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Developmental Toxicity of Diethylene Glycol Monomethyl Ether (diEGME)

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Developmental Toxicity of Diethylene Glycol Monomethyl Ether (diEGME). HARDIN, B. D., GOAD, P. T., AND BURG, J. R. (1986). *Fundam. Appl. Toxicol.* **6**, 430–439. Diethylene glycol monomethyl ether (diEGME) was one of 15 glycols tested in CD-1 mice using a short-term *in vivo* reproductive toxicity assay (Chernoff/Kavlock test). Because results were strongly suggestive of potential reproductive toxicity, a teratology study was conducted in Sprague–Dawley rats. Time-mated females were orally dosed on Days 7–16 of gestation with diEGME in distilled water. Doses of 0, 1000, 1495, 2235, 3345, and 5175 mg/kg/day were used in a preliminary dose-finding study. At 5175 mg/kg/day, two of nine rats died, five of five litters were totally resorbed, and maternal extra gestational body weight gain was reduced. At 3345 mg/kg/day, six of nine litters were resorbed but there were no deaths and extra gestational body weight gain was not affected. Visceral and skeletal examinations revealed a dose-related increase in malformations, primarily of the ribs and cardiovascular system. Subsequently, pregnant rats were similarly dosed with 0, 720, or 2165 mg/kg/day. Neither dose was maternally toxic, but fetal body weights and the number of live implantations were significantly reduced at 2165 mg/kg/day. Rib malformations were seen in 9.1% (control), 42.9% (720 mg/kg/day, $p < 0.05$), and 80.0% (2165 mg/kg/day, $p < 0.001$) of litters. Cardiovascular malformations occurred in 0.0, 4.8, and 71.4% ($p < 0.001$) of litters. Diethylene glycol monomethyl ether thus was teratogenic in rats at all doses tested, producing a dose-dependent series of malformations similar to those produced by other members of the glycol ether family. © 1986 Society of Toxicology.

Diethylene glycol monomethyl ether (Methyl Carbitol, CAS No. 111-77-3) is a colorless, hygroscopic liquid used as a high-boiling solvent in printing inks and pastes, stamp pad inks, textile dye pastes, lacquers, and synthetic resin surface coatings. It is completely miscible with water, ketones, alcohol, ethers, aromatic hydrocarbons, and halogenated hydrocarbons (Mellan, 1977).

Several alkyl ether derivatives of ethylene glycol have recently been reported to be testicular or developmental toxins (reviewed by Hardin, 1983). As part of a program to evaluate the reproductive toxicity of other ethylene glycol ethers, 15 members of this chemical family were tested (Schuler *et al.*, 1984) in a short-term reproductive toxicity assay (Chernoff and Kavlock, 1982). Five methyl ether derivatives were included: ethylene glycol monomethyl (EGME) and dimethyl (EGdiME) ethers, diethylene glycol monomethyl (diEGME), and dimethyl (diEGdiME) ethers, and triethylene glycol dimethyl ether (triEGdiME). Teratogenicity had previously been demonstrated for both EGME (Nagano *et al.*, 1981) and EGdiME (Uemura, 1980). The three remaining methyl ethers were otherwise untested in female animals.

In the Chernoff/Kavlock assay (Smith, 1983; Schuler *et al.*, 1984), 4000 mg (33.3 mmol) diEGME/kg/day was administered by gavage in distilled water to 50 time-mated CD-1 mice on Days 7–14 of gestation, after which females were left undisturbed in nesting boxes. The number of live-born pups was noted as

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soon as possible after birth, and live pups were weighed as a litter. They were then returned to their mothers until the third postnatal day, when the number of live pups and the total litter weight were again recorded. Five of 50 treated mice died as a result of diEGME toxicity. Among the survivors, 32 were pregnant (31 of 50 controls were pregnant). Viable litters (one or more live-born pups) were produced by 5/32 diEGME-treated dams (31/31 controls). Still-born litters were delivered by 9/32, and litters were completely resorbed in 18/32 pregnant diEGME-treated mice. The number of live-born pups per viable litter, per-pup body weight at birth, neonatal body weight gain, and survival to 3 days of age all were adversely affected by diEGME treatment.

