

Developmental Toxicity of Four Glycol Ethers Applied Cutaneously to Rats

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Previous NIOSH studies demonstrated the embryo- and fetotoxicity and teratogenicity of ethylene glycol monoethyl ether (EGEE) applied to the shaved skin of pregnant rats. In the present study ethylene glycol monoethyl ether acetate (EGEEA), ethylene glycol monobutyl ether (EGBE), and diethylene glycol monoethyl ether (diEGEE) were tested in the same experimental model, using distilled water as the negative control and EGEE as a positive control. Water or undiluted glycols were applied four times daily on days 7 to 16 of gestation to the shaved interscapular skin with an automatic pipetter. Volumes of EGEE (0.25 mL), EGEEA (0.35 mL), and diEGEE (0.35 mL) were approximately equimolar (2.6 mmole per treatment). EGBE at 0.35 mL four times daily (approximately 2.7 mmole per treatment) killed 10 of 11 treated rats, and was subsequently tested at 0.12 mL (0.9 mmole) per treatment. EGEE- and EGEEA-treated rats showed a reduction in body weight relative to water controls that was associated with completely resorbed litters and significantly fewer live fetuses per litter. Fetal body weights were also significantly reduced in those groups. Visceral malformations and skeletal variations were significantly increased in EGEE and EGEEA groups over the negative control group. No embryotoxic, fetotoxic, or teratogenic effects were detected in the EGBE- or diEGEE-treated litters.

Introduction

The alkyl ether derivatives of ethylene and diethylene glycol are an important class of solvents with numerous applications in consumer products as well as in industrial processes. Several members of this chemical family have recently been shown to be potent reproductive toxins in several animal species, as reviewed in detail elsewhere (1,2). Teratogenicity has been reported for ethylene glycol monomethyl ether (EGME) in mice (3) and rats (4); for ethylene glycol dimethyl ether (EGdiME) in mice (5); for ethylene glycol monoethyl ether (EGEE) in rabbits (6) and rats (6-8); and for ethylene glycol monoethyl ether acetate (EGEEA) in rats (4). Evidence of testicular toxicity has been reported for EGME in mice (9-11), rats (10-12), and rabbits (12); for ethylene glycol monomethyl ether acetate (EGMEA) in mice (9); for EGEE in mice (9), rats (8), and dogs (8); for EGEEA in mice (9); and for diethylene glycol dimethyl ether (diEGdiME) in mice and rats (13). In contrast, ethylene glycol monobutyl ether (EGBE) and diethylene glycol monoethyl ether (diEGEE) both have been tested once by inhalation exposure for teratogenicity (4) with negative results. EGBE and ethylene glycol monophenyl ether (EGPhE)

have been tested for testicular toxicity with negative results (9).

The glycol ethers do not have especially high vapor pressures (14), but they penetrate the skin readily and are commonly used in applications that are conducive to skin contact. Hardin et al. (7) applied undiluted EGEE to the skin of pregnant rats to investigate its potential for developmental toxicity by this route of exposure. When 0.5 mL was applied four times daily on days 7 to 16 of gestation, all pregnant females had completely resorbed litters. Treatment with 0.25 mL on the same schedule induced significant embryotoxic, fetotoxic, and teratogenic effects (7). The cardiovascular system was the primary target for visceral malformations, as had been previously reported in rats (4,6) and rabbits (6) exposed to EGEE by inhalation. The present study was conducted to evaluate the reproductive and developmental toxicity of three other alkyl glycol ethers in the same cutaneous exposure model.

Methods and Materials

Purified glycol ethers were purchased from Fisher Scientific: EGEE, Catalog No. E-180, Lot 70107; EGEEA, Catalog No. E-181, Lot 794501; EGBE, Catalog No. E-179, Lot 703549; and diEGEE, Catalog No. D-51, Lot 792796. Time-mated primigravida SPF Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, MA. Rats were shipped on day 4 and received on day 5 of gestation

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(sperm = day 1), when they were weighed and marked for individual identification by toe clipping. The interscapular region was shaved with electric clippers on day 5 or 6, and rats were formally randomized by weight to one of five groups. All rats were singly housed throughout the study in suspended stainless steel cages in a ventilated cage rack (Hazelton Systems, Aberdeen, MD) with free access to food and water. These cages are designed such that room air was drawn through the wire mesh floors of each cage and into an overhead plenum from which the exhaust air was ducted directly to a fume hood. Light was on a 12-hr light-dark cycle.

