
| Rat (M, F) | Inhalation: 0, 20, 86, or 245 ppm, 6 hr/day for 9 days | Reduced RBC counts, Hb levels, MCHC; increases in MCV, nucleated erythrocytes, reticulocytes, and (in males only) lymphocytes (245 ppm); reduced Hb in males (86 ppm); reduced Hb and MCHb, and increased Hct and MCV in females (86 ppm) | Dodd et al. 1983 |
| | Inhalation: 0, 5, 25, or 77 ppm, 6 hr/day for 90 days | Decreased RBC counts and Hb, and increased MCHb in females (77 ppm); 5% decrease in RBC counts in males (77 ppm) | Dodd et al. 1983 |

(Continued)

Table 4–6 (Continued).—Hematologic effects of EGBE and EGBEA

| Species and sex | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| Rat (F) | Inhalation: 25, 50, 100, or 200 ppm, 6 hr/day, g.d. 6-15 | Reduced RBC count (100, 200 ppm); increased Hb and Hct (200 ppm); increased MCV and MCHb, reduced MCHC (100 and 200 ppm) | Tyl et al. 1984 |
| Rabbit (F) | Inhalation: 25, 50, 100, or 200 ppm, 6 hr/day, g.d. 6-18 | No effects | Tyl et al. 1984 |
| Rat (F) | Dermal: 200, 260, 320, 375 or 500 mg/kg | Increased MCV, decreased RBC and Hb, hemoglobinuria (500 mg/kg) | Bartnik et al. 1987 |
| Rat (M)<br>Man (M) | In vitro: 100, 125, 150, 175, 200, 225, or 250 mmol/liter added to blood cultures | Complete hemolysis of rat erythrocytes (175-250 mmol/liter).<br>Complete hemolysis of human erythrocytes (200-250 mmol/liter). | Bartnik et al. 1987 |
| Man (M) | In vitro: 5, 10, or 20 mm added to human blood | Decrease in Hct followed by hemolysis (20 mM) | Ghanayem 1989 |
| | | | EGBEA: |
| Rat (M, F) | Inhalation: 400 ppm for 4 hr | No effect | Truhaut et al. 1979 |
| Rabbit (M, F) | Inhalation: 400 ppm for 4 hr | Transient hemoglobinuria and hematuria | Truhaut et al. 1979 |
| Rat (M, F) | Inhalation: 400 ppm, 4 hr/day, 5 d/wk for 1 mo | Slight hematuria and hemoglobinuria | Truhaut et al. 1979 |
| Rabbit (M, F) | Inhalation: 400 ppm, 4 hr/ day, 5 d/wk for 1 mo | Hematuria and hemoglobinuria; severe decrease in RBC counts and Hb in 2 out of 4 rabbits | Truhaut et al. 1979 |
| Rat (M, F) | Inhalation: 100 ppm, 4 hr/day, 5 d/wk for 10 mo | No effects | Truhaut et al. 1979 |
| Rabbit | Inhalation: 100 ppm, 4 hr/day, 5 d/wk for 10 mo | No effects | Truhaut et al. 1979 |
| | Dermal: 1,500 mg/kg | Reduced RBC counts and Hb levels | Truhaut et al. 1979 |

#### *4.3.3.1 Oral Administration*

In a study by Nagano et al. [1979], groups of five JCL-ICR male mice were treated orally with various doses of EGBE (500, 1,000, or 2,000 mg/kg per day, 5 days/wk for 5 wk). The mice treated with 2,000 mg EGBE/kg died. The day after the final administration of EGBE, the remaining mice were sacrificed and dissected. Weight of the testes was not significantly affected by treatment with EGBE.

Krasavage [1986] administered EGBE by gavage to groups of 10 male rats at doses of 0, 222, 443, or 885 mg/kg per day, 5 days/wk for 6 wk. The animals were sacrificed at the end of the exposure period. Organs were removed, weighed, and examined histopathologically. No adverse effects on the testes were observed.

