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# Teratologic Evaluation of Ethylene Glycol Monobutyl Ether in Fischer 344 Rats and New Zealand White Rabbits Following Inhalation Exposure

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**Timed-pregnant Fischer 344 rats and New Zealand White rabbits were exposed to ethylene glycol monobutyl ether vapors by inhalation on gestational days 6 through 15 (rats) or 6 through 18 (rabbits) at concentrations of 0, 25, 50, 100 or 200 ppm. The animals were sacrificed on gestational day 21 (rats) or 29 (rabbits). In rats, exposure to 200 or 100 ppm resulted in maternal toxicity (clinical signs, decreased body weight and weight gain, decreased absolute and relative organ weights, decreased food and water consumption and evidence of anemia), embryotoxicity (increased number of totally resorbed litters and decreased number of viable implantations per litter) and fetotoxicity (reductions in skeletal ossification). No increase in fetal malformations was observed in any exposure group relative to controls. At 50 or 25 ppm, there was no maternal, embryo or fetal toxicity (including malformations) in rats. In rabbits, exposure to 200 ppm resulted in maternal toxicity (apparent exposure-related increases in deaths and abortions, clinical signs, decreased weight during exposure and reduced gravid uterine weight at sacrifice) and embryotoxicity (reduced number of total and viable implantations per litter). No treatment-related fetotoxicity was seen. No treatment-related increases in fetal malformations or variations were seen at any exposure concentration tested. There was no evidence of maternal, embryo, or fetal toxicity (including malformations) at 100, 50 or 25 ppm in rabbits.**

## Introduction

As part of a program evaluating the potential toxicity of ethylene glycol monobutyl ether (EGBE), studies were initiated to evaluate the teratogenic potential of EGBE in test animals under the sponsorship of the Chemical Manufacturers Association Glycol Ethers Program Panel. The initial rat study employed exposure of timed-pregnant Fischer 344 rats to EGBE at 300, 200, or 100 ppm or to 0 ppm (air control) for 6 hr per day during gestational days 6 through 15. Maternal clinical signs, food and water consumption, and body weights were recorded. Animals were sacrificed on gestational day 21 after obtaining blood samples for clinical hematology. Following a complete ovarian and uterine examination, all live fetuses were weighed, sexed, and examined externally. Approximately one-half of the fetuses in each litter were examined for visceral and craniofacial abnormalities, and the remaining fetuses were examined for skeletal abnormalities.

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Exposures to 100, 200 and 300 ppm EGBE produced statistically significant reductions in maternal body weight gain and food consumption. Water consumption was reduced during exposure at 300 or 200 ppm. Signs of maternal toxicity (decrease in red blood cell count; ocular, nasal, and urogenital discharges; hypoactivity; etc.) were observed predominantly at 300 and 200 ppm of EGBE. One animal exposed to 300 ppm EGBE died. Exposures to 300 ppm EGBE resulted in an increased incidence of embryo and fetal mortality (16 of 23 females pregnant at sacrifice had 100% resorptions at 300 ppm). No treatment-related fetal external or skeletal malformations were noted at any concentration of EGBE. Visceral examinations revealed cardiovascular defects, specifically ventricular septal defect in two fetuses in two litters (out of seven litters examined) at 300 ppm and one fetus in one litter at 100 ppm. These findings were not statistically significant relative to controls. Incidence of absent and/or severely shortened innominate artery, considered variations, was significantly elevated at 300 ppm relative to controls. Craniofacial examination of fetuses revealed a concentration-related incidence of enlarged lateral ventricles of the brain,

considered a variation indicative of embryo/fetal toxicity, at all concentrations of EGBE. Results from the skeletal examinations included concentration-related increases in the incidence of extra ribs (fourteenth, rudimentary, considered a variation), significant only at 300 ppm, and in the incidence of reduced ossification in those skeletal districts indicative of fetal toxicity at 200 and 300 ppm, relative to controls.

Since the exposure employed in this initial study produced maternal and fetal toxicity at all exposure concentrations, did not identify a "no observable effect level" (NOEL), and excessive embryo/fetal loss was observed at 300 ppm, another rat study and a teratogenic evaluation of EGBE in another mammalian species were undertaken. This report details the evaluation of EGBE for teratogenic potential in Fischer 344 rats and New Zealand White rabbits.

## Materials and Methods

### Generation of Atmospheres and Chamber Parameters

A 55-gal container of EGBE test material (Lot Number 5804213, BRRC Sample Number 45-235) was received from Union Carbide Corporation on July 30, 1982. Test material from the original container was placed in 1 gal glass bottles and blanketed with nitrogen. The purity and stability were verified by gas chromatographic analysis both before and after the study. No significant compositional changes occurred while the study progressed. The test material remained 99.6% EGBE throughout the study. Fisher certified hexanes was used as the solvent for preparation of standards. The gases for the chromatograph were obtained from Linde Corporation. The nitrogen and hydrogen were Ultra High Purity grade and the air was breathing quality.

A Perkin-Elmer Model 3920B gas chromatograph (GC) equipped with a flame ionization detector was used to monitor the EGBE vapor concentrations in the chambers. The GC column was a 6-ft × 4 mm (internal diameter) glass column packed with 0.15% Fluorad FC431 (3M Company, St. Paul, MN) on glass beads, 80–120 mesh. A Spectra-Physics series 4000 central processor, a data interface, and a Perkin-Elmer automatic gas sampling system (station and valve programmer units and a gas sampling valve) were used for the analyses.

Calibration of the gas chromatograph was done with liquid injections of standard solutions of EGBE in hexanes prepared volumetrically. The series of standard solutions encompassed the entire range of vapor concentrations generated in the exposure chambers. A linear calibration curve was obtained when areas (integration counts) were plotted versus the gas equivalent concentrations of the standards. The calibration factor (KF) was calculated as the ratio between area $A$ and concentration $C$:

$$KF = A/C$$

The mean KF for all valid injections of standards was used in subsequent microprocessor calculations. This calibration factor was checked at least once each week during the exposure period and did not require adjustment.

Each chamber atmosphere was analyzed for EGBE approximately once every hour during each 6-hr exposure. Daily nominal concentrations (an estimated concentration calculated from the amount of test material delivered and the chamber airflow during the exposure period) were also calculated for each chamber.

Liquid EGBE was metered from a piston pump into a heated glass evaporator similar in design as described by Carpenter et al. (*1*). The temperature in the evaporator was maintained at the lowest level sufficient to vaporize the liquid. The resultant vapor was carried into the chamber by passage of conditioned air through the evaporator. Chamber atmospheres containing EGBE were filtered before leaving an exhaust stack.

The five chambers employed in this study were rectangular in shape, constructed of glass and stainless steel (Wahmann Manufacturing Company, Timonium, MD), and measured approximately 2.1 m × 1 m × 2.1 m (height). Total volume in each chamber was approximately 4350 L. An orifice plate was positioned in the exhaust duct of the chamber and was connected to a Dwyer Magnehelic Pressure Gauge. Excellent chamber distribution for EGBE vapors has been demonstrated. The coefficient of variation of concentrations measured at eight locations in the chamber in the breathing zones of the animals was 1%.

Airflow in each chamber was approximately 1000 L/min (14 air changes per hour) with a $t_{99}$ (theoretically derived time required for the chamber to reach 99% of the equilibrium concentration) of approximately 20 min.

The chambers were illuminated with artificial room light. Temperature (Markson Science, Inc., Model #13177) and relative humidity (Airguide Instrument Company) gauges were placed inside each chamber during exposures. Chamber temperature, relative humidity, and airflow rate were recorded at least four times during each 6-hr exposure.

Within each chamber, the animal cages were rotated daily to compensate for any possible, but undetected, variation in chamber exposure conditions (i.e., concentration, temperature, relative humidity).

Target concentrations were 0, 25, 50, 100 and 200 ppm. These exposure concentrations were based on a range-finding study in pregnant rats and rabbits which employed concentrations of 450, 200, 75 or 0 ppm, and an initial rat teratology study with concentrations of 300, 200, 100 and 0 ppm. Maternal mortality was seen at 450 ppm (100% in both species) and maternal toxicity was seen at all exposure concentrations employed (except at 75 ppm for rabbits) in the two studies. Since a

no-observable effect level (NOEL) could not be ascertained from these studies, the current exposure concentrations were selected for both rats and rabbits in the present study.

## Rats

Virgin male and female Fischer 344 rats [NIH:(F-344)/HlaBR(F141 + 3)] (100 days old upon arrival) were purchased from Hilltop Lab Animals, Inc., Scottdale, PA. They were quarantined for 2 weeks during which time representative animals were subjected to fecal sampling, aerobic bacteriologic cultures of lung tissue, histologic examination of selected organs and to serum viral antibody examination for sialodacryoadenitis. Rats were housed in stainless steel wire-mesh cages with food (Certified Ground Rodent Chow, Ralston Purina Co., Richmond, IN) and water (Municipal Authority of Westmoreland County, Greensburg, PA) available *ad libitum* except during exposures. Deotized Animal Cage Board (Shepherd Specialty Papers, Inc., Kalamazoo, MI) was placed beneath the cages and changed daily. Animals were kept on a 12-hr photoperiod and room temperatures and humidity were recorded continuously (Cole-Parmer Hygrothermograph Seven-Day Continuous Recorder, Model #8368-00, Cole Parmer Instrument Co., Chicago, IL).

Rats were mated 1:1 (one male:one female) in stainless steel wire-mesh cages and the paperboard beneath the cages was checked twice daily for dropped copulation plugs. Successfully mated (plug-positive) females were housed singly for the duration of the study. The day a copulation plug was found was designated gestational day (gd) 0. Thirty-six plug-positive females were randomly assigned to each experimental group.

For exposures, plug-positive females were transferred, one per cage, to stainless steel wire-mesh cages and the cage carriers, with stainless steel shelves beneath each row of cages, were moved into the chambers. Food and water were withheld during exposures. Exposures were for 6 hr/day, gd 6 through 15. After each exposure, animals were returned to their original cages. The animals were observed daily for clinical signs throughout the study (gd 0 through 21). Food and water consumption was measured for the intervals gd 0–3, 3–6, 6–9, 9–12, 12–15, 15–18, and 18–21. Maternal body weights were taken on gd 0, 6, 9, 12, 15 and 21.

At scheduled sacrifice on gd 21, rats were anesthetized by carbon dioxide inhalation for collection of blood samples. Following blood collections, animals were sacrificed by carbon dioxide asphyxiation.

## Rabbits

Virgin New Zealand White rabbits (3.0 to 3.5 kg, approximately 5 to 5.5 months old) were purchased from Hazleton Dutchland Laboratories, Inc., Denver, PA. They were quarantined for 2 weeks, during which they were examined by a veterinarian and fecal samples taken for detection of intestinal parasites. The rabbits were individually housed in stainless steel wire-mesh cages with food (Big Red Rabbit Food, Agway, Inc., St. Marys, OH) and water (Municipal Authority of Westmoreland County, Greensburg, PA) available *ad libitum* except during exposures. Paperboard was placed beneath the cages, and photoperiod, temperature and relative humidity were monitored.

Rabbits were bred, using in-house breeding colony males. The date of copulation was designated gd 0. Twenty-four mated females were randomly assigned to each experimental group.

For exposures, mated females were transferred to stainless steel wire-mesh cages in cage carriers which were moved into the chambers. Food and water were withheld during exposures. Exposures were for 6 hr/day, gd 6 through 18, in the same chambers as the rats. After each exposure, animals were returned to their original cages. The animals were observed daily for clinical signs throughout the study (gd 0 through 29). Maternal body weights were taken on gd 0, 6, 9, 12, 15, 18, 21 and 29.

At scheduled sacrifice on gd 29, blood was collected from each doe after she was placed in a rabbit restrainer. Following blood collection, rabbits were sacrificed by cervical dislocation.