Rats were received in two separate shipments in consecutive weeks and the experiment was conducted in two replicates. Treatment volumes were selected based on results of the earlier study of the teratogenicity of EGEE when applied to the skin of rats (7). A volume of EGEE (0.25 mL four times daily) that was teratogenic in that study was used as the positive control. Approximately equimolar volumes (0.35 mL four times daily, for a total daily dose of 1.4 mL) of EGEEA, EGBE, and diEGEE were employed initially. Because the rats treated with 0.35 mL of EGBE showed marked toxic effects, the treatment volume was reduced in the second replicate to 0.12 mL EGBE four times daily. Undiluted glycols or water (control) were applied to the shaved interscapular region using an automatic pipetter (Finnpipette) four times daily at 2.5-hr intervals on days 7 to 16 of gestation.

In addition to the randomization body weights (day 5 of gestation), body weights were recorded on the first and sixth days of treatment and on the first and fifth post-treatment days (gestation days 7, 12, 17 and 21, respectively). On day 21, females were killed by CO₂ asphyxiation and gravid uterus weights were recorded. Fetuses were weighed and examined for external

malformations. Alternate fetuses were preserved in Bouin's fluid or 70% ethanol for subsequent visceral examination by the Wilson (15) method or skeletal examination according to Staples and Schnell (16). Uteri from apparently nonpregnant females were placed in 10% sodium sulfide to reveal early termination of pregnancy.

All maternal and fetal body weights, maternal organ weights were analyzed by analysis of variance techniques. If significant ($p < 0.05$) differences between groups were detected, *a posteriori* Waller-Duncan *K*-ratio *t*-tests were conducted to describe in more detail the source of variation. Implantation data (total and numbers of live and dead) were analyzed using the Kruskal-Wallis test. Frequencies of malformations and of variations were analyzed by Fisher-Irwin exact test of the numbers of affected litters. Because repeated contrasts were made with the water control group, the level of significance was set at $p < 0.01$. All procedures were carried out using SAS 82.3 and BMDP statistical packages on the Parklawn Computer Center, Rockville, MD.

Results

Toxic signs were not noted in EGEE-, EGEEA-, or diEGEE-treated rats. However, red-stained urine was observed after only two treatments with 0.35 mL EGBE. By the end of the first treatment day, EGBE-treated rats produced burgundy-colored urine. Continued treatment with EGBE led to ataxia progressing to moderate to marked inactivity and rough haircoats with dark stains around the muzzle and ano-genital area. Several rats' tails blackened distally and were gradually eaten away as the apparent necrosis progressed. Deaths occurred on the third to seventh days of treatment (days

Table 1. Gestational body weights (mean \pm SD) of pregnant rats in various treatment groups.

