APPENDIX B

GLYCOL ETHER TOXICITY IN ANIMALS

B.1 MALE REPRODUCTIVE EFFECTS

B.1.1 EGEE and EGEEA

B.1.1.1 Subcutaneous and Intravenous Administration

Reports in the literature indicate that EGEE and EGEEA exert adverse effects on the male reproductive system. Histopathological testicular changes were reported in rats treated subcutaneously (s.c.) with varying doses of EGEE (93, 186, 372, or 744 mg/kg per day) [Stenger et al. 1971].* Treatment of five rats/group for 4 wk with 372 mg EGEE/kg per day caused testicular damage. The interstitium of the testes was edematously disintegrated; parent spermatophores were found in the tubuli between typical Sertoli cells, and in some instances, powdery spermatophores and spermatocytes were found in several layers. In most instances, there were no additional maturation stages; polynuclear cells were found occasionally. Limited changes in the liver and kidneys were also observed. Subcutaneous administration of 744 mg EGEE/kg per day caused occasional edema and hemorrhaging at the injection site. The histopathological changes described in the 372 mg EGEE/kg per day group were more pronounced in the 744 mg EGEE/kg per day group. Stenger et al. [1971] also noted that treating dogs (two per group) i.v. for 22 days with 93 mg EGEE/kg per day resulted in inflammation at the injection site, and treatment with 465 mg EGEE/kg per day caused pronounced thrombophlebitis.

B.1.1.2 Oral Administration

Twenty male albino rats were fed 1.45% EGEE in their basic diet for 2 yr [Morris et al. 1942]. Upon histological examination, testicular enlargement and edema and tubular atrophy were observed in two-thirds of the animals that had received EGEE. These changes were not seen in untreated controls. The testicular lesions were more often bilateral than unilateral and consisted of marked interstitial edema.

Oral administration of 46.5 or 93 mg EGEE/kg per day for 13 weeks to male dogs (three per group) had no adverse effect [Stenger et al. 1971]. On the other hand, oral administration of 186 mg EGEE/kg per day for 13 wk caused degenerative changes in the testes of all

*References for Appendix B can be found beginning on page 262.

three animals. In one dog, the lumen of the tubuli appeared to be expanded, and the last maturation stages of the seminal epithelium clearly were absent in many of the tubuli. In the second dog, tubuli were constricted, and parent and powdery spermatophores had been retained. In the third dog, there was a conspicuous flattening of germinal epithelium with complete absence of upper layers; the parent epithelium was, in some cases, absent in these tubuli. Slight kidney changes were observed in two of the three dogs; the lumen in the region of the tubuli contorti was expanded, and the epithelium was flattened.

In another set of experiments by Stenger et al. [1971], groups of five rats per dosage level were orally administered 46.5 or 93 mg EGEE/kg per day for 13 wk or 93 mg EGEE/kg per day for 59 days followed by an oral dose of 372 mg EGEE/kg per day for the remainder of the 13-wk period. No adverse effects were observed at these doses. Following oral administration of 186 mg EGEE/kg per day for 13 wk, the testicular interstitium was occasionally broken down edematously, and there was a lack of mature cells in the canals. The oral administration of 186 mg EGEE/kg per day for 59 days, followed by oral administration of 744 mg EGEE/kg per day for the 32 days remaining in the 13-wk period, also caused testicular changes that corresponded to findings following a 4-wk s.c. application of 744 mg EGEE/kg per day. The diameters of tubuli were also reduced.

Nagano et al. [1979] treated groups of five male JCL-ICR mice orally with various doses (500, 1,000, 2,000, or 4,000 mg/kg per day) of EGEE or EGEEA 5 days/wk for 5 wk. Testicular atrophy was observed and assessed in terms of testicular weight, both absolute and relative to body weight. Statistically significant decreases in the testicular weights of exposed animals in comparison to control animals were noted in those given doses of at least 1,000 mg/kg per day of either EGEE or EGEEA ($P<0.05$). Histologically, varying dosage-related degrees of seminiferous tubule atrophy were observed. In the 2,000 mg EGEE/kg per day and the 4,000 mg EGEEA/kg per day groups, the diameter of the seminiferous tubules decreased, spermatozoa and spermatids completely vanished, and spermatocytes existed in extremely small numbers in only some of the tubules; interstitial tissue also increased. When expressed as moles/kg per day, EGEE and EGEEA exerted the same degree of testicular toxicity [Nagano et al. 1979].

Foster et al. [1983] administered EGEE (250, 500, or 1,000 mg/kg per day) or EGEEA at 727 mg/kg per day (equimolar to 500 mg EGEE/kg per day) orally for 11 days to 36 male Sprague-Dawley rats/group. Animals treated with equivalent volumes of the water vehicle served as controls. A statistically significant decrease in testes weights was noted on day 11 in the 500 ($P<0.01$) and 1,000 mg ($P<0.05$) EGEE/kg per day groups. Although the degree of spermatocyte degeneration and depletion was similar for both dosage groups, the onset of degeneration was more rapid with the 500 mg EGEE/kg per day dose than with 1,000 mg/kg per day. Testicular degeneration was restricted to the later stages of primary spermatocyte development and secondary spermatocytes. Partial maturation depletion of early stage spermatids occurred, whereas Sertoli cells, Leydig cells, spermatogonia, and pre-pachytene spermatocytes were unaffected. Animals treated with EGEEA at a dose equimolar to 500 mg EGEE/kg per day showed a similar pattern of testicular damage [Foster et al. 1983]. These findings were confirmed in a similar set of experiments using

EGEE in which testicular lesions were examined at sequentially timed intervals (1/4, 1, 2, 4, 7, and 11 days) during the dosing period of 11 days [Creasy and Foster 1984]. EGEE exerted no adverse testicular effect at 250 mg/kg per day, but it did at doses of 500 and 1,000 mg/kg per day. Although no testicular abnormalities were observed in any of the groups 6 hr after dosing, degenerative spermatocytes were frequently seen 24 hr after dosing with EGEE. Dose levels of 500 and 1,000 mg EGEE/kg per day produced degeneration of the dividing and early-pachytene spermatocytes but had no effect on the middle and late stage of pachytene development. Although the 500 mg EGEE/kg per day induced a more extensive lesion than did 1,000 mg EGEE/kg per day after 48 hr of dosing, this trend was reversed with prolonged dosing. The authors concluded that primary spermatocytes undergoing pachytene development constitute the initial and major site of morphological damage [Creasy and Foster 1984].

Ethoxyacetic acid (EAA) and its glycine conjugate are known metabolites of EGEE [Jonsson et al. 1982; Cheever et al. 1984]. Foster et al. [1987] undertook the following study to support the contention that the testicular toxicity of EGEE [Foster et al. 1983] is due to the toxicity exerted by EAA. Foster et al. [1987] exposed groups of six male Alpk/AP (Wistar-derived) rats to a single oral dose of EAA (137, 342, or 684 mg/kg) to determine the initial target for testicular toxicity. Rats were sacrificed at 1, 2, 4, and 14 days post-treatment. Histological examination revealed that dosing with EAA induced testicular damage at the highest dose level only; diplotene, diakinetic, secondary, and early pachytene spermatocytes were affected at 24 hr, with effects on round spermatids seen at day 14. The pachytene spermatocytes had previously been identified [Foster et al. 1983] as the target of EGEE toxicity.

In a 2-yr study, groups of 50 Fischer 344/N rats and 50 B6C3F_1 mice of both sexes were administered EGEE by gavage at dose levels of 0, 500, 1,000, and 2,000 mg/kg per day [Melnick 1984]. Repeated administration of EGEE at the 2,000 mg/kg dose level was lethal to rats and mice, and death appeared to result from stomach ulcers. As a consequence of the high mortality rate, the high dose (2,000 mg/kg per day) group was terminated at wk 17 to 18. Gross and microscopic examinations at the end of the study revealed testicular atrophy in male rats and mice at all doses of EGEE.

The effect of EGEE on spermatogenesis was studied by Oudiz et al. [1984]. Groups of 16 Long-Evans hooded male rats were treated by gavage on 5 consecutive days with 0, 936, 1,872, or 2,808 mg EGEE/kg per day. The animals were then mated weekly for the following 14 wk with ovariectomized females, and ejaculated semen samples recovered from females immediately following copulation were analyzed at selected timepoints over a 16-wk period. The males were killed at wk 16, and the testes and epididymides were submitted for histological examination. Exposure to 936 mg EGEE/kg per day impaired testicular function as reflected in an increased percent of abnormal sperm forms as well as in decreased sperm count; azoospermia and oligozoospermia were observed in the higher-dose groups (1,872 and 2,808 mg EGEE/kg per day). Although there appeared to be no effect on motility, a significant decline in sperm count, relative to that of the controls, occurred as early as wk 4 post-exposure in the groups receiving 1,872 mg EGEE/kg per day ($P \leq 0.001$) and 2,808 mg EGEE/kg per day ($P \leq 0.01$). The most dramatic effects were noted at wk 7

postexposure; at this time, the low-dose group (936 mg EGEE/kg per day) also exhibited significantly decreased ($P \leq 0.01$) sperm counts and increased ($P \leq 0.05$) abnormal forms in the semen. Partial recovery of sperm count was evident in semen samples collected at wk 14 in the group receiving doses of 1,872 mg EGEE/kg per day where sperm counts were 40% of the baseline value of 58.8×10^6 sperm/ml of recovery fluid. Animals in the group receiving the high dose (2,808 mg EGEE/kg per day) manifested total recovery of sperm counts by wk 14. Insult resulting from EGEE treatment also occurred on the epididymis. Epididymal weights in the group receiving 1,872 mg EGEE/kg per day were significantly lower than those of the controls ($P \leq 0.05$), whereas differences between the high-dose (2,808 mg EGEE/kg per day) and control groups only approached significance ($P \leq 0.10$).

In a later study by Oudiz and Zenick [1986], the time course of effects on rat sperm parameters was examined. Male rats were treated orally with 936 mg EGEE/kg per day, 5 days/wk for 6 wk. Semen samples were collected on a weekly basis during the exposure period from ovariectomized, hormonally primed females 15 min after mating. The samples were analyzed for sperm count, sperm morphology, and sperm motility. All males were sacrificed 3 days after cessation of treatment. The weights of testes, epididymides, vas deferens, prostates, and seminal vesicles were recorded at termination. The EGEE-treated males had significantly decreased sperm counts at wk 5 and 6 when compared with those of the controls ($P<0.001$). A 30% to 40% decline in sperm counts was noted at wk 5, and by wk 6, 3 of the 10 EGEE-treated males were azoospermic. The remaining rats had severely reduced sperm counts ranging from 5 to 30 million compared with counts of 70 to 78 million sperm in the unexposed group. At wk 5 and 6, there were significant increases in abnormal sperm morphology for the EGEE-treated males ($P<0.01$), and at wk 6, a significant decrease in percent sperm motility ($P<0.01$) was seen in the EGEE-treated males. There were also significant decreases in the weights of testes and epididymides ($P \leq 0.01$), although there was no effect on the weights of vas deferens [Oudiz and Zenick 1986].

B.1.1.3 Inhalation

In two separate reports of a single study, Terrill and Daly [1983a,b] and Barbee et al. [1984] exposed Sprague-Dawley CD rats (15 per group) and New Zealand white rabbits (10 per group) of both sexes to EGEE vapor at 0, 25, 100, or 400 ppm for 6 hr/day, 5 days/wk for 13 wk. The only significant alterations noted in the rats were decreased pituitary weights in males exposed to 400 ppm ($P<0.05$) and reduced spleen weights in females exposed to 100 or 400 ppm EGEE ($P<0.01$). The rabbit was more sensitive to EGEE exposure. Mean body weights decreased for low (25 ppm EGEE, $P<0.05$) and high exposure groups (400 ppm EGEE, $P<0.01$), whereas animals in the middle (100 ppm EGEE) group showed no change. However, pathological changes supportive of these organ weight changes were not observed. The testis weights of rabbits were decreased significantly at 400 ppm EGEE ($P<0.01$). Microscopic examination of testes in this group revealed slight focal seminiferous tubule degeneration in 3 of 10 rabbits. The authors concluded that no biologically significant effects were observed in rats exposed at 400 ppm EGEE and in rabbits exposed at 100 ppm EGEE.

B.1.2 EGME and EGMEA

Toxicity of EGME on the male reproductive system was first demonstrated in rabbits by Wiley et al. [1938]. Two male rabbits received two or three injections of unspecified doses of EGME; both animals developed degeneration of the germinal epithelium.

B.1.2.1 Oral Administration

Nagano et al. [1979] treated groups of five male JCL-ICR mice by gastric intubation 5 days/wk for 5 wk with EGME or EGMEA (62.5, 125, 250, 500, 1,000, and 2,000 mg/kg). Testicular atrophy was assessed in terms of testicular weight, both absolute and relative to body weight. Statistically significant decreases ($P<0.01$) in testes weights were seen in animals given doses of 250 mg EGME/kg per day or greater or 500 mg EGMEA/kg per day or greater when compared with controls. Graphs of testes body weight ratios per dose were almost identical for EGME and EGMEA when doses were expressed as mmoles per kg body weight. Dose-related seminiferous tubular atrophy was observed in the mice with decreased testicular weight, with the 1,000 mg EGME/kg per day and 2,000 mg EGMEA/kg per day groups having no germ cells present. Histologically, varying dosage-related degrees of testicular seminiferous tubule atrophy were noted. At 250 mg of EGME and 500 mg of EGMEA, spermatozoa and spermatids were seen in small numbers in some of the tubules and spermatocyte numbers were reduced. At 500 mg EGME/kg and 1,000 mg EGMEA/kg, the diameter of the seminiferous tubules decreased and spermatozoa and spermatids completely vanished although extremely small numbers of spermatocytes existed in some of the tubules; interstitial tissue also increased.

The relationship between oral administration of EGME and testicular damage was also investigated by Foster et al. [1983]. Groups of 36 male Sprague-Dawley rats received EGME orally at dosages of 0, 50, 100, 250, or 500 mg/kg per day. Six animals from each group were sacrificed at 6 and 24 hr, and at 2, 4, 7, and 11 days. Significant decreases in testicular weight ($P<0.05$) were evident at day 2 in the 500 mg EGME/kg per day group and became more pronounced ($P<0.01$) with increasing total dose (day 4, 7, and 11). At days 7 and 11, statistically significant decreases in testicular weight ($P<0.01$) were also seen in the 250 mg EGME/kg per day group.

Foster et al. [1983] also conducted a recovery study in which groups of male Sprague-Dawley rats received 500 mg EGME/kg per day orally for 4 days. After cessation of treatment, six animals from treated and control groups were sacrificed at 0 (the day after the last treatment), 2, 4, and 8 wk. A statistically significant decrease ($P<0.001$) in relative testicular weights was noted at 0, 2, and 4 wk, with testes weights returning to control values 8 wk following treatment. Seminal vesicle weights were significantly increased ($P<0.05$) at wk 8; the authors suggested this might have been due to increased testosterone levels [Foster et al. 1983].