## Thoracolaparotomy, Maternal and Fetal Examinations

The maternal body cavities were opened by midline thoracolaparotomy. The gravid uteri, ovaries (including corpora lutea), cervices, vagina, and peritoneal and thoracic cavities were examined grossly. Ovarian corpora lutea of pregnancy were counted. Maternal liver, kidney, thymus, spleen and uterus weights were determined. The uteri were immediately ligated at their cervical end to prevent the expulsion of conceptuses by myometrial peristalsis. Uteri were externally examined for signs of hemorrhage. The ligated uteri were removed from the peritoneal cavity and dissected longitudinally to expose their contents. All live and dead fetuses, placentae, and resorption sites were noted and recorded. Uteri from females that appeared nongravid were placed in a 10% ammonium sulfide solution for detection of early resorptions (*2*).

Each rabbit fetus was euthanized immediately upon removal from the uterus by intraperitoneal injection of sodium pentobarbital. All live fetuses were weighed and sexed. All fetuses were examined for external malformations including cleft palate. One-half of the fetuses (selected at random) in each litter were examined for thoracic and peritoneal visceral abnormalities by modification of methods described by Staples (*3*). These fetuses were decapitated and their heads were fixed in Bouin's solution for examination of craniofacial structures by sectioning methods modified from Wilson (*4,5*) and van Julsingha and Bennett (*6*). The remaining

fetuses in each litter were eviscerated, fixed in ethanol, and then processed for skeletal staining with Alizarin Red S (*7,8*), and examined for skeletal malformations.

## Hematologic Analyses

Blood samples for erythrocyte osmotic fragility and hematologic evaluation were obtained from female rats via retro-orbital bleeding immediately prior to sacrifice. Approximately 700 μL of blood was collected into B-D Microtainer Brand capillary whole blood collection tubes containing sodium heparin as anticoagulant (Becton-Dickinson Co., Rutherford, NJ) for the osmotic fragility assay. Approximately 200 μL of blood was collected into B-D Microtainer Brand Capillary whole blood collection tubes with EDTA as anticoagulant (Becton-Dickinson Company, Rutherford, NJ) for hematologic determinations.

Blood samples for erythrocyte osmotic and hematologic evaluation were obtained from rabbit does via cardiac puncture immediately prior to sacrifice. Approximately 2.0 mL of blood was collected into tubes with sodium heparin as anticoagulant (Vacutainer Brand, Becton-Dickinson Company, Rutherford, NJ) for the osmotic fragility assay. Approximately 2.0 mL of blood was collected into tubes containing EDTA as anticoagulant (Vacutainer Brand, Becton-Dickinson Company, Rutherford, NJ) for hematologic determinations.

A 20 μL portion of blood from each female rat and rabbit was added to each of ten Unopette Reservoirs (Becton-Dickinson Co., Rutherford, NJ) containing a specific concentration of buffered saline. Each saline solution containing red cells was incubated for 20 min and was then centrifuged for 5 min at 2000 rpm. The supernatant of each sample was decanted and the percentage of hemolysis was read using a Trace III Clinical Chemistry System (Beckman Instruments, Inc., Fullerton, CA). A precision check of the Trace III System was performed immediately prior to sample analysis.

The following hematologic parameters were measured on all rat and rabbit samples: erythrocyte count, hemoglobin, and hematocrit. These analyses were performed on a Coulter Counter Model S-Plus IV (Coulter Electronics, Inc., Hialeah, FL). Commercially available quality control samples (4C Plus II Coulter Counter Cell Control, Coulter Diagnostics, Hialeah, FL) were run prior to the rat and rabbit samples. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from the measured hematologic values.

## Statistical Analyses

The unit of comparison was the pregnant female (*9*). Results of the quantitative continuous variables (e.g., maternal body weights, liver weights) were intercompared for the four exposure groups and a control group by use of Levene's test for equal variances (*10*), analysis of variance (ANOVA), and *t*-tests with Bonferroni probabilities. The *t*-tests were used when the *F* value from the ANOVA was significant. When Levene's test indicated homogeneous variances and the ANOVA was significant, the pooled *t*-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances (*11*) followed, when necessary, by the separate variance *t*-test.

Nonparametric data obtained following laparohysterectomy were statistically treated by using the Kruskal-Wallis test (*12*) followed by the Mann-Whitney *Ú* test (*12*) when appropriate. Incidence data were compared by using Fisher's exact test (*12*). For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for significance.

# Results

## Generation of Atmospheres and Chamber Parameters

The means of the daily means for EGBE chamber concentration, temperature and humidity are presented in Table 1. All EGBE analyses were within ± 10% of the

**Table 1. Summary of chamber concentrations and exposure conditions.**

| | Ethylene glycol monobutyl ether (EGBE) target concentrations | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Analytical concn, ppm | 201 ± 6[a] | 98 ± 2 | 50 ± 1 | 25 ± 1 | < MDL[b] |
| A/T ratio[c] | 1.005 | 0.98 | 1.00 | 1.00 | — |
| Nominal concn, ppm | 220 | 110 | 49 | 26 | — |
| A/N ratio[d] | 0.92 | 0.89 | 1.01 | 0.94 | — |
| Temperature, °F | 79.3 ± 1.4[a] | 78.9 ± 2.0 | 78.9 ± 2.0 | 79.0 ± 1.8 | 77.9 ± 2.2 |
| Relative humidity, % | 41.2 ± 6.3[a] | 41.4 ± 5.6 | 40.0 ± 5.8 | 40.7 ± 6.8 | 42.8 ± 6.2 |

[a]Grand mean of daily means ± SD.
[b]< MDL = below minimum detection limit of 2 ppm.
[c]Analytical/target concentration ratio.
[d]Analytical/nominal concentration ratio.

target concentrations. Since generation of the EGBE atmosphere required evaporation under heat which elevated the temperature in the EGBE-exposure chambers, the control chamber temperature was also elevated to keep the temperature conditions consistent in all groups (78–79°F). Relative humidity was maintained at 40 to 43%.

## Rats

The distribution and fate of all plug-positive rats on study are presented in Table 2. A small number (five or six) of rats in each treatment group were inadvertently exposed to EGBE for one additional day (gd 16). They were subsequently removed from study but all their in-life, necropsy and fetal evaluation data were recorded and retained in the Bushy Run Research Center Archives. (For discussion of the results from these animals, please see the Addendum to this paper.) Pregnancy rate, as determined at scheduled sacrifice, was equivalent across all groups. There was a significant increase in the number of totally resorbed litters at 200 ppm relative to controls.

Clinical signs observed in a dose-related incidence in dams during the exposure period (Table 3) included evidence of hematuria (or hemoglobinuria) especially early in the exposure period, pale and cold extremities and necrosis of the tail tip.

Maternal toxicity was also expressed as significant reductions in body weight during the exposure period (gd 9, 12, 15) and at sacrifice at 200 ppm, as significantly reduced weight gain during the exposure period (gd 6–9, 6–12, and 6–15) at 100 and 200 ppm and for the gd 6–21 period for dams exposed to 200 ppm (Table 4). Food consumption, expressed as grams/dam/day, was statistically reduced during the exposure period (gd 6–9, 9–12 and 6–15) for dams exposed to 100 and 200

**Table 2. Distribution and fate of F-344 rats exposed to EGBE by inhalation on gestational days 6 through 15.**

| | EGBE target concentrations | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| No. in study | 36 | 36 | 36 | 36 | 36 |
| No. removed[a] | 6 | 6 | 5 | 5 | 5 |
| No. early deliveries | 0 | 0 | 0 | 0 | 1 |
| No. dead | 0 | 0 | 0 | 0 | 0 |
| No. nonpregnant at sacrifice | 5 | 7 | 9 | 9 | 7 |
| No. (%) pregnant at sacrifice | 25 (83.3) | 23 (76.7) | 22 (71.0) | 22 (71.0) | 23 (76.7) |
| No. litters totally resorbed | 9[b†] | 0 | 1[c] | 0 | 0 |
| No. litters examined | 16 | 23 | 21 | 22 | 23 |

[a]These animals were removed because they were inadvertently exposed for one additional day (gestational day 16).
[b]Nine dams had only resorption sites at scheduled sacrifice, seven of which were found only after ammonium sulfide staining.
[c]One dam had only resorption sites at scheduled sacrifice, detected after ammonium sulfide staining.
[†]$p < 0.01$ versus controls, Fisher's exact test.

**Table 3. Clinical observations in Fischer 344 dams exposed to EGBE by inhalation on gestational days 6 through 15.[a]**

| | EGBE concentrations | | | | |
|---|---|---|---|---|---|
| | 0 ppm | 25 ppm | 50 ppm | 100 ppm | 200 ppm |
| Urogenital area stained red, red black | 0 | 0 | 0 | + | + + + |
| Urogenital wetness | + | + | + | + | + + + |
| Fur stained (red) | + | + | + | + | + + + |
| Urogenital encrustation | + | + | + | + | + + + |
| Fluid on tray (positive for occult blood) | 0 | 0 | 0 | + | + + + |
| Red fluid on tray | 0 | 0 | 0 | + | + + + |
| Periocular wetness | 0 | + | + | + + | + + + |
| Perinasal encrustation | + | + | + | + + | + + + |
| Extremities cold | 0 | 0 | 0 | 0 | + + + |
| Extremities pale | 0 | 0 | 0 | 0 | + + + |
| Tail tip discolored | 0 | 0 | 0 | 0 | + + + |
| Tail tip ulcerated | 0 | 0 | 0 | 0 | + + + |
| Tail tip missing | 0 | 0 | 0 | 0 | + + + |

[a]Code: + + + = majority of dams (75% or more) exhibited the clinical sign sometime during the exposure period; + + = approximately 50% of the dams (30–70%) exhibited the clinical sign sometime during the exposure period; + = up to 25% of the dams exhibited the clinical sign sometime during the exposure period; 0 = no dams exhibited the clinical sign during the exposure period.

**Table 4. Maternal weights and weight gain of F-344 rats exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Maternal body weight, g | | | | | |
| gd 0 | 194.7 ± 5.3[a] | 195.6 ± 7.3 | 195.5 ± 7.2 | 195.7 ± 7.3 | 191.8 ± 6.1 |
| gd 6 | 207.3 ± 5.6 | 207.1 ± 7.1 | 206.1 ± 7.8 | 206.5 ± 7.0 | 205.2 ± 6.7 |
| gd 9 | 181.9 ± 6.5[‡] | 204.3 ± 10.5 | 208.4 ± 8.7 | 210.2 ± 7.5 | 207.8 ± 7.9 |
| gd 12 | 191.3 ± 9.1[‡] | 216.8 ± 8.1 | 219.0 ± 7.2 | 218.1 ± 15.0 | 218.7 ± 8.5 |
| gd 15 | 203.5 ± 18.0[‡] | 224.8 ± 13.1 | 226.7 ± 10.0 | 230.6 ± 11.3 | 230.1 ± 9.1 |
| gd 21 | 244.5 ± 30.9[‡] | 277.1 ± 19.0 | 272.4 ± 20.2 | 277.6 ± 19.5 | 275.6 ± 17.8 |
| Corrected body weight[b] | 210.4 ± 13.9 | 215.1 ± 11.0 | 215.5 ± 7.9 | 214.5 ± 11.8 | 215.7 ± 8.3 |
| Maternal body weight gain, g | | | | | |
| gd 0–6 | 12.6 ± 4.1 | 11.5 ± 2.9 | 10.6 ± 5.4 | 10.8 ± 4.0 | 13.4 ± 3.6 |
| gd 6–9 | − 25.4 ± 3.7[‡] | − 2.9 ± 6.5[‡] | 2.3 ± 4.0 | 3.7 ± 2.9 | 2.6 ± 4.2 |
| gd 6–12 | − 16.0 ± 6.8[‡] | 9.7 ± 2.8[†] | 12.9 ± 3.7 | 11.6 ± 12.1 | 13.5 ± 4.5 |
| gd 6–15 | − 3.8 ± 17.1[‡] | 17.7 ± 9.2[*] | 20.6 ± 7.9 | 24.1 ± 8.0 | 24.9 ± 6.0 |
| gd 6–21 | 37.2 ± 30.0[‡] | 70.0 ± 15.8 | 66.3 ± 19.5 | 71.1 ± 16.1 | 70.4 ± 15.3 |
| gd 15–21 | 41.0 ± 19.7 | 52.3 ± 14.6 | 45.7 ± 15.6 | 47.0 ± 11.5 | 45.5 ± 10.4 |

[a]Values represent mean ± standard deviation; units are in grams.
[b]Corrected body weight = body weight on gestational day 21 minus gravid uterine weight.
[*]$p < 0.05$ compared to control ($p = 0.0033$ Bonferroni $t$-test, adjusted).
[†]$p < 0.01$ compared to control.
[‡]$p < 0.001$ compared to control.

