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Comparative Inhalation Teratogenicity of Four Glycol Ether Solvents and an Amino Derivative in Rats*

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Previous research demonstrated the inhalation teratogenicity of the solvent 2-ethoxyethanol in rats and rabbits. As this is one of a class of widely used industrial solvents, we investigated the teratogenicity of five structurally related compounds. Each chemical was vaporized and administered to approximately 15 pregnant rats in one to three concentrations for 7 hr/day on gestation days 7 to 15, and dams were sacrificed on day 20. Fetuses were individually weighed, and two-thirds of them were fixed in Bouin's solution and examined for soft-tissue anomalies. The other one-third were fixed in alcohol, stained with Alizarin Red and examined for skeletal defects. Data were analyzed on a litter basis; three solvents were compared with a pooled group (N = 34) of sham-exposed controls, and the remaining two were compared with a group of 15 controls. At concentrations which were apparently not maternally toxic, 2-methoxyethanol was highly embryotoxic, producing complete resorptions at 200 ppm; increased resorptions, reduced fetal weights and skeletal and cardiovascular defects occurred at both 100 and 50 ppm. 2-ethoxyethyl acetate at 600 ppm induced complete resorption of litters; 390 ppm reduced fetal weights and induced skeletal and cardiovascular defects, but only a single defect was observed at 130 ppm. 2-Butoxyethanol evidenced slight maternal toxicity at 200 ppm but produced no increase in congenital defects at that concentration. Neither 2-(2-ethoxyethoxy)ethanol (100 ppm) nor 2-methylaminoethanol (150 ppm) was maternally toxic or embryotoxic. In summary, shorter alkyl chained glycol ethers produced greater embryotoxicity than those having longer chains, and the ester produced effects equivalent to the ether, both patterns predictable from the biochemical literature.

Of the thousands of chemicals to which workers are exposed, only a few have even minimal experimental animal data from which to evaluate or predict reproductive toxicity. One class of chemicals that has demonstrated reproductive toxicity is a class of mono- and dialkyl ethers of ethylene glycol and their derivatives, collectively referred to as cellosolves or glycol ethers. Approximately 700 million pounds (318 thousand metric tons) of glycol ethers were produced in 1977, with the ethylene glycol monoethers representing about 78% of the total production (*1*). Ethoxyethanol, the prototype of this class, has the largest production volume, followed by butoxyethanol and methoxyethanol (*1*). The glycol ethers are widely used in industry as solvents for nitrocellulose, lacquers, inks, dyes, enamels, varnishes, varnish removers, and dry cleaning compounds. In addition, they are used in some commercial products such as cosmetics and liquid household cleaners. Produced and distributed by at least six major American chemical companies, the glycol ethers have a combined potential exposure population of around 1.5 million workers in the United States.

The earliest evidence of reproductive toxicity of this chemical group was observed when skeletal defects were reported in offspring of rats following 100 μg/kg/day IP injections of ethoxyethanol throughout gestation, although no defects were noted in mice or rabbits (*2*). The same investigators administered ethoxyethanol at 400 μL/kg/day for 4 weeks or 200 μL/kg/day for 13 weeks to male rats and observed testicular damage after both exposure regimens. Recently increased skeletal variations were observed following intubation of doses as low as 31.25 mg/kg/day methoxyethanol on gestation days 7 to 14 in mice (*3*).

Only recently, however, have there been reports of teratogenic effects produced by the glycol ethers after

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administration using the common routes of occupational exposure, inhalation and cutaneous absorption. Skeletal and cardiovascular defects were observed in rats after dermal exposure to 1.0 mL (0.25 mL, four times/day) ethoxyethanol on gestation days 7 to 16, with nearly half of the litters entirely resorbed at that level, and all litters completely resorbed at twice that level (*4*). Gestational exposure via inhalation to 600 to 750 ppm ethoxyethanol produced complete embryomortality in rats and rabbits, whereas 200 or 160 ppm ethoxyethanol induced skeletal and cardiovascular defects in rats and rabbits, respectively (*5*). Increased neonatal mortality following inhalation exposure of pregnant rats to ethoxyethanol at the current Federal occupational standard (200 ppm) and behavioral and neurochemical alterations in the offspring of pregnant rats exposed to one-half that concentration (viz. 100 ppm) have been reported (*6*).

Dose-dependent increases in testicular atrophy have been reported in mice following oral administration of various glycol ethers at 250 to 4000 mg/kg/day, 5 days/week for 5 weeks (*7*). The lowest levels causing significant deviations from controls were as follows: methoxyethanol 250 mg/kg, methoxyethyl acetate 500 mg/kg; ethoxyethanol 1000 mg/kg, ethoxyethyl acetate 1000 mg/kg; butoxyethanol and phenoxyethanol produced mortality in all rats at 2000 mg/kg but had no effect on testicular weight at 1000 mg/kg. The most important implication of these results is the indication that the chemicals having shorter attached alkyl groups produced the most damage and that reproductive toxicity decreases with increasing chain length. Esterification of the compounds (i.e, the acetate forms) resulted in less testicular atrophy when the dose was calculated on a weight per body weight (mg/kg) basis (at least with methoxyethanol), but produced effects equivalent to those of the alcohol form when calculated on a molar basis (thus implying that the acetate was removed prior to the toxic action).