The purpose of this short-term assay (Schuler *et al.*, 1984) was to test chemicals quickly for potential reproductive toxicity and, on the basis of observations in that assay, to assign relative priorities for additional reproductive or developmental toxicity testing. Because of the marked embryotoxicity observed and the similarity of the effects produced by all the methyl derivatives tested, the three untested ethylene glycol methyl ethers (diEGME, diEGdiME, and triEGdiME) were high-priority candidates for conventional teratology tests. Results of testing diEGME are presented here.

METHODS

Chemical. Diethylene glycol monomethyl ether was purchased from the Eastman Kodak Company (Lot C8X).

Animals. Time-mated SPF Sprague–Dawley rats (Crl: CD (SD) BR) were ordered from Charles River Breeding Laboratories' Kingston colony and used in initial dose-finding and subsequent teratology studies. Rats were shipped on Day 4 and received on Day 5 of gestation (day of sperm = Day 1). The teratology phase of the study was conducted in two identical replicates. There were no differences by replicate, and all subsequent discussion treats the combined replicates. Upon receipt, 5 to 10 rats from each shipment were arbitrarily taken for pathogen screening. In all cases, no internal or external parasites were detected, and serum tested negative for *Mycoplasma* and a panel of 10 rat viruses. All rats were singly housed in stainless-steel wire mesh cages with free access to food and water from receipt until sacrifice on Day 21 of gestation.

Procedures. In the dose-finding study, rats were weighed on gestation Day 6 and randomly assigned to one of six groups, nine rats per group. Weights were recorded daily on gestation Days 7 to 16 and diEGME in distilled water was administered by gavage in a standard volume of 5 ml/kg at doses of 0 (control), 1000, 1495, 2235, 3345, or 5175 mg (5 ml, undiluted)/kg/day (8.3, 12.4, 18.6, 27.8, or 43.1 mmol/kg/day). Food consumption was determined over the gestation Days 7–12, 12–17, and 17–21 intervals. Dams were killed and weighed on Day 21, and fetuses were weighed, examined for gross external defects, then preserved in alcohol or Bouin's fluid for subsequent internal examinations. Based on observations in the range-finding study, doses of 720 and 2165 mg/kg/day (6.0 and 18.0 mmol/kg/day) were selected for use in the subsequent teratology study.

For each replicate teratology experiment, time-mated rats were randomly assigned to one of three groups, 12–13 rats per group. diEGME in distilled water was administered by gavage on gestation Days 7 to 16 at doses of 0 (control), 720, or 2165 mg (0, 6, or 18 mmol)/kg/day. Body weights and food consumption were determined and fetuses were processed on gestation Day 21 as in the dose-finding study.

Statistical analysis. All food consumption, maternal and fetal body weight data, numbers of implantations, and percentage of live implants were analyzed by nonparametric m-ranking procedures, with corrections for multiple comparisons to the control. The proportion of litters affected was analyzed by χ^2 test for independence among treatment groups, with pairwise comparisons between control and individual treatment groups by one-tail Fischer's exact test. All statistical procedures were conducted using the Statistical Analysis System (SAS Institute, Cary, N.C.).

RESULTS

In the initial dose-finding study, the high dose (5175 mg/kg/day) killed two of nine treated females (Table 1). Food consumption was significantly reduced at that dose and at 3345 mg/kg, but only in the first 5 days of treatment. Extra gestational body weight gain, another indicator of maternal toxicity, was significantly reduced only at 5175 mg/kg/day. The reduced Day 21 gross body weight at 3345 mg/kg (Table 1) is attributed to the significant reduction in the number of live fetuses at that dose (Table 2). Maternal toxicity at 3345 mg/kg was therefore judged to have been minimal, and not a contributing factor at doses below 3345 mg/kg/day.

TABLE 1
DOSE-FINDING STUDY MATERNAL DATA (MEAN ± SD)

	Dose (mg/kg/day)					
	0	1000	1495	2235	3345	5175
No. survivors/treated	9/9	9/9	9/9	9/9	9/9	7/9
No. live litters/preg.	9/9	8/8	4/4	8/8	3/9*	0/5*
Maternal body weight (g)						
Day 7	207 ± 14	216 ± 12	215 ± 14	211 ± 10	210 ± 15	208 ± 14
12	246 ± 17	251 ± 11	254 ± 15	247 ± 16	240 ± 22	228 ± 36
16	275 ± 23	276 ± 16	282 ± 17	278 ± 16	261 ± 23	244 ± 29*
21	337 ± 34	327 ± 30	337 ± 30	325 ± 19	279 ± 28*	239 ± 23*
Extra gestational weight gain (g)[a]	77 ± 18	76 ± 22	77 ± 17	72 ± 10	70 ± 18	54 ± 19*
Food consumption (g)						
Days 7–12	123 ± 18	119 ± 11	123 ± 12	116 ± 11	96 ± 20*	79 ± 21*
Days 12–17	133 ± 21	134 ± 13	134 ± 15	134 ± 13	120 ± 14	109 ± 14
Days 17–21	107 ± 10	111 ± 14	114 ± 18	116 ± 9	109 ± 14	92 ± 16