**Table 5. Food consumption of F-344 rats exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Food consumption, g/dam/day | | | | | |
| gd 0–3 | 12.0 ± 1.3[a] | 11.6 ± 1.9 | 11.4 ± 2.4 | 11.6 ± 1.7 | 12.6 ± 1.7 |
| gd 3–6 | 12.8 ± 1.0 | 12.8 ± 1.2 | 12.2 ± 1.9 | 12.8 ± 1.0 | 13.3 ± 1.5 |
| gd 6–9 | 2.9 ± 1.0[‡] | 10.9 ± 1.7[‡] | 13.7 ± 1.4 | 14.0 ± 0.9 | 14.4 ± 1.0 |
| gd 9–12 | 9.4 ± 2.4[‡] | 15.0 ± 1.2[*] | 15.4 ± 1.2 | 15.3 ± 3.2 | 16.0 ± 1.4 |
| gd 12–15 | 14.9 ± 3.4 | 15.1 ± 2.4 | 15.6 ± 3.3 | 16.0 ± 2.0 | 16.7 ± 1.2 |
| gd 15–18 | 19.0 ± 2.5 | 18.6 ± 1.7 | 17.4 ± 2.0 | 18.3 ± 3.3 | 19.1 ± 2.9 |
| gd 18–21 | 19.7 ± 2.2 | 19.2 ± 1.9 | 17.9 ± 2.8 | 18.2 ± 1.1 | 18.6 ± 1.4 |
| gd 0–6 (prior to treatment) | 12.4 ± 0.9 | 12.2 ± 1.3 | 11.8 ± 1.8 | 12.2 ± 1.1 | 12.9 ± 1.5 |
| gd 6–15 (during treatment) | 9.0 ± 2.0[‡] | 13.7 ± 1.3[‡] | 14.9 ± 1.6 | 15.1 ± 1.7 | 15.7 ± 0.9 |
| gd 15–21 (following treatment) | 19.4 ± 2.2 | 18.9 ± 1.6 | 17.6 ± 2.0 | 18.2 ± 2.0 | 18.9 ± 1.9 |

[a]Values represent mean ± standard deviation; units are in grams per dam per day.
[*]$p < 0.05$ compared to control.
[‡]$p < 0.001$ compared to control.

**Table 6. Water consumption of F-344 rats exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Water consumption, g/dam/day | | | | | |
| gd 0–3 | 16.9 ± 2.9(25)[a] | 17.4 ± 3.3(23) | 15.8 ± 4.4(21) | 16.6 ± 2.0(21) | 17.7 ± 2.1(22) |
| gd 3–6 | 17.3 ± 2.0(25) | 18.4 ± 2.4(23) | 17.6 ± 2.5(21) | 17.3 ± 2.5(21) | 17.3 ± 1.9(22) |
| gd 6–9 | 6.4 ± 2.7(25)[‡] | 18.1 ± 5.6(23) | 17.9 ± 2.6(22) | 18.2 ± 1.6(22) | 19.1 ± 2.7(22) |
| gd 9–12 | 20.1 ± 5.1(23) | 25.3 ± 7.2(23) | 21.9 ± 2.9(22) | 22.1 ± 4.9(21) | 22.0 ± 2.8(23) |
| gd 12–15 | 30.1 ± 5.9(23)[*] | 27.1 ± 7.3(23) | 23.7 ± 5.3(22) | 24.6 ± 4.4(21) | 25.5 ± 2.9(23) |
| gd 15–18 | 31.5 ± 6.5(25) | 33.7 ± 3.4(22) | 30.3 ± 4.8(22) | 28.7 ± 6.4(22) | 31.4 ± 4.0(23) |
| gd 18–21 | 31.2 ± 7.7(21) | 33.3 ± 3.4(21)[b*] | 30.0 ± 5.3(22) | 29.7 ± 2.5(21) | 30.4 ± 3.7(23) |
| gd 0–6 (prior to treatment) | 17.1 ± 2.0(25) | 17.9 ± 2.4(23) | 16.6 ± 2.4(20) | 16.8 ± 1.6(21) | 17.6 ± 1.9(21) |
| gd 6–15 (during treatment) | 19.1 ± 3.8(21)[†] | 23.5 ± 4.0(23) | 21.2 ± 2.8(22) | 21.8 ± 1.9(20) | 22.2 ± 2.2(23) |
| gd 15–21 (following treatment) | 31.6 ± 6.9(21) | 33.6 ± 3.1(21)[*] | 30.1 ± 4.8(22) | 29.1 ± 3.5(21) | 30.9 ± 3.6(23) |

[a]Values represent mean ± standard deviation; units are in grams per dam per day; values in parentheses indicate $N$ for each group. Reduced $N$ values were due to spillage.
[b]The statistical significance for this value was assessed using the Welch ANOVA test statistic. This value was not statistically significant when the Brown-Forsythe test (ANOVA) was employed.
[*]$p < 0.05$ compared to control.
[†]$p < 0.01$ compared to control.
[‡]$p < 0.001$ compared to control.

**Table 7. Hematologic determinations for Fischer 344 rat dams exposed to EGBE by inhalation on gestation days (gd) 6–15.[a]**

| Target concentration, ppm | Hemolysis, % | | | Hematologic parameters[b] | | |
|---|---|---|---|---|---|---|
| | 0.55% saline | 0.50% saline | 0.45% saline | RBC $\times 10^{-6}/mm^3$ | Hgb, g/dL | Hct, % |
| 200 | 8.2 | 39.5 | 82.4 | 5.83[‡] | 13.8[‡] | 42.0[‡] |
| | (7.1) | (14.2) | (11.2) | (0.60) | (1.4) | (4.8) |
| 100 | 4.9 | 33.5 | 76.8 | 5.77[‡] | 11.9 | 35.2 |
| | (5.1) | (10.4) | (8.8) | (0.47) | (0.9) | (2.9) |
| 50 | 7.3 | 38.4 | 77.0 | 6.36 | 12.0 | 35.0 |
| | (7.5) | (12.7) | (10.3) | (0.55) | (1.0) | (3.0) |
| 25 | 5.1 | 32.4 | 74.4 | 6.37 | 12.0 | 35.0 |
| | (5.4) | (9.2) | (7.2) | (0.34) | (0.6) | (1.9) |
| 0 | 8.3 | 38.0 | 77.7 | 6.36 | 12.1 | 35.1 |
| | (6.7) | (12.1) | (6.5) | (0.41) | (0.8) | (2.3) |

[a]Data are presented as mean (standard deviation).
[b]RBC = red blood cells ($\times 10^6/mm^3$); Hgb = hemoglobin (g/dL); Hct = hematocrit (%).
[‡]$p < 0.001$ versus controls.

ppm (Table 5). Water consumption, expressed as grams/dam/day, was also significantly reduced for the periods gd 6–9, 12–15 and 6–15 for dams at 200 ppm. Water consumption was elevated relative to controls for dams exposed to 100 ppm during the postexposure periods gd 18–21 and 15–21 (Table 6).

Hematologic determinations on dams at scheduled sacrifice (Tables 7 and 8) indicated no alterations in osmotic fragility of erythrocytes but significant reductions in erythrocyte (RBC) count, and significant increases in hemoglobin and hematocrit (packed red blood cell volume) at 200 ppm. RBC count was also reduced at 100 ppm. The size of the red blood cell (MCV) was significantly enlarged at 200 and 100 ppm, and the hemoglobin per cell (MCH) was significantly increased. The mean corpuscular hemoglobin concentration (MCHC) was significantly increased. The mean corpuscular hemoglobin concentration (MCHC) was significantly reduced at 100 and 200 ppm relative to controls.

**Table 8. Erythrocyte indices for Fischer 344 rat dams exposed to EGBE by inhalation on gestation days 6–15.**

| Target concentration, ppm | Erythrocyte indices[a] MCV, $\mu m^3$[b] | MCH, pg[c] | MCHC, g/dL[c] |
|---|---|---|---|
| 200 | 71.4‡ [1.2] | 23.6‡ (0.8) | 32.8‡ (0.8) |
| 100 | 60.8‡ [0.9] | 21.0‡ (2.0) | 33.8† (0.7) |
| 50 | 55.0 [0.2] | 19.0 (0.4) | 34.4 (0.6) |
| 25 | 54.9 [0.5] | 18.9 (0.3) | 34.5 (0.6) |
| 0 | 55.0 [0.6] | 18.9 (0.3) | 34.4 (0.6) |

[a]MCV = mean corpuscular volume ($\mu m^3$), MCH = mean corpuscular hemoglobin (pg), MCHC = mean corpuscular hemoglobin concentration (g/dL).
[b]Median [quartile deviation].
[c]Mean (standard deviation).
†$0.01 \geq p \geq 0.001$ versus controls.
‡$p < 0.001$ versus controls.

At scheduled sacrifice, maternal gravid uterine weight was significantly reduced, and absolute and relative spleen weight and relative kidney weight were elevated compared to controls at 200 ppm (Table 9).

Reproductive toxicity was also evident at 200 ppm (Table 10). The number of viable implants and the percent live fetuses per litter were reduced relative to controls and the number of nonviable implants, primarily due to the elevated number of early embryonic resorptions, was elevated at 200 ppm relative to controls. Preimplantation loss was also elevated, but the difference was not statistically significant. Sex ratio (% males) and the body weight of male or female fetuses per litter were unaffected by treatment (Table 10).

The results of fetal evaluations are presented in Tables 11 and 12. There were no statistically significant increases in the incidence of external, visceral, skeletal or total malformations due to treatment (Table 11). The incidence of variations observed in rat fetuses in the present study is presented in Table 12. Evidence for retarded skeletal ossification was seen at 100 and 200 ppm. At 200 ppm, there was a significant increase in the number of litters with one or more fetuses exhibiting unossified skeletal elements, e.g., the anterior arch of the atlas, cervical centra 5 and 6, and poorly ossified skeletal elements, e.g., cervical arches, sternebrae 1, 3, 4 and 6 and proximal phalanges in the forelimbs. At 100 ppm, there was a significant increase in the incidence of

**Table 9. Maternal organ weights of F-344 rats exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15[a] 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
|---|---|---|---|---|---|
| Maternal body weight (gd 21), g | 244.5 ± 30.9 | 277.1 ± 19.0 | 272.4 ± 20.2 | 277.6 ± 19.5 | 275.6 ± 17.8 |
| Organ weights, g | | | | | |
| Uterus | 34.09 ± 28.62† | 62.04 ± 17.91 | 56.94 ± 20.44 | 63.10 ± 11.76 | 59.90 ± 17.56 |
| Liver | 10.42 ± 2.09 | 11.30 ± 1.04 | 10.67 ± 1.06 | 11.19 ± 1.22 | 11.17 ± 0.88 |
| Relative liver, %[b] | 4.95 ± 0.97 | 5.25 ± 0.39 | 4.96 ± 0.48 | 5.20 ± 0.41 | 5.18 ± 0.38 |
| Thymus | 0.25 ± 0.10 | 0.26 ± 0.06 | 0.25 ± 0.08 | 0.27 ± 0.08 | 0.26 ± 0.07 |
| Relative thymus, %[b] | 0.12 ± 0.04 | 0.12 ± 0.03 | 0.12 ± 0.03 | 0.12 ± 0.04 | 0.12 ± 0.03 |
| Spleen | 0.65 ± 0.09* | 0.57 ± 0.06 | 0.60 ± 0.26 | 0.53 ± 0.07 | 0.54 ± 0.05 |
| Relative spleen %[b] | 0.31 ± 0.04‡ | 0.27 ± 0.03 | 0.28 ± 0.12 | 0.25 ± 0.03 | 0.25 ± 0.02 |
| Kidney | 1.54 ± 0.17 | 1.44 ± 0.08 | 1.39 ± 0.10 | 1.42 ± 0.10 | 1.46 ± 0.14 |
| Relative kidney, %[b] | 0.74 ± 0.09* | 0.68 ± 0.04 | 0.65 ± 0.04 | 0.66 ± 0.05 | 0.68 ± 0.05 |

[a]Values represent mean ± standard deviation.
[b]Relative organ weight = organ weight expressed as a percent of corrected body weight.
*$p < 0.05$ as compared to control.
†$p < 0.01$ as compared to control.
‡$p < 0.001$ as compared to control.