The present research evaluated the teratogenic effects of structurally related glycol ethers in an attempt to determine the structure activity relationships for teratogenicity. Table 1 presents the glycol ethers evaluated for teratogenicity in the present research, along with the same data for ethoxyethanol. As seen from the table, a chemical having a shorter alkyl chain was selected (methyl rather than ethyl), as was one with a longer chain (butyl), and were the diethylene glycol ethyl ether and the ethyl acetate. The latter was chosen rather than the ether in order to verify that the acetate would produce effects equivalent to the ether, as would be predicted from the ubiquitous nature of esterases within the body and as shown for the male reproductive effects cited above. Finally, the amino analog of methoxyethanol was tested to determine the structural specificity required to produce teratogenic effects.

Materials and Methods

Subjects

Virgin female and male Sprague-Dawley rats specified to be free of mycoplasma and Sendai virus and of internal and external parasites (Charles River Breeding Laboratories, Wilmington, MA) were acclimated to a 12-hr light–dark cycle (lights on at 6 am) and to a temperature of 24 ± 2°C for 2 weeks. The humidity, not controlled, typically was in the range of 40 ± 20%. Purina Lab Chow and tap water were available *ad libitum* except when pregnant animals were in exposure chambers. Bedding consisted of cleaned, heat-treated sawdust from a local supplier (Absorb-Dri, Tasty Foods, Cincinnati, OH).

Apparatus and Procedures

Males weighing over 300 g were placed individually into a cage with three females weighing 200 to 300 g.

Table 1. General information on the glycol ethers included in the present research.

Common name and formula	Skeleton formula	Rat toxicity data: Oral, g/kg LD_{50}	Rat toxicity data: Inhalation, ppm/hr LC_{LO}	OSHA Standard (TWA), ppm	NIOSH estimated exposure population
2-Methoxyethanol $CH_3OC_2H_4OH$	C-O-C-C-OH	2.46	2000/4H	25	97,285
2-Ethoxyethanol[a] $C_2H_5OC_2H_4OH$	C-C-O-C-C-OH	3.0	4000/4H	200	359,790
2-Ethoxyethyl acetate $C_2H_5OC_2H_4O_2C_2H_3$	C-C-O-C-C-O-C-C (O)	5.1	3000/4H	100	231,970
2-Butoxyethanol $C_4H_9OC_2H_4OH$	C-C-C-C-O-C-C-OH	1.48	500/4H	50	1,279,707
2-(2-Ethoxyethoxy)-ethanol (carbitol) $C_2H_5OC_2H_4OC_2H_4OH$	C-C-O-C-C-O-C-C-OH	6.5	None	None	20,000
2-Methylaminoethanol $CH_3NHC_2H_4OH$	C-N-C-C-OH	2.34	None	None	Unknown

[a]Investigated in previous research.

Vaginal smears were taken each morning, and the presence of sperm marked day zero of gestation. These females were placed alone in 38 × 33 × 17-cm polycarbonate cages with filter tops. Feed and water intake and maternal weight were recorded weekly (i.e., on days 7, 14, and 21); any other signs of maternal toxicity were noted daily.

The glycol ethers (technical grade, 98 to 99.5% pure; Eastman Kodak Co., Rochester, NY) were heated in a flask set in hemispherical mantles. Vaporization was achieved by bubbling air through each solvent which was heated to approximately 40°C (for the higher concentrations). This vapor was then mixed with filtered room air to obtain the desired concentration and introduced into 0.5 m^3 exposure chambers (Charles Spengler and Associates, Cincinnati, OH). The concentration was continually monitored by a Miran I infrared spectrophotometer (Foxboro Analytical, South Norwalk, CT) with the concentration recorded hourly. Typically, two or three charcoal tube samples (silica gel tubes for 2-methylaminoethanol) were taken weekly from the chamber air for independent verification of the concentration by gas chromatograph. Two exceptions are noted below where more were taken (*9*). Throughout this paper concentrations are expressed in parts of solvent per million parts of air; hence values for the different chemicals are comparable on a molar equivalent basis. Air flow through the chambers provided approximately four air changes per minute. Exposures were conducted sequentially in one or two chambers, with a third chamber for sham exposure of control subjects.