Histological examination of testes from rats exposed to EGME at 100, 250, and 500 mg/kg per day revealed degeneration of pachytene spermatocytes as early as 24 hr after a single

dose, whereas dosing with 50 mg EGME/kg per day for 11 days produced no testicular abnormalities (no-effect level) [Foster et al. 1983]. The proportion of the spermatocyte population affected at 24 hr was related to dose. Progressive depletion of spermatocytes and maturation depletion of early spermatids were observed with continued dosing. Degenerative changes (cellular shrinkage, increased eosinophilia, and nuclear pyknosis) were restricted to secondary spermatocytes and to pachytene, diplotene, diakinetic, and dividing stages of primary spermatocyte development. Preleptotene, leptotene, and zygotene spermatocytes were unaffected. After 4 days of treatment with 500 mg EGME/kg per day and 7 days of treatment with 250 mg EGME/kg per day, degenerative changes (chromatin margination) were evident in the spermatid population. After 11 days of treatment with 250 and 500 mg EGME/kg per day, spermatid and late spermatocyte populations were absent, and zygotene spermatocytes showed an increase in number; these events were indicative of maturation arrest. Treatment for 11 days with 100 mg EGME/kg per day produced partial depletion and continued degeneration of spermatocytes and partial maturation depletion of early spermatids. Ultrastructural examination of testes 24 hr after a single dose of 100 mg EGME/kg per day showed spermatocytes with mitochondrial swelling and disruption, cytoplasmic vacuolation, and early condensation of nuclear chromatin [Foster et al. 1983].

In the recovery study, the animals sacrificed 2 wk after 4 days of dosing with 500 mg EGME/kg per day showed maturation depletion of middle and late stage spermatids and maturation arrest of pachytene spermatocytes. At 4 wk after exposure, recovery was evident by the presence of maturation phase spermatids, and by 8 wk, full spermatogenesis was present in the majority of tubules from all animals [Foster et al. 1983]. The authors, in a separate publication [Creasy and Foster 1984], concluded that the data demonstrated a defined order of spermatocyte sensitivity: dividing spermatocytes (Stage 14) > early pachytene spermatocytes (Stages 1 through 3) > late pachytene spermatocytes (Stages 9 through 13) > mid-pachytene spermatocytes (Stages 4 through 8) > leptotene/zygotene spermatocytes (Stages 9 through 14).

Similar studies were conducted by Chapin and Lamb [1984] using a different age and strain of rat. Forty adult male F344 rats were treated orally with 150 mg EGME/kg per day for 5 days/wk for up to 10 days. Controls received daily doses of distilled water. Animals were killed on days 1, 2, 4, 7, and 10 after the start of dosing. As previously observed [Foster et al. 1983], degeneration of spermatocytes appeared in treated animals 24 hr after a single dose of EGME. Subsequently, a more consistent, progressive degeneration of spermatocytes and epithelial disruption were accompanied by a statistically significant reduction ($P<0.05$) in testicular weight. In contrast to the work of Foster et al. [1983], a broader range of spermatocytes was affected, including leptotene and zygotene stages as well as pachytene stage spermatocytes and spermatids. An additional purpose of this research was to correlate histologic changes with androgen binding protein (ABP), a Sertoli cell secretion, found in fluid collected at the rete testis after ligation of the efferent testicular ducts. Six animals were treated with EGME as previously described and were sacrificed on days 2, 4, 7, and 10. No significant difference in production of testis fluid was observed between the ligated animals from treated and control groups; total protein and ABP activity in this fluid were unchanged by EGME treatment. The authors concluded that early and late spermatocytes

are targets for EGME in the testes and that Sertoli cell functions, as measured by ABP levels, fluid production, and total protein profile, were unaffected [Chapin and Lamb 1984].

The following study was undertaken to assess possible effects of EGME on late stage and epididymal spermatids and on spermatogonia [Chapin et al. 1985a]. Male F344 rats (20 per group) were treated orally with EGME at 0, 50, 100, or 200 mg/kg per day for 5 days and then allowed to mate with two female F344 rats/week for 8 wk. At the end of the 8-wk period, the male rats were housed singly for an additional 8 wk, and then allowed to mate again for 5 days. The percentage pregnancies decreased significantly ($P<0.05$) during wk 4 for females mated to high-dose males (200 mg EGME/kg per day) and remained significantly lower than controls for the duration of the study. At the 100 mg EGME/kg per day dosage, males demonstrated significantly reduced fertility at week 5 only ($P<0.05$). The fertility rate of males dosed with 50 mg EGME/kg per day was not affected by treatment. The mean number of live fetuses per pregnant female was significantly decreased ($P<0.05$) in the high-dose animals during wk 4 through 16 when compared with controls. Mid-dose (100 mg EGME/kg per day) males sired significantly fewer pups ($P<0.05$) at wk 5 only, whereas the number of live young sired by the 50 mg EGME/kg per day males was not significantly different from that of the controls. Statistically significant increases ($P<0.05$) in resorptions in females were found only in the high-dose group at wk 5 and 6. The high-dose group also demonstrated significantly increased ($P<0.05$) preimplantation losses during wk 3 through 16. A significant increase ($P<0.05$) in preimplantation loss was also seen in the 100 mg EGME/kg per day group during wk 2 and 5 [Chapin et al. 1985a].

In addition to the above mating studies, Chapin et al. [1985a] also conducted sperm assessments in the same investigation using groups of 96 male F344 rats treated orally with EGME at the same doses as above. At weekly intervals for 8 wk, bilateral efferent duct ligation was performed on nine animals/group and the following day each was sacrificed. A dose and time-dependent change in the number of sperm/gram cauda epididymis was seen in the 100 and 200 mg EGME/kg per day groups. Both groups had significantly fewer ($P<0.05$) sperm/gram cauda at wk 2, and sperm counts remained significantly lower than did those of the controls for the 8-wk study. High-dose animals had lower sperm counts than mid-dose animals. Rats treated with 50 mg EGME/kg per day had lower sperm counts at wk 5 only. Sperm motility was also significantly decreased ($P<0.05$) for high- and mid-dose animals: high-dose (200 mg EGME/kg per day) rats were significantly affected from wk 3 through 8, and sperm of mid-dose (100 mg EGME/kg per day) rats showed decreased motility from wk 4 through 8. Motility depression for both groups reached a maximum at wk 5 and 6, and then began to recover. The percentage of morphologically abnormal sperm was significant ($P<0.05$) at wk 3 for the high-dose group and at wk 5 for the mid-dose group, and remained significantly high for both throughout the study, reaching a maximum at wk 6 (mean 80% $\pm$ 10%, high-dose group) and falling thereafter.

The authors [Chapin et al. 1985a] concluded that the fertility decline from wk 4 through wk 8 in the high-dose group suggested an effect of EGME on elongating testicular spermatids and cells at least as immature as intermediate or B spermatogonia. At 16 wk, fertility in these males was 70% that of controls, indicating a relatively prolonged recovery process. This slow recovery demonstrates that A spermatogonia were also affected by

EGME treatment at 200 mg/kg per day. They also concluded that, as the dose was increased, the number of types of affected cells increased and that EGME was a very weak inducer of dominant lethal mutations [Chapin et al. 1985a].

In a separate study, Chapin et al. [1985b] attempted to correlate the above noted fertility indices with changes in testicular histology, the activity of cell specific enzymes, and protein in fluid collected from the ligated rete testis. Adult F344 rats were treated orally with 0, 50, 100, or 200 mg EGME/kg per day for 5 days. Three days later (wk 1), and at weekly intervals for the next 7 wk, nine rats/group were subjected to bilateral efferent duct ligation and sacrificed 16 hr later. In the 50 mg EGME/kg per day group, no change in the morphology of the testes was seen until wk 4, when condensed spermatids lacking tails were seen close to the basement membrane of some tubules in some rats. At wk 5 through 7, 20% to 40% of stage 9 or 10 tubules contained these condensed spermatid nuclei near the basement membrane. By wk 8, none of the animals sacrificed had treatment-related lesions.

In the 100 mg EGME/kg per day group, numerous spermatid heads were seen near the basement membrane and pachytene spermatocyte death was frequent in stages 10 to 12 at wk 1. By wk 3, 100% of the tubules were affected by early and late stage spermatid and pachytene spermatocyte loss, delayed spermiation, or numerous spermatid heads near the basement membrane. These effects persisted through wk 6, and by wk 8, 50% of stage 1 to 5 tubules and some tubules of each stage were unaffected; delayed spermiation was less prominent [Chapin et al. 1985b].

All animals dosed with 200 mg EGME/kg per day showed severe testicular effects at wk 1. By wk 5, 10% to 30% of the tubules were indistinguishable from controls. Fifty percent appeared normal by wk 7; the remaining 50% were severely depopulated with delayed spermiation, and basally located spermatid heads were common in 50% to 80% of stage 9 to 11 tubules [Chapin et al. 1985b].

The effects of EGME on the epididymis were limited to tubular contents: high- and mid-dose groups had fewer sperm and many more immature germ cells than did the controls, whereas low-dose rats showed only a transient mild increase in the number of immature germ cells and decreased sperm density at wk 2. The amount of protein in rete testis fluid was elevated in the high-dose group at wk 2 through 5 and in the mid-dose group at wk 4 and 6.

The authors [Chapin et al. 1985b] concluded that although the low dose of EGME was designed to be a no-effect level, there were slight, previously noted [Chapin et al. 1985a] changes in epididymal sperm concentration and morphology, that is, a delay in spermiation and the presence of tailless, basally located spermatid heads. In the 100 mg EGME/kg per day group, the observed histologic effects tended to be more severe and to diminish with increased time after dosing, until many tubules appeared normal by wk 8. Previous fertility data [Chapin et al. 1985a] showed that the pregnancy rate and number of live pups were similar for the 100 mg EGME/kg per day group and controls at wk 8 also. The most widespread and persistent testicular damage was produced by 200 mg EGME/kg per day. Chapin et al. [1985b] also concluded that the elevation of fluid protein levels suggests first

that the ability of the testes to secrete protein is not inhibited by EGME and second that the lack of germ cells that normally take up this protein may have contributed to the elevated protein levels.

Anderson et al. [1987] investigated the stage-specific effect of EGME on spermatogenesis. Adult male CD rats and CD-1 mice were given single oral doses of EGME at 0, 500, 750, 1,000, or 1,500 mg/kg. Groups of 10 rats and 10 mice were sacrificed at weekly intervals, after dosing for a period of 8 wk, for analysis of epididymal sperm counts and morphology or testicular histology; additional groups of 10 EGME-treated animals were sequentially mated to pairs of virgin females to test for dominant lethality or gross fetal malformations in the F_1 generation (F_1 abnormalities). In the rat, a reduction in testes weights was observed at all dose levels at wk 3, 4, and 5, but this effect disappeared in all but the 1,500 mg EGME/kg group by wk 6. At wk 4, 5, 6, and 7, the sperm counts were significantly lower ($P<0.001$) in the EGME-treated groups compared with those of the controls. A dose-dependent increase in abnormal sperm morphology was noted at all dose levels ($P<0.01$). In the EGME-treated mice, the mean testes weights were significantly lower than those of the control groups at wk 2 to 5, and appeared to increase again towards the end of the study (statistics not given); there was also a tendency towards a dose-response relationship in the incidence of abnormal sperm (statistics not given).

In the rat dominant lethal study, the total implant numbers among females mated at wk 5 to EGME-dosed rats were reduced in a dose-dependent manner ($P<0.001$). Although there was a rise in preimplantation loss rate, there was no statistically significant evidence for the induction of dominant lethality. All rats were infertile at wk 6 after dosing except for those given the lowest dose (500 mg EGME/kg). No induction of gross abnormalities in the offspring was noted (data not given). The histological study in the rats revealed a dose-dependent response to EGME-treatment. One day after treatment with 500 mg EGME/kg, primary spermatocytes undergoing pachytene development were either degenerate or absent. Other stages of spermatocytes, including those in midpachytene, zygotene, and leptotene, were affected with increasing doses of EGME. Depletion of early pachytene spermatocytes 2 and 4 wk after dosing with 1,000 or 1,500 mg EGME/kg suggested early spermatogonial damage. In the mouse, however, the sensitive cells were the late spermatocytes and spermatids [Anderson et al. 1987].

B.1.2.2 Inhalation

Inhalation exposure to EGME has also caused testicular damage [Miller et al. 1981]. Groups of five male Fischer 344 rats and five male $B6C3F_1$ mice were exposed to EGME (0, 100, 300, or 1,000 ppm) 6 hr/day for 9 days in an 11-day interval. EGME at concentrations of 100 or 300 ppm exerted no adverse effects on rat or mouse testes. At 1,000 ppm, however, EGME exerted statistically significant decreases ($P<0.05$) in testes weights when compared with controls. Histopathologic changes in this group included severe degeneration of the testicular germinal epithelium with necrosis of all spermatogenic elements.

Miller et al. [1983a] continued their investigation of the inhalation toxicity of EGME by exposing groups of 10 male Sprague-Dawley rats and 5 male New Zealand white rabbits to

0, 30, 100, or 300 ppm EGME 6 hr/day, 5 days/wk, for a total of 13 wk. The mean testes weights of the rats and rabbits in the 300-ppm group were significantly reduced ($P<0.05$). Testes weights of rabbits in the 100-ppm group were also decreased when compared with those of the controls, but not in a statistically significant manner. Gross pathology showed small, flaccid testes in the males of both species at 300 ppm. Microscopic lesions were found in rats only at the 300-ppm EGME-exposure level; these lesions included bilateral, diffuse, and moderate-to-severe degeneration of the tubular germinal epithelium and reduced numbers of spermatozoa or degenerating spermatozoa. Rabbits demonstrated a dose-related increase in the incidence and severity of the testicular degeneration. In the three surviving rabbits at 300 ppm EGME, severe degeneration affected every tubule, with only Sertoli cells and occasional spermatogonia remaining. At 100 ppm EGME, three of five rabbits had some normal tubules and some tubules contained no germinal elements. Two animals from this group had normal testes. Microscopic degenerative changes were seen in one rabbit from the 30-ppm group. The authors concluded that rabbits were apparently more sensitive than rats to EGME [Miller et al. 1983a].