**Table 10. Reproductive toxicity evaluation of F-344 rats exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15[a] | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Corpora lutea | 15.7 ± 5.4 | 13.2 ± 1.2 | 13.2 ± 5.3 | 13.0 ± 1.7 | 12.9 ± 1.8 |
| Total implants | 8.7 ± 3.8 | 10.3 ± 3.2 | 9.1 ± 3.6 | 10.3 ± 2.2 | 10.0 ± 3.5 |
| Viable implants | 4.7 ± 4.5[‡] | 9.9 ± 3.3 | 8.9 ± 3.7 | 10.0 ± 2.2 | 9.4 ± 3.2 |
| Nonviable implants | 4.0 ± 4.6[†] | 0.4 ± 1.1 | 0.2 ± 0.4 | 0.3 ± 0.5 | 0.5 ± 0.7 |
| Embryonic resorptions | 2.8 ± 4.7[b†] | 0.0 ± 0.0 | 0.1 ± 0.3[c] | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Embryonic resorptions with placenta | 1.2 ± 2.2 | 0.3 ± 0.9 | 0.1 ± 0.4 | 0.2 ± 0.4 | 0.4 ± 0.7 |
| Dead and macerated fetuses | 0.0 ± 0.2 | 0.1 ± 0.3 | 0.0 ± 0.0 | 0.0 ± 0.2 | 0.1 ± 0.3 |
| Live fetuses, % | 52.7 ± 45.1[†] | 94.4 ± 12.9 | 93.3 ± 21.4 | 97.5 ± 4.3 | 95.7 ± 5.9 |
| Preimplantation loss, % | 39.7 ± 30.6 | 22.2 ± 22.7 | 25.2 ± 29.2 | 19.6 ± 18.0 | 23.7 ± 26.2 |
| Sex ratio | 43.8 ± 17.2 | 51.8 ± 20.0 | 51.2 ± 15.9 | 50.6 ± 19.8 | 50.5 ± 23.2 |
| Mean litter weight, g | | | | | |
| Male fetus weight | 4.4 ± 0.4 | 4.5 ± 0.2[d] | 4.6 ± 0.4[d] | 4.5 ± 0.1[d] | 4.5 ± 0.2[d] |
| Female fetus weight | 4.0 ± 0.5 | 4.2 ± 0.2 | 4.3 ± 0.4 | 4.2 ± 0.2 | 4.3 ± 0.2[e] |

[a]Values represent mean ± standard deviation on a per litter basis.
[b]Nine of twenty-five dams had embryonic resorptions, seven of which were found to be gravid by ammonium sulfide staining only.
[c]One of twenty-two dams had embryonic resorptions, detected only by ammonium sulfide staining.
[d]$N$ = 22, 20, 21, and 22 for exposure groups 100, 50, 25 and 0 ppm, respectively. One litter from each group had only female fetuses.
[e]$N$ = 22; one litter had only male fetuses.
[†]$p < 0.01$ versus controls.
[‡]$p < 0.001$ versus controls.

**Table 11. Malformations observed in Fischer 344 rat fetuses exposed to EGBE in utero.[a]**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Number examined externally[d] | 217 | 221 | 196 | 228 | 117 | 23 | 22 | 21 | 23 | 16 |
| Umbilical hernia | | | | | | | | | | |
| No. | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| % | (0.0) | (0.5) | (0.5) | (0.0) | (0.0) | (0.0) | (4.5) | (4.8) | (0.0) | (0.0) |
| Number examined viscerally[e] | 112 | 115 | 104 | 119 | 64 | 23 | 22 | 21 | 23 | 16 |
| Right aortic arch | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.6) | (0.0) | (0.0) | (0.0) | (0.0) | (6.2) |
| Ventricular septal defect | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (1.6) | (0.0) | (4.5) | (0.0) | (0.0) | (6.2) |
| Situs inversus | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.6) | (0.0) | (0.0) | (0.0) | (0.0) | (6.2) |
| Left carotid artery emerges from arch on right | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Dorsal deflected aortic arch | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.6) | (0.0) | (0.0) | (0.0) | (0.0) | (6.2) |
| Retroesophageal aortic arch | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Microphthalmia, right | | | | | | | | | | |
| No. | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| % | (0.9) | (0.0) | (0.0) | (0.8) | (1.6) | (4.3) | (0.0) | (0.0) | (4.3) | (6.2) |

**Table 11. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Microphthalmia, left | | | | | | | | | | |
| No. | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.8) | (0.0) | (0.0) | (0.0) | (4.8) | (4.3) | (0.0) |
| Incomplete septation of aorta and pulmonary artery | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.6) | (0.0) | (0.0) | (0.0) | (0.0) | (6.2) |
| Number examined skeletally[f] | 105 | 106 | 92 | 109 | 49 | 23 | 22 | 20[g] | 22[g] | 15[h] |
| Thoracic vertebrae curved | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Unossified rib, unilateral | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Sternebrae misshapen | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Digit, curved and abnormal size | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Tympanic annuli abnormal | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Microagnathia | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Total malformations | | | | | | | | | | |
| External[d] | | | | | | | | | | |
| No. | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| % | (0.0) | (0.5) | (0.5) | (0.0) | (0.0) | (0.0) | (4.5) | (4.8) | (0.0) | (0.0) |
| Visceral[e] | | | | | | | | | | |
| No. | 1 | 1 | 2 | 2 | 3 | 1 | 1 | 2 | 2 | 3 |
| % | (0.9) | (0.9) | (1.9) | (1.7) | (4.7) | (4.3) | (4.5) | (9.5) | (8.7) | (18.7) |
| Skeletal[f] | | | | | | | | | | |
| No. | 0 | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | 1 |
| % | (0.0) | (2.8) | (0.0) | (0.0) | (2.0) | (0.0) | (13.6) | (0.0) | (0.0) | (6.7) |
| Total | | | | | | | | | | |
| No. | 1 | 5 | 3 | 2 | 4 | 1 | 5 | 2 | 2 | 4 |
| % | (0.5) | (2.3) | (1.5) | (0.9) | (3.4) | (4.3) | (22.7) | (9.5) | (8.7) | (25.0) |

[a]A single fetus may be represented more than once in listing individual defects.
[b]Only live fetuses were examined for malformations.
[c]Includes litters with one or more malformed fetuses.
[d]All fetuses were examined externally.
[e]Approximately 50% of each litter were examined viscerally (*3*) and for soft tissue craniofacial malformations (*4*).
[f]Approximately 50% of each litter were examined for skeletal malformations after staining with Alizarin Red S.
[g]One litter contained only one live fetus. By convention this fetus was subjected to visceral and craniofacial examination.
[h]The skeletal preparations from one litter were macerated and could not be evaluated.

unossified cervical centrum 6. At 100 and 200 ppm, there was a decreased incidence of bilobed cervical centrum 5 (primarily because at these exposure concentrations this skeletal element was largely unossified). At 200 ppm there was also a decreased incidence of bilobed thoracic centra 9 and 13 and of poorly ossified proximal phalanges of the hindlimb relative to controls. Rudimentary rib and/or bone island at the first lumbar

**Table 12. Variations observed in Fischer 344 rat fetuses exposed to EGBE in utero.[a]**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Number examined externally[d] | 217 | 221 | 196 | 228 | 117 | 23 | 22 | 21 | 23 | 16 |
| Ecchymoses, trunk | | | | | | | | | | |
| No. | 21 | 22 | 27 | 29 | 16 | 15 | 12 | 12 | 16 | 8 |
| % | (9.7) | (10.0) | (13.8) | (12.7) | (13.8) | (65.2) | (54.5) | (57.1) | (69.6) | (50.0) |
| Ecchymoses, head | | | | | | | | | | |
| No. | 5 | 3 | 3 | 3 | 4 | 4 | 3 | 3 | 3 | 3 |
| % | (2.3) | (1.4) | (1.5) | (1.3) | (3.4) | (17.4) | (13.6) | (14.3) | (13.0) | (18.7) |
| Ecchymoses, extremities | | | | | | | | | | |
| No. | 4 | 5 | 5 | 8 | 4 | 3 | 5 | 5 | 5 | 3 |
| % | (1.8) | (2.3) | (2.6) | (3.5) | (3.4) | (13.0) | (22.7) | (23.8) | (21.7) | (18.7) |
| Number examined viscerally[e] | 112 | 115 | 104 | 119 | 64 | 23 | 22 | 21 | 23 | 16 |
| Thymus, hemorrhagic | | | | | | | | | | |
| No. | 3 | 6 | 6 | 6 | 4 | 3 | 6 | 5 | 5 | 4 |
| % | (2.7) | (5.2) | (5.8) | (5.0) | (6.2) | (13.0) | (27.3) | (23.8) | (21.7) | (25.0) |
| Shortened innominate artery | | | | | | | | | | |
| No. | 0 | 2 | 2 | 0 | 2 | 0 | 2 | 2 | 0 | 2 |
| % | (0.0) | (1.7) | (1.9) | (0.0) | (3.1) | (0.0) | (9.1) | (9.5) | (0.0) | (12.5) |
| Missing innominate artery | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.6) | (0.0) | (0.0) | (0.0) | (0.0) | (6.2) |
| White nodule, caudate lobe of liver | | | | | | | | | | |
| No. | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 1 |
| % | (0.9) | (1.7) | (1.9) | (1.7) | (3.1) | (4.3) | (4.5) | (9.5) | (8.7) | (6.2) |
| Liver discoloration | | | | | | | | | | |
| No. | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| % | (0.0) | (0.9) | (1.0) | (0.0) | (0.0) | (0.0) | (4.5) | (4.8) | (0.0) | (0.0) |
| Liver hemorrhagic | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Red foci, right kidney | | | | | | | | | | |
| No. | 1 | 2 | 0 | 2 | 1 | 1 | 2 | 0 | 2 | 2 |
| % | (0.9) | (1.7) | (0.0) | (1.7) | (1.6) | (4.3) | (9.1) | (0.0) | (8.7) | (6.2) |
| Red foci, both kidneys | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Dark red coagulation in abdominal cavity | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Lungs hemorrhagic | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Fetal atelectasis | | | | | | | | | | |
| No. | 2 | 4 | 3 | 2 | 8 | 1 | 2 | 2 | 2 | 4 |
| % | (1.8) | (3.5) | (2.9) | (1.7) | (12.5) | (4.3) | (9.1) | (9.5) | (8.7) | (25.0) |
| Fourth cusp on aortic semilunar valve | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.3) | (0.0) | (0.0) | (0.0) | (0.0) |
| Red foci on right atrium | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (0.8) | (0.0) | (0.0) | (0.0) | (0.0) | (4.3) | (0.0) |
| Lateral ventricles dilated (cerebrum) | | | | | | | | | | |
| No. | 2 | 1 | 0 | 1 | 0 | 2 | 1 | 0 | 1 | 0 |
| % | (1.8) | (0.9) | (0.0) | (0.8) | (0.0) | (8.7) | (4.5) | (0.0) | (4.3) | (0.0) |
| Third ventricles dilated (diencephalon) | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (4.3) | (0.0) | (0.0) | (0.0) | (0.0) |
| Enlarged fourth ventricle (cerebellum) | | | | | | | | | | |
| No. | 21 | 14 | 19 | 14 | 8 | 13 | 9 | 11 | 9 | 7 |
| % | (18.7) | (12.2) | (18.3) | (11.8) | (12.5) | (56.5) | (40.9) | (52.4) | (39.1) | (43.7) |
| Nasal septum or passage distorted | | | | | | | | | | |
| No. | 2 | 8 | 8 | 7 | 1 | 2 | 5 | 5 | 4 | 1 |
| % | (1.8) | (7.0) | (7.7) | (5.9) | (1.6) | (8.7) | (22.7) | (23.8) | (17.4) | (6.2) |