[a] (Day 21 body weight)—(gravid uterus weight)—(Day 6 body weight).
* Significantly reduced relative to control group ($p < 0.05$).

There were no live fetuses in five of five pregnant survivors of 5175 mg/kg/day di-EGME treatment, or in six of nine pregnant rats dosed at 3345 mg/kg/day (Table 1). Live fetuses as a percentage of implants declined with increasing dose, and was statistically significantly reduced at 3345 mg/kg/day. Similarly, fetal body weight consistently fell with

TABLE 2
DOSE-FINDING STUDY LITTER DATA (MEAN ± SD)

	Dose (mg/kg/day)					
	0	1000	1495	2235	3345	5175
Implants/litter	13.2 ± 2.4	10.5 ± 3.6	13.0 ± 4.8	12.4 ± 1.6	13.1 ± 2.4	14.2 ± 1.3
Live/litter						
Number	12.1 ± 3.0	9.5 ± 3.3	11.5 ± 4.4	10.8 ± 1.7	3.3 ± 0.6*	0*
Percent	91.2 ± 11.9	90.8 ± 7.9	89.7 ± 12.6	87.1 ± 9.8	9.2 ± 13.8*	0*
Fetal weight (g)						
Male	4.0 ± 0.6	3.8 ± 0.8	3.6 ± 0.6	3.5 ± 0.8	2.3 ± 1.3*	NA
Female	3.8 ± 0.5	3.5 ± 0.8	3.3 ± 0.7	3.2 ± 0.6*	2.4 ± 0.5*	NA
Gross malformations: litters (fetuses)/mean of percent fetuses per litter						
No. examined	9 (109)	8 (76)	4 (46)	8 (86)	3 (11)[a]	NA
Malformations	1 (1)/1[b]	0	1 (1)/1[c]	2 (3)/4[d]	0	NA

[a] Includes one dead fetus.
[b] Exencephaly.
[c] Short tail (about half normal length).
[d] One litter had one fetus with acaudia and imperforate anus, and one edematous fetus. A second litter had one fetus with acaudia and imperforate anus.
* Significantly reduced relative to control group ($p < 0.05$).

increasing dose. Differences were statistically significant for both sexes at 3345 mg/kg, and for female fetuses at 2235 mg/kg/day.

One or more fetuses had a skeletal malformation in 11.1% (control), 12.5% (1000 mg/kg/day), 50% (1495 mg/kg/day), 75% (2235 mg/kg/day), and 33.3% (3345 mg/kg/day) of litters. Only the 2235-mg/kg/day group differed significantly ($p < 0.05$) from controls (Table 3). The majority of skeletal malformations were bilateral wavy ribs, occasionally accompanied by fused ribs, but no individual malformation was statistically significantly increased. Skeletal ossification was impaired at 1495 mg/kg/day and higher doses. Cardiovascular malformations (Table 4) were signif-