Foster et al. [1987] exposed groups of six male Alpk/AP (Wistar-derived) rats to single oral doses (174, 434, or 868 mg/kg) of BAA, the metabolite of EGBE (see Section 4.2), to determine the initial target for testicular toxicity. Rats were sacrificed on days 1, 2, 4, and 14 after treatment. No testicular damage was induced by BAA at any dose for any length of exposure, but rats receiving the high dose of BAA did show evidence of hematuria throughout the study. The addition of 5 mM BAA to Sertoli male germ cell culture systems did not produce any specific changes in testicular cell populations.

#### *4.3.3.2 Inhalation*

In an inhalation study by Doe [1984], male Alpk/Ap Wistar-derived rats were exposed to 800 ppm EGBE for 3 hr; the rats were then observed throughout the next 14 days. The animals were sacrificed on day 15 and subjected to gross macroscopic postmortem examination, which included the weighing of the testes. EGBE had no effect on testicular weights.

#### *4.3.3.3 Summary of Reproductive Effects in Males*

Studies using EGBE [Nagano et al. 1979; Doe 1984; Krasavage 1986] and BAA [Foster et al. 1987], the metabolite of EGBE (see Section 4.2), have demonstrated no adverse effects on the male reproductive system. No studies exist for EGBEA. However, because EGBEA would be metabolized to EGBE (see Section 4.2), it would also be expected to cause no reproductive effects.

### 4.3.4 Effects on the Female Reproductive System and the Embryo

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

#### *4.3.4.1 Oral Administration*

Schuler et al. [1984] exposed CD-1 mice orally to 4,000 mg EGBE/kg once per day on g.d. 7 through 14. This treatment resulted in 20% maternal mortality and in 77% viable litters, which differed significantly from 100% viability in the control group ($P<0.05$).

#### 4.3.4.2 Inhalation

In an inhalation study, Nelson et al. [1984] exposed Sprague-Dawley rats on g.d. 7 through 15 to 150 or 200 ppm EGBE for 7 hr/day. The investigators had reduced the doses from 250 and 500 ppm EGBE because of maternal toxicity. Three of four nonpregnant rats exposed to 500 ppm EGBE for 6.5 hr died within 36 hr after termination of EGBE exposure; of three nonpregnant rats exposed to 250 ppm EGBE for 7 hr, one died within 18 hr after exposure ended, and a second died 2 days after exposure. Some hematuria was observed on the first day of exposure in the group exposed to 200 ppm EGBE. No other adverse effects were observed in the dams or the pups in either treatment group. The number of resorptions, fetal weights, and incidence of malformations did not differ from the controls.

Tyl et al. [1984] examined the effects of EGBE administered via inhalation to Fischer 344 rats and New Zealand white rabbits. The animals were exposed to 0, 25, 50, 100, or 200 ppm EGBE, 6 hr/day, on g.d. 6 through 15 (rats) or g.d. 6 through 18 (rabbits). Rats were then sacrificed on g.d. 21 and rabbits on g.d. 29. In rats, the pregnancy rate was equivalent across all groups. A significant increase occurred in the number of totally resorbed litters at 200 ppm EGBE relative to controls ($P<0.01$). Maternal toxicity in rats was indicated by reductions in body weight on g.d. 9, 12, 15, and 21 at 200 ppm EGBE, and by reduced body weight gain on (1) g.d. 6 through 15 at 100 ppm EGBE ($P<0.05$) and (2) g.d. 6 through 15 and 6 through 21 at 200 ppm EGBE ($P<0.001$).

When rats were exposed to 200 ppm EGBE, the number of viable implants ($P<0.001$) and the percentage of live fetuses ($P<0.01$) per litter were reduced relative to controls [Tyl et al. 1984]. However, treatment with EGBE resulted in no statistically significant increases in the incidence of external, visceral, skeletal, or total malformations. Evidence of retarded skeletal ossification was seen at 100 and 200 ppm EGBE. At 100 and 200 ppm EGBE, a significant increase ($P<0.05$) occurred in the number of litters containing one or more fetuses with unossified or poorly ossified skeletal elements.