**Table 12. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Gel-like material between skin and nasal bone | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.8) | (0.0) | (0.0) |
| Number examined skeletally[f] | 106 | 106 | 92 | 109 | 50 | 23 | 22 | 20[g] | 22[g] | 15[h] |
| Anterior arch of Atlas, unossified | | | | | | | | | | |
| No. | 1 | 5 | 2 | 3 | 10 | 1 | 4 | 2 | 2 | 7* |
| % | (0.9) | (4.7) | (2.2) | (2.8) | (20.0) | (4.3) | (18.2) | (10.0) | (9.1) | (46.7) |
| Cervical centra 1–3 and/or 4, poorly ossified | | | | | | | | | | |
| No. | 57 | 73 | 58 | 62 | 9 | 22 | 22 | 20 | 22 | 7* |
| % | (53.8) | (68.9) | (63.0) | (56.9) | (18.0) | (95.7) | (100.0) | (100.0) | (100.0) | (46.7) |
| Cervical centra #5, unossified | | | | | | | | | | |
| No. | 13 | 17 | 11 | 22 | 24 | 9 | 10 | 9 | 14 | 12* |
| % | (12.3) | (16.0) | (12.0) | (20.2) | (48.0) | (39.1) | (45.5) | (45.0) | (63.6) | (80.0) |
| Cervical centra #5, bilobed | | | | | | | | | | |
| No. | 19 | 11 | 13 | 6 | 1 | 14 | 9 | 11 | 4* | 1* |
| % | (17.9) | (10.4) | (14.1) | (5.5) | (2.0) | (60.9) | (40.9) | (55.0) | (18.2) | (6.7) |
| Cervical centra #6, unossified | | | | | | | | | | |
| No. | 2 | 4 | 5 | 11 | 12 | 2 | 4 | 4 | 10* | 10* |
| % | (1.9) | (3.8) | (5.4) | (10.1) | (24.0) | (8.7) | (18.2) | (20.0) | (45.5) | (66.7) |
| Cervical centra #6, bilobed | | | | | | | | | | |
| No. | 40 | 33 | 27 | 20 | 6 | 19 | 17 | 13 | 13 | 5* |
| % | (37.7) | (31.1) | (29.3) | (18.3) | (12.0) | (82.6) | (77.3) | (65.0) | (59.1) | (33.3) |
| Cervical arches 1–6 and/or 7, poorly ossified | | | | | | | | | | |
| No. | 2 | 4 | 7 | 2 | 7 | 2 | 4 | 4 | 2 | 7* |
| % | (1.9) | (3.8) | (7.6) | (1.8) | (14.0) | (8.7) | (18.2) | (20.0) | (9.1) | (46.7) |
| Throacic centra #1, bilobed | | | | | | | | | | |
| No. | 47 | 30 | 46 | 34 | 22 | 20 | 12* | 18 | 16 | 12 |
| % | (44.3) | (28.3) | (50.0) | (31.2) | (44.0) | (87.0) | (54.5) | (90.0) | (72.7) | (80.0) |
| Thoracic centra #9, bilobed | | | | | | | | | | |
| No. | 15 | 16 | 10 | 10 | 4 | 11 | 10 | 9 | 7 | 2* |
| % | (14.2) | (15.1) | (10.9) | (9.2) | (8.0) | (47.8) | (45.5) | (45.0) | (31.8) | (13.3) |
| Thoracic centra #13, bilobed | | | | | | | | | | |
| No. | 31 | 24 | 22 | 17 | 6 | 18 | 16 | 13 | 11 | 3* |
| % | (29.2) | (22.6) | (23.9) | (15.6) | (12.0) | (78.3) | (72.7) | (65.0) | (50.0) | (20.0) |
| Sternebra #1, poorly ossified | | | | | | | | | | |
| No. | 4 | 12 | 5 | 5 | 12 | 3 | 8 | 3 | 5 | 7* |
| % | (3.8) | (11.3) | (5.4) | (4.6) | (24.0) | (13.0) | (36.4) | (15.0) | (22.7) | (46.7) |
| Sternebra #3, poorly ossified | | | | | | | | | | |
| No. | 4 | 11 | 4 | 11 | 10 | 3 | 8 | 4 | 6 | 7* |
| % | (3.8) | (10.4) | (4.3) | (10.1) | (20.0) | (13.0) | (36.4) | (20.0) | (27.3) | (46.7) |
| Sternebra #4, poorly ossified | | | | | | | | | | |
| No. | 13 | 23 | 14 | 25 | 16 | 7 | 13 | 9 | 13 | 11* |
| % | (12.3) | (21.7) | (15.2) | (22.9) | (32.0) | (30.4) | (59.1) | (45.0) | (59.1) | (73.3) |
| Sternebra #6, poorly ossified | | | | | | | | | | |
| No. | 28 | 24 | 19 | 28 | 35 | 11 | 14 | 12 | 14 | 14* |
| % | (26.4) | (22.6) | (20.7) | (25.7) | (70.0) | (47.8) | (63.6) | (60.0) | (63.6) | (93.3) |
| Sternebra #5, bilobed | | | | | | | | | | |
| No. | 14 | 34 | 18 | 16 | 9 | 9 | 17* | 14 | 11 | 9 |
| % | (13.2) | (32.1) | (19.6) | (14.7) | (18.0) | (39.1) | (77.3) | (70.0) | (50.0) | (60.0) |
| Proximal phalanges poorly ossified (hindlimb) | | | | | | | | | | |
| No. | 66 | 53 | 35 | 56 | 10 | 22 | 20 | 15 | 22 | 8* |
| % | (62.3) | (50.0) | (38.0) | (51.4) | (20.0) | (95.7) | (90.9) | (75.0) | (100.0) | (53.3) |
| Proximal phalanges unossified (forelimb) | | | | | | | | | | |
| No. | 12 | 18 | 14 | 23 | 21 | 7 | 12 | 8 | 10 | 10* |
| % | (11.3) | (17.0) | (15.2) | (21.1) | (42.0) | (30.4) | (54.5) | (40.0) | (45.5) | (66.7) |
| Rudimentary rib lumbar-1, unilateral | | | | | | | | | | |
| No. | 3 | 5 | 3 | 5 | 4 | 3 | 5 | 3 | 4 | 4 |
| % | (2.8) | (4.7) | (3.3) | (4.6) | (8.0) | (13.0) | (22.7) | (15.0) | (18.2) | (26.7) |
| Rudimentary rib lumbar-1, bilateral | | | | | | | | | | |
| No. | 0 | 1 | 2 | 1 | 3 | 0 | 1 | 2 | 1 | 3 |
| % | (0.0) | (0.9) | (2.2) | (0.9) | (6.0) | (0.0) | (4.5) | (10.0) | (4.5) | (20.0) |
| Extra rib, unilateral | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (4.0) | (0.0) | (0.0) | (0.0) | (0.0) | (13.3) |
| Bone island at lumbar-1 | | | | | | | | | | |
| No. | 2 | 2 | 5 | 7 | 4 | 2 | 2 | 5 | 7 | 4 |
| % | (1.9) | (1.9) | (5.4) | (6.4) | (8.0) | (8.7) | (9.1) | (25.0) | (31.8) | (26.7) |

**Table 12. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Rudimentary rib, thoracic area | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |

[a]A single fetus may be represented more than once in listing individual defects.
[b]Only live fetuses were examined for defects.
[c]Includes litters with one or more affected fetuses.
[d]All fetuses were examined externally.
[e]Approximately 50% of each litter were examined viscerally (*3*) and for soft tissue craniofacial defects (*4*).
[f]Approximately 50% of each litter were examined for skeletal defects after staining with Alizarin Red S. Only those parameters whose incidence differed significantly from that of controls at $p < 0.05$, Fisher's exact test (two-tailed) and related parameters are indicated.
[g]One litter contained only one live fetus. By convention this fetus was subjected to visceral and craniofacial examination.
[h]The skeletal preparations from one litter were macerated and could not be evaluated.
*$p < 0.05$ versus controls, Fisher's exact test (two-tailed).

**Table 13. Distribution and fate of NZW rabbits exposed to EGBE by inhalation on gestational days 6 through 18.**

| | EGBE target concentrations | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| No. in study | 24 | 24 | 24 | 24 | 24 |
| No. removed | 0 | 0 | 1[a] | 0 | 0 |
| No. abortions | 4[b] | 1 | 0 | 0 | 0 |
| No. dead | 4[c] | 0 | 0 | 0 | 0 |
| No. sacrificed (gd 29) | 16 | 23 | 23 | 24 | 24 |
| No. nonpregnant at sacrifice | 1 | 3 | 1 | 1 | 4 |
| No. (%) pregnant at sacrifice | 15 (93.8) | 20 (87.0) | 22 (95.7) | 23 (95.8) | 20 (83.3) |
| No. litters examined | 15 | 19[d] | 22 | 22[e] | 20 |

[a]Animal removed from study because it died immediately following cardiac puncture; all fetuses were dead when cesarean section was performed.
[b]The number of abortions in this group was not significantly elevated relative to that in controls by Fisher's exact test.
[c]All females were confirmed pregnant at postmortem necropsy. The number of deaths was not significantly elevated relative to controls.
[d]One rabbit was found gravid at cesarean section with only one embryonic resorption with placenta.
[e]One rabbit was found gravid at cesarean section with 10 dead and macerated fetuses only.

**Table 14. Clinical observations in NZW rabbit does exposed to EGBE on gestational days 6 through 18.[a]**

| | EGBE concentration | | | | |
|---|---|---|---|---|---|
| | 0 ppm | 25 ppm | 50 ppm | 100 ppm | 200 ppm |
| Deaths | | | | | |
| By gd 8 | | | | | 2[b] |
| By gd 10 | | | | | 1 |
| Sacrificed *in extremis* (gd 9) | | | | | 1 |
| Behavior | | | | | |
| Hypoactive | | | | | 1 |
| Listless | | | | | 1 |
| Lethargic | | | | | 1 |
| Alopecia | + | + | + | + | + |
| Urogenital area stained | + | + | + | + | + + |
| Fur stained | + | + | + | + | + + |
| Periocular wetness | + | + | + | + + | + + |
| Periocular encrustation | + | + | + | + | + |
| Perinasal wetness | + | + | 0 | 0 | + + |
| Perinasal discharge | 0 | 0 | 0 | 0 | + |
| Red fluid on tray or paperboard | 0 | 0 | 0 | + | + |
| Abortion (placental/fetal tissue on paperboard; gd 21–25) | | | | 1[a] | 4[a] |

[a]Code: + + = approximately 50% of the does (30–70%) exhibited the clinical sign sometime during the exposure period; + = up to 25% of the does exhibited the clinical sign sometime during the exposure period; 0 = no does exhibited the clinical sign during the exposure period.
[b]Number of does exhibiting the clinical sign sometime during the exposure or postexposure period.