In reproductive and dominant lethal studies, Rao et al. [1983] exposed Sprague-Dawley rats to EGME vapor. Male rats were exposed to EGME (30 per group at 0 and 30 ppm; 20 per group at 100 and 300 ppm) 6 hr/day, 5 days/wk for 13 consecutive wk. Immediately after the 13-wk exposure period, the males were paired with unexposed female rats for breeding. The fertility index (number of fertile males per number housed with unexposed females) was significantly decreased ($P<0.05$) only in males exposed to 300 ppm EGME. To assess the recovery of reproductive function in males exposed to 300 ppm EGME, additional breedings were conducted at 13 and 19 wk after the termination of exposure. A continued significant decrease ($P<0.05$) in the fertility index was found (50% of males were infertile), although it was not as great as that found in this group immediately after exposure (when 80% of males were infertile). Data suggested that the decreased reproductive function induced by EGME was partially reversible [Rao et al. 1983]. Reproductive parameters examined were normal for males exposed to 30 or 100 ppm EGME; male rats exposed to 300 ppm EGME failed to sire any litters. No dominant lethal effects were found in male rats exposed to 30 or 100 ppm EGME for 13 wk. It was not possible to assess dominant lethality in male rats exposed to 300 ppm due to the complete infertility of these animals. All implantations from the 20% fertile group were nonviable. There was no indication of an increased incidence of resorptions when males exposed to 300 ppm EGME were bred again with unexposed virgin females 26 wk and 32 wk post-exposure after fertility had partially recovered. The authors concluded that the no-adverse-effect level of EGME for fertility and reproduction was 100 ppm in male rats [Rao et al. 1983].

Doe et al. [1983] reported the same 100 ppm EGME no-adverse-effect level for male rats. Groups of 10 male Wistar-derived, Alderly Park strain rats were exposed to 0, 100, or 300 ppm EGME 6 hr/day for 10 consecutive days. The testes of the 300 ppm group were flaccid, reduced in size, and lighter than controls, whereas testes of rats exposed to 100 ppm EGME did not differ from controls. Histological examination of the testes revealed pronounced tubular atrophy in all the rats exposed to 300 ppm EGME, with 70% to 100% of the tubules affected. In contrast, the testes of rats exposed to 100 ppm EGME could not be distinguished from those of controls.

The same laboratory also investigated the effects of a single inhalation exposure of EGME in male rats [Samuels et al. 1984]. Groups of 20 SPF Alpk/AP male albino rats were exposed to 150, 300, 625, 1,250, 2,500, or 5,000 ppm EGME for 4 hr. The control group consisted of 40 rats. Following the single exposure, they were returned to their cages for 13 days and were sacrificed on day 14. Statistically significant reductions ($P<0.01$) in testes weights were seen in the 1,250-, 2,500-, and 5,000-ppm exposure groups when compared with controls. Histological examination revealed severe bilateral tubular atrophy with disordered spermatogenesis in the 5,000-ppm group. Many tubules showed only stem cells and Sertoli cells. Similar but less marked changes were seen in the 2,500- and 1,250-ppm exposure groups, and at 625 ppm, testes weights were not reduced but maturing spermatids showed unspecified evidence of damage.

Samuels et al. [1984] conducted a second study in which groups of 90 SPF Alpk/AP male albino rats were exposed to 0, 1,000, or 2,500 ppm EGME for a single 4-hr period. Ten rats per group were sacrificed on each of days 1, 2, 3, 4, 5, 8, 10, 15, and 19 following exposure. Forty-eight hours following exposure, testes weight reduction was observed in both exposed groups. Damage to the germinal epithelium was observed 24 hr postexposure with primary spermatocytes the target cells for EGME. At day 19, recovery was not evident in the 2,500-ppm EGME group and the germinal epithelium remained disordered. Cytoplasmic retraction and swollen mitochondria in Sertoli cells were observed 4 days following exposure using electron microscopy. The authors concluded that even a relatively brief exposure to EGME vapor can cause marked testicular atrophy [Samuels et al. 1984].

B.1.2.3 Dermal Exposure

EGME has been shown to penetrate human skin in vitro [Dugard et al. 1984]. To determine if EGME produced toxicity following subchronic dermal exposure, six male Hartley guinea pigs were dermally dosed with 1 g EGME/kg per day, 5 days/wk for 13 wk [Hobson et al. 1986]. EGME was applied to 2 × 2 cm gauze patches that were affixed to the shaved backs of the guinea pigs and held in place for 6 hr with a stockinette bandage. At the end of 13 wk, the mean and relative testicular weights were significantly decreased ($P<0.01$) when compared with those of the controls. All animals had severe testicular atrophy, with moderate to severe segmental degeneration of the seminiferous tubules characterized by complete loss of spermatogenic cells. Sertoli cells and Leydig cells remained largely unaffected.

B.2 EFFECTS ON THE FEMALE REPRODUCTIVE SYSTEM AND THE DEVELOPING EMBRYO

B.2.1 EGEE and EGEEA

B.2.1.1 Subcutaneous Administration

The effects of EGEE-treatment on pregnant rats, mice, and rabbits were examined in a study by Stenger et al. [1971]. Groups of 20 pregnant rats were treated s.c. with varying

concentrations of EGEE (0, 23, 46.5, 93 mg/kg per day) on gestation days (g.d.) 1 through 21; pregnant mice (20 per group) were treated s.c. on g.d. 1 through 18 with 0, 46.5, or 93 mg/kg per day; and rabbits (15 per group) were treated s.c. on g.d. 7 through 16 with 0 or 23 mg/kg per day. At the highest doses used, no adverse effects were noted in mice (93 mg EGEE/kg per day) and rabbits (23 mg EGEE/kg per day), but fetal skeletal defects were observed in rats treated s.c. with 93 mg EGEE/kg per day.

B.2.1.2 Oral Administration

In the Stenger et al. study [1971], groups of 20 pregnant rats were treated orally with EGEE (0, 11, 23, 46.5, 93, 186, or 372 mg/kg per day) on g.d. 1 through 21. A significant increase in embryonic and fetal deaths occurred in rats treated orally with doses of 46.5 mg EGEE/kg per day and higher; at oral doses of 93 to 372 mg EGEE/kg per day, the incidence of skeletal aberrations increased in a dose related pattern (no statistical treatment given).

Using an in vivo mouse screening bioassay, a group of 10 pregnant CD-1 mice was treated orally with 3,600 mg EGEE/kg per day on g.d. 7 through 14. Results indicated 10% maternal mortality and no viable litters [Schuler et al. 1984].

The in vitro culture system of Yonemoto et al. [1984] was used by Rawlings et al. [1985] to demonstrate the fetotoxicity of EAA, the alkoxy acid metabolite of EGEE [Jonsson et al. 1982; Cheever et al. 1984]. Conceptuses were explanted from pregnant Wistar-Porton rats at embryonic age 9.5 days and cultured for 48 hr with 2 mM or 5 mM EAA. 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. EAA at the 5 mM concentration had an adverse effect on fetal development. EAA-exposed embryos had statistically significant reductions ($P<0.01$) in morphological score, crown-rump length, head length, and yolk sac diameter. Additionally, EAA produced statistically significant reductions ($P<0.01$) in somite number and in protein content of the embryo. No statistically significant reductions in growth parameters were seen at the 2-mM level. Irregularity of the neural suture line was found in 100% of the EAA-exposed embryos. Other abnormalities seen in the EAA groups were abnormal otic and somite development, turning failure, open cranial folds, and abnormal yolk sac [Rawlings et al. 1985].

B.2.1.3 Inhalation

In inhalation studies [Andrew et al. 1981; Hardin et al. 1981], pregnant New Zealand white rabbits were exposed 7 hr/day on g.d. 1 through 18 to 0, 160, or 615 ppm of EGEE. Five of 29 rabbits died at the high dose (615 ppm); the other 24 suffered severe anorexia and weight loss. Maternal toxicity was mild in rabbits exposed at 160 ppm; a statistically significant reduction in food consumption and body weight gain and increased maternal liver weight was noted ($P<0.05$). In the 615-ppm group, all litters were totally resorbed, and in the 160-ppm group, the number of live fetuses was significantly reduced and resorptions were increased ($P<0.001$). Fetal morphological examinations revealed a significantly increased incidence ($P<0.05$) of renal, cardiovascular, and ventral body wall

defects in fetuses from the 160-ppm exposure group; there were also increases in certain minor skeletal variations.

In the same studies [Andrew et al. 1981; Hardin et al. 1981], female Wistar rats were exposed to 0, 150, or 650 ppm EGEE for 7 hr/day, 5 days/wk for 3 wk before breeding, and then for 7 hr/day on g.d. 1 through 19 to 0, 200, or 765 ppm EGEE. Exposure to EGEE under these conditions exerted no effect on fertility (i.e., mating success or the establishment of pregnancy). A statistically significant ($P<0.05$) reduction in maternal liver weight and an increase in lung and kidney weight ($P<0.05$) occurred with the higher EGEE-exposure regimen (i.e., 650 ppm before breeding followed by 765 ppm on g.d. 1 through 19), and no maternal toxicity occurred at the lower EGEE-exposure regimen (i.e., 150 ppm before breeding followed by 200 ppm on g.d. 1 through 19). Although the incidence of resorptions was not significantly increased in the lower exposure group, all the litters were totally resorbed in the higher exposure group. Fetal toxicity was evident in the lower exposure group as a significant reduction ($P\leq0.05$) in fetal body weight and crown-rump length. Morphological examinations of fetuses from the lower EGEE-exposure group revealed a significantly increased incidence ($P<0.05$) in cardiovascular and skeletal defects.

Nelson et al. [1981] also conducted an inhalation study in which EGEE was evaluated for possible functional effects in the offspring of Sprague-Dawley rats allowed to deliver litters following exposures during gestation to 0, 100, 200, or 900 ppm. The pilot dose-finding study revealed complete resorption of litters in dams exposed to 900 ppm EGEE for 7 hr/day during g.d. 7 through 13, and no live pups in litters of dams exposed on g.d. 14 through 20. There was 34% mortality of pups after exposure of dams to 200 ppm EGEE on g.d. 7 through 13 or 14 through 20. Exposure to 900 ppm EGEE during days 14 through 20 of gestation also produced a consistent pattern of a 48-hr extended gestation period. The only maternal effect observed in rats exposed to 100 ppm EGEE for 7 hr/day on g.d. 7 through 13 and 14 through 20 was a slightly prolonged gestation period (0.7 day, $P<0.001$) in the rats exposed on g.d. 14 through 20. Six behavioral tests were selected to assess central nervous system functions in the control group (filtered air) and in the 100-ppm EGEE-treated groups: neuromuscular ability (ascent and rotorod tests), exploratory activity (open field test), circadian activity (activity wheel test), aversive learning (avoidance conditioning test), and operant conditioning (appetitively motivated test of learning). Behavioral testing of offspring from dams exposed on days 7 through 13 of gestation revealed: (1) impaired performance on a rotorod test ($P=0.002$); (2) prolonged latency of leaving the start of an open field ($P=0.009$); and (3) marginal superiority in avoidance conditioning begun on day 34 of age (not significant, $P=0.061$). Offspring from dams exposed on g.d. 14 through 20 were less active than were controls in a running wheel (not significant, $P=0.32$); they also received an increased number and duration of shocks in avoidance conditioning begun on day 60 of age ($P=0.004$).

Neurochemical alterations also occurred in newborn and 21-day-old rats from dams exposed prenatally to 100 ppm EGEE. Levels of norepinephrine in offspring from both exposure periods (g.d. 7 through 13 and 14 through 20) were decreased significantly ($P<0.01$). In 21-day-old offspring of dams exposed to 100 ppm EGEE on g.d. 7 through 13, the cerebrum had significant elevations in acetylcholine ($P<0.01$), norepinephrine ($P<0.01$), and

dopamine ($P<0.05$); the cerebellum had nearly a threefold increase in acetylcholine ($P<0.01$); the brainstem had an increase in norepinephrine ($P<0.01$); and the midbrain had excesses of acetylcholine ($P<0.01$), norepinephrine ($P<0.05$) and protein ($P<0.05$). In 21-day-old offspring from dams exposed on g.d. 14 through 20, the cerebrum had significant elevations in acetylcholine, dopamine, and 5-hydroxytryptamine ($P<0.05$) [Nelson et al. 1981].

Neuromotor ability of offspring, assessed by ascent and rotorod tests, was reduced ($P<0.05$) when pregnant Sprague-Dawley rats were exposed by inhalation to 200 ppm EGEE 7 hr/day on g.d. 7 through 13; the 200 ppm treatment group was also less active than controls in exploratory activity in the open field and in the shuttle box [Nelson et al. 1982a]. Exposure of pregnant Sprague-Dawley rats to 200 ppm EGEE 7 hr/day on g.d. 7 through 13 altered neurochemical transmitter levels in all brain regions except for the brainstem of 21-day-old offspring. Dopamine levels increased significantly in the cerebrum and midbrain ($P<0.01$ and $P<0.05$, respectively). Norepinephrine levels increased significantly ($P<0.01$) in the cerebrum and cerebellum [Nelson et al. 1982b].

Female Dutch rabbits were exposed to 0, 50, 150, or 400 ppm EGEE on g.d. 6 through 18 [Tinston 1983]. On g.d. 21, animals were sacrificed and necropsied. Although maternal, mean body-weight gain and food consumption of the 400 ppm EGEE-exposure group were markedly lower than those of the controls, they were not statistically different. At 400 ppm EGEE, the group mean number of live fetuses ($P<0.01$), gravid uterus weight ($P<0.01$), and litter weight ($P<0.01$) were statistically lower than they were in the control group. The group mean percentage post implantation loss ($P<0.01$), percentage of early fetal deaths ($P<0.01$), and percentage of late deaths ($P<0.05$) were statistically higher in the 400 ppm EGEE-exposure group compared with controls. No adverse effects were observed in the groups exposed to either 50 or 150 ppm EGEE. No macroscopic pathological abnormalities or external fetal abnormalities could be attributed to EGEE exposure.

EGEE and EGEEA were examined in an inhalation study [Doe 1984a] in which groups of 24 pregnant Alpk/AP rats were exposed to 0, 10, 50, or 250 ppm EGEE 6 hr/day on g.d. 6 through 15, and groups of 24 pregnant Dutch rabbits were exposed either to 0, 10, 50, or 175 ppm EGEE or to 0, 25, 100, or 400 ppm EGEEA on g.d. 6 through 18. Animals were sacrificed on g.d. 21 (rats) or 29 (rabbits), and fetuses were examined for external, visceral, and skeletal malformations. In the rat study, the only sign of maternal toxicity was an effect on the hematopoietic system; reductions in Hb, Hct, and MCV were observed in the group exposed to 250 ppm and are reported in Chapter 4, Section 4.3.4.1. Although there was a higher incidence of preimplantation losses in all exposure groups, this was statistically significant only in the 10- and 50-ppm groups ($P<0.05$). Fetal weights were significantly reduced ($P<0.05$) in the 250-ppm group. In addition, reduced ossification and an increased incidence of skeletal variants ($P<0.05$) were observed in this exposure group. A small and statistically insignificant number of these changes (unossified cervical centra, partial ossification of the second sternebra, extra ribs) also occurred at 50 ppm EGEE. No statistically significant increase in visceral malformations was observed. Data indicated that although EGEE was not teratogenic in rats at the concentrations tested, it was fetotoxic in rats at 250 ppm (98% of fetuses affected) and slightly fetotoxic (51% of fetuses affected) at 50 ppm

($P<0.05$). Exposure of rabbits to 10, 50, or 175 ppm EGEE resulted in no evidence of maternal toxicity. The incidence of skeletal defects and variants at the 175-ppm dose was statistically greater than in the control group ($P<0.05$). This was a result of retarded skeletal ossification, an increased incidence of presacral vertebrae, and an increased number of fetuses with extra ribs, both of short and of normal length.