Significant reductions in maternal body weight, gravid uterine weight, and numbers of total implants and viable implants were noted at 200 ppm EGBE relative to controls ($P<0.05$). No statistically significant increases in the number of fetuses or litters with malformations were observed in any treatment group. Two significant variations were seen in rabbit fetuses. At 200 ppm EGBE, there was a significant reduction in unossified sternebrae and in rudimentary rib ($P<0.05$). The occurrence of unossified skeletal elements in both rats and rabbits was considered an indication of delayed development. None of the observations indicated abnormal development in rats or rabbits exposed to EGBE in this study [Tyl et al. 1984].

#### 4.3.4.3 Dermal Exposure

Hardin et al. [1984] applied EGBE to the skin of pregnant Sprague-Dawley rats to investigate its potential for developmental toxicity. Four daily doses of 106 mg EGBE (total daily dose of 424 mg) were applied to shaved interscapular skin of rats on g.d. 7 through 16. No maternal, embryotoxic, fetotoxic, or teratogenic effects were detected in litters of the EGBE-exposed dams.

#### *4.3.4.4 In Vitro*

The in vitro culture system of Yonemoto et al. [1984] was used by Rawlings et al. [1985] to study the mechanism of teratogenicity of EGBE. Conceptuses were explanted from pregnant Wistar-Porton rats at the embryonic age of 9.5 days and cultured for 48 hr with 2 or 5 mmol BAA/liter. At the end of the culture period, crown-rump length, head length, and yolk sac diameter were measured, and the degree of differentiation and development was evaluated by a morphological scoring system. BAA at the 5mM concentration had an adverse effect on all parameters except crown-rump length. BAA produced statistically significant reductions in somite number ($P<0.01$), head length ($P<0.01$), yolk sac diameter ($P<0.05$), and protein content of the embryo ($P<0.05$). No statistically significant reductions in growth parameters were seen at the 2-mM level. Irregularity of the neural suture line was seen in 29% of the embryos exposed to 5 mM BAA. BAA-exposed embryos also showed abnormal otic and somite development. None of the observations indicated abnormal development.

#### *4.3.4.5 Summary of Reproductive Effects in Females*

The preceding studies demonstrated that EGBE administered by a variety of routes in a variety of species did not have teratogenic effects on litters of EGBE-exposed dams [Schuler et al. 1984; Tyl et al. 1984; Nelson et al. 1984; Hardin et al. 1984]. Signs of maternal toxicity included decreased body weight and body weight gain [Tyl et al. 1984]. At the maternal $LD_{20}$ (lethal dose for 20% of the test animals), EGBE induced fetal death [Schuler et al. 1984]. BAA, the metabolite of EGBE (see Section 4.2), did not adversely affect fetal development in vitro [Rawlings et al. 1985].

No studies are reported for EGBEA. However, because it would be metabolized to EGBE (see Section 4.2), it would also be expected to cause no effects on the female reproductive system and the embryo. Table 4-7 summarizes the studies of reproductive and developmental effects of EGBE.

### 4.3.5 Carcinogenicity

Prechronic carcinogenicity studies of EGBE are currently in progress [NTP 1988].

### 4.3.6 Mutagenicity

A limited number of studies concerning the potential mutagenicity of EGBE have been performed. Most of these involved tests with microorganisms or mammalian cell cultures in vitro. EGBE does not appear to be mutagenic. No data are available concerning the mutagenicity of EGBEA.

EGBE was tested for mutagenic activity by using an assay of unscheduled DNA synthesis (UDS) in rat primary hepatocytes; total radioactivity was measured by scintillation counting [McGregor 1984]. In this study, EGBE was tested at concentrations up to 0.1% of the culture medium for 2 hr. The results suggested that EGBE might be inhibiting UDS, but further