**Table 15. Maternal weights and weight gain of NZW rabbits exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–15 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Maternal body weight, g | | | | | |
| gd 0 | 3892.1 ± 520.6[a] | 3910.5 ± 517.0 | 3748.3 ± 563.5 | 4086.6 ± 529.7 | 4077.8 ± 612.2 |
| gd 6 | 4047.9 ± 535.2 | 4071.0 ± 505.8 | 3958.2 ± 641.5 | 4249.1 ± 530.3 | 4242.2 ± 599.1 |
| gd 9 | 3844.5 ± 449.6[b] | 4007.6 ± 477.7 | 3877.7 ± 596.9 | 4190.4 ± 525.7 | 4164.3 ± 583.9 |
| gd 12 | 3786.5 ± 393.6[c] | 4009.7 ± 451.1 | 3889.1 ± 586.5 | 4195.7 ± 526.0 | 4196.7 ± 536.5 |
| gd 15 | 3811.3 ± 406.2[c*] | 4022.7 ± 436.1 | 3945.0 ± 590.3 | 4227.7 ± 506.1 | 4221.8 ± 540.8 |
| gd 21 | 3878.1 ± 422.2[c] | 4099.0 ± 443.1 | 4050.6 ± 593.7 | 4316.9 ± 500.7 | 4290.9 ± 383.1[d] |
| gd 29 | 3994.4 ± 426.0[e] | 4163.5 ± 445.3[f] | 4047.7 ± 514.9 | 4314.7 ± 502.7[h] | 4363.1 ± 455.0 |
| Corrected body weight[g] | 3553.5 ± 380.6 | 3692.1 ± 413.7 | 3507.5 ± 456.6 | 3784.8 ± 474.0[h] | 3795.2 ± 434.0 |
| Maternal body weight gain, g | | | | | |
| gd 0–6 | 155.8 ± 101.4 | 160.5 ± 72.2 | 209.9 ± 145.3 | 162.5 ± 104.7 | 164.4 ± 126.2 |
| gd 6–9 | −134.2 ± 144.1[b] | −63.4 ± 66.5 | −80.4 ± 82.3 | −58.7 ± 60.4 | −77.9 ± 80.8 |
| gd 6–12 | −102.6 ± 58.0[c] | −61.3 ± 89.9 | −69.1 ± 90.5 | −53.4 ± 94.1 | −45.6 ± 99.9 |
| gd 6–15 | −77.7 ± 70.0[c] | −48.3 ± 119.5 | −13.1 ± 113.7 | −21.4 ± 133.5 | −20.4 ± 129.5 |
| gd 6–21 | −11.0 ± 152.3[c] | 28.0 ± 247.9 | 92.4 ± 146.4 | 67.8 ± 202.5 | 74.2 ± 233.4 |
| gd 6–29 | 85.7 ± 162.7[e] | 66.3 ± 347.3[f] | 89.6 ± 275.7 | 55.3 ± 199.4[h] | 120.9 ± 246.9 |
| gd 21–29 | 33.1 ± 123.8[e] | 28.9 ± 204.7[f] | −2.8 ± 190.1 | −3.5 ± 135.6[h] | 41.9 ± 177.8[d] |

[a]Values represent mean ± standard deviation; units are in grams.
[b]$N = 21$ – Two rabbits dead prior to gestational day 9.
[c]$N = 19$ – Four rabbits dead prior to gestational day 12.
[d]$N = 16$ – Four rabbits were accidentally not weighed.
[e]$N = 15$ – Four rabbits dead prior to gestational day 12 and four rabbits aborted prior to gestational day 29.
[f]$N = 20$ – One rabbit aborted prior to gestational day 29.
[g]Corrected body weight = body weight on gd 29 minus gravid uterine weight.
[h]$N = 22$ – One rabbit died immediately following cardiac puncture on gestational day 29; a final body weight was not obtained.
[*]$p < 0.05$ versus controls.

**Table 16. Hematologic determinations for NZW rabbit does exposed to EGBE by inhalation on gestational days 6–18.[a]**

| Target concentration, ppm | Hemolysis, % | | | Hematologic parameters[b] | | |
|---|---|---|---|---|---|---|
| | 0.55% saline | 0.50% saline | 0.45% saline | RBC $\times 10^{-6}/mm^3$ | Hgb, g/dL | Hct, % |
| 200 | 20.2 | 54.2 | 88.1 | 5.87 | 13.1 | 40.6 |
| | (14.0) | (19.8) | (10.7) | (0.40) | (0.8) | (2.7) |
| 100 | 23.8 | 56.1 | 87.3 | 6.13 | 13.5[†] | 41.5[†] |
| | (19.4) | (21.4) | (12.5) | (0.62) | (1.2) | (4.0) |
| 50 | 19.2 | 47.7 | 81.4 | 5.94 | 13.1 | 40.0 |
| | (13.2) | (18.8) | (14.0) | (0.43) | (0.9) | (2.8) |
| 25 | 22.5 | 53.0 | 82.2 | 5.71 | 12.6 | 38.5 |
| | (12.3) | (16.7) | (11.9) | (0.70) | (1.5) | (4.4) |
| 0 | 25.4 | 57.0 | 86.0 | 5.66 | 12.4 | 38.1 |
| | (11.6) | (13.5) | (8.2) | (0.52) | (1.1) | (3.3) |

[a]Data expressed as mean (standard deviation).
[b]RBC = red blood cells ($\times 10^6/mm^3$); Hgb = hemoglobin (g/dL); Hct = hematocrit (%).
[†]$0.01 > p > 0.001$ versus controls.

vertebra was observed in fetuses from all groups with no treatment-related changes in incidence.

## Rabbits

The distribution and fate of all mated New Zealand White rabbits on study are presented in Table 13. There was an apparent, but not statistically significant increase in spontaneous abortions and deaths at 200 ppm relative to controls. All dead females were pregnant. Pregnancy rate at scheduled sacrifice on gd 29 was equivalent across treatment groups. Clinical observations (Table 14) included periocular wetness, perinasal wetness and discharge, red fluid on tray, and stained fur as possible treatment-related clinical signs.

Reduced maternal body weight at gd 15 was seen at 200 ppm, but there were no significant weight gain depressions in any exposure group relative to controls (Table 15).

Hematologic determinations (Tables 16 and 17) indicated no apparent treatment-related changes in any parameter evaluated. Statistically significant increases in hemoglobin content and hematocrit were seen at 100 ppm but not at 200 ppm.

The only treatment-related change in maternal organ weights was a reduction in gravid uterine weight at 200 ppm (Table 18). Evaluation of reproductive parameters (Table 19) indicated a significant reduction in number of total implants and viable implants per litter at 200 ppm relative to controls. There were no treatment-related effects on the number of nonviable implants, preimplantation loss, percent live fetuses, sex ratio or male and female fetal body weight per litter.

The type and incidence of malformations and variations observed in rabbit fetuses are presented in Tables 20 and 21, respectively. There was no statistically significant increase in the number of fetuses or of litters with one or more affected fetuses with pooled external, visceral, skeletal or total malformations in any treatment group relative to controls. For individual malformations, there was an increase in the number of litters at 100 ppm with one or more fetuses exhibiting fusion of papillary muscles in the left ventricle relative to controls. Five fetuses in 4 litters out of 19 litters examined exhibited this malformation at 100 ppm. There was no concentration-response, i.e., no other fetuses at any other exposure group exhibited this malformation (Table 20). This malformation was therefore not considered treatment-related.

Variations seen in rabbit fetuses are presented in Table 21. There was a significant reduction in unossified sternebra 6 and in rudimentary rib at the first lumbar vertebra, bilaterally, both at 200 ppm relative to controls. Except for these two findings, there were no treatment-related changes in the incidence or type of variations found across treatment groups. Rudimentary rib or extra rib was seen across all groups at relatively high frequency with no exposure-related incidence.

**Table 17. Erythrocyte indices for NZW rabbit does exposed to EGBE by inhalation on gestational days 6–18.**

| Target concentration, ppm | Erythrocyte indices[a] | | |
|---|---|---|---|
| | MCV, $\mu m^3$[b] | MCH, pg[c] | MCHC, g/dL[c] |
| 200 | 68.6 | 22.4 | 32.4 |
| | [0.9] | (0.8) | (0.4) |
| 100 | 67.8 | 22.1 | 32.5 |
| | [2.0] | (0.9) | (0.6) |
| 50 | 67.1 | 22.0 | 32.7 |
| | [1.0] | (0.8) | (0.6) |
| 25 | 67.4 | 22.1 | 32.7 |
| | [1.2] | (0.7) | (0.6) |
| 0 | 67.3 | 22.0 | 32.6 |
| | [1.1] | (0.6) | (0.4) |

[a]MCV = mean corpuscular volume ($\mu m^3$); MCH = mean corpuscular hemoglobin (pg); MCHC = mean corpuscular hemoglobin concentration (g/dL).
[b]Median [quartile deviation].
[c]Mean (standard deviation).

## Discussion

The present study, evaluating the effects of EGBE administered by inhalation during organogenesis, indicates that EGBE produces maternal and embryo/fetal toxicity in Fischer 344 rats at 100 and 200 ppm, and maternal toxicity and embryotoxicity but no fetal toxicity in New Zealand White rabbits at 200 ppm. No teratogenicity was seen in either species at any of the exposure concentrations employed. In rats at 100 and 200 ppm, the finding of maternal hematuria and/or hemoglobinuria seen early in the exposure period and evidence of anemia confirms and extends the work done previously in rats (*13,14*). The reduced red blood cell count, elevated hemoglobin and hematocrit, increased cell size and increased hemoglobin per cell found at sacrifice in rats at 100 and 200 ppm are consistent with destruction of mature erythrocytes and release of immature and/or young erythrocytes into the peripheral circulation (*13,15*). Increased absolute and relative

**Table 18. Maternal organ weights of NZW rabbits exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–18 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Maternal body weight (gd 29), g | 3994.4 ± 426.0[a] | 4163.5 ± 445.3 | 4047.7 ± 154.9 | 4314.7 ± 502.7 | 4363.1 ± 455.0 |
| Organ weights, g | | | | | |
| Uterus | 440.83 ± 99.84* | 417.43 ± 187.89 | 540.20 ± 120.40 | 526.62 ± 103.07 | 567.87 ± 157.00 |
| Liver | 83.92 ± 11.94 | 97.52 ± 21.99 | 88.12 ± 16.80 | 93.21 ± 17.25 | 95.53 ± 21.25 |
| Relative liver, %[b] | 2.37 ± 0.32 | 2.64 ± 0.54 | 2.51 ± 0.36 | 2.45 ± 0.42 | 2.52 ± 0.48 |
| Thymus | 3.57 ± 1.96 | 2.82 ± 1.10 | 3.17 ± 1.60 | 3.30 ± 1.47 | 3.89 ± 2.21 |
| Relative thymus, %[b] | 0.10 ± 0.05 | 0.08 ± 0.03 | 0.09 ± 0.04 | 0.09 ± 0.04 | 0.10 ± 0.05 |
| Spleen | 1.45 ± 0.30 | 1.66 ± 0.91 | 1.66 ± 0.70 | 1.51 ± 0.42 | 1.72 ± 0.75 |
| Relative spleen, %[b] | 0.04 ± 0.01 | 0.04 ± 0.02 | 0.05 ± 0.02 | 0.04 ± 0.01 | 0.04 ± 0.02 |
| Kidney | 17.13 ± 2.03 | 19.19 ± 3.13 | 17.24 ± 2.45 | 18.20 ± 2.17 | 18.96 ± 2.96 |
| Relative kidney, %[b] | 0.48 ± 0.06 | 0.52 ± 0.07 | 0.49 ± 0.05 | 0.48 ± 0.06 | 0.50 ± 0.05 |

[a]Values represent mean ± standard deviation.
[b]Relative organ weight = organ weight expressed as percent of corrected body weight.
*$p < 0.05$ compared to control.