Exposure of rabbits to 400 ppm EGEEA in the same study [Doe 1984a] caused reduced maternal weight gain and food consumption, whereas no adverse maternal effects were noted in the groups exposed to 25 or 100 ppm EGEEA. At 400 ppm EGEEA, there was an increase in resorptions and a reduction in fetal body weight per litter ($P<0.05$). Reduced fetal body weight also occurred at 100 ppm EGEEA ($P<0.05$). There was no effect on fetal number or weight at 25 ppm EGEEA. Retarded ossification was seen at 400 ($P<0.05$) and 100 ppm ($P<0.05$) but not at 25 ppm EGEEA. Major malformations of the vertebral column were noted at 400 ppm EGEEA, and the incidence of minor defects and variants was elevated at both 400 and 100 ppm EGEEA ($P<0.05$). The investigator concluded that EGEEA was teratogenic in rabbits exposed at 400 ppm, slightly fetotoxic at 100 ppm, and exerted no effect at 25 ppm [Doe 1984a].

In another series of experiments, groups of 15 pregnant Sprague-Dawley rats were exposed 7 hr/day to 0, 130, 390, or 600 ppm EGEEA on g.d. 7 through 15 and sacrificed on g.d. 20 [Nelson et al. 1984b]. All implantations from dams exposed to 600 ppm EGEEA were resorbed, and there was a 56% increase in resorptions at 390 ppm. At 390 and 130 ppm EGEEA, fetal weights were significantly reduced compared with those of the controls ($P<0.05$). Visceral malformations of the heart and umbilicus occurred in fetuses of the 390 ppm group ($P<0.01$). One fetus from the 130 ppm group had a heart defect. The authors concluded that both 130 ppm and 390 ppm EGEEA were teratogenic in the rat [Nelson et al. 1984b].

Tyl et al. [1988] evaluated the teratogenic potential of EGEEA. Pregnant Fischer 344 rats (30 per group) and New Zealand white rabbits (24 per group) were exposed to EGEEA vapor by inhalation 6 hr/day on g.d. 6 through 15 (rats) or 6 through 18 (rabbits) at concentrations of 0, 50, 100, 200, or 300 ppm; the animals were then sacrificed on g.d. 21 (rats) or 29 (rabbits). This study indicated that exposure of rabbits to EGEEA during organogenesis resulted in maternal toxicity at 100 to 300 ppm. Signs of this included significantly decreased weight gain and reduced gravid uterine weight ($P<0.001$) and elevated absolute liver weight ($P<0.05$). In rats, significantly ($P<0.001$) reduced weight gain and reduced food consumption were noted at 200 and 300 ppm EGEEA; significantly elevated relative liver weights were noted at 100, 200, and 300 ppm EGEEA (no statistics given). In rabbits, an increased incidence of totally resorbed litters at 200 ppm ($P<0.05$) and 300 ppm ($P<0.001$), an increase in nonviable fetuses at 300 ppm ($P<0.05$), and a decrease in viable fetuses per litter at 200 ppm and 300 ppm EGEEA ($P<0.05$) were observed. Fetotoxicity (reduced ossification) was observed at 100, 200, and 300 ppm EGEEA. The incidence of external visceral and skeletal malformations was increased at 200 ppm and 300 ppm ($P<0.05$). In rats, embryo/fetotoxicity was observed at 100, 200, and 300 ppm EGEEA. Observations included increased nonviable implantations/litter at 300 ppm ($P<0.05$), reduced fetal body weight/litter at 200 and 300 ppm ($P<0.05$), and increased incidence

($P<0.05$) of external variations at 300 ppm and visceral and skeletal variations at 100, 200, and 300 ppm. There was no evidence of maternal, embryonic, or fetal toxicity (including teratogenicity) at 50 ppm EGEEA in either species. Tyl et al. [1988] concluded that 50 ppm EGEEA was the no observable effect level.

B.2.1.4 Dermal Exposure

The effects of dermal exposure to EGEE on pregnant Sprague-Dawley rats have been investigated by Hardin et al. [1982]. Applications of EGEE (0.25 mL or 0.5 mL) were made 4 times per day on g.d. 7 through 16 to the shaved interscapular region of pregnant rats (20 per group); control rats were treated similarly with water. The only signs of maternal toxicity were ataxia and significantly reduced body weight gain in the last half of gestation following treatment with 0.5 mL EGEE ($P<0.001$). All litters were completely resorbed in the high exposure group, and the incidence of resorptions (76%) was significantly increased in the low exposure group ($P<0.001$). There was a significant reduction in fetal body weight ($P<0.001$), and both cardiovascular malformations (ventricular septal defects) and skeletal variations were significantly increased ($P<0.05$) in the litters treated with 0.25 mL EGEE.

Using the preceding experimental design, equimolar volumes of EGEE (0.25 mL) and EGEEA (0.35 mL) were applied cutaneously to pregnant rats [Hardin et al. 1984]. Data demonstrated that EGEE and EGEEA treatment reduced maternal body weight gain, and at days 17 and 21, body weight gain in the EGEEA group was significantly lower than that in the controls ($P<0.001$). Gravid uterus weights were also significantly reduced in both treatment groups compared with those of the controls ($P<0.001$). Although extragestational body weights did not differ significantly ($P<0.1$), extragestational body weight gain was significantly reduced ($P<0.05$) in the treated groups. A reduction in body weight, which was associated with completely resorbed litters and significantly fewer live fetuses per litter ($P<0.01$), was noted in EGEE- and EGEEA-treated rats relative to that of the controls. Fetal body weights were also significantly decreased ($P<0.001$). Cardiovascular malformations and skeletal variations were significantly increased compared with untreated controls ($P<0.001$) in both EGEE- and EGEEA-treated groups.

B.2.2 EGME and EGMEA

B.2.2.1 Oral Administration

Female JCL-ICR mice were mated with males of the same strain and assigned to experimental and control groups of 21 to 24 animals [Nagano et al. 1981]. EGME was administered by gavage on g.d. 7 through 14 at doses of 0, 31.25, 62.5, 125, 250, 500, or 1,000 mg/kg per day, and the animals were sacrificed on g.d. 18. The incidence of dead fetuses was significantly increased at 250, 500, and 1,000 mg EGME/kg per day ($P<0.01$). Only one fetus survived in the 500 mg EGME/kg per day group, and none survived in the 1,000 mg EGME/kg per day group. Fetal weights were significantly reduced at the 125 and 250 mg EGME/kg per day doses ($P<0.01$). The incidence of gross anomalies in the 250 mg EGME/kg per day exposure group was significantly increased when compared with the controls and included exencephaly, abnormal digits, and umbilical hernia ($P<0.01$). The

one live fetus from the 500 mg EGME/kg per day group also had exencephaly and abnormal digits. The incidence of skeletal malformations was also significantly higher ($P<0.01$) in the 250 mg EGME/kg per day group than in the control group. All fetuses examined in this group had skeletal malformations, including fusion and/or agenesis (nondevelopment) of vertebrae or ribs, spina bifida occulta, syndactyly (fusion of digits), oligodactyly (fewer than five digits), and polydactyly (more than five digits). A significant increase ($P<0.01$) in skeletal malformations was seen also in the 125 mg EGME/kg per day exposure group (fused ribs, fusion and/or agenesis of vertebrae, and spina bifida occulta) and the 62.5 mg EGME/kg per day exposure group (spina bifida occulta). Bifurcated or split cervical vertebrae ($P<0.05$) were observed in the 31.25 mg EGME/kg per day exposure group. The ossification of fetuses was significantly retarded ($P<0.05$) in all treatment groups when compared with the controls, as indicated by decreased numbers of proximal and middle phalanges of fore and hind limbs. The authors concluded that the severity and frequency of the malformations noted following administration of EGME at doses greater than 31.25 mg/kg per day were dose dependent [Nagano et al. 1981].

Pregnant CD-1 mice dosed by gavage with 1,400 mg EGME kg/day on days 7 to 14 of gestation produced no viable litters. EGME caused 14% maternal mortality [Schuler et al. 1984].

The developmental phase-specific and dose-related embryotoxic effects of EGME were investigated by Horton et al. [1985]. Initially, 250 mg EGME/kg per day was administered orally on g.d. 7 through 14 to 10 pregnant CD-1 strain mice, which were subsequently sacrificed on g.d. 18. This group demonstrated gross malformations (exencephaly and paw lesions) similar to those reported by Nagano et al. [1981]. The treatment period was then reduced to 250 mg/kg on g.d. 7 through 9, 8 through 10, 9 through 11, or 250 mg/kg on g.d. 7 and 8, 9 and 10, 10 and 11, or 500 mg EGME/kg on g.d. 9, 10, 11, 12, or 13 to define the developmental phase specificity of the embryotoxic effects observed. Multiple doses of 250 mg EGME/kg per day or single doses of 500 mg EGME/kg significantly reduced fetal weights in all dose groups ($P<0.05$) and significantly increased embryolethality (percentage of implantations resorbed) in all groups except the single 500 mg EGME/kg exposure on g.d. 12 or 13 ($P<0.05$).

Studies with mice dosed during different gestational stages also demonstrated phase-specific teratogenic effects [Horton et al. 1985]. Groups of 9 or 10 pregnant mice treated on g.d. 7 through 9 or 8 through 10 with 250 mg EGME/kg per day had significantly more exencephalic fetuses than did the controls ($P<0.05$). Exposure during later stages of development did not result in excess exencephaly. The incidence of digit malformations (syndactyly, oligodactyly, and polydactyly) increased significantly after three doses on days 8 through 10, or 9 through 11, as well as with two doses on days 10 and 11 ($P<0.05$).

A single oral administration of 500 mg EGME/kg to groups of 9 to 12 mice on g.d. 9, 10, 11, or 12 produced significant increases in paw malformations ($P<0.05$) [Horton et al. 1985]. Peak susceptibility to paw malformations occurred on g.d. 11 and 12 and included syndactyly and oligodactyly. Treatment with EGME on g.d. 9, 10, or 11 produced a prevalence of forepaw anomalies; treatment on g.d. 12 shifted the higher incidence to hind paw syndactyly.

Horton et al. [1985] also investigated the dose dependence of digit anomalies in groups of 9 to 11 mice orally dosed with 100, 175, 250, 300, 350, 400, or 450 mg EGME/kg on g.d. 11. Exposure to 100 mg EGME/kg did not induce digit anomalies. Digit anomalies occurred at 175 mg EGME/kg (not statistically significant), and their frequency increased in a statistically significant dose-related manner ($P<0.05$) to a maximum incidence at 350 mg EGME/kg, with intermediate responses at 250 and 300 mg EGME/kg. The authors concluded a no observed effect level of 100 mg EGME/kg for digit malformations after a single oral dose of EGME [Horton et al. 1985].

The role of cytotoxicity in digital maldevelopment in CD-1 mouse embryos was examined following oral treatment of dams with 100, 250, or 350 mg EGME/kg on g.d. 11 [Greene et al. 1987]. Pregnant mice were sacrificed 6 or 24 hr later. The embryos were removed and incubated for 15 min in Nile blue A stain. The right forelimb buds of EGME-treated embryos were compared with the right forelimb buds from control embryos of the same gestational age. Right forelimbs were examined for the pattern of cell death as determined by uptake of the dye into the tissue, and the overall shape and conformation were recorded by photography and drawings. None of the treatment regimens produced maternal toxicity. Forelimb buds collected 6 or 24 hr after administration of EGME showed marked cytotoxic responses, which were dose related. Cell death was induced in the mesenchymal tissue and to some extent in the limb bud ectoderm. Forelimb buds from the dams treated with 350 mg EGME were consistently malformed in the preaxial region; virtually all of the limb buds examined were extremely altered in appearance. Necrosis was evident in forelimb buds from the dams treated with 250 mg EGME, but the lesions were less severe. In the embryos from the dams treated with 100 mg EGME only slight increases in cell death were noted in approximately 50% of the limb buds from embryos collected 24 hr after EGME treatment [Greene et al. 1987].

Pregnant mice that had been treated with 350 mg EGME/kg on g.d. 11 were sacrificed 2, 6, 24, or 48 hr later [Greene et al. 1987]; a single untreated mouse was included for each time point. Forelimb buds from at least five embryos/dam were excised and prepared for examination by light or electron microscopy. Microscopic evaluations of forelimb buds revealed the presence of phagocytic vacuoles and condensed, fragmented cytoplasm, indicative of cytotoxicity, as early as 2 hr after EGME treatment. The maximum effect was observed 6 hr after EGME treatment, and the severity of the effect appeared to be dose-related.

In the same study [Greene et al. 1987], pregnant mice were given a single oral dose of EGME (100, 175, 250, 300, 350, 400, 450, or 500 mg) on g.d. 11 and were sacrificed on g.d. 18. Near-term fetuses were removed and examined for digit malformations. Although digital malformations were not detected in near-term fetuses following treatment with 100 mg EGME, they were induced in all other treatment groups in a dose-dependent manner (statistics not given). The percentage of fetuses with paw malformations ranged from 12% to 93%. The primary anomalies observed were preaxial syndactyly (fusion of digits of No. 2 and No. 3) and ectrodactyly (absence of digit No. 1).

Hardin and Eisermann [1987] studied the potency of dimethyl-substituted ethylene glycol ethers relative to EGME in inducing paw malformations. EGME, ethylene glycol dimethyl ether (EGdiME), diethylene glycol dimethyl ether (diEGdiME), and triethylene glycol dimethyl ether (triEGdiME) were administered orally in single equimolar doses (304, 361, 537, and 713 mg/kg, respectively) to CD mice on g.d. 11. On g.d. 18, fetuses were collected, weighed, and examined for gross external malformations. None of the treatment regimens produced maternal toxicity. In the fetuses, only paw malformations were observed; they occurred with significantly increased frequency ($P<0.05$) in litters of mice treated with EGME, EGdiME, diEGdiME, but not in litters of triEGdiME-treated dams. The average percent of fetuses affected per litter was 69% (EGME), 34% (EGdiME), and 40% (diEGdiME). Only in litters of dams treated with EGME was polydactyly observed with significantly increased frequency ($P<0.05$) in forepaws. Syndactyly appeared in increased frequency ($P<0.05$) in hindpaws of EGME, EGdiME, and diEGdiME litters. The frequency of short digits was significantly increased ($P<0.05$) in both forepaws and hindpaws of EGME litters but only in hindpaws of diEGdiME-treated litters. Oligodactyly appeared in both forepaws and hindpaws of EGME litters, forepaws of EGdiME litters, and hindpaws of diEGdiME litters more often ($P<0.01$) than in controls. It was suggested by Hardin and Eisenmann [1987] that these paw malformations were attributable to in vivo conversion of these glycol ethers to a common teratogen, MAA.