Pregnant females were transported from the animal quarters to the inhalation chambers in their home cages with filter tops (Hazleton Systems, Aberdeen, MD). They were placed individually in 13 × 25 × 189-cm stainless steel wire mesh cages within exposure chambers. Control animals were placed in similar chambers for the same hours as the exposed animals; a pooled group of controls (N = 34) served as the comparison group for the first three chemicals examined. Another group of 15 controls served as the comparison group for the last two chemicals examined, as these groups were exposed at a later time (approximately 6 months later) than the first three. Exposures, as outlined above, were conducted 7 hr/day, and the animals were left in the chamber for at least one additional hour blow-off time after vapor generation terminated. They were then returned in their individual housing cages to the animal quarters, where water bottles were replaced. Exposures were conducted on gestation days 7–15. On day 20, the females were individually weighed and euthanized by chloroform asphyxiation. The entire uterus was removed and numbers of resorption sites (classified as early, middle or late) and live fetuses were determined. Fetuses were serially removed, blotted of excess fluids, weighed, examined for external malformations and external sex determined. One third of the fetuses were randomly selected and placed in 95% ethanol, and the remaining fetuses were placed in Bouin's solution. After being in the Bouin's solution for at least 1 week, these fetuses were examined for visceral abnormalities using Wilson's razor blade sectioning technique (*10*). The viscera were examined with the aid of a dissecting microscope. A representative sample of sections with malformations was identified by dam number and saved in 70% alcohol.

Fetuses were examined for skeletal defects by using a modified Staples technique (*11*). They were fixed in 95% alcohol, eviscerated and macerated in 2% KOH/Alizarin Red S solution. The fetuses were further macerated and cleared in the appropriate solutions of 2% KOH/glycerin (60:40, 40:60, 20:80) and stored in 100% glycerin. A crystal of thymol was added to each storage vial to retard fungal growth. Storage vials were individually identified by dam number.

Statistical Analysis

Numbers of implants and proportions of resorptions were independently analyzed by using a Kruskal-Wallis

Table 2. Concentrations of vapors generated in inhalation study.

	Days of exposure	Vapors generated, ppm: By infrared ($\bar{X}$ ± SD)	Vapors generated, ppm: By gas chromatography ($\bar{X}$ ± SD)
Methoxyethanol	11	199.2 ± 0.98	[b]
	29	101.4 ± 2.2	99.3 ± 16.3
	19	50.6 ± 1.0	47.4 ± 5.9
Butoxyethanol	11	194.4 ± 6.1	[b]
	20	153.3 ± 6.8	176.8 ± 8.3[c]
Ethoxyethyl acetate	12	601.0 ± 3.47	594.9 ± 109.9
	21	[a]	387.2 ± 8.7
	12	[a]	128.9 ± 1.5
2-(2-Ethoxyethoxy) ethanol	29	[a]	102.2 ± 15.6
2-Methylaminoethanol	28	[a]	150.0 ± 15.2

[a]Due to equipment problems, suitable data for concentrations from the infrared analyzer were not available for these chemicals.
[b]Gas chromatograph samples were not taken in these exposures.
[c]This charcoal tube result is approximately 15% higher than that from the infrared analyzer (IR); since the IR provided an essentially continuous daily record of exposures, we have used the figures (usually rounded to the nearer 10 ppm) from the IR throughout the text for all chemicals where suitable data from the IR were available. Where IR data were not available, the charcoal tube results were used within the text.

test corrected for ties, with subsequent multiple comparisons to determine where the differences occurred (*12*). Analysis of pup weights involved a mixed model analysis of covariance (with the number of live pups in the litter as the covariate) using maximum likelihood estimation (*13,14*). The model was mixed, since there was both within-litter and between-litter variation. Subsequently, pairwise comparisons between the pooled control group and each treatment group were performed. Incidence of total defects and of total variants were compared using a Kruskal-Wallis test with multiple comparisons (*12*) with the litter as the experimental unit and the level of significance at $p < 0.05$.

Results

The concentrations within the exposure chambers as measured by the infrared analyzer were relatively close to those obtained from gas chromatography (Table 2).

Control

No visceral or skeletal malformations were observed in fetuses from control dams. The types of visceral and skeletal variants observed in fetuses from the control treated dams were similar to those variants observed in chemically exposed groups.

Methoxyethanol (ME)

Concentrations of ME (Table 2) were easily generated and were rapidly cleared from the exposure chambers. There were no overt signs of toxicity in the pregnant rats in any of the groups. The only weight decrements in the maternal animals were likely due to increased resorptions. As shown in Table 3, all litters at the highest concentration (200 ppm) were entirely resorbed. At 100 ppm, about half of the litters (18 of 34) were entirely resorbed, and all litters had some resorptions; the mean weight of the live fetuses was reduced by approximately one-third from the control value. At 50 ppm there was approximately a 3-fold increase in resorptions and fetal weights were reduced by 20% compared to the control values.