**Table 19. Reproductive toxicity evaluation of NZW rabbits exposed to EGBE by inhalation.**

| | EGBE target exposure concentrations, gestational days (gd) 6–18 | | | | |
|---|---|---|---|---|---|
| | 200 ppm | 100 ppm | 50 ppm | 25 ppm | 0 ppm |
| Corpora lutea | 10.1 ± 2.1[a] | 11.3 ± 2.7 | 10.5 ± 2.5 | 10.8 ± 2.9 | 11.7 ± 2.1 |
| Total implants | 8.4 ± 1.9* | 9.0 ± 3.3 | 9.2 ± 2.7 | 9.5 ± 2.3 | 9.8 ± 3.2 |
| Viable implants | 7.2 ± 2.4* | 7.6 ± 3.4 | 8.5 ± 2.2 | 8.4 ± 3.0 | 9.0 ± 2.9 |
| Nonviable implants | 1.2 ± 1.7 | 1.4 ± 2.7 | 0.7 ± 1.6 | 1.1 ± 2.1 | 0.8 ± 1.2 |
| Embryonic resorptions | 0.1 ± 0.4 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.4 |
| Embryonic resorptions with placenta | 0.9 ± 1.7 | 0.8 ± 2.7 | 0.3 ± 1.1 | 0.3 ± 0.5 | 0.3 ± 0.8 |
| Dead and macerated fetuses | 0.2 ± 0.6 | 0.6 ± 0.9 | 0.5 ± 0.9 | 0.8 ± 2.1 | 0.4 ± 0.9 |
| Live fetuses, % | 85.8 ± 20.0 | 83.5 ± 28.7 | 94.0 ± 11.8 | 88.3 ± 20.9 | 93.4 ± 9.6 |
| Preimplantation loss, % | 16.0 ± 11.8 | 22.2 ± 27.0 | 13.2 ± 15.2 | 12.4 ± 14.1 | 17.3 ± 24.1 |
| Sex ratio | 58.4 ± 13.1 | 58.8 ± 21.9[b] | 48.9 ± 17.4 | 52.5 ± 16.1[c] | 58.2 ± 15.0 |
| Fetal body weights, g | | | | | |
| Male | 39.7 ± 6.6 | 39.6 ± 6.4[d] | 41.4 ± 6.1[e] | 40.6 ± 4.9[f] | 41.8 ± 6.3 |
| Female | 39.2 ± 6.0 | 38.7 ± 7.1[g] | 41.6 ± 6.7 | 40.5 ± 6.4[f] | 40.1 ± 6.0[h] |

[a]Values represent mean ± standard deviation.
[b]$N = 19$ – One pregnant female had only nonviable uterine implantation sites.
[c]$N = 22$ – One pregnant female had only nonviable uterine implantation sites.
[d]One rabbit was found gravid at C-section with one embryonic resorption with placenta only ($N = 19$).
[e]One rabbit had only female fetuses ($N = 21$).
[f]One rabbit was found gravid at C-section with 10 dead and macerated fetuses only ($N = 22$).
[g]Two rabbits had only male fetuses and one rabbit had an embryonic resorption with placenta only ($N = 17$).
[h]One rabbit had only male fetuses ($N = 19$).
*$p < 0.05$ versus controls.

**Table 20. Malformations observed in NZW rabbit fetuses exposed to EGBE in utero.[a]**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Number examined externally[d] | 181 | 195 | 187 | 152 | 108 | 20 | 22 | 22 | 19 | 15 |
| Imperforate anus | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Severely shortened tail | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.9) | (0.0) | (0.0) | (0.0) | (0.0) | (13.3) |
| Gastroschisis | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (0.9) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Malrotated limb | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (0.5) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Midfacial hypoplasia | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.5) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Shortened limbs | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.5) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Number examined viscerally[e] | 94 | 102 | 100 | 81 | 59 | 20 | 22 | 22 | 19 | 15 |
| Papillary muscles in left ventricle fused | | | | | | | | | | |
| No. | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 4* | 0 |
| % | (0.0) | (0.0) | (0.0) | (6.2) | (0.0) | (0.0) | (0.0) | (0.0) | (21.1) | (0.0) |
| Papillary muscles in right ventricle fused | | | | | | | | | | |
| No. | 1 | 0 | 2 | 2 | 2 | 1 | 0 | 2 | 2 | 2 |
| % | (1.1) | (0.0) | (2.0) | (2.5) | (3.4) | (5.0) | (0.0) | (9.1) | (10.5) | (13.3) |
| Right atrioventricular valve not closed | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.2) | (0.0) | (0.0) | (0.0) | (0.0) | (5.3) | (0.0) |

**Table 20. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Left carotid branches and off innominate | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Small constricted pulmonary artery, channel to aorta from rt. ventricle, extra branches off aorta, vestigal rt. ventricle, absent tricuspid valve and associated papillary muscles | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Excessive fluid within pericardium | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Ventricular septal defect | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Ventricular walls of heart thin | | | | | | | | | | |
| No. | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0 |
| % | (0.0) | (0.0) | (0.0) | (3.7) | (0.0) | (0.0) | (0.0) | (0.0) | (10.5) | (0.0) |
| Missing papillary muscle(s) | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Two urinary bladders | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Fused ureters | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Horseshoe kidney (same fetus as above) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Hydronephrosis, right | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.2) | (0.0) | (0.0) | (0.0) | (0.0) | (5.3) | (0.0) |
| Hydronephosis, bilateral | | | | | | | | | | |
| No. | 0 | 4 | 0 | 1 | 1 | 0 | 2 | 0 | 1 | 1 |
| % | (0.0) | (3.9) | (0.0) | (1.2) | (1.7) | (0.0) | (9.1) | (0.0) | (5.3) | (6.7) |
| Liver misshapen | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Liver necrotic (same fetus as above) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Midfacial hypoplasia (repeat) | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Number examined skeletally[f] | 87 | 93 | 87 | 71 | 49 | 20 | 22 | 22 | 18[g] | 15 |
| Cervical centra fused | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Cervical centra malaligned | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Cervical centra irregularly shaped (same fetus as above) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Cervical arches irregularly shaped (same fetus as above) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Cervical vertebrae malaligned (same fetus as above) | | | | | | | | | | |
| No | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Caudal vertebrae fused (same fetus as above) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Cervical rib (bilateral) | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |

**Table 20. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Duplication of clavicle | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.4) | (0.0) | (0.0) | (0.0) | (0.0) | (5.6) | (0.0) |
| Missing cervical centra | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.4) | (0.0) | (0.0) | (0.0) | (0.0) | (5.6) | (0.0) |
| Thoracic centra malaligned | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Thoracic centra fused (same as fetus above) | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Thoracic arches malaligned (same as fetus above) | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Thoracic-12 fused to lumbar-1 centra and arch | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Rib branched | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| All ribs poorly developed | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (2.0) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Total malformations | | | | | | | | | | |
| External[d] | | | | | | | | | | |
| No. | 0 | 1 | 1 | 0 | 3 | 0 | 1 | 1 | 0 | 2 |
| % | (0.0) | (0.5) | (0.5) | (0.0) | (2.8) | (0.0) | (4.5) | (4.5) | (0.0) | (13.3) |
| Visceral[e] | | | | | | | | | | |
| No. | 2 | 6 | 3 | 11 | 6 | 2 | 4 | 3 | 7 | 5 |
| % | (2.1) | (5.9) | (3.0) | (13.6) | (10.2) | (10.0) | (18.2) | (13.6) | (36.8) | (33.3) |
| Skeletal[f] | | | | | | | | | | |
| No. | 1 | 1 | 1 | 2 | 4 | 1 | 1 | 1 | 2 | 3 |
| % | (1.1) | (1.1) | (1.1) | (2.8) | (8.2) | (5.0) | (4.5) | (4.5) | (11.1) | (20.0) |
| Total | | | | | | | | | | |
| No. | 3 | 7 | 5 | 13 | 10 | 3 | 5 | 5 | 7 | 7 |
| % | (1.7) | (3.6) | (2.7) | (8.6) | (9.3) | (15.0) | (22.7) | (22.7) | (36.8) | (46.7) |

[a]A single fetus may be represented more than once in listing individual defects.
[b]Only live fetuses were examined for malformations.
[c]Includes litters with one or more malformed fetuses.
[d]All fetuses were examined externally.
[e]Approximately 50% of each litter were examined viscerally (*3*), and for soft tissue craniofacial malformations.
[f]Approximately 50% of each litter were examined for skeletal malformations after staining with Alizarin Red S.
[g]One litter contained only one live fetus. By convention this fetus was subjected to visceral and craniofacial examination.
$^{*}p < 0.05$ versus controls, Fisher's exact test (two-tailed).

**Table 21. Variations observed in NZW rabbit fetuses exposed to EGBE in utero.[a]**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Number examined externally[d] | 181 | 195 | 187 | 152 | 108 | 20 | 22 | 22 | 19 | 15 |
| Ecchymoses, limbs | | | | | | | | | | |
| No. | 10 | 2 | 13 | 5 | 15 | 3 | 2 | 6 | 4 | 3 |
| % | (5.5) | (1.0) | (7.0) | (3.3) | (13.9) | (15.0) | (9.1) | (27.3) | (21.1) | (20.0) |
| Ecchymoses, head | | | | | | | | | | |
| No. | 11 | 6 | 20 | 14 | 6 | 4 | 3 | 9 | 7 | 4 |
| % | (6.1) | (3.1) | (10.7) | (9.2) | (5.6) | (20.0) | (13.6) | (40.9) | (36.8) | (26.7) |
| Ecchymoses, trunk | | | | | | | | | | |
| No. | 3 | 3 | 18 | 4 | 8 | 3 | 3 | 9 | 3 | 4 |
| % | (1.7) | (1.6) | (9.6) | (2.6) | (7.4) | (15.0) | (13.6) | (40.9) | (15.8) | (26.7) |