TABLE 3
DOSE-FINDING STUDY SKELETAL MALFORMATIONS

	Affected litters (fetuses)/percent[a] dose (mg/kg/day)				
	0	1000	1495	2235	3345
Number examined	9 (55)	8 (38)	4 (23)	8 (42)	3 (6)
Malformations					
Vertebrae					
Missing thoracic	0	0	1 (1)/3	0	0
Abnormal cervical arch	1 (1)/3	0	1 (1)/3	0	0
Total	1 (1)/3	0	1 (2)/6	0	0
Ribs					
Rudimentary cervical	1 (1)/3	0	0	4 (6)/16	1 (1)/17
Missing	0	0	1 (1)/3	0	0
Wavy/fused: unilateral	0	0	0	1 (1)/3	1 (1)/17
bilateral	0	1 (2)/5	2 (2)/7	4 (6)/15	0
Total	1 (1)/3	1 (2)/5	2 (3)/10	5 (12)/31	1 (1)/17
Cleft sternebrae	0	0	0	1 (1)/3	0
Total malformations	1 (1)/3	1 (2)/5	2 (4)/13	6* (13)/34	1 (1)/17
Variations					
Reduced cranial ossification	2 (2)/3	3 (8)/23	4* (13)/60	6* (15)/36	3* (5)/83
Sternebrae					
Misaligned	2 (4)/6	4 (5)/14	2 (3)/13	6 (8)/18	2 (2)/33
Reduced ossification	1 (1)/2	0	2 (5)/16	2 (2)/5	3 (4)/67
Total	3 (5)/7	4 (5)/14	3 (8)/29	6 (10)/24	3 (5)/83
Vertebrae					
Misaligned	0	2 (2)/5	1 (1)/8	8*** (15)/36	3** (4)/67
Reduced ossification	2 (2)/3	1 (1)/4	2 (5)/31	6* (15)/36	3* (6)/100
Total	2 (2)/3	3 (3)/9	2 (5)/31	8** (20)/48	3* (6)/100
Thoraco-lumbar ribs	4 (4)/8	1 (1)/2	2 (4)/24	6 (8)/17	0
Appendicular skeleton					
Reduced ossification	2 (2)/3	1 (1)/2	3 (10)/38	5 (13)/30	2 (3)/50
Total variations	6 (11)/18	5 (12)/33	4 (18)/80	8 (32)/76	3 (6)/100

[a] Mean of percent fetuses per litter.
Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 4
DOSE-FINDING STUDY VISCERAL MALFORMATIONS

	Affected litters (fetuses)/percent[a] dose (mg/kg/day)				
	0	1000	1495	2235	3345
Number examined	9 (54)	8 (38)	4 (23)	8 (44)	3 (5)
Malformations					
Cardiovascular					
Double aortic arch[b]	0	0	0	3 (4)/10	0
Right aortic arch	0	1 (1)/2	0	1 (1)/3	2 (2)/33
Right ductus arteriosis	0	1 (1)/2	0	1 (1)/3	2 (2)/33
Ventricular septal defect	0	1 (1)/2	0	2 (2)/5	2 (2)/33
Total	0	1 (1)/2	0	4* (7)/17	2 (3)/50
Brain					
Hydrocephalus	0	1 (2)/8	0	3 (8)/17	2 (2)/50
Eye					
Folded retina	0	0	0	1 (1)/3	2 (2)/50
Anophthalmia	1 (1)/2	0	0	0	0
Urinary					
Hydroureter	1 (1)/2	0	0	0	0
Hydronephrosis	1 (1)/2	1 (3)/6	3 (4)/25	1 (1)/3	1 (1)/33
Ectopic kidney	0	0	0	1 (1)/2	0
Fused kidneys	0	0	0	1 (1)/2	0
Total	2 (2)/3	1 (3)/6	3 (4)/25	2 (3)/7	1 (1)/33
Miscellaneous					
Tracheo–esophageal transposition	0	0	0	1 (1)/2	0
Ectopic stomach	0	0	0	1 (1)/2	0
Total Malformations	2 (2)/3	3 (6)/16	3 (4)/25	6 (15)/23	3 (5)/100
Variations					
Cardiovascular					
Missing innominate	0	0	0	4* (6)/15	0
Brain					
Hemorrhage	0	0	1 (1)/5	0	0
Urinary					
Dilated renal pelvis	2 (2)/3	4 (4)/11	4 (6)/30	4 (6)/14	1 (1)/33
Dilated ureter	2 (2)/3	3 (3)/10	1 (1)/8	1 (1)/2	0
Total	4 (4)/6	5 (6)/17	4 (7)/38	4 (6)/14	1 (1)/33
Total variations	4 (4)/6	5 (6)/17	4 (8)/43	7 (11)/26	1 (1)/33

[a] Mean of percent fetuses per litter.
[b] Ascending aorta bifurcated to form a vascular ring around the trachea and esophagus, then reformed as a single descending aorta.
* Differs significantly from corresponding control group, $p < 0.05$.

icantly increased at 2235 mg/kg/day ($p < 0.05$), and an overall dose-related pattern of visceral malformations was evident with 22.2, 37.5, 75, 75, and 100%, respectively, of litters affected. However, these differences for total visceral defects were not statistically significant ($\chi^2 = 9.2$, $df = 4$, $p < 0.10$). Combining the gross, skeletal, and visceral observations, the

percentages of litters with at least one malformed fetus were 22.2%, 37.5%, 100% ($p = 0.05$), 100% ($p < 0.01$), and 100% ($p < 0.10$).