| | Treatment group | | | | |
|--|----------------------------|--------------------------|--------------------------------|---------------------------|---------------------------|
| | EGEE (0.25 mL) | EGEEA (0.35 mL) | EGBE ^a (0.12 mL) | diEGEE (0.35 mL) | Water (control) |
| Group size | 18 | 18 | 9 | 13 | 17 |
| Body weight, g | | | | | |
| Day 5 | 188 \pm 8 | 192 \pm 9 | 192 \pm 10 | 193 \pm 7 | 189 \pm 7 |
| Day 7 | 210 \pm 7 | 212 \pm 12 | 219 \pm 12 | 213 \pm 10 | 210 \pm 7 |
| Day 12 | 239 \pm 10 | 240 \pm 14 | 239 \pm 13 | 242 \pm 12 | 246 \pm 11 |
| Day 17 | 262 \pm 15 | 256 \pm 17 | 268 \pm 16 | 269 \pm 17 | 278 \pm 14 |
| Day 21 | 284 \pm 32 | 268 \pm 29 | 336 \pm 30 | 323 \pm 31 | 331 \pm 22 |
| Body weight gain, g | | | | | |
| Day 5-7 | 22 \pm 8 | 20 \pm 7 | 27 \pm 6 | 19 \pm 8 | 21 \pm 5 |
| Day 5-12 [*] | 51 \pm 9 ^{x,y} | 48 \pm 9 ^y | 48 \pm 12 ^y | 49 \pm 7 ^{x,y} | 57 \pm 10 ^x |
| Day 5-17 [†] | 74 \pm 13 ^y | 65 \pm 12 ^z | 77 \pm 18 ^y | 76 \pm 13 ^y | 89 \pm 11 ^x |
| Day 5-21 [†] | 97 \pm 29 ^y | 76 \pm 27 ^z | 144 \pm 31 ^x | 130 \pm 27 ^x | 142 \pm 19 ^x |
| Gravid uterus weight, g [†] | 23 \pm 21 ^y | 9 \pm 12 ^z | 59 \pm 22 ^x | 65 \pm 20 ^x | 60 \pm 15 ^x |
| Extragestational body weight, g ^b | 261 \pm 18 | 259 \pm 24 | 276 \pm 18 | 258 \pm 18 | 271 \pm 15 |
| Extragestational body weight gain, g ^{c*} | 74 \pm 15 ^{x,y} | 67 \pm 20 ^y | 85 \pm 18 ^x | 65 \pm 16 ^y | 82 \pm 15 ^x |

^aReplicate 2 only. All other groups are combined data for replicates 1 and 2.

^bDay 21 body weight minus gravid uterus weight.

^cExtragestational body weight minus day 5 body weight.

^{*}Significant difference ($p < 0.05$). Groups with the same superscript do not differ from each other by *a posteriori* test.

[†]Significant difference ($p < 0.001$). Groups with the same superscript do not differ from each other by *a posteriori* test.

9–13 of gestation). In replicate 1, only one of 11 rats treated with 0.35 mL EGBE survived. In the second replicate, the EGBE treatment volume was reduced to 0.12 mL four times daily (0.48 mL EGBE/day) and no toxic signs were noted.

Body weights of pregnant females are summarized in Table 1, along with gravid uterus and extragestational body weight (day 21 body weight minus gravid uterus weight). Day 5 (randomization) body weights did not differ between replicates and body weight data were therefore combined across replicates for analysis. Analysis of variance confirmed that body weights on day 5 did not differ across treatment groups. Body weights on days 7, 12, 17, and 21 were analyzed by multivariate analysis of the change relative to day 5. Treatment with EGEE and EGEEA profoundly reduced maternal body weight gain, and at days 17 and 21 body weight gain in the EGEEA group was significantly lower than in the EGEE positive controls. Gravid uterus weights were also markedly reduced in those groups, accounting for much of the difference in body weight. Extragestational body weights did not differ significantly ($p < 0.10$), but extragestational body weight gain was significantly different across groups ($p < 0.05$). Extragestational weight gain in EGEEA and diEGEE groups was significantly less than in the water controls by *a posteriori* test.

Data in Table 2 reveal that, relative to the water controls, both EGEE and EGEEA treatments were strongly embryotoxic as reflected in significantly higher frequencies of completely resorbed litters and increased numbers of dead implants per litter. The number of dead implants per litter was also significantly higher in EGEEA than in the positive control EGEE-treated litters. Considering only litters with at least one live fetus, there was a significant reduction in the number of live fetuses per litter in the EGEE- and EGEEA-treated groups. Similarly, the body weights of live fetuses in these two groups were significantly reduced relative to the water-treated controls. No evidence of

embryo- or fetotoxicity was noted in the EGBE and diEGEE groups. On gross examination of fetuses, the only malformations noted were three fetuses from EGEE-treated litters with acaudia and imperforate anus (Table 2).