**Table 21. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Placental membrane filled with red fluid | | | | | | | | | | |
| No. | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.3) | (0.0) | (0.0) | (0.0) | (0.0) | (5.3) | (0.0) |
| Placental membrane filled with yellow fluid | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (0.7) | (0.0) | (0.0) | (0.0) | (0.0) | (5.3) | (0.0) |
| Right hind leg extended | | | | | | | | | | |
| No. | 0 | 1 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| % | (0.0) | (0.5) | (1.1) | (0.0) | (0.0) | (0.0) | (4.5) | (4.5) | (0.0) | (0.0) |
| Right hind leg inverted | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (0.5) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Umbilical cord split | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (0.5) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Number examined viscerally[e] | 94 | 102 | 100 | 81 | 59 | 20 | 22 | 22 | 19 | 15 |
| Spleen misshapen | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 |
| % | (0.0) | (1.0) | (0.0) | (0.0) | (1.7) | (0.0) | (4.5) | (0.0) | (0.0) | (6.7) |
| Accessory spleen | | | | | | | | | | |
| No. | 1 | 3 | 1 | 1 | 0 | 1 | 2 | 1 | 1 | 0 |
| % | (1.1) | (2.9) | (1.0) | (1.2) | (0.0) | (5.0) | (9.1) | (4.5) | (5.3) | (0.0) |
| Spleen short | | | | | | | | | | |
| No. | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| % | (0.0) | (0.0) | (0.0) | (1.2) | (0.0) | (0.0) | (0.0) | (0.0) | (5.3) | (0.0) |
| Red masses attached to gall bladder membrane | | | | | | | | | | |
| No. | 3 | 6 | 2 | 1 | 2 | 3 | 5 | 2 | 1 | 2 |
| % | (3.2) | (5.9) | (2.0) | (1.2) | (3.4) | (15.0) | (22.7) | (9.1) | (5.3) | (13.3) |
| Liver discolored | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Liver nodule | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Liver, reticular pattern | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Stomach wall, red in color | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Excessive fat | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Thymus hemorrhagic | | | | | | | | | | |
| No. | 0 | 2 | 0 | 2 | 0 | 0 | 2 | 0 | 1 | 0 |
| % | (0.0) | (2.0) | (0.0) | (2.5) | (0.0) | (0.0) | (9.1) | (0.0) | (5.3) | (0.0) |
| Body cavity filled with milky white fluid | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Kidney discoloration, left milky white fluid | | | | | | | | | | |
| No. | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| % | (0.0) | (0.0) | (1.0) | (1.2) | (0.0) | (0.0) | (0.0) | (4.5) | (5.3) | (0.0) |
| Renal pelvis dilation | | | | | | | | | | |
| No. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| % | (0.0) | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) |
| Heart indented and/or darkened at apex | | | | | | | | | | |
| No. | 0 | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 1 | 2 |
| % | (0.0) | (1.0) | (1.0) | (1.2) | (3.4) | (0.0) | (4.5) | (4.5) | (5.3) | (13.3) |
| Papillary muscle(s) in right ventricle shortened | | | | | | | | | | |
| No. | 3 | 1 | 0 | 2 | 1 | 3 | 1 | 0 | 2 | 1 |
| % | (3.2) | (1.0) | (0.0) | (2.5) | (1.7) | (15.0) | (4.5) | (0.0) | (10.5) | (6.7) |
| Papillary muscle(s) in left ventricle shortened | | | | | | | | | | |
| No. | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 | 0 |
| % | (0.0) | (0.0) | (0.0) | (3.7) | (0.0) | (0.0) | (0.0) | (0.0) | (10.5) | (0.0) |

**Table 21. (continued)**

| | Fetuses[b] | | | | | Litters[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE | 0 ppm EGBE | 25 ppm EGBE | 50 ppm EGBE | 100 ppm EGBE | 200 ppm EGBE |
| Red markings on heart surface | | | | | | | | | | |
| No. | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| % | (1.1) | (0.0) | (0.0) | (0.0) | (0.0) | (5.0) | (0.0) | (0.0) | (0.0) | (0.0) |
| Atria engorged | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Atria discolored | | | | | | | | | | |
| No. | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| % | (0.0) | (0.0) | (0.0) | (0.0) | (1.7) | (0.0) | (0.0) | (0.0) | (0.0) | (6.7) |
| Fetal atelectasis | | | | | | | | | | |
| No. | 19 | 17 | 13 | 5 | 9 | 10 | 13 | 8 | 4 | 5 |
| % | (20.2) | (16.7) | (13.0) | (6.2) | (15.3) | (50.0) | (59.1) | (36.4) | (21.1) | (33.3) |
| Pulmonary artery larger than normal | | | | | | | | | | |
| No. | 2 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 1 |
| % | (2.1) | (0.0) | (1.0) | (0.0) | (1.7) | (5.0) | (0.0) | (4.5) | (0.0) | (6.7) |
| Granulation in nasal passage and/or on palate | | | | | | | | | | |
| No. | 2 | 6 | 9 | 6 | 3 | 2 | 4 | 3 | 4 | 1 |
| % | (2.1) | (5.9) | (9.0) | (7.4) | (5.1) | (10.0) | (18.2) | (13.6) | (21.1) | (6.7) |
| White substance in nasal passage | | | | | | | | | | |
| No. | 4 | 1 | 2 | 0 | 0 | 2 | 1 | 2 | 0 | 0 |
| % | (4.3) | (1.0) | (2.0) | (0.0) | (0.0) | (10.0) | (4.5) | (9.1) | (0.0) | (0.0) |
| Cochlea dark | | | | | | | | | | |
| No. | 4 | 3 | 3 | 11 | 0 | 2 | 2 | 2 | 3 | 0 |
| % | (4.3) | (2.9) | (3.0) | (13.6) | (0.0) | (10.0) | (9.1) | (9.1) | (15.8) | (0.0) |
| Indentations on soft and/or hard palate | | | | | | | | | | |
| No. | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| % | (0.0) | (1.0) | (0.0) | (0.0) | (0.0) | (0.0) | (4.5) | (0.0) | (0.0) | (0.0) |
| Gap between skull plates and skin | | | | | | | | | | |
| No. | 0 | 6 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 |
| % | (0.0) | (5.9) | (0.0) | (1.2) | (0.0) | (0.0) | (4.5) | (0.0) | (5.3) | (0.0) |
| Lateral ventricles dilated (cerebrum) | | | | | | | | | | |
| No. | 13 | 5 | 7 | 12 | 5 | 8 | 4 | 4 | 9 | 2 |
| % | (13.8) | (4.9) | (7.0) | (14.8) | (8.5) | (40.0) | (18.2) | (18.2) | (47.4) | (13.3) |
| Number examined skeletally[f] | 87 | 93 | 87 | 71 | 49 | 20 | 22 | 22 | 18[g] | 15 |
| Rudimentary rib lumbar-1, unilateral | | | | | | | | | | |
| No. | 18 | 22 | 12 | 11 | 6 | 14 | 15 | 11 | 7 | 6 |
| % | (20.7) | (23.7) | (13.8) | (15.5) | (12.2) | (70.0) | (68.2) | (50.0) | (38.9) | (40.0) |
| Rudimentary rib lumbar-1, bilateral | | | | | | | | | | |
| No. | 9 | 10 | 4 | 3 | 0 | 6 | 8 | 3 | 3 | 0* |
| % | (10.3) | (10.8) | (4.6) | (4.2) | (0.0) | (30.0) | (36.4) | (13.6) | (16.7) | (0.0) |
| Extra rib lumbar-1, unilateral | | | | | | | | | | |
| No. | 10 | 16 | 11 | 6 | 7 | 10 | 10 | 10 | 6 | 7 |
| % | (11.5) | (17.2) | (12.6) | (8.5) | (14.3) | (50.0) | (45.5) | (45.5) | (33.3) | (46.7) |
| Extra rib lumbar-1, bilateral | | | | | | | | | | |
| No. | 36 | 37 | 49 | 35 | 34 | 16 | 16 | 19 | 17 | 14 |
| % | (41.4) | (39.8) | (56.3) | (49.3) | (69.4) | (80.0) | (72.7) | (86.4) | (94.4) | (93.3) |
| Sternebra #5, poorly ossified | | | | | | | | | | |
| No. | 32 | 49 | 42 | 30 | 30 | 15 | 21 | 19 | 14 | 14 |
| % | (36.8) | (52.7) | (48.3) | (42.3) | (61.2) | (75.0) | (95.5) | (86.4) | (77.8) | (93.3) |
| Sternebra #6, poorly ossified | | | | | | | | | | |
| No. | 35 | 26 | 28 | 17 | 14 | 15 | 15 | 14 | 9 | 9 |
| % | (40.2) | (28.0) | (32.2) | (23.9) | (28.6) | (75.0) | (68.2) | (63.6) | (50.0) | (53.3) |
| Sternebra #5, unossified | | | | | | | | | | |
| No. | 11 | 6 | 6 | 10 | 7 | 6 | 3 | 4 | 6 | 4 |
| % | (12.6) | (6.5)( | 6.9) | (14.1) | (14.3) | (30.0) | (13.6) | (18.2) | (33.3) | (26.7) |
| Sternebra #6, unossified | | | | | | | | | | |
| No. | 7 | 9 | 9 | 3 | 0 | 6 | 5 | 5 | 2 | 0* |
| % | (8.0) | (9.7) | (10.3) | (4.2) | (0.0) | (30.0) | (22.7) | (22.7) | (11.1) | (0.0) |

[a]A single fetus may be represented more than once in listing individual defects.
[b]Only live fetuses were examined for defects.
[c]Includes litters with one or more affected fetuses.
[d]All fetuses were examined externally.
[e]Approximately 50% of each litter were examined viscerally (*3*), and for soft tissue craniofacial defects (*4,6*).
[f]Approximately 50% of each litter were examined for skeletal defects after staining with Alizarin Red S. Only those parameters whose incidence was significantly different from that of controls ($p < 0.05$, Fisher's exact test) and related parameters are indicated.
[g]One litter contained only one live fetus. By convention this fetus was subjected to visceral and craniofacial examination.
*$p < 0.05$ versus controls, Fisher's exact test (two-tailed).

spleen weights and relative kidney weight at 200 ppm in rats also indicate stimulation of hematopoiesis. That the homeostatic mechanisms of compensation were not entirely successful is indicated by the significant reduction in mean corpuscular hemoglobin concentration seen at 100 and 200 ppm.

The early rat conceptus appeared vulnerable to insult by EGBE since 9 of 25 dams pregnant at sacrifice at 200 ppm had totally resorbed litters, 7 of 9 detected only after staining of the uteri. This embryotoxic effect is responsible for the significant elevations in nonviable implants and resorptions and the reduction in viable (i.e., live) fetuses at scheduled necropsy at 200 ppm, and occurred in the presence of maternal toxicity. Fetal body weights were unaffected by treatment but fetotoxicity was indicated by the increased incidence of poorly ossified or unossified skeletal elements seen in gd 21 fetuses. These skeletal districts were those examined by Aliverti et al. (*16*), who suggested that retardation of skeletal ossification, especially on gd 21 in rats, provided a reliable index of delayed development in teratogenicity studies. Khera (*17*) has interpreted reductions in ossification as indications of toxicity and suggested that the gd 21 fetal skeleton is the most uniform and therefore the most appropriate to examine.

Khera (*17*) included as fetal "aberrations" retardations, variations and deviations. He includes supernumerary ribs as a fetal variation, a designation also followed in this study which demonstrated this finding across all groups. Heart and great vessel malformations, seen with increased frequency after exposure of rats to ethylene glycol monomethyl and monoethyl ether (*18,19*) were not seen with increased frequency in this study. The lack of teratogenicity in the presence of maternal toxicity in rats after exposure to EGBE, seen in this study, was also reported briefly by Nelson et al. (*18*).

New Zealand White rabbit does exhibited toxicity to EGBE at 200 ppm. Treatment-related increases in maternal deaths and spontaneous abortions, depressed body weight during the exposure period and some relatively nonspecific clinical signs were observed at this exposure concentration. Embryotoxicity was indicated by reduced gravid uterine weight and a concomitant reduction in total and viable fetuses at scheduled sacrifice only at 200 ppm. No evidence of fetal toxicity was observed at any exposure concentration (i.e., no treatment-related reduction in fetal body weight, no increase in malformations or variations, including reduced ossification in skeletal elements). Supernumerary ribs, presenting as rudimentary or extra ribs, and observed across all groups, is the most commonly occurring skeletal variation in New Zealand White rabbits (*20*).

## Conclusions

The present study indicates that inhalation exposure to EGBE at 100 ppm (rats) or 200 ppm (rats and rabbits) during organogenesis results in maternal toxicity and toxicity to the conceptus (embryotoxicity in rats and rabbits; fetotoxicity in rats), but no teratogenicity. Exposure to 100 ppm (rabbits), 50, or 25 ppm (rats and rabbits) results in no observable maternal, embryo or fetal toxicity.

## Addendum

Analysis of the data generated from the five to six rats per exposure group exposed one additional day (gd 16) to EGBE indicated the same profile of maternal toxicity: clinical signs, decreased weight and weight gains during exposure, elevated relative spleen weights, decreased food and water consumption and evidence of anemia. With the small number of litters in each group, no alterations in status of implantation sites were seen. Only one fetal malformation was observed: missing median lung lobe at 25 ppm. Fetal variations seen were similar to those reported herein, i.e., treatment-related reduced ossification of skeletal districts, and rudimentary rib and/or bone island at the first lumbar vertebra distributed across all treatment groups.

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