Visceral malformations (Table 4) were found in fetuses from both the 50 ppm and 100 ppm ME-treated dams, but only the incidence of those from the 100 ppm group was significantly different from the controls ($p \leq 0.025$). Heart abnormalities were the predominant malformations observed in both the 100 ppm and 50 ppm ME groups. Malformations of the retina, eye, umbilicus and lungs of ME-exposed fetuses were also observed but at a frequency lower than the aforementioned.

Wavy, fused, and absent ribs, plus extra vertebrae were observed in the 100 and 50 ppm ME treatment groups (Table 5). Additionally, tail malformations were observed in the 100 ppm group. Statistical analyses of these pooled data indicate significant differences from controls for both the 50 ppm ($p < 0.025$) and 100 ppm ($p < 0.05$) treatment groups. Fetuses in the 50 ppm group demonstrated more rib malformations than fetuses in the 100 ppm group. The failure of the incidence of malformations to increase with dose may be the result of increased embryo lethality observed in the high dose group. These data indicate that inhalation of 50 or 100 ppm ME is teratogenic in the rat.

Table 3. Effects of maternal exposure to glycol ethers on pregnancy outcome.

		2-Methoxyethanol			2-Butoxyethanol		2-Ethoxyethyl acetate		
	Controls	50 ppm	100 ppm	200 ppm	150 ppm	200 ppm	130 ppm	390 ppm	600 ppm
Number pregnant/number mated	34/41	14/17	34/38	8 /11	16/19	15/18	15/15	15/20	9/9
Implants/dam ($\bar{X}$)	12.7	12.5	12.5	12.6	12.9	15.1[a]	14.5[a]	12.9	14.7[a]
Litters with resorptions, %	14	9	34	8	4	9	7	9	9
	(42)	(64)	(100)	(100)	(25)	(60)	(47)	(56)	(100)
Resorptions/litter ($\bar{X}$)	0.64	1.6[a]	9.8[a]	12.6[a]	0.23	1.2	0.49	2.3[a]	14.7[a]
Resorptions									
Early	21	15	192	—	4	16	8	20	78
Middle	1	2	77	—	0	1	0	7	19
Late	1	2	61	—	0	1	0	8	6
Total	23	19	330	101	4	18	8	35	103
(% of implants)	(5)	(11)	(77)	(100)	(2)	(8)	(4)	(17)	(100)
Total live fetuses	407	156	96	0	206	203	211	175	0
Live fetuses/litter ($\bar{X}$)	12.2	11.1	2.8	0	12.6	13.9	14.0	10.8	0
(% of implants)	(95)	(89)	(23)		(98)	(92)	(96)	(83)	
Live fetal weights ($\bar{X}$)									
Female	3.46	2.84[a]	2.29[a]	—	3.28[a]	3.49	3.31[a]	2.74[a]	—
Male	3.64	2.91[a]	2.49[a]	—	3.55[a]	3.66	3.46[a]	2.85[a]	—
Standard error of mean[b]	0.07	0.07	0.28		0.10	0.10	0.10	0.10	

[a]Significantly different from controls at $p < 0.05$ (only data on implants, $\bar{X}$ resorptions, and fetal weights were analyzed).
[b]In no case was there a significant difference between sexes on this variable; hence, only the larger is presented.

Table 4. Visceral malformations.

		ME		BU		EEAC	
	Controls	50 ppm	100 ppm	150 ppm	200 ppm	130 ppm	390 ppm
Number of litters	34	14	11	16	15	15	15
Number of fetuses examined	270	103	65	135	135	142	116
Cardiac malformation, no. (%)							
IV septal defect	0	4(4)	13(20)	0	0	0	6(5)
Ringed aortic arch	0	1(1)	4(6)	0	0	1(1)	2(2)
Rt.-sided aortic arch	0	1(1)	1(2)	0	0	0	0
Rt.-sided ductus arteriosus	0	0	0	0	0	0	1(1)
Pulmonary valve stenosis	0	0	1(2)	0	0	0	0
Truncus arteriosus communus	0	0	1(2)	0	0	0	0
Levocardia	0	0	1(2)	0	0	0	0
Pulmonary malformations							
Hypoplastic lungs	0	0	2(3)	0	0	0	0
Optical malformation							
Deformed retina	0	1(1)	0	0	0	0	0
Reduced eye size	0	0	1(2)	0	0	0	0
Miscellaneous							
Umbilical hernia	0	1(1)	0	0	0	0	2(2)
Total fetuses normal[a]	191	55	27	104	99	95	55
Total fetuses malformed	0	8	19	0	0	1	7
Total litters affected	0	6	10	0	0	1	3
Statistical significance		NS[b]	$p < 0.025$	NS	NS	NS	$p < 0.01$

[a]Normal defined as having no visceral variations or malformations.
[b]NS = not significant at $p = 0.05$.