Discussion

The results of this study confirmed the previously reported embryo- and fetotoxicity and teratogenicity of EGEE applied to the skin of pregnant rats. As was previously reported, cardiovascular defects were the predominant visceral malformation. A wide spectrum of skeletal variants was also seen both in the present and the earlier skin teratology evaluation of EGEE. Vertebral malformations and reduced skeletal ossification also occurred with increased frequency in EGEE-treated litters but were not statistically significant. The only gross malformations noted in the study were three EGEE-treated fetuses with acaudia and imperforate anus, a defect also seen in the previous evaluation of cutaneously applied EGEE.

The spectrum and frequency of malformations and variations in the EGEEA-treated litters was remarkably similar to that described for the EGEE-treated group. Statistically significant increases in cardiovascular malformations and skeletal variations were detected. Similar responses to EGEE and EGEEA are not unexpected since EGEEA should be rapidly hydrolyzed *in vivo* to EGEE and acetate. At an equimolar dose, in fact, EGEEA in this study caused even more severe maternal, embryo, and fetal toxicity than did EGEE. This was reflected in significantly reduced body weight gain (Table 1) and increased dead implants per litter (Table 2) in EGEEA-treated litters relative to the EGEE litters.

Neither EGBE nor diEGEE was associated with embryo- or fetotoxicity, and the incidence of visceral and skeletal defects was not increased in either group.

Table 2. Observations at necropsy.

| | Treatment group | | | | |
|--|------------------------|-------------------------|--------------------------------|-------------------------|-------------------------|
| | EGEE (0.25 mL) | EGEEA (0.35 mL) | EGBE ^a (0.12 mL) | diEGEE (0.35 mL) | Water (control) |
| Group size | 18 | 18 | 9 | 13 | 17 |
| No. (%) litters totally resorbed | 7 (38.9) | 13 (72.2) | 0 | 0 | 0 |
| No. implants per litter ^b | 11.6 ± 2.9 | 11.7 ± 2.7 | 11.2 ± 4.3 | 11.8 ± 1.7 | 11.6 ± 2.5 |
| No. dead implants per litter ^{b†} | 7.7 ± 5.1 ^y | 10.6 ± 3.3 ^x | 1.2 ± 1.0 ^z | 1.2 ± 1.2 ^z | 1.2 ± 1.2 ^z |
| No. live fetuses per litter with live fetuses ^{b†} | 6.5 ± 4.1 ^y | 4.2 ± 3.4 ^y | 10.0 ± 3.8 ^x | 10.6 ± 2.3 ^x | 10.4 ± 2.6 ^x |
| (no. litters) | (11) | (5) | (9) | (13) | (17) |
| Fetal body weight, g ^{b†} | 3.0 ± 0.6 ^y | 2.6 ± 1.0 ^y | 4.2 ± 0.7 ^x | 3.9 ± 0.8 ^x | 3.9 ± 0.6 ^x |
| Gross malformations litters (fetuses) | 3(3) ^c | 0 | 0 | 0 | 0 |

^aReplicate 2 only. All other groups are combined data for replicates 1 and 2.

^bMean ± SD.

^cAcaudia and imperforate anus.

[†]Differs significantly ($p < 0.01$) from water controls.

[†]Significant differences ($p < 0.001$). Groups with the same superscript do not differ from each other ($p > 0.05$) by *a posteriori* test.

EGBE is a potent hemolytic agent in rodents, as reflected in the rapid onset of red-stained urine (within 5 hr of first dosing) and subsequent death of rats receiving 0.35-mL doses. Equimolar volumes of EGEE, EGEEA and diEGEE caused no mortality and no clinical signs of toxicity. The extragestational body weight gain (Table 1) of diEGEE-treated rats was significantly reduced, to a degree comparable to the reduction the EGEEA-treated rats. Thus, while diEGEE was not embryo- or fetotoxic, it was maternally toxic under these test conditions.