Toraason et al. [1985] studied the effect of EGME on the developing cardiovascular system using electrocardiography. Pregnant Sprague-Dawley rats were treated by gavage with 0, 25, 50, or 100 mg EGME/kg per day on g.d. 7 through 13. All fetuses from the eight rats treated with 100 mg EGME/kg per day were resorbed. There was a dose-dependent increase in cardiovascular defects in fetuses exposed to EGME, including ventricular septal defects and right ductus arteriosus in the 50 mg EGME/kg per day exposure group. No cardiovascular malformations were seen in control fetuses. Significantly more litters in the 25 and 50 mg EGME/kg per day exposure groups had fetuses with aberrant heart QRS intervals than did the controls ($P<0.05$). The most prevalent abnormality was a prolonged QRS complex, which the authors suggest indicated the presence of an intraventricular conduction delay. There was no association between abnormal electrocardiograms (EKGs) and any morphological defect.

To assess the risk for women of childbearing age exposed to EGME, Scott et al. [1989] exposed nonhuman primates, *Macaca fasicularis* females, orally to 12, 24, or 36 mg EGME/kg on g.d. 20 through 45 (8, 11, and 13 animals/group, respectively). The fetuses were collected by Caesarean section at day 100. Two groups of three monkeys served as controls. One of the control groups was treated orally on g.d. 20 through 45 with 15 mL of ethanol. Although no statistics were presented, the data indicated that EGME caused a dose-related loss of maternal body weight. This was accompanied by anorexia, the severity of which was dose-related.

Because the loss of appetite was so severe at times, especially in the high-dose animals, the investigators administered gruel and/or electrolytes by gavage to prevent serious physical deterioration of the adult animals. After the cessation of treatment at g.d. 45, the animals

regained their appetites, and body weights were similar to control body weights at the time of Caesarean section on g.d. 100 [Scott et al. 1989].

Hematologic analysis of the EGME-treated monkeys did not reveal any dose-related effects. Embryotoxicity of EGME in these monkeys was manifest mainly in the form of embryonic death. At the 36 mg/kg dose, all eight pregnancies ended in death. One of these dead embryos, judged to have been about 40 days old at the time of death, was missing one digit on each forelimb. This malformation has never been seen in macaque pregnancies, and EGME has caused the same type of defect in mice [Horton et al. 1985], rats [Ritter et al. 1985], and rabbits [Hanley et al. 1984a]. The authors [Scott et al. 1989] therefore attributed this malformation to EGME treatment. Three of 10 pregnancies (30%) at the 24-mg/kg dose and 3 of 13 pregnancies (23%) at the 12-mg/kg dose ended in embryonic death. An additional pregnancy in each of the 12- and 24-mg/kg groups was lost to abortion, but both were thought to be spontaneous (spontaneous abortions occur in 10% to 20% of untreated macaque pregnancies). All surviving fetuses were free from malformation. Despite the maternal toxicity associated with the higher doses of EGME, the authors concluded that EGME acted directly on the embryo to cause its demise [Scott et al. 1989].

B.2.2.2 Inhalation

A rapid assessment of the effect of inhaled EGME on the developing embryo was conducted by Doe et al. [1983]. Groups of 20 pregnant female Wistar-derived, Alderly Park strain rats were exposed to 0, 100, or 300 ppm EGME for 6 hr/day on g.d. 6 through 17. The rats were allowed to deliver their litters, which were observed for 3 days. Maternal body weight gain was significantly reduced in the 300 ppm group ($P<0.05$), and none of these rats produced litters. The gestation period was significantly increased for the nine rats producing litters in the 100 ppm EGME group when compared with that of the controls (23.6 vs. 22 days, $P<0.05$). This group also showed a significant reduction in the total number of pups ($P<0.001$), the proportion of live pups at birth ($P<0.01$), and the proportion surviving to 3 days ($P<0.01$). All the pups were normal externally [Doe et al. 1983].

Hanley et al. [1984a] conducted a study to compare the teratogenic potential of exposure to low vapor concentrations of EGME in F344 rats, CF-1 mice, and New Zealand white rabbits. Groups of 24 to 32 pregnant mice, rats, and rabbits were exposed to EGME via inhalation for 6 hr/day on g.d. 6 through 15 (rats and mice) or g.d. 6 through 18 (rabbits). Exposure concentrations were 0, 3, 10, or 50 ppm EGME for rats and rabbits, and 0, 10, or 50 ppm EGME for mice. Mice were sacrificed on g.d. 18, rats on g.d. 21, and rabbits on g.d. 29. Statistically significant increases ($P<0.05$) in lumbar spurs and delayed ossification of the ventral centra (minor skeletal variations) were seen in rats at 50 ppm. Maternal effects found in rats were minimal. At 50 ppm, a slight transient decrease in body weight gain was observed, and decreases in mean Hb, packed cell volume (PCV), and RBC values were found. Hanley et al. [1984a] concluded that these decreases were of no toxicologic significance. The mice demonstrated a pattern of effects similar to that observed in rats. The only maternal effect was a statistically lower ($P<0.05$) body weight gain on g.d. 12 through 15. There was a significant increase ($P<0.05$) in the incidence of extra ribs, and the

incidence of unilateral testicular hypoplasia in animals exposed to 50 ppm indicated slight fetotoxicity [Hanley et al. 1984a].

Rabbits demonstrated a greater sensitivity to EGME at these concentrations than did rats or mice [Hanley et al. 1984a]. At 50 ppm EGME, significant decreases in maternal body weight gain during exposure ($P<0.05$) and significant increases in the absolute weights of the liver ($P<0.05$) occurred. Changes in reproductive and developmental parameters in the 50 ppm EGME group included a significant increase in the resorption rate ($P<0.05$) and a significant decrease in the mean fetal body weights ($P<0.05$) when compared with those of the controls. Examination of fetuses revealed a statistically significant increase ($P<0.05$) in the total incidence of malformations in the 50 ppm group. External malformations involved the extremities and included persistent joint contracture (arthrogryposis) and digit anomalies such as absence of nails (anonychia), shortness of digits (brachydactyly), and absence of digits (ectrodactyly). Visceral examination of rabbit fetuses revealed ventricular septal defects and coarctation (segmental constriction) of the aortic arch, splenic hypoplasia, and severe dilation of the renal pelvis. Malformations seen at skeletal examination of the 50-ppm EGME exposure group included missing bones of the paws and a variety of rib malformations. Numerous minor variations considered evidence of fetotoxicity at 50 ppm EGME included patent ductus arteriosus (delayed development), pale spleen, and convoluted or dilated ureters. Other variations were delayed ossification of the hyoid, tarsals, and sternebrae and an irregularity in the pattern of the palatine rugae. At 3 or 10 ppm EGME, there were no differences among fetal rabbits suggesting any adverse developmental effect. Hanley et al. [1984a] concluded that these results established no observed effect levels of 10 ppm EGME in these three species.

Nelson et al. [1984a] determined how offspring were affected when male or female rats were exposed at 25 ppm EGME. Pregnant female Sprague-Dawley rats were exposed either on g.d. 7 through 13 or 14 through 20 and then allowed to deliver their young. Males were exposed to EGME 7 hr/day, 7 days/wk for 6 wk and then mated with unexposed females. Six behavioral tests were selected to evaluate CNS functions in offspring: ascent on a wire mesh, rotorod, open field, activity wheel, avoidance conditioning, and operant conditioning. Offspring from the group exposed on g.d. 7 through 13 showed significant differences ($P<0.05$) in performance of avoidance conditioning (aversive learning) when compared with the control animals. No other significant behavioral differences were seen.

Chemical analyses were performed on whole brain samples from newborns of each group, and on cerebrum, cerebellum, brain stem, and midbrain samples from 21-day-old animals. Concentrations of total brain protein and four neurotransmitters (acetylcholine [Ach], dopamine [DA], norepinephrine [NE], and 5-hydroxytryptamine [5-HT]) were measured. Significant differences in concentrations of Ach, DA, NE, and 5-HT were apparent in 21-day-old rats from all three exposed groups when compared with the controls ($P<0.05$). Protein levels were significantly ($P<0.05$) different only in 21-day-old offspring exposed from day 14 through 20 of gestation. In whole brain samples from newborns, increases were found in Ach and 5-HT for the offspring of exposed males, but no other differences were significant when compared with the controls [Nelson et al. 1984a].

B.2.2.3 Dermal Exposure

The teratogenic potential of dermal exposure to EGME was estimated using the Chernoff and Kavlock in vivo assay. Groups of 10 pregnant Alpk/AP (Wistar derived) rats were exposed to 3%, 10%, 30%, or 100% EGME in physiological saline at 10 mL/kg [Wickramaratne 1986]. The test compound was applied for 6 hr (occluded exposure) on g.d. 6 through 17. Rats were then allowed to deliver litters normally and rear their litters until day 5 post-partum. The application of 100% EGME was lethal to all dams and 30% was lethal to all developing fetuses. At 10% EGME, the litter size was reduced by 26% as was survival at day 5 (neither statistically significant). No adverse effects were seen in the 3% group. The results were evaluated using "rules" generated from the Chernoff and Kavlock in vivo teratology screen assay. The authors concluded that the data demonstrated a clear dose-response and that a 10% solution of EGME is likely to be a rat teratogen [Wickramaratne 1986].

Feuston et al. [1990] studied the effect of a single dermal application of EGME as a function of both gestation day administered and dose level. Pregnant Sprague-Dawley rats (8 to 10 rats/group) received a single dermal application of 0, 250, 500, 1,000, or 2,000 mg EGME/kg on g.d. 12. In the other part of the study, pregnant Sprague-Dawley rats (8 to 10 rats/group) received a single dermal application of 2,000 mg EGME/kg on g.d. 10, 11, 12, 13, or 14. The control group was sham treated on g.d. 10 through 14 to correspond to all of the days of 2,000 mg EGME/kg exposure. Dose levels were based on a pilot study at this facility (data not presented). Each female rat was observed daily; body weights were measured on g.d. 0, 6, 10 through 15, and 20. On g.d. 20, each female rat was necropsied and the fetuses were examined for normal development.

Maternal toxicity consisted of a statistically significant ($P<0.05$) decrease in mean body weight gain for female rats on the day following their exposure. This occurred at all EGME exposure concentrations on g.d. 10 through 14, except for animals exposed to 250 mg EGME/kg on g.d. 12. This weight loss was transient.

Adverse reproductive effects were noted in the female rats who had received 2000 mg EGME/kg on g.d. 10. These effects included a statistically significant increase ($P<0.05$) in both the mean number of resorptions and the mean percentage resorptions. The number of dams in this group with resorptions was also higher than the number of dams with resorptions in the untreated group.

There was a statistically significant decrease ($P<0.05$) in fetal body weights in the female rats exposed to 1,000 mg EGME/kg on g.d. 12 or 2,000 mg EGME/kg on g.d. 10 and 12. In general, female fetuses were more affected than were male fetuses.

Application of 500, 1,000, or 2,000 mg EGME/kg on g.d. 12 caused statistically significant ($P<0.05$) increases in external, visceral, and skeletal malformations. Cardiovascular and urinary system defects were the prominent visceral malformations. The most frequently observed skeletal defects were limb (primarily of the digits) and vertebral column (primarily of the tail) defects. The application of 250 mg EGME/kg caused no adverse developmental

effects and was considered to be the no observable adverse effect level (NOAEL) by the investigators [Feuston et al. 1990]. Examination of the effects on the various days of exposure indicated that application of 2,000 mg EGME/kg on g.d. 11, 12, or 13 produced the highest incidence and greatest variety of fetal visceral malformations. The application of EGME on g.d. 12 or 13 resulted in a predominance of external and skeletal malformations. Exposure to EGME on g.d. 14 produced minimal developmental effects.

B.3 HEMATOLOGY

B.3.1 EGEE and EGEEA

An early report [von Oettingen and Jirouche 1931] indicated that adding 1 cc of EGEE or EGEEA to 5 cc suspensions of dog or beef red blood cells in Ringer solution caused hemolysis. The investigators noted that hemolysis was more marked with EGEEA than with EGEE.

B.3.1.1 Oral Administration

In a study by Stenger et al. [1971], EGEE administered orally 7 days/wk for 13 wk to dogs (186 mg/kg per day) and rabbits (186, 372, or 744 mg/kg per day) decreased the Hb and Hct values. No statistical analysis was presented. Hemosiderin accumulation and isolated hematopoietic foci were observed in the spleens of all dogs and rabbits treated with EGEE.

In a study by Nagano et al. [1979], oral administration to mice of 2,000 mg EGEE or EGEEA/kg per day, 5 days/wk for 5 wk significantly reduced WBC counts compared with control values ($P<0.05$). No disturbances of erythrocytic parameters were observed following administration of 500, 1,000, or 2,000 mg EGEE/kg per day. However, administration of 4,000 mg EGEEA/kg per day, reduced the MCV ($P<0.01$).

B.3.1.2 Inhalation

When rats were exposed to 370 ppm EGEE 7 hr/day, 5 days/wk for 5 wk, an adverse effect on both the RBC and WBC populations was evident from (1) the increase in the hemosiderin content and the decrease in myeloid cells in the spleen, (2) fat replacement in the bone marrow, and (3) an increase in the proportion of circulating immature granulocytes [Werner et al. 1943a]. Hemosiderin was still present in the 3-wk interval following termination of EGEE exposure. Exposure of dogs 7 hr/day, 5 days/wk for 12 wk to 840 ppm EGEE also increased the numbers of circulating immature granulocytes [Werner et al. 1943b]. Although the hemosiderin content in the spleen was increased, RBC counts, Hb concentration, and MCV were only marginally reduced in the exposed dogs. These blood changes occurred at exposure concentrations not sufficiently severe to cause overt signs of toxicity. These studies demonstrated hematologic changes from EGEE exposure that were not severe and were reversible.

In a study by Carpenter et al. [1956], a single 4-hr inhalation exposure of rats to either EGEE or EGEEA increased erythrocyte osmotic fragility. The lowest concentrations causing osmotic fragility were 125 ppm EGEE and 62 ppm EGEEA. In another study, a single 4-hr exposure of rats and rabbits to 2,000 ppm EGEEA caused transient hematuria and/or hemoglobinuria only in rabbits [Truhaut et al. 1979]. In the same study [Truhaut et al. 1979], exposure of rats and rabbits of both sexes to 200 ppm EGEEA 4 hr/day, 5 days/wk for 10 months caused no effect on RBC or Hb levels.

Adverse effects on hematologic parameters were observed in rats and rabbits exposed to 0, 25, 100, or 400 ppm EGEE 6 hr/day, 5 days/wk for 13 wk [Terrill and Daly 1983a,b; Barbee et al. 1984]. In the rabbit study, Hct and Hb levels and erythrocyte counts were reduced significantly in males ($P<0.05$, $P<0.01$, $P<0.05$, respectively) and females ($P<0.05$) exposed to 400 ppm EGEE. No hematologic changes were observed in either sex at lower concentrations. In the rat study, WBC counts were significantly reduced ($P<0.05$) in females exposed to 400 ppm EGEE; no effects were observed in male rats.