Table 5. Skeletal malformations.

		ME		BU		EEAC	
	Control	50 ppm	100 ppm	150 ppm	200 ppm	130 ppm	390 ppm
Number of litters	34	14	9	16	15	15	15
Number of fetuses examined	137	53	31	68	71	69	59
Vertebra malformations, no. (%)							
Extra	0	0	2(6)	0	0	0	0
Ribs							
Rudimentary 13th[a]	0	0	0	1(1)	0	0	0
Wavy	0	15(28)	5(16)	0	0	0	2(4)
Fused	0	2(4)	0	0	0	0	1(2)
Absent	0	1(2)	0	0	0	0	0
Tail							
Shortened	0	0	4(6)	0	0	0	0
Absent	0	0	1(2)	0	0	0	0
Total fetuses normal[b]	137	2	1	37	52	30	2
Total fetuses malformed	0	16	10	0	0	0	4
Total litters affected	0	8	4	0	0	0	3
Statistical significance		$p < 0.025$	$p < 0.05$	NS[c]	NS	NS	$p < 0.01$

[a]Considered a minor malformation.
[b]Normal defined as having no skeletal malformations or deviations.
[c]NS = not significant at $p = 0.05$.

Table 6. Visceral variations.

		ME		BU		EEAC	
	Control	50 ppm	100 ppm	150 ppm	200 ppm	130 ppm	390 ppm
Number of litters	34	14	11	16	15	15	15
Number of fetuses examined	270	103	65	135	135	142	116
Cranial variations, no. (%)							
Enlarged lateral ventricles	5(2)	21(20)	24(37)	0	5(4)	7(5)	22(19)
Cardiac							
Enlarged atria	0	0	1(2)	0	0	0	0
Urogenital							
Hydronephrosis	4(2)	1(1)	2(3)	2(1)	3(2)	6(4)	6(5)
Bilateral	1	0	1	2	1	1	5
Unilateral	3	1	1	0	2	5	1
Dilated renal pelvis	39(14)	23(22)	2(3)	13(10)	19(14)	25(18)	14(12)
Bilateral	13	16	2	7	9	13	7
Unilateral	26	7	0	6	10	12	7
Low set kidney	6(2)	0	0	0	1(1)	2(1)	8(7)
Hypoplastic kidney	0	0	0	0	0	0	1(1)
Hydroureter	52(19)	8(8)	3(5)	17(13)	12(9)	34(24)	19(16)
Bilateral	21	5	1	9	3	25	11
Unilateral	31	3	2	8	2	9	8
Misplaced testes	20(7)	11(11)	5(8)	10(7)	12(9)	5(4)	10(9)
Bilateral	5	1	1	0	1	1	2
Unilateral	15	10	4	10	11	4	8
Total fetuses normal	191	55	27	104	99	95	55
Total fetuses with variations	79	44	31	31	36	47	58
Total litters affected	26	13	10	12	13	14	15
Statistical significance		$p < 0.01$	$p < 0.01$	NS[a]	NS	NS	$p < 0.01$

[a]NS = not significant at $p = 0.05$.

Visceral variants observed in fetuses from both ME-treated groups were similar to those variants observed in control fetuses (Table 6); however, their frequency in exposed groups was significantly increased above that for the control group ($p < 0.01$). Skeletal variants were significantly more numerous in fetuses from both the 50 ($p < 0.01$) and 100 ppm ($p < 0.025$) ME-exposed dams than were variants in fetuses from control dams (Table 7). These data indicate that inhalation of ME did cause growth retardation in the rat.

Butoxyethanol (BU)

Concentrations of BU were more difficult to generate in that the solvent required heat to generate even the low concentrations. Clearance time from the chambers was also longer than for ME. This was the only one of the chemicals which produced apparent maternal toxicity [ranging from hematuria, as has been reported in the literature (*15*), to death at concentrations from 250 to 500 ppm.* Based on the maternal toxicity, exposure levels of 150 and 200 ppm were chosen for the teratology study.

Pregnant rats exposed to 200 ppm BU showed some hematuria, but only on the first day of exposure. With that exception, the dams showed no adverse effects. There was a significant increase in the mean number of implants in the 200 ppm BU group, but since no treatment began until day 7, this increase was not treatment-related. There was also no apparent adverse effect upon the pups, as the number of resorptions, fetal weights, and incidence of malformations did not differ from controls (Tables 3–7). (There was a statistically significant decrease in fetal weights in the 150 ppm BU groups, but since the differences were slight and there were no effects at the higher level, these differences