For the subsequent teratology study, doses of 720 and 2165 mg (6.0 and 18 mmol) diEGME/kg/day were selected on the basis of the dose-related patterns of embryo/fetal toxicity and malformations in the preliminary study. At the higher dose, maternal food consumption was reduced in the first 5 days of dosing and gross maternal body weight was reduced on Day 21 (Table 5). However, extra gestational weight gain was not influenced by diEGME treatment (Table 5), and maternal toxicity was not considered significant. Nevertheless, both fetal weight and litter size were significantly reduced at 2165 mg/kg/day (Table 6), and two of 23 litters were completely resorbed at that dose. None were so affected in the control and 720 mg/kg/day groups. There was no gross evidence of fetotoxicity at 720 mg/kg/day, although the average fetal body weight was slightly lower than the control average.

Skeletal malformations included rudimentary cervical ribs and bilateral wavy ribs (Table 7), both of which were significantly increased ($p < 0.001$) at 2165 mg/kg/day. Individually, neither rib defect was significantly increased at 720 mg/kg, but the combined rib malformations were significantly elevated ($p < 0.05$). Abnormal vertebrae and unilateral wavy ribs were also suggestive of a treatment effect ($\chi^2 = 6.8$, $df = 2$, $p < 0.05$), but control and 2165-mg/kg/day groups did not differ significantly by Fischer's exact test ($p < 0.10$). Ossification deficiencies were apparent in litters receiving the higher dose ($p < 0.01$), and less marked but still significant ($p < 0.05$) delayed ossification was also present in the 720 mg/kg/day group. Visceral malformations (Table 8) again were predominantly in the cardiovascular system, and were significantly increased ($p < 0.001$) at 2165 mg/kg/day. Combining the gross, skeletal, and visceral observations, the percentages of litters with at least one malformed fetus were significantly increased in both treatment groups: 22.7% of control litters versus 52.4% ($p < 0.05$) of litters at 720 mg/kg/day, and 90.5% ($p < 0.001$) of litters at 2165 mg/kg/day.

DISCUSSION

There have been few previous studies of diEGME toxicity. Hobson *et al.* (1984) ex-

TABLE 5
MATERNAL DATA (MEAN ± SD)

	Dose (mg/kg/day)		
	0	720	2165
No. survivors/treated	25/25	25/25	25/25
No. live litters/preg.	22/22	21/21	21/23
Maternal body weight (g)			
Day 7	213 ± 7	213 ± 9	212 ± 11
12	248 ± 10	251 ± 13	245 ± 13
16	278 ± 14	279 ± 17	273 ± 17
21	332 ± 18	332 ± 24	308 ± 29*
Extra gestational weight gain (g)[a]	47 ± 13	53 ± 14	46 ± 11
Food consumption (g)			
Days 7–12	120 ± 13	122 ± 14	111 ± 13*
Days 12–17	128 ± 14	127 ± 25	128 ± 12
Days 17–21	105 ± 10	109 ± 12	106 ± 23

[a] (Day 21 body weight)—(gravid uterus weight)—(Day 6 body weight).
* Significantly reduced relative to the control group ($p < 0.05$).

TABLE 6

LITTER DATA (MEAN ± SD)

	Dose (mg/kg/day)		
	0	720	2165
Implants/litter	12.6 ± 1.9	11.8 ± 2.5	12.0 ± 2.7
Live/litter			
Number	11.4 ± 2.0	10.8 ± 2.8	7.4 ± 3.9*
Percent	90.7 ± 8.8	90.5 ± 10.0	60.5 ± 31.5*
Fetal weight (g)			
Male	4.6 ± 0.8	4.5 ± 0.8	3.5 ± 0.8*
Female	4.4 ± 0.7	4.2 ± 0.7	3.2 ± 0.9*
Gross malformations: litters (fetuses)/mean of percent fetuses per litter			
No. Examined	22 (252)	21 (226)	21 (171)
Malformations[a]	1 (1)/0.4[b]	0	5 (5)/3[c]

[a] Litters with gross malformations differed significantly across groups ($\chi^2 = 7.93$, $df = 2$, $p < 0.05$), but control and 2165 mg/kg/day groups did not differ significantly by Fischer's exact test ($p < 0.10$).

[b] Acaudia, imperforate anus.