Exposure of pregnant rats and rabbits during gestation to EGEE or EGEEA also affected hematologic parameters [Doe 1984a]. Pregnant Alpk/AP rats were exposed to 0, 10, 50, or 250 ppm EGEE 6 hr/day on g.d. 6 through 15. In the 250 ppm EGEE-exposure group, there were reductions in Hb, Hct, and MCV in erythrocytes. There were no effects at either 50 or 10 ppm EGEE in rats. No hematologic effects were observed in pregnant Dutch rabbits exposed to 0, 10, 50, or 175 ppm EGEE 6 hr/day on g.d. 6 through 18. In the same study, pregnant rabbits were exposed to 0, 25, 100, or 400 ppm EGEEA 6 hr/day on g.d. 6 through 18. Doe [1984a] concluded that a significant reduction in Hb concentration and a slight reduction in the associated RBC parameters were seen in the 400 ppm EGEEA-exposure group but provided no specific statistical data. No effects were observed in rabbits at the lower exposure concentrations.

Exposure of New Zealand white rabbits and Fischer 344 rats to 0, 50, 100, 200, or 300 ppm EGEEA 6 hr/day on g.d. 6 through 18 (rabbits) and 6 through 15 (rats) resulted in adverse hematologic parameters in both species [Tyl et al. 1988]. In rabbits, there was evidence of enlarged erythrocytes (elevated MCV) at 300 ppm EGEEA ($P<0.01$) and significant dose-related decreases in the number of platelets at 100 ($P<0.05$), 200 ($P<0.01$) and 300 ppm EGEEA ($P<0.001$). In rats, the WBC count was significantly increased ($P<0.001$) at 200 and 300 ppm EGEEA. Statistically significant reductions in rat RBC count ($P<0.0$), Hb level ($P<0.01$), and Hct and RBC volume (0.05) were seen at the three highest exposures (100, 200, and 300 ppm EGEEA). Platelet counts were also significantly reduced at 200 ppm ($P<0.001$) and 300 ppm EGEEA ($P<0.01$) in the rat.

B.3.1.3 Dermal Exposure

In a study by Truhaut et al. [1979], rabbits were exposed to a single 24-hr dermal application (10,500 mg/kg) of EGEEA; death followed between 1 and 4 days after application. The reduction in RBC count did not exceed 15% to 20% and blood Hb levels showed little variation; however, a 50% to 70% decrease in WBC count was noted.

B.3.2 EGME and EGMEA

B.3.2.1 Oral Administration

Oral administration of EGME and EGMEA has induced hematologic changes in laboratory animals. Nagano et al. [1979] found a statistically significant decrease ($P<0.01$) in WBC counts following oral administration of 500 mg EGME/kg or 1,000 mg EGMEA/kg to male JCL-ICR mice 5 times/wk for 5 wk. Statistically significant decreases were also observed in RBC and Hb values for the 1,000 mg EGME/kg group ($P<0.01$) and in Hb values for the 2,000 mg EGMEA/kg group ($P<0.01$). Treatment of female JCL-ICR mice with 1,000 mg EGME/kg on g.d. 7 through 14 also significantly decreased the leukocyte counts ($P<0.01$) [Nagano et al. 1981].

Grant et al. [1985] investigated the effects of subchronic oral exposure to EGME on the hematopoietic system of rats and the reversibility of such effects. Groups of 24 male F344 rats were orally dosed with EGME at 0, 100, or 500 mg/kg per day for 4 consecutive days. Six animals from each group were then sacrificed 1, 4, 8, and 22 days after the last treatment. Rats in the high-dose group displayed severely hemorrhagic femoral bone marrow with major loss of the normal nucleated tissue and damage of sinus endothelial cells on day 1. The normal architecture of the marrow was restored by day 4 post-treatment. Treatment with 500 mg EGME/kg for 4 days abolished extramedullary hemopoiesis (EMH) in the spleen; partial recovery was seen on day 4, followed by marked improvement on day 8, and a return to control values by day 22. The high-dose group also showed mild anemia characterized by reductions of the Hct and Hb values at day 4 ($P<0.05$ and $P<0.001$, respectively), and RBC, Hct, and Hb values at day 8 ($P<0.05$, $P<0.05$, and $P<0.01$, respectively). Leukocyte counts (neutrophils and lymphocytes) were significantly reduced in this group on day 1 ($P<0.001$) and did not return to control values by the end of the recovery period. Low dose (100 mg EGME/kg per day) rats also had reduced leukocyte counts on day 1 ($P<0.05$). The authors [Grant et al. 1985] concluded that the major hematological effect of EGME was leukopenia characterized by reductions in lymphocytes and neutrophils. Changes in the circulating blood together with reduced splenic EMH and bone marrow toxicity suggested an inhibitory action on erythropoiesis.

B.3.2.2 Inhalation

Hematologic effects from exposure to EGME were first reported by Werner et al. [1943b] who exposed two dogs by inhalation to 750 ppm EGME for 7 hr/day, 5 days/wk for 12 wk. This exposure to EGME resulted in microcytic anemia as indicated by depressed erythrocyte and Hb values, which appeared at 4 to 6 wk of exposure; these values remained depressed throughout the exposure period. Recovery, as measured by Hb and Hct values, was gradual in proportion to the severity of the anemia. RBC were found to have increased osmotic fragility at the end of 11 and 12 wk of exposure. When Werner et al. [1943a] exposed Wistar rats to 310 ppm EGME 7 hr/day, 5 days/wk for 5 wk, increased levels of hemosiderin and immature granulocytes were observed; this indicated destruction of blood cell populations.

In a study by Carpenter et al. [1956], hemolytic effects in the rat erythrocyte were demonstrated by a single 4-hr inhalation exposure of six female rats to EGME or EGMEA. The lowest concentrations causing significant osmotic fragility were 2,000 ppm EGME and 32 ppm EGMEA.

In a study by Miller et al. [1981], Fischer 344 rats and B6C3F$_1$ mice were exposed by inhalation to 0, 100, 300, or 1,000 ppm EGME 6 hr/day for a total of 9 days in an 11-day period. WBC counts of both rats and mice exposed to 1,000 ppm EGME were statistically lower than those of the controls ($P<0.05$). MCV, RBC counts, and Hb levels of male and female rats and male mice in the 1,000 ppm EGME-exposure group were also statistically depressed ($P<0.05$). At 300 ppm EGME, similar but less severe effects were seen in rats; statistically lower ($P<0.05$) WBC counts in both sexes and Hb and RBC counts in females were noted. Hematologic parameters in mice exposed at 300 ppm EGME were stated to be similarly affected, but data were not presented. Histopathology was performed on rats only, revealing reduced bone marrow cellularity, lymphoid depletion in the cortex of the thymus, and reduced numbers of lymphoid cells in the spleen and in the mesenteric lymph nodes in the 1,000-ppm EGME group. Both myeloid and erythroid elements of the bone marrow were markedly reduced in all rats exposed to 1,000 ppm EGME, and megakaryocytes were present in decreased numbers and were smaller than those of controls. The entire thymic cortical lymphoid population was depleted, with less dramatic reductions in the lymph nodes and spleen. Lymphoid organ toxicity persisted at 300 ppm EGME, but to a much lesser extent. In addition, serum total protein, albumin (males only), and globulin values in the 1,000 ppm EGME-exposure group (rats) were significantly reduced ($P<0.05$).

In a longer inhalation study, Miller et al. [1983a] exposed Sprague-Dawley rats and New Zealand white rabbits to 0, 30, 100, or 300 ppm EGME 6 hr/day, 5 days/wk for 13 wk. Hematologic analyses were performed after 4 or 12 wk of exposure. After 12 wk, both rats and rabbits from the 300 ppm EGME-exposure groups had significantly decreased mean WBC counts, platelet counts, MCV, and Hb concentrations ($P<0.05$); RBC counts were significantly reduced only in the 300 ppm EGME-exposed rabbits. These same hematologic changes were also seen after 4 wk (data not given). Mean values for total protein, albumin, and globulins were significantly lower than those of the controls in rats exposed to 300 ppm EGME ($P<0.05$) but were normal in rabbits. Microscopic lesions in rats occurred in the 300-ppm group only as a decrease in cortical lymphocytes indicating thymic atrophy. No histopathologic changes were seen in bone marrow. In rabbits, microscopic lesions were also present at 300 ppm EGME, including lymphoid atrophy of the thymus and gut-associated lymphoid organs and a decrease in the size of hepatocytes [Miller et al. 1983a].

B.3.2.3 Dermal Exposure

Subchronic dermal exposure of male guinea pigs to EGME demonstrated adverse hematologic effects [Hobson et al. 1986]. Six male Hartley guinea pigs were exposed to 1,000 mg EGME/kg per day, 5 days/wk for 13 wk. EGME was applied to gauze patches affixed to the shaved backs of the guinea pigs and held in place with a stockinette bandage

6 hr/day, 5 days/wk for 13 wk. Statistically significant decreases in RBC counts and increases in MCV were noted when compared with the control values ($P<0.05$). Differential white cell counts demonstrated significant ($P<0.05$) lymphopenia with neutrophilia. Additionally, significantly increased serum creatinine kinase (CK) and lactate dehydrogenase (LDH) activity were noted in this group ($P<0.01$).

B.4 METABOLISM, UPTAKE, AND ELIMINATION

B.4.1 EGEE/EGEEA

Jonsson et al. [1982] investigated the biotransformation of EGEE in albino rats exposed either to 10 ppm EGEE via inhalation for 1 hr or to single doses of 9.3 or 93 mg EGEE by gastric intubation. Ethoxyacetic acid (EAA) and N-ethoxyacetyl glycine were the two major metabolites present in the urine of animals dosed by either route. In the oral-dosing study, urine was collected after dosing for 48 hr, in 24-hr portions. The combined excretion of the two metabolites amounted to 30% of the administered oral dose for both dose groups. No recovery was given for the animals dosed by inhalation.

The biotransformation and excretion of EGEE was studied by Cheever et al. [1984] in Sprague-Dawley rats. The animals received a single oral dose of 230 mg/kg of EGEE [ethanol or ethoxy labeled ^{14}C]. The animals were sacrificed for assay of tissue radioactivity at the end of a 96-hr experimental period. Rats treated with the ethanol-labeled material excreted 81% of the radioactive dose in urine over a 96-hr period, whereas rats treated with the ethoxy-labeled compound excreted 76% of the dose in urine. Within the first 24 hr, 72% of the ethanol label and 70% of the ethoxy label were excreted in urine. The major urinary metabolites were EAA and N-ethoxyacetyl glycine, which accounted for 73% to 76% of the administered radioactivity. Results of this study confirmed previous work by Jonsson et al. [1982] and indicated that, in the rat, metabolism of EGEE proceeds primarily through oxidation via alcohol dehydrogenase to EAA, with some subsequent conjugation of the acid metabolite with glycine. It is noteworthy that in this study, 2 hr after administration of EGEE, EAA was found in the rat testes; these data suggest that adverse testicular effects of EGEE may be due to EAA [Cheever et al. 1984].

Absorption and elimination of EGEEA were studied by Guest et al. [1984] in beagle dogs following inhalation, intravenous (i.v.), or dermal exposure. EGEEA was rapidly absorbed through the lungs during exposure to 50 ppm EGEEA for 5 hr. After 10 min of exposure, the concentration of EGEEA in expired breath was 9 ppm (80% absorption), and it reached the plateau value of 16 ppm at 3 hr, indicating that 68% of the inhaled compound was absorbed. The breath concentration of EGEEA decreased to 2 ppm at 3 hr post-exposure. Following single i.v. dosing with 1 mg/kg, 20% and 61% of the dose appeared in urine in 4 and 24 hr, respectively. The blood elimination half-life was 7.9 hr. Estimated over a 60-min period, the percutaneous absorption rate of EGEEA following dermal application to the dog's thorax was 110 mM/cm^2 per min. The rate of absorption of EGEEA through dog skin in vitro was 292 nmol/cm^2 per hr (2.3 mg/cm^2 per hr).

Groeseneken et al. [1986a] developed a method for measuring urinary EAA. Five healthy male volunteers were exposed at rest via face mask to air containing 5.4 ppm EGEE during four 50-min periods. Experimental conditions are described in Groeseneken et al. [1986b]. Urinary EAA concentration rose significantly 1 hr after the exposure period. Urinary EAA concentration rose from 0.07 mg/liter before exposure to 2.39 ± 1.03 mg/liter 1 hr after the exposure period ($P<0.005$). One of the subjects showed measurable levels of EAA before exposure. Questioning of the subject indicated that he may have had occupational exposure to EGEE some days before the experiment. Preliminary results of the excretion of EAA in urine suggested that measurement of EAA could be a specific and sensitive parameter for monitoring worker exposure to EGEE.

Groeseneken et al. [1986b] next investigated the respiratory uptake and elimination of EGEE in 10 male volunteers under controlled experimental conditions of exposure concentration and physical workload. The subjects were divided into two groups, five subjects per group, and were exposed to EGEE for 4 hr, the equivalent of half a workshift. At the end of each 50 min during exposure, a short break of 10 min was inserted. All subjects participated in three experiments according to their group assignment. Experimental conditions are presented in Table B-1.

Table B-1.—Experimental conditions for Groeseneken et al. [1986b]

Group	Exposure concentration		Workload
	ppm	mg/m^3	(W)
I	2.7	10	0
	5.4	20	0
	10.8	40	0
II	5.4	20	0
	5.4	20	30
	5.4	20	60

This study showed that in man, EGEE is rapidly absorbed through the lungs. About 64% of the inhaled vapor was retained at rest, and retention increased as physical exercise was performed during exposure. The absorbed dose was apparently proportional to the inhaled concentration, and a linear relation was observed between uptake rate and exposure concentration. The rate of uptake increased when physical exercise was performed during exposure. The rate of uptake was higher as exposure concentration, or pulmonary ventilation rate, or both increased. Individual uptake rates seemed dependent only on transport mechanisms (pulmonary ventilation, or cardiac output, or both) and not on anthropometric data or body fat content. Respiratory elimination of unchanged EGEE accounted for ≤0.4% of the total body uptake and occurred rapidly after cessation of exposure, followed by a much slower decrease. This slow decrease indicated that two pharmacological compartments were involved.