In summary, the results of this study confirm that EGEE can be absorbed in embryotoxic, fetotoxic, and teratogenic quantities through the intact skin of pregnant rats. EGEEA was shown to share these properties

and to be at least as active as EGEE by this route. It has been previously reported that inhalation exposure of pregnant rats to EGBE resulted in no developmental toxicity, and none was detected here at approximately one-third the molar dose used for EGEE and EGEEA. A treatment volume of EGBE equimolar to the EGEE and EGEEA volume was strongly hemolytic and ultimately lethal, demonstrating that EGBE also penetrates the skin readily. There also was no measurable developmental toxicity in rats treated with diEGEE, despite administration at a dose causing slight maternal toxicity. These data demonstrate that reproductive and developmental toxicity, like other manifestations of glycol ether toxicity, are possible following cutaneous exposure.

Table 3. Visceral malformations and variations: number of litters (fetuses) affected.

| | Treatment Group | | | | |
|---------------------------|---------------------|--------------------|--------------------------------|---------------------|--------------------|
| | EGEE (0.25 mL) | EGEEA (0.35 mL) | EGBE ^a (0.12 mL) | diEGEE (0.35 mL) | Water (control) |
| Total examined | 10(34) | 3(10) | 8(45) | 13(70) | 17(87) |
| Malformations | | | | | |
| Cardiovascular: total | 8(9) [†] | 3(5) [†] | 0 | 0 | 0 |
| Right aorta | 4(4) ^b | 2(2) | 0 | 0 | 0 |
| Double aorta | 1(1) | 1(1) | 0 | 0 | 0 |
| Right ductus | 3(3) ^b | 1(1) | 0 | 0 | 0 |
| Ventricular septal defect | 5(5) | 3(5) | 0 | 0 | 0 |
| Abnormal subclavian | 1(1) | 0 | 0 | 0 | 0 |
| Renal: total | 7(8) [†] | 1(2) | 0 | 2(3) | 3(3) |
| Hydronephrosis | 4(4) | 1(1) | 0 | 1(2) | 0 |
| Hydroureter | 4(4) | 0 | 0 | 1(2) | 3(3) |
| Ectopic kidney | 1(2) | 1(1) | 0 | 1(1) | 0 |
| Hypoplastic kidney | 0 | 1(1) | 0 | 0 | 0 |
| Fused kidneys | 1(1) | 0 | 0 | 0 | 0 |
| Brain: total | 4(4) [†] | 1(2) | 0 | 2(3) | 0 |
| Hydrocephalus | 2(2) | 1(2) | 0 | 2(3) | 0 |
| Hemorrhage | 2(2) | 0 | 0 | 0 | 0 |
| Eye: total | 2(2) | 2(2) | 0 | 0 | 0 |
| Microphthalmia | 1(1) | 0 | 0 | 0 | 0 |
| Abnormal optic nerve | 1(1) | 0 | 0 | 0 | 0 |
| Folded retina | 2(2) | 2(2) | 0 | 0 | 0 |
| Total malformations | 10(21) [†] | 3(8) | 0(0) | 3(5) | 3(3) |
| Variations | | | | | |
| Cardiovascular | | | | | |
| Missing innominate | 2(2) | 1(1) | 0 | 0 | 0 |
| Renal: total | 5(7) | 0 | 4(5) | 6(13) | 10(15) |
| Dilated renal pelvis | 2(3) | 0 | 0 | 3(6) | 6(7) |
| Dilated ureter | 4(5) | 0 | 4(5) | 4(9) | 7(10) |
| Total variations | 5(8) | 1(1) | 4(5) | 6(13) | 10(15) |
| Testicular defects | | | | | |
| Males examined | 21 | 6 | 23 | 40 | 39 |
| Testicular: total | 3(3) | 1(1) | 1(1) | 4(4) | 1(1) |
| Ectopic (slight) | 2(2) | 1(1) | 1(1) | 4(4) | 1(1) |
| Undescended | 1(1) | 0 | 0 | 0 | 0 |
| Agenesis | 1(1) | 0 | 0 | 0 | 0 |

^aReplicate 2 only. All other groups are combined data for replicates 1 and 2.

^bIncludes one fetus with situs inversus.

[†]Differs significantly from water controls ($p < 0.01$) by two-tail Fisher-Irwin exact test of litters affected.

[†]Differs significantly from water controls ($p < 0.001$) by two-tail Fisher-Irwin test of litters affected.