Table 4–7.—Reproductive and developmental effects of EGBE and BAA

| Species and sex | Route of administration and dose | Observed effects | Compound studied and reference |
|---|---|---|---|
| | | | EGBE: |
| Mouse (M)* | Oral: 500, 1,000, or 2,000 mg/kg per day, 5 day/wk for 5 wk | No testicular effects | Nagano et al. 1979 |
| Mouse (F)† | Oral: 4,000 mg/kg per day on g. d. 7–14 | 20% maternal mortality, 23% nonviable litters | Schuler et al. 1984 |
| Mouse (M) | Oral: 222, 443, or 885 mg/kg per day, 5 day/wk for 6 wk | No testicular effects | Krasavage 1986 |
| Rat (F) | Inhalation: 25, 50, 100, or 200 ppm, 6 hr/day on g. d. 6–15; sacrificed on g. d. 21 | Increase in number of litters resorbed, reduced maternal body weight and body weight gain, reduced live fetuses (200 ppm), retarded skeletal ossification (100, 200 ppm) | Tyl et al. 1984 |
| Rabbit (F) | Inhalation: 25, 50, 100, or 200 ppm, 6 hr/day on g. d. 6–18; sacrificed on g. d. 29 | Reduced maternal body weight and gravid uterine weight (200 pm), reduced numbers of total viable implants (200 pm), two skeletal variations (200 ppm) | Tyl et al. 1984 |
| Rat (F) | Inhalation: 250 or 500 ppm, 7 hr/day on g. d. 7–15 | Death | Nelson et al. 1984 |
| | Inhalation: 150 or 200 ppm, 7 hr/day on g. d. 7–15 | Hematuria (200 ppm) | Nelson et al. 1984 |
| Rat (M) | Inhalation: 800 ppm for 3 hr | Hematuria; no testicular effects | Doe 1984 |
| Rat (F) | Dermal: 0.9 mmol, 4 times/day on g. d. 7–16 | No effects on offspring | Hardin et al. 1984 |
| | Dermal: 2.7 mmol, 4 times/day on g. d. 7–16 | Hematuria, death of 10/11 treated rats | Truhaut et al. 1979 |
| | | | BAA: |
| Rat (M) | Oral: single dose of 174, 434, or 868 mg/kg | No testicular effects | Foster et al. 1987 |
| | In vitro: 2 or 5 mmol/liter | No effect on embryonic development | Rawlings et al. 1985 |

*Male.
†Female.

testing is needed to confirm this possibility. McGregor [1984] also examined the ability of EGBE to induce point mutations in vitro. EGBE was not mutagenic at the HGPRT locus of Chinese hamster ovary (CHO) cells in either the presence or absence of rat S9 mix at concentrations up to 1% of the culture medium for 5 hr, followed by a 7-day expression period. Data from the McGregor [1984] study are presented in Table 4-8.

### 4.3.7 Cytotoxicity

The in vitro cytotoxicity of EGBE and BAA was studied using CHO cells [Jackh et al. 1985]. CHO cells were seeded into culture flasks, and after 4 to 5 hr, test material was added to the medium. After 16 hr, the medium was renewed and the cells were allowed to grow in colonies for 6 to 7 days before counting. Cloning efficiency was used as an indication of cytotoxicity. Concentrations that allowed approximately 50% of the seeded cells to form colonies ($EC_{50}$) were calculated. EGBE was cytotoxic ($EC_{50}$ = 0.05 mmol/ml or 6.9 mg/ml). The $EC_{50}$ for BAA was 0.04 mmol/ml (5.3 mg/ml).

Chinese hamster V79 cells display a specific form of cell-to-cell communication called metabolic cooperation, which is characterized by the exchange of molecules between cells through permeable junctions formed at sites of cell contact [Hooper and Subak-Sharpe 1983]. The effects of EGBE on cell-to-cell communication in Chinese hamster V79 cells were demonstrated in two separate studies [Welsch and Stedman 1984; Loch-Caruso et al. 1984]. In both studies, EGBE was able to block metabolic cooperation in vitro.

**Table 4-8.—Mutagenic effects**

| Type of test | Compound | Test species | Results | Reference |
|---|---|---|---|---|
| Mammalian in vitro unscheduled DNA syntheis | EGBE | CHO cells with and without rat S9 mix | No response | McGregor 1984 |
| Mammalian in vitro point mutations | EGBE | CHO cells, HGRPT locus, with and without rat S9 mix | No response | McGregor 1984 |