In a dose finding study conducted at the outset of the project, nonpregnant rats (250–300 g) were exposed to 250, 350 or 450 to 500 ppm BU (no charcoal tube verification). Three of four rats exposed to 450 to 500 ppm of BU for 6.5 hr died within 36 hr after termination of exposure, and two of three nonpregnant rats died within 18 hr after exposure to approximately 350 ppm BU for 7 hr. Of three nonpregnant rats exposed for 7 hr to 250 ppm BU, one died within 18 hr after exposure, and a second died after day 2 of exposure. With each of these concentrations, the exposed rats began showing various degrees of hematuria 4 to 6 hr after onset of exposure, as has been reported elsewhere (*15*). From each of these concentrations, one rat survived, and these three animals were placed together in a 41 × 32 × 18-cm steel cage. Approximately 1 week after exposure, the distal portion of the tail (about one-half the tail length) became necrotic and fell off or was chewed off. Observations of a small number of singly housed rats exposed cutaneously to butoxyethanol would suggest that each rat chewed its own tail off.

Table 7. Skeletal variations.

		ME		BU		EEAC	
	Control	50 ppm	100 ppm	150 ppm	200 ppm	130 ppm	390 ppm
Number of litters	34	14	9	16	15	15	15
Number of fetuses examined	137	53	31	68	71	69	59
Skull variations, no. (%)							
Incomplete ossification							
Frontal	1(1)	16(30)	4(13)	0	1(1)	1(1)	12(21)
Parietal	3(2)	16(30)	15(48)	0	1(1)	1(1)	12(21)
Interparietal	3(2)	15(28)	12(39)	1(1)	0	1(1)	10(17)
Supraoccipital	2(2)	17(32)	18(58)	0	0	1(1)	11(19)
Maxilla	0	0	5(16)	0	0	0	0
Vertebra							
Absent							
Sacral/caudal	0	35(66)	3(10)	4(6)	0	0	2(4)
Incomplete ossification							
Thoracic	0	0	0	0	0	0	0
Lumbar	0	1(2)	0	0	0	0	0
Sacral/caudal	20(15)	21(40)	12(39)	9(13)	7(10)	22(32)	26(46)
Centra							
Absent							
Thoracic	0	14(26)	16(52)	0	0	1(1)	17(30)
Lumbar	0	0	1(3)	0	0	0	0
Sacral/caudal	0	23(43)	5(16)	0	0	0	1(2)
Incomplete ossification							
Thoracic	0	0	0	0	0	0	0
Lumbar	0	0	0	0	0	0	0
Sacral/caudal	0	1(2)	0	0	0	5(7)	8(14)
Abnormal							
Thoracic	6(4)	40(75)	21(68)	15(22)	7(10)	11(16)	32(56)
Lumbar	0	16(30)	16(52)	0	1(1)	0	17(30)
Sacral/caudal	0	3(6)	3(10)	0	0	0	0
Sternebrae							
4-incomplete ossification	2(2)	17(32)	23(74)	3(4)	0	1(1)	12(21)
Abnormal	1(1)	11(21)	5(16)	1(1)	0	0	5(9)
Ribs							
Rudimentary 14th	5(4)	4(8)	14(45)	3(4)	9(13)	10(14)	36(63)
Pelvic girdle							
Pubis							
Absent (not ossified)	1(1)	9(17)	6(19)	0	0	1(1)	2(4)
Incomplete ossification	0	3(6)	5(16)	0	0	0	0
Illium							
Absent (not ossified)	0	0	12(39)	0	0	0	0
Incomplete ossification	0	0	5(16)	0	0	1(1)	7(12)
Ischium							
Absent (not ossified)	0	1(2)	9(29)	0	0	0	0
Incomplete ossification	2(2)	11(21)	6(19)	0	0	1(1)	3(5)
Other							
Metacarpals (3 ossified)	0	19(36)	12(39)	0	0	1(1)	11(19)
Total fetuses normal	100	2	1	37	52	30	2
Total fetuses with variations	37	50	30	31	19	39	56
Total litters affected	19	13	9	10	8	14	15
Statistical significance		$p < 0.01$	$p < 0.025$	NS[a]	NS	$p < 0.05$[b]	$p < 0.01$

[a]NS = not significant at $p = 0.05$.
[b]130 ppm vs. 390 ppm, $p < 0.05$.

Table 8. Effects of maternal exposure to 2-(2-ethoxyethoxy)ethanol (2-EEE) or 2-methylaminoethanol (2-MAE) on pregnancy outcome.

	Controls	2-EEE	2-MAE
Number pregnant/number mated	15/16	20/21	18/24
Implants/dam ($\bar{X}$)	13.1	13.4	11.9
Litters with resorptions (%)	10(67)	12(60)	10(56)
Resorptions/litter ($\bar{X}$)	1.3	0.9	0.7
Total live fetuses	174	250	212
Live fetuses/litter ($\bar{X}$)	11.6	12.5	11.8
(% of implants)			
Live fetal weights ($\bar{X}$)			
Female	3.26	3.19	3.59
Male	3.33	3.25	3.69
Standard deviation	0.30	0.25	0.31
Number (%) of litters with abnormal fetuses[a]	5(33)	6(30)	7(39)

[a]Litter with at least one fetus having either skeletal or visceral anomalies; detailed in Tables 9 and 10.