Table 4. Skeletal malformations and variations: number of litters (fetuses) affected.

| | Treatment group | | | | |
|-----------------------------|---------------------|--------------------|--------------------------------|---------------------|--------------------|
| | EGEE (0.25 mL) | EGEEA (0.35 mL) | EGBE ^a (0.12 mL) | diEGEE (0.35 mL) | Water (control) |
| Total examined | 11(36) | 5(11) | 9(45) | 13(67) | 17(90) |
| Malformations | | | | | |
| Ribs: total | 2(2) | 2(2) | 1(1) | 2(4) | 1(1) |
| Missing ribs | 2(2) ^b | 0 | 0 | 2(3) | 1(1) |
| Fused, malformed | 1(1) ^b | 2(2) | 0 | 1(2) | 0 |
| Cervical | 0 | 0 | 1(1) | 0 | 0 |
| Vertebrae: total | 4(5) | 0 | 0 | 2(4) | 0 |
| Fused arches | 2(2) ^b | 0 | 0 | 1(1) | 0 |
| Extra lumbar | 2(2) | 0 | 0 | 1(3) | 0 |
| Reduced lumbar | 2(3) ^b | 0 | 0 | 0 | 0 |
| Reduced thoracic | 1(1) ^b | 0 | 0 | 1(1) | 0 |
| Absent arch | 0 | 0 | 0 | 1(1) | 0 |
| Scoliosis | 0 | 1(1) | 0 | 0 | 0 |
| Missing tympanic ring | 0 | 0 | 0 | 1(1) | 0 |
| Total malformations | 4(5) | 2(2) | 1(1) | 3(5) | 1(1) |
| Variations | | | | | |
| Ribs: total | 6(13) [*] | 5(6) [‡] | 3(4) | 5(12) | 1(1) |
| Extra thoracic | 2(6) | 0 | 0 | 0 | 0 |
| Extra lumbar | 5(7) ^b | 5(6) | 3(4) | 5(12) | 1(1) |
| Vertebrae: total | 10(32) [‡] | 5(11) [‡] | 1(1) | 3(6) | 0 |
| Reduced sacral (< 6) | 2(2) ^b | 0 | 0 | 0 | 0 |
| Missing lumbo-sacral arch | 2(2) | 1(1) | 1(1) | 0 | 0 |
| Increased thoracic | 1(3) | 0 | 0 | 0 | 0 |
| Centra: misshaped | 7(16) ^b | 5(10) | 0 | 2(3) | 0 |
| Centra: absent, incomplete | 7(23) | 5(11) | 0 | 2(5) | 0 |
| Sternebrae: total | 11(32) | 4(9) | 6(13) | 7(18) | 13(28) |
| Misaligned | 6(8) | 0 | 0 | 1(3) | 2(2) |
| Split | 0 | 1(1) | 0 | 0 | 0 |
| Decreased number (< 6) | 9(29) | 4(9) | 6(13) | 7(17) | 13(27) |
| Reduced ossification: total | 5(14) | 5(11) [‡] | 3(3) | 3(3) | 1(1) |
| Skull | 2(8) | 5(11) | 3(3) | 2(2) | 0 |
| Pelvic girdle | 4(8) ^b | 4(6) | 0 | 0 | 0 |
| Decreased metatarsals (< 4) | 2(3) ^b | 4(7) | 0 | 1(1) | 0 |
| Decreased metacarpals (< 3) | 2(5) | 2(5) | 1(1) | 0 | 1(1) |
| Total variations | 11(34) | 5(11) | 7(16) | 9(22) | 13(29) |

^aReplicate 2 only. All other groups are combined data for replicates 1 and 2.

^bIndicates two fetuses with acaudia and imperforate anus.

^{*}Differs significantly from water controls ($p < 0.01$) by two-tail Fisher-Irwin exact test of litters affected.

[‡]Differs significantly from water controls ($p < 0.001$) by two-tail Fisher-Irwin exact test of litters affected.

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Mention of a company name or product does not constitute endorsement by the National Institute for Occupational Safety and Health.

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