Groeseneken et al. [1986c] also studied the urinary excretion of EAA in the 10 male volunteers who inhaled various concentrations of EGEE for 4 hr both at rest and during physical exercise in the previously described study [Groesenken et al. 1986b]. The subjects, divided into two groups of five, were either exposed at rest to concentrations of 2.7, 5.4, or 10.8 ppm EGEE (10, 20, or 40 mg/m^3, respectively) or were exposed to 5.4 ppm EGEE at rest and during physical exercises (see Table B-2). Urine samples were collected at hourly intervals during exposure and up to 4 hr after exposure ended. Additional urine samples were collected at 2-hr intervals for the rest of the day; four 8-hr samples were collected during the next 2 days. Urine samples were analyzed for EAA using the method of Groeseneken et al. [1986a]. During both experimental protocols, the rate of excretion of EAA increased and continued to do so to a maximum level 3 to 4 hr after the end of the exposure period. After attaining the maximum level, a slow decrease began with a biological half-life between 21 and 24 hr. In both the resting condition under increasing EGEE concentrations and during physical exercise at a constant EGEE concentration, the rate of urinary excretion of EAA increased ($P<0.001$ and $P<0.005$, respectively) and appeared to be related to the rate of uptake of EGEE. The rate of uptake increased under both experimental conditions in a statistically significant manner ($P<0.001$).

The total amount of EAA excreted within 42 hr was significantly related ($P<0.001$) to the EGEE concentration in inspired air, uptake rate, pulmonary ventilation rate, oxygen consumption during exposure, and heart rate during and after exposure. EAA was negatively related ($P<0.05$) to height, body weight, and lean body mass. Multiple linear regression analysis revealed that only the relations between EAA excretion and EGEE uptake rate ($P<0.001$), heart rate ($P<0.001$), oxygen consumption during exposure ($P<0.05$), and height ($P<0.001$) were significant. Respiratory frequency was a contributing factor to EAA excretion. On the average, 23% of inhaled EGEE was recovered as EAA within 42 hr in both experiments at rest and during physical work. The authors concluded that good correlations between EAA excretion and EGEE uptake were found following the exposure period. Although biological monitoring of exposed workers is usually based on urinary metabolite levels in samples taken immediately after the end of a workshift, a maximal excretion rate of EAA may be reached several hours later. In addition, as a result of the long biological half-life of EAA, EAA will not be cleared from the urine the next morning, and accumulation can be expected through repetitive exposures [Groeseneken et al. 1986c].

The urinary excretion of EAA was studied in a group of five female silk-screen printing operators during repeated daily inhalation exposure to a mixture of EGEE and EGEEA [Veulemans et al. 1987]. The subjects worked in weekly, alternating morning and evening shifts. They agreed to wear rubber gloves at all times to avoid occasional skin contact with inks and thinners. Air and urine samples were collected each day during 5 days of normal production and 7 days after a 12-day production stop. Urine samples were collected immediately before and after work; air samples were collected as individual half-shift samples. Urinary EAA excretion increased during the workweek, and elimination proved to be far from complete over the weekends. In the last observation period, urinary EAA values on Monday morning still attained about half the urinary concentration on Friday evening (30 mg EAA/g creatinine versus 64 mg EAA/g creatinine). Even after a prolonged nonexposure period of 12 days, traces of the metabolite (1.2 to 2.6 mg EAA/g creatinine)

were still detectable. On a number of days, the preshift EAA concentrations were even higher than the immediate postshift values on the preceding and same day. For this reason, and because of the more constant exposure profiles, an estimation of the maximum EAA levels after prolonged daily exposure was made on the basis of the results of the first exposure period. A linear correlation (r=0.92) was found between average exposure to EGEE and EGEEA over the 5 exposure days and EAA excretion at the end of the 5-day workweek. EAA estimate of 150 mg ± 35 mg/g creatinine was found to correspond with repeated 5-day full-shift exposures to 5 ppm of EGEE or 5 ppm of EGEEA.

Groeseneken et al. [1988] compared urinary EAA excretion in man and the rat after experimental exposure to EGEE. The human data were drawn from the previously mentioned inhalation study [Groeseneken et al. 1986c] in which five subjects had been exposed to 2.7 ppm, 5.4 ppm, or 10.8 ppm EGEE. Urine samples collected at short intervals were pooled into 12-hr groups for comparison with rat data. Groups of five male Wistar rats were treated by oral intubation with 0.5, 1, 5, 10, 50, or 100 mg EGEE/kg. Rat urine samples were collected before EGEE exposure and then at 12-hr intervals up to 60 hr after the dosing. The maximal excretion rate of EAA in human and rat urine was found within 12 hr after exposure or dosing. Afterwards, the decline of urinary EAA was much slower in man than in the rat. In man, the half-life of EAA was on average 42.0 ± 4.7 hr, a longer half-life than reported in the original study (21 to 24 hr) [Groeseneken et al. 1986c]. The author attributes the longer half-life to the use of 12-hr pooled urine specimens in this study [Groeseneken et al. 1988] rather than to specimens collected at 1- to 2-hr intervals in the previous study [Groeseneken et al. 1986c]. In the previous study, half-lives were calculated from the peak exposure time (8 hr after the start of exposure). Examination of the excretion curves from the previous paper showed that elimination between 8 and 12 hr was more rapid than at longer time intervals, leading to a shorter calculated elimination half-life [Groeseneken et al. 1986c]. The authors concluded that the longer elimination half-life was more consistent with half-lives seen in occupational exposure, which were as high as 48 hr [Groeseneken et al. 1988; Veulemans et al. 1987]. The recovery of EAA in human urine after 48 hr averaged 23%. Based on the half-life of EAA elimination of 42 hr, the authors estimated total recovery of EAA as 30% to 35% of the absorbed dose.

In the rat, the half-life of EAA was 7.20 ± 1.54 hr. On the average, 27.6% ± 6.1% of urinary EAA in rats was present as the glycine conjugate, with the extent of conjugation being independent of the dose. The extent of conjugation demonstrated a diurnal variation; the lowest extent of conjugation was found during the night. EAA glycine conjugates were absent in human urine. In man, the recovery of EAA was higher than in the rat for equivalent low doses of EGEE (0.5 and 1 mg/kg). When urinary excretion data for the lower dose range were normalized for body weight in both species, rats excreted EAA at a higher rate than did man for equivalent doses. The authors concluded, that although nonlinear kinetics had been observed in some animal studies at high doses, the elimination kinetics seen at low doses in this study were not dose-dependent in either rats or humans [Groeseneken et al. 1988].

Groeseneken et al. [1987a] studied the pulmonary absorption and elimination of EGEEA in 10 male subjects under various conditions of exposure and physical workload; subjects were

assigned into two groups, 5 per group. Exposures were by mask for 50 min/hr. Experimental conditions are presented in Table B-2.

Table B-2.—Experimental conditions for Groeseneken et al. [1987a]

Group	Exposure concentration		Workload
	ppm	mg/m^3	(W)
I	2.6	14	0
	5.2	28	0
	9.3	50	0
II	5.2	28	0
	5.2	28	30
	5.2	28	60

All subjects performed three experiments according to their group assignment. The subjects remained unexposed for at least 1 wk between experimental sessions. The pharmacokinetics of respiratory uptake are more complicated for EGEEA than for EGEE. Retention, atmospheric clearance, and uptake rate decreased with time and reached steady-state levels at 3 to 4 hr; retention increased with exposure and workload. Retention at steady state for the three exposure concentrations was 53%, 57%, and 62%, respectively. Although retention of EGEEA increased proportional to the workload, no further increase was noticed for EGEE after 30 w [Groeseneken 1986b]. Individual uptake of EGEEA was determined by pulmonary ventilation, cardiac output, height, and fat content, whereas uptake of EGEE seemed mainly determined by the cardiopulmonary transport parameters alone [Groeseneken 1986b]. The hypothesis that EGEEA is first converted to EGEE by plasma esterases was confirmed by the observation of partial respiratory elimination of EGEE. The amount of EGEE expired during steady state conditions correlated with the uptake rate of EGEEA rather than with EGEEA exposure concentrations. Respiratory elimination of unmetabolized EGEEA accounted for ≤0.5% of total body uptake. This slow decrease could be represented as a regression equation with two exponential terms, indicating at least two pharmacologic compartments were involved. The author speculated that the complex pulmonary kinetics may be due to metabolic competition in the conversion of EGEEA to EGEE and to possible redistribution into the fat soluble compartment.

Groeseneken et al. [1987b] examined urinary EAA excretion in the experimental groups exposed to EGEEA in the previous study [Groeseneken et al. 1987a]. Urine samples were taken at the beginning of the exposure period and at every hour until the fourth hour after exposure. Then three 2-hr samples followed by four 8-hr samples were collected. Urine samples were analyzed for EAA by the method of Groeseneken et al. [1986a]. EAA levels appeared with a half-life of 2.3 ± 0.1 hr during the 4-hr EGEEA exposure period. Maximal EAA excretion rate was attained 3 to 4 hr after the exposure period. The half-life was

23.6 ± 1.8 hr. However, 3 hr after the first peak EAA excretion, a second maximum excretion of EAA was observed; this second peak was especially pronounced after exposure during physical exercise. Redistribution of EGEEA, or EAA, or both from a peripheral to central compartment could explain this phenomenon. Urinary EAA excretion was dependent on the EGEEA uptake rate, as a consequence of higher exposure ($P<0.001$), and on the uptake rate of EGEEA at constant exposure, as a consequence of physical workload ($P<0.001$). On average, 22.2% ± 0.9% of the absorbed EGEEA was metabolized and excreted in the urine as EAA within 42 hr. The total excretion of EAA in 42 hr was related both to total uptake from increasing concentrations of EGEEA ($P<0.001$) and to total uptake, at constant exposure, with increasing workload ($P<0.001$). Total EAA excretion was correlated to EGEEA concentration, uptake rate, and transport mechanisms (pulmonary ventilation, oxygen consumption, respiratory rate, etc.). In addition, EAA excretion was correlated to body fat ($r=0.40$, $P<0.001$). Groeseneken et al. [1987b] concluded that the metabolism of EGEEA proceeded through EGEE via esterases and then continued through the same excretion pathway as EGEE. Indeed, the kinetics of EAA excretion after exposure to EGEEA were very similar to those found after exposure to EGEE [Groeseneken et al. 1987a].

Workers from a shipyard painting operation who applied paint containing EGEE were evaluated for EGEE exposure [Lowry 1987]. Work conditions and practices varied considerably between brush and spray painters. Some workers were in confined spaces below deck, whereas others were in the open. The study was conducted in the winter, and the temperatures varied greatly depending on the painters' work areas. Information on work practices such as the number of hours spent painting, the type of paint used, the work area locations, and the use of personal protective equipment was gathered from questionnaires.

Environmental breathing zone samples were collected for each worker for 3 days. Urine samples were collected every day for 1 wk, at the beginning and end of each workday, and EAA levels were measured. A wide range of EAA levels was noted in workers using EGEE-containing paints; this was probably due to variation in work assignments, work areas, and use of personal protective equipment. This study has not gone through extensive evaluation to determine the importance of the many variables on the levels of EAA in urine. The author could only conclude that there appeared to be a relationship between urinary EAA excretion and the use of paints containing EGEE [Lowry 1987].

Clapp et al. [1987] investigated EGEE exposure of workers engaged in casting precision metal parts. The 8-hr TWAs of EGEE ranged from nondetectable to 23.8 ppm. EGEE was not detected in any of the blood samples from the EGEE-exposed workers, but exposed workers were found to have measurable levels of EAA in urine (163 mg/g creatinine). EAA was not detected in the urine of unexposed control subjects.

B.4.2 EGME and EGMEA

EGME has been shown to be a possible substrate for alcohol dehydrogenase (ADH) [Tsai 1968; Blair and Vallee 1966], and thus oxidation of EGME via ADH and aldehyde dehydrogenase to methoxyacetic acid (MAA) is a potential route of metabolism [Miller et al. 1982].

The toxicity of MAA will be discussed next to evaluate the importance of metabolism as a detoxification or bioactivation mechanism for EGME. In a study by Miller et al. [1982], groups of five male F344 rats were given daily doses of 0, 30, 100, or 300 mg MAA/kg per day orally on 8 days out of 10 and were then sacrificed 24 hr after the final dose. Rats given the high dose had significantly lower body weights on the fifth day and again when recorded on the tenth day ($P<0.05$). Absolute and relative weights of spleen, thymus, and testes were also significantly reduced in the 300 mg MAA/kg per day exposure group ($P<0.05$). In the 100 mg MAA/kg per day exposure group, relative thymus weight was significantly reduced ($P<0.05$). Hematology revealed significantly lower RBC, Hb concentration, MCV, and WBC in the group dosed with 300 mg MAA/kg per day ($P<0.05$). Significant but less pronounced reductions in RBC, Hb, and MCV were seen in those receiving 100 mg MAA/kg per day ($P<0.05$). Testicular atrophy and a decrease in the size of the thymus were seen in the 300 mg MAA/kg per day group; thymus size was decreased in the 100 mg MAA/kg per day group also. Histological evaluation revealed diffuse, severe depletion of cortical lymphoid elements in the thymus of all rats treated with 300 mg MAA/kg per day and a slight reduction in the same cell population in all rats treated with 100 mg MAA/kg per day. All rats treated with 300 mg MAA/kg per day had severe degenerative changes in the germinal epithelium of the seminiferous tubules, and slight degenerative changes were observed in the rats treated with 100 mg MAA/kg per day [Miller et al. 1982].

Miller et al. [1983b] used radiolabeled EGME to isolate and identify urinary metabolites in rats. Groups of three male F-344 rats were given a single oral dose of approximately 76.1 mg/kg or 660 mg/kg of ^{14}C EGME; animals were sacrificed 48 hr after dosing. Urine was the major route of elimination (50% to 60% of ^{14}C) at both dose levels, with approximately 18% and 12% of the radioactivity remaining in the carcasses of low- and high-dose animals, respectively. Target organs of EGME such as testes, thymus, and spleen did not show an accumulation of EGME or its metabolites. Blood had the greatest amount of ^{14}C per gram of tissue at 48 hr postexposure. The profile of radioactivity in a composite sample of urine collected during the 0 to 12 hr interval for both high- and low-dose groups was very similar. The majority of the ^{14}C (83% to 95%) was found in one major peak identified as MAA. The authors [Miller et al. 1983b] proposed that oxidation to MAA is a major route for elimination of EGME and occurs via ADH to methoxyaldehyde and, thereafter, via aldehyde dehydrogenase to MAA.

Foster et. al. [1983] exposed six male Sprague-Dawley rats orally to 592 mg MAA/kg per day (equimolar to 500 mg EGME/kg per day) for 4 days. Significant decreases in actual and relative liver and testes weights were seen ($P<0.01$). The severity and nature of testicular changes were essentially similar to those of the corresponding dosage of EGME given for the same period [Foster et al. 1983].