Table 9. Summary of visceral observations after exposure to 2-(2-ethoxyethoxy)ethanol or 2-methylaminoethanol.

	Number of litters (fetuses)/percent[a]		
	Control	2-EEE	2-MAE
Number of litters	15	19	18
Number of fetuses examined	116	166	143
Malformations			
Cardiovascular:			
Double aortic arch	0	1(1)/.53	0
Missing innominate	0	1(1)/.53	0
Brain:			
Hydrocephalus (mild)	3(6)/8	1(4)/4	3(5)/4
Variations			
Testicular: (number males examined)	62	76	74
Undescended testes	0	0	1(1)/1
Position variation	10(22)/39	13(17)/22	9(13)/17
Urinary:			
Dilated renal pelvis	3(4)/3	7(13)/7	5(5)/3
Dilated ureter	3(6)/5	9(19)/11	6(18)/11
Hydronephrosis	2(2)/2	3(4)/3	0
Hydroureter	3(3)/2	4(7)/4	5(6)/4
Ectopic kidney	1(1)/1	2(2)/1	0
Other:			
Abdominal hemorrhage	1(1)/1	1(1)/1	0
Liver hemorrhage	0	1(1)/1	0
Number of fetuses with variations	30	43	27
Percent normal[b,c]	89 ± 26	91 ± 21	92 ± 15
Number of fetuses	107	153	131
Percent abnormal[b]	11 ± 26	9 ± 21	8 ± 15
Number of fetuses	9	13	12

[a]Mean of litter mean percent.
[b]Mean of litter mean percent ± SD.
[c]Includes fetuses with variations.

were likely not of biological significance.) After exposure to 150 ppm BU, rats occasionally showed slight hematuria on the first day of exposure, but no other adverse effects were noted in the dams or pups; one fetus with cephalothoracomphalopagus was observed, but this was likely a chance occurrence and not treatment-related.

2-Ethoxyethyl Acetate (EEAC)

Concentrations of EEAC were relatively easy to generate and cleared rapidly from the chamber. Maternal animals showed no overt toxicity; a weight reduction was observed at higher concentrations, but this was likely due to increased resorptions. As shown in Table 3, all offspring from dams exposed to 600 ppm EEAC were resorbed. At 390 ppm there was a significant increase in resorptions and fetal weights were reduced by 21%, and at 130 ppm there were slight but significant decreases in fetal weights.

Visceral malformations of the heart and umbilicus were observed in fetuses from the 390 ppm EEAC-treated dams (Table 4). Only one fetus from the 130 ppm group had a heart defect. Statistical analysis of the data

Table 10. Summary of skeletal observations after exposure to 2-(2-ethoxyethoxy)ethanol or 2-methylaminoethanol.

	Number of litters (fetuses)/percent[a]		
	Control	2-EEE	2-MAE
Number of litters	14	19	18
Number of fetuses examined	58	84	69
Malformations			
Missing ribs	0	1(1)/1	0
Decreased thoracic vertebrae	0	1(1)/1	0
Variations			
Ribs:			
Rudimentary 14th	1(1)/2	5(7)/8	1(2)/4
Rudimentary lumbar	0	0	1(1)/1
Skull:			
Incomplete ossification			
Supraoccipital	0	1(1)/1	1(1)/1
Interparietal	1(1)/2	2(2)/3	1(2)/2
Parietal	1(1)/2	3(3)/4	0
Frontal	0	2(2)/3	0
Sternebrae:			
Decreased number (< 4)	1(1)/2	1(1)/1	0
Split	0	0	1(1)/1
Misaligned	1(1)/2	6(6)/7	5(5)/7
Centra			
Thoracic			
Missing, half-sized	0	4(4)/5	0
Misshapen	1(1)/2	3(3)/3	5(5)/8
Appendages			
Decreased metatarsals (< 4)	0	3(3)/4	0
Decreased metacarpels (< 3)	0	1(1)/1	0
Pelvic girdle			
Pubis, missing, pinpoint	0	1(1)/1	2(2)/3
Ischium, missing, reduced size	0	1(1)/1	1(1)/1
Number of fetuses with variations	4	23	16
Number of fetuses with no variations or malformations	14(54)/93	19(61)/73	18(53)/77
Percent normal[b,c]	100 ± 0	99 ± 6	100 ± 0
Number of fetuses	58	83	69
Percent abnormal	0	1 (6)	0
Number of fetuses	0	1	0

[a]Mean of litter mean percent.
[b]Mean of litter mean percent ± SD.
[c]Includes fetuses with variations.

indicated significant differences between EEAC treated and control groups ($p < 0.01$); furthermore, since heart defects rarely occur spontaneously, and none were observed in control fetuses, these data indicate that inhalation of either 130 ppm of 390 ppm EEAC is teratogenic in the rat.