[c] Acaudia, imperforate anus (four fetuses); gross edema (one fetus)

* Significantly reduced relative to control group ($p < 0.05$).

posed male guinea pigs cutaneously to EGME or diEGME at up to 1.0 ml/kg/day for 13 weeks. EGME treatments caused dose-related lymphocytic leukopenia, increased serum LDH activity, testicular atrophy, and reduced body weight gain. Reduced growth was the only toxic sign detected in the diEGME-treated animals. Nagano *et al.* (1984) administered several glycol ethers in drinking water to male mice. EGME was given at 2.5% for 18 days, and diEGME at 2.0% for 25 days. EGME induced significant reductions in mean body weight, white blood counts, testis weights, and weights of combined seminal vesicles and coagulating glands. Similar toxicity was not seen in the diEGME-treated mice. In a modification of the Chernoff/Kavlock assay, pregnant rats were injected subcutaneously on gestation Days 6–20 with 0.25, 0.5, or 1.0 ml (approximately 2.1, 4.2, or 8.5 mmol) diEGME/kg/day (Doe, 1984). No maternal toxicity was detected after this treatment, and there were no statistically significant fetal effects. However, neonatal survival declined in a dose-related pattern. It is possible that functional deficits or visceral malformations were induced which contributed to neonatal mortality. A considerably higher dose (4000 mg/kg/day) administered to pregnant mice produced 10% maternal mortality and a very high incidence of intrauterine death with reduced neonatal survival of the few pups born alive (Schuler *et al.*, 1984).

The spectrum of malformations reported here is very similar to that previously reported as induced in this strain of rat by ethylene glycol monomethyl ether (Nelson, *et al.*, 1984) and ethylene glycol monoethyl ether (EGEE) and monoethyl ether acetate (EGEEA) (Hardin, *et al.*, 1982; 1984). This marked similarity of EGME and diEGME fetal effects suggests a mechanistic commonality, but the potency of diEGME on a molar basis is considerably less than that of EGME. A metabolite of EGME, methoxy acetic acid (and possibly methoxy acetaldehyde), rather than EGME itself is believed to be the proximate teratogen (Brown *et al.*, 1984). The metabolism of diEGME has not been investigated, but if it is a substrate for the alcohol dehydrogenase system, 2-methoxyethoxy acetaldehyde and 2-methoxyethoxy acetic acid are the presumptive metabolites and could act much like the corresponding EGME metabolites. In fact, 2-

TABLE 7

SKELETAL MALFORMATIONS

	Affected litters (fetuses)/percent[a] dose (mg/kg/day)		
	0	720	2165
Number examined	22 (123)	21 (111)	20 (89)
Malformations			
Vertebral			
Abnormal thoracic arch	0	0	1 (1)/2
Missing sacrococcygeal	0	0	2 (2)/2
Total	0	0	3 (3)/4
Ribs			
Rudimentary cervical	1 (2)/2	5 (9)/8	11*** (16)/18
Wavy/fused: unilateral	0	0	3 (3)/3
bilateral	1 (4)/3	4 (6)/6	13*** (32)/36
Total	2 (6)/4	9* (15)/15	16*** (43)/48
Total malformations	2 (6)/4	9* (15)/15	16*** (45)/51
Variations			
Reduced cranial ossification	4 (6)/4	10* (17)/16	16*** (51)/56
Sternebrae			
Reduced ossification	1 (1)/1	1 (1)/1	11*** (22)/28
Misaligned	9 (13)/10	11 (13)/12	14 (23)/25
Total	9 (13)/10	12 (14)/13	17** (40)/47
Vertebrae			
Reduced ossification	0	0	15*** (44)/58
Misaligned centra	0	2 (4)/4	19*** (61)/74
Extra	1 (1)/1	0	10** (15)/21
Total	1 (1)/1	2 (4)/4	19*** (68)/81
Ribs			
Reduced ossification	0	0	3 (4)/5
Thoraco-lumbar	8 (10)/7	3 (3)/4	15* (35)/42
Total	8 (10)/7	3 (3)/4	16** (38)/45
Appendicular skeleton			
Reduced ossification	1 (1)/1	6* (13)/12	15*** (41)/53
Total variations	14 (24)/18	15 (33)/30	20** (82)/94

[a] Mean of percent fetuses per litter.

Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

ethoxyethoxy acetic acid has been reported in the urine of humans exposed to diEGEE (Kamerling *et al.*, 1977). The greater length of the diEGME molecule, relative to EGME, could inhibit this metabolic pathway or reduce the activity of the putative metabolites, or both, either of which would reduce the relative potency of diEGME. On the other hand, cleavage of the ether linkage has been demonstrated in EGEE (Cheever *et al.*, 1984) and ethylene glycol monopropyl ether (EGPE) (Hutson and Pickering, 1971). Ether cleavage thus may

TABLE 8

VISCERAL MALFORMATIONS

	Affected litters (fetuses)/percent[a] dose (mg/kg/day)		
	0	720	2165
Number examined	22 (129)	21 (115)	21 (82)
Malformations			
Cardiovascular			
Double aortic arch[b]	0	0	7** (9)/12
Right aortic arch	0	1 (1)/1	6** (6)/10
Right ductus arteriosis	0	0	1 (1)/5
Ventricular septal defect	0	0	14*** (27)/39
Total	0	1 (1)/1	15*** (33)/46
Brain			
Hydrocephalus	1 (1)/1	0	0
Eye			
Folded retina	0	0	1 (1)/1
Anophthalmia	0	1 (1)/1	0
Microphthalmia	1 (1)/2	0	0
Urinary			
Hydroureter	1 (1)/1	1 (1)/1	0
Hydronephrosis	0	2 (2)/2	5 (6)/7
Total	1 (1)/1	2 (2)/2	5 (6)/7
Total malformations	3 (3)/3	4 (4)/3	16*** (37)/50
Variations			
Cardiovascular			
Missing innominate	0	1 (1)/1	1 (1)/1
Urinary			
Dilated renal pelvis	2 (4)/3	8* (11)/14	12** (17)/23
Dilated ureter	4 (4)/3	3 (5)/5	1 (1)/1
Total	5 (7)/6	9 (13)/16	12* (18)/24
Total variations	5 (7)/6	10 (14)/17	12* (19)/25

[a] Mean of percent fetuses per litter.

[b] Ascending aorta bifurcated to form a vascular ring around the trachea and esophagus, then reformed as a single descending aorta.

Differs significantly from corresponding control group: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

contribute to the developmental toxicity of diEGME, depending upon the rate of endogenous EGME production.

Bilateral wavy ribs and cardiovascular malformations predominated in both the dose-finding and teratology studies. A dose-related pattern of response was also evident in both. A "no observable effect level" for developmental toxicity was not included in this series, since statistically significant effects were seen at the lowest dose tested, 720 mg/kg/day. Slight maternal toxicity was suggested at doses of 2165 mg/kg/day and higher by transient reductions in food consumption. However, materal body weight gain as reflected in extra gestational weight gain was significantly affected only at 5175 mg/kg/day, a dose that was lethal to two of nine treated rats. Using

2165 and 5175 mg/kg/day as lower and upper limits for the appearance of maternal toxicity and 720 mg/kg/day as the lowest effective dose for developmental toxicity, the A/D (adult to developmentally toxic) ratio for diEGME is 3.0 to 7.2. In contrast, Nagano *et al.* (1981) reported skeletal malformations in mice treated on gestation Days 8–15 with as little as 62.5 mg (0.8 mmol) EGME/kg/day. The only reported evidence of maternal toxicity was reduced lymphocyte counts at 1000 mg/kg/day, suggesting an A/D ratio of 16.0 for EGME in mice.

In the present study, diEGME was distinctly teratogenic, embryotoxic, and fetotoxic in rats, producing all these responses at doses that apparently were not toxic to the maternal rat. The Chernoff/Kavlock assay in mice thus correctly predicted the developmental toxicity of diEGME (Schuler *et al.*, 1984). The highest dose used in a Chernoff/Kavlock assay in rats (Doe, 1984) was only slightly higher than the lowest dose used here (1000 versus 720 mg/kg). That study was designed primarily to model skin absorption of diEGME, which is a more probable route of human exposure than inhalation. The vapor pressure of EGME is 9.7 mm Hg at 25°C, versus only 0.18 mm Hg for diEGME. EGME also penetrates the skin much more readily (2.8 ± 2.6 mg/cm^2/hr) than does diEGME (0.2 ± 0.16 mg/cm^2/hr) (Dugard *et al.*, 1984). EGME and diEGME thus are both developmental toxicants, but EGME, with its higher vapor pressure, more rapid skin penetration, and higher A/D ratio, clearly poses the greater risk.

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