Foster et al. [1987] exposed groups of six male Alpk/AP (Wistar-derived) rats to a single oral dose of MAA to determine the initial target for testicular toxicity. Dose levels were administered equimolar with 100, 250, or 500 mg EGME/kg (i.e., 118, 296, or 592 mg MAA/kg). Rats were sacrificed at 1, 2, 4, and 14 days post-treatment. A significant decrease in testes weight relative to body weight was seen only in the high dose MAA group at days

4 and 14 ($P<0.05$). Histological examination of testes revealed damage 24 hr after dosing in all groups treated with MAA. Pyknosis and nuclear condensation were seen in late pachytene, diplotene, diakinetic, and secondary spermatocytes in high-dose rats at 24 hr. At 2 days, these effects were more extensive and included the loss of early and late pachytene, diplotene, and secondary spermatocytes; some signs of degeneration to zygotene spermatocytes (precursor cells to pachytene spermatocytes); and partial loss of the succeeding generation of early round spermatids. By day 4, the lesion had progressed to midpachytene spermatocytes, although by day 14, resolution of the damage had begun.

Degeneration seen in the mid-dose MAA-exposed rats included fewer stages of spermatogenesis with some loss of affected cells and early round spermatids at day 2. Low-dose MAA-exposed rats demonstrated minimal effects in diplotene secondary spermatocytes and early pachytene spermatocytes [Foster et al. 1987].

Wistar-Porton rats were given single injections of 224 mg MAA/kg on g.d. 8, 10, 12, or 14, and then sacrificed on g.d. 20 to study the teratogenicity of MAA [Brown et al. 1984]. Embryotoxicity was indicated by increased embryo-fetal death, structural malformations, and decreased fetal weight (no statistical treatment given). Embryo-fetal mortality was greatest after MAA administration on g.d. 8 (93%), decreasing to 3% for g.d. 14. The highest incidence of fetal malformations followed exposure on g.d. 12, although malformations were induced on each day of treatment. Defects included skeletal malformations, hydrocephalus, and urogenital abnormalities [Brown et al. 1984].

The teratogenicity of MAA compared with that of EGME was further studied in pregnant Wistar rats (six, seven, or eight per group). The rats received single oral doses of 186 or 373 mg MAA/kg or 158 or 315 mg EGME/kg on g.d. 12 [Ritter et al. 1985]. Pregnancy was terminated on g.d. 20, and total embryotoxicity was calculated as the sum of dead, resorbed, and malformed fetuses as percent of total implantations. MAA demonstrated a dose response at 186 mg MAA/kg (58% total embryotoxicity) and at 373 mg MAA/kg (99%); these responses were not significantly different from that produced by EGME doses of 158 (54%) and 315 mg EGME/kg (100%). The authors noted that between 80% and 96% of the total defects found in any of the treatment groups were classified as hydronephrosis and heart, tail, and limb defects. These defects included dilated ductus arteriosus, dilated aortic arch, and ventral polydactyly; the authors report these are rarely seen with any other teratogenic agent.

The role of metabolism in EGME-induced testicular toxicity was investigated by Moss et al. [1985] using groups of nine male Sprague-Dawley rats pretreated i.p. with 400 mg pyrazole/kg, an alcohol dehydrogenase (ADH) inhibitor, or pretreated with 300 mg disulfiram/kg, an aldehyde dehydrogenase inhibitor. One hr after pretreatment with pyrazole or 24 hr after pretreatment with disulfiram, animals were injected i.p. with 250 mg of labeled ^{14}C EGME/kg. Controls with no pretreatment also received 250 mg(^{14}C) EGME/kg i.p. Urinary excretion was the major route of elimination of EGME metabolites after 24 hr (40.4% $\pm$ 3.6% of dose) and 48 hr (14.8% $\pm$ 0.6% of dose) in controls. High performance liquid chromatography (HPLC) analysis identified MAA as the major urinary metabolite at 0 to 24 hr (63% of the radioactivity) and at 24 to 48 hr (50% of the radioactivity). The

second major urinary metabolite was identified as methoxyacetylglycine (approximately 20% of radioactivity). Analysis of radioactivity in the plasma demonstrated rapid disappearance (t1/2 = 0.56 hr) of EGME between 0 and 4 hr after dosing with a corresponding appearance of MAA. Radioactivity clearance from plasma (t1/2) was estimated to be 19.7 hr.

In the rats pretreated with pyrazole, the metabolism of EGME to MAA was inhibited. Analysis of radioactivity in plasma showed a slower disappearance of EGME (t1/2 = 42.6 $\pm$ 5.6 hr) and radioactivity clearance from plasma (t1/2 = 51.0 $\pm$ 7.8 hr) than in the controls. The percentage of the dose found in urine after 24 hr (9.8% $\pm$ 2.4%) and 48 hr (7.9% $\pm$ 2.2%) showed urinary excretion not to be the major route of elimination. MAA was not a major urinary component, and methoxyacetylglycine was not found in urine from these rats. Pretreatment with the aldehyde dehydrogenase inhibitor disulfiram had no significant effect on plasma or urinary metabolic profiles. Administration of EGME by i.p. injection demonstrated extensive degeneration and necrosis of rat primary spermatocytes in the early and late pachytene stages of development. Pretreatment with pyrazole appeared to protect against spermatocyte damage, whereas pretreatment with disulfiram had no effect on the degree of spermatocyte damage observed [Moss et al. 1985].

Ritter et al. [1985] investigated the effect of an ADH inhibitor, 4-methylpyrazole (4-MP), on EGME-induced teratogenicity in pregnant Wistar rats. Groups of seven animals were administered 315 mg EGME/kg i.p. on g.d. 12 with or without a concurrent dose of 100 mg 4-MP/kg. Pregnancy was terminated on g.d. 20. Coadministration of EGME and 4-MP resulted in significantly decreased total embryotoxicity: 16.8% compared with 100% with EGME alone (P<0.05). Ritter et al. [1985] stated that 4-MP inhibits ADH and might interfere with the metabolism of EGME to MAA and consequently prevent the embryotoxicity or teratogenicity dependent on the production of MAA.

Sleet et al. [1988] conducted a series of experiments on the role of EGME metabolism in the induction of paw malformations in CD-1 mice. Pregnant dams were dosed on g.d. 11 and sacrificed on g.d. 18. A comparison was made of dose-dependent digit anomalies produced by oral exposure to a single dose of EGME (99 to 463 mg/kg) or of MAA (99 to 693 mg/kg). MAA and EGME were equipotent in producing digit anomalies (syndactyly, oligodactyly, and polydactyly) as expressed on the basis of percent of litters affected and percent of fetuses affected. Fetal body weights and incidence of resorbed implants were not significantly different between EGME- and MAA-exposed animals.

The effects of gavage and i.v. injection on the teratogenicity of MAA were also compared by Sleet et al. [1988]. MAA was administered to pregnant mice on g.d. 11 by gavage or tail vein injection at doses of 261 mg/kg or 342 mg/kg. The incidence of digit malformations in both i.v. groups was statistically lower than that of the corresponding gavage group (P<0.05). These results are expected since gavage and i.v. routes of administration differ with respect to the metabolic fate of MAA [Sleet et al. 1988].

Sleet et al. [1988] administered EGME (251 or 350 mg/kg) orally to pregnant CD-1 mice on g.d. 11 in combination with ethanol (2,901 mg/kg) to investigate competition for

oxidation by ADH to teratogenic metabolites of EGME. Three experimental conditions were used: (1) ethanol concomitantly with EGME (251 and 350 mg/kg) and again 5 hr and 10 hr later; (2) ethanol 5 hr and 10 hr after 251 mg EGME/kg; and (3) single doses of ethanol concomitantly with 251 mg EGME/kg or 5 hr later. Single and multiple dosings of ethanol attenuated EGME teratogenicity as expressed by digit malformations. Experimental condition No. 1 provided the greatest reductions in incidences of total anomalies ($P \leq 0.05$) when compared with controls exposed to EGME only. At 251 and 350 mg EGME/kg, ethanol administration decreased the incidence from 47% to 6% and 94% to 36%, respectively. The single dose of ethanol at 0 hr (condition No. 3) significantly decreased, from 47% to 23%, the occurrence of digit anomalies caused by 251 mg/kg of EGME ($P \leq 0.05$). Reduction of paw malformations decreased when ethanol was administered following EGME exposure. Condition No. 2 lowered paw malformations from 47% to 35% ($P \leq 0.05$), but condition No. 3 of a single dose of ethanol 5 hr after EGME treatment had no protective effect.

Levels of EGME, MAA, and (^{14}C) in maternal and conceptus compartments were quantitated by isotope dilution analysis for up to 6 hr after oral administration of (^{14}C) EGME (251 mg/kg) and ethanol (2,901 mg/kg) [Sleet et al. 1987]. HPLC measurements demonstrated that ethanol caused only a transient delay in EGME metabolism to MAA and embryonal accumulation of ^{14}C MAA. Using (^{14}C) EGME only, approximately 90% of the radioactivity in the maternal plasma and in the embryo was (^{14}C) MAA at 1 hr and 100% at 6 hr after treatment. Additionally, the (^{14}C) level of the embryo was greater than that of maternal blood at both times. Concomitant dosing with ethanol reduced the proportion of (^{14}C) MAA in maternal plasma and embryo. At 1 hr, MAA represented 17% of radioactivity in both compartments, and at 2 hr, it increased to 40% in plasma and 32% in the embryo. At 3 hr, plasma levels of EGME and MAA were equivalent to the 1-hr levels following EGME administration alone; 80% of the total radioactivity in the embryo was MAA.

In light of the previous results, Sleet et al. [1988] investigated the possibility that further metabolism of MAA was necessary for expression of embryotoxicity by coadministering metabolic intermediates common to alcohol oxidation with EGME (251 mg or 350 mg/kg) on g.d. 11 and sacrificing the dams on g.d. 18. The incidence of paw malformations induced by EGME at either dosage was significantly lowered ($P<0.05$) by coadministration of sodium acetate (43 mmol/kg), formic acid (4.3 mmol/kg), or glycine (43 mmol/kg). The authors concluded that the marked decline in the incidence of digit malformations demonstrated that EGME teratogenicity is dependent on events subsequent to the formation of MAA [Sleet et al. 1988].

Yonemoto et al. [1984] used an in vitro culture system [New 1978] to determine the effects of EGME and MAA on the development of post-implantation rat embryos. On g.d. 9, conceptuses were removed from the dams (Wistar-Porton strain) and placed in pairs in bottles that contained 3 mL of heat-inactivated male rat serum and 1 mL of test compound. Ten to 15 conceptuses per group were cultured for 48 hr in 381 mg EGME or in 90, 180, 270, or 450 mg MAA and then examined under a stereoscopic dissecting microscope. EGME had no significant effects on embryonic growth and development when compared with that of the controls. MAA, however, produced statistically significant reductions in morphological development, crown-rump length, head length, number of somites, and yolk

sac diameter when compared with those of the controls ($P<0.001$). These effects demonstrated a dose response, as all were seen at 450 mg MAA; all but yolk sac diameters were affected at 270 mg, and only head length and morphological development were affected at 180 mg. No significant effects were seen at 90 mg MAA. The predominant abnormalities seen in affected conceptuses were irregular fusion of the neural tube (wavy or open neural suture line) and irregular segmentation of the somites. The authors [Yonemoto et al. 1984] concluded that the data demonstrated that MAA or its metabolites are the proximal toxins in vivo and that, at the organogenesis stage, the rat fetus in vitro lacks alcohol dehydrogenase activity.

The in vitro culture system used by Yonemoto et al. [1984] was used by Rawlings et al. [1985] to study the mechanism of teratogenicity of EGME. Conceptuses were explanted from pregnant Wistar-Porton rats at embryonic age 9.5 days and cultured for 48 hr with 2 or 5 mM MAA. 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. MAA at the 5 mM concentration had an adverse effect on fetal development. MAA-exposed embryos had statistically significant reductions ($P<0.01$) in morphological score, crown-rump length, head length, and yolk sac diameter compared with those of the controls. MAA also produced statistically significant reductions ($P<0.05$) in the protein content of the embryo. No statistically significant reductions in growth parameters were seen at the 2 mM level. However, irregularity of the neural suture line was seen in 100% of the MAA-exposed embryos. Other abnormalities observed in the MAA-exposed groups included abnormal otic and somite development, turning failure, open cranial folds, and abnormal yolk sac [Rawlings et al. 1985].

As has been demonstrated, the induction of paw malformations following in utero [Brown et al. 1984; Ritter et al. 1985] as well as in vitro [Yonemoto et al. 1984] exposure to EGME appears to depend on the oxidation of EGME to MAA. Sleet et al. [1988] investigated the relationship between the induction of paw malformations and the disposition of EGME in the maternal and embryonal compartments. Pregnant CD-1 mice were dosed by gavage on g.d. 11 with either EGME (1.3 to 6.6 mmol/kg, 100 to 500 mg/kg, or 5.2 µl/g) or MAA (1.1 to 7.7 mmol/kg, 100 to 693 mg/kg, or 4.9 µl/g) and were sacrificed on g.d. 18. Fetuses were delivered by laparotomy and weighed before external examination for paw defects. The embryotoxic potencies of EGME and MAA were determined by comparing the dose-dependent incidence of digit anomalies. EGME and MAA were equipotent in causing digit malformations. The ADH inhibitor 4-methylpyrazole administered orally 1 hr before EGME reduced the incidence of malformations 60% to 100%, depending on the dosing regimen. Oxidation of EGME to MAA was nearly complete after 1 hr when approximately 90% of (^{14}C) in maternal compartment and conceptus coeluted with authentic (^{14}C)-MAA on HPLC. Embryonic (^{14}C)-MAA levels were 1.2 times those in plasma 1 hr and 6 hr after dosing; by 6 hr, however, concentrations in the embryo had declined to approximately 50% of 1-hr values. Dams treated i.v. with (^{14}C) MAA had higher (^{14}C) blood levels than did dams treated orally, but the offspring of the former had fewer digit malformations. The authors concluded that peak and steady-state plasma levels of MAA, as well as embryonic MAA levels, do not appear to determine the embryotoxic outcome whereas further metabolism of MAA does [Sleet et al. 1988].

EGME uptake and urinary MAA excretion were examined in seven male subjects exposed at rest to 5.1 ppm EGME (16 mg/m^3) by mask for four 50-min periods [Groeseneken et al. 1989a]. There was a short 10-min break at the end of each 50-min period to allow for urine collection. Urine samples were collected immediately before the beginning of the experiment and at hourly intervals until the fourth hour after exposure. Collections were taken until the morning of the fifth day after the exposure period (four 2-hr collections, one 8-hr collection, and eight 12-hr collections). Urinary MAA was then measured by the method of Groeseneken et al. [1989b]. Retention of EGME was 76% during the 4-hr exposure period. The uptake rate showed no significant variation because of constant pulmonary ventilation and a fixed exposure concentration. On average, 19.4 $\pm$ 2.1 mg EGME was inhaled during the 4-hr exposure period. MAA was detected in the urine during and up to 120 hr after exposure. The elimination half-life averaged 77.1 $\pm$ 9.5 hr. On average, 54.9% $\pm$ 4.5% of inhaled EGME was excreted within 120 hr of the start of exposure; half of this amount was excreted within 48 hr. By extrapolation, the total amount of MAA was estimated at 85.5% $\pm$ 4.9% of inhaled EGME.