Malformations of the ribs were observed in three fetuses from 390 ppm EEAC-exposed dams (Table 5); no skeletal malformations were observed in fetuses from the 130 ppm EEAC group. Statistical analysis of these data indicated significant differences from the controls ($p < 0.01$); however, due to the low frequency of occurrence of these malformations, treatment-induced cause cannot be positively concluded.

Visceral variants observed in fetuses from EEAC-treated dams were similar to those variants observed in control fetuses (Table 6); however, the frequency of variants in the exposed groups was significantly different from the control-treated group Significant statistical differences in skeleltal variants (Table 7) were observed between control and 130 ppm ($p < 0.05$) and 390 ppm ($p < 0.01$) and also between 130 ppm and 390 ppm treatment groups ($p < 0.01$). ($p < 0.05$). These data indicate that inhalation of EEAC at either dose level during the period of organogenesis can cause growth retardation in the rat.

2-(2-Ethoxyethoxy)ethanol (Carbitol, 2-EEE)

Due to the low vapor pressure of 2-EEE, higher concentrations could not be tested due to probable formation of an aerosol (this was observed during chamber calibration). No toxicity was observed in maternal animals or in the offspring (Tables 8–10) after exposure to 100 ppm 2-EEE (mean concentration from 65 charcoal tubes or 2 to 3 per day = 102 ppm). Consequently, 2-EEE is likely not a teratogenic hazard after inhalation exposure. Nor is it of teratogenic risk when given in comparable molar equivalents to a cutaneously teratogenic (4) level of ethoxyethanol (Nelson, unpublished observations).

2-Methylaminoethanol (2-MAE)

Low vapor pressure also prevented our generating high concentrations of 2-MAE. At 150 ppm 2-MAE (mean concentration from 28 silica gel tubes, one per day, analyzed in duplicate = 150.0 ppm), no maternal or fetal toxicity was observed (Tables 8–10).

Discussion

In this study of the inhalation teratogenic effects of four glycol ethers and an amino derivative, methoxyethanol was the most embryotoxic; ethoxyethyl acetate was less potent; but butoxyethanol, 2-(2-ethoxyethoxy)-ethanol, and 2-methylaminoethanol were not embryotoxic at the concentrations tested. Congenital malformations in mice have been reported after the mice were intubated with various levels of ME on gestation days 7–14 (*3*). Comparing the frequency of resorptions in that study with the frequency of resorptions we observed, it appears that our level of 100 ppm ME would roughly correspond to their dose of 250 mg/kg. At one half that level (50 ppm and 125 mg/kg) in both studies, there was little increase in resorptions, but malformations were observed. Since the study in mice (*3*) found malformations at levels lower than this (even at their lowest dose of 31.25 mg/kg), it is possible that malformations could be induced by concentrations lower than our low level of 50 ppm.

The results from ethoxyethyl acetate were as expected. Based upon the ubiquitous nature of the esterases within the body, and upon work with testicular atrophy (*7*), we had predicted at the outset of the study that, calculated on a molar basis, EEAC would have precisely the same effects as ethoxyethanol (EE). Consequently, 600 ppm EEAC was expected to, and did, induce complete embryomortality as 600 ppm EE had done (*5,16*). At 390 ppm EEAC, resorptions were only slightly increased, though fetal weights were depressed and malformations were observed. At 130 ppm EEAC, fetal weights were still depressed and there was one defective fetus, indicating that this level is near the teratogenic threshold but should still be considered teratogenic in the rat. Thus, there is a relatively steep dose-effect curve with EEAC, and the same can be said of 2-ethoxyethanol and ME.

Butoxyethanol appears to be rather unique in this series, in that it produces maternal toxicity at levels below those which produce embryotoxicity. Its toxicity and hemolytic effects have been described (*15*), with a recent study verifying and extending the previous report (*17*). The same investigators also indicated (*15*) that the likely metabolic product is butoxyacetic acid, and that the majority of the metabolite is excreted within 24 hr. That the excretion is fairly rapid is also suggested from our results in that the hematuria was observed only on the first day of exposure. As is apparently true with most of the glycol ethers, BU is readily absorbed through the skin (*18*). Our incidental observation of necrosis in the tails of rats exposed to BU was not noted in the literature. Whether the necrosis was due to damage in the extremity of the peripheral nervous system (in the tail) or to the lack of oxygen and nutrients provided by the blood of the region is unknown, though the known effects of BU on the blood suggest the latter as a strong possibility.

Theoretically, one of the biotransformation products of 2-EEE would be 2-ethoxyethanol (or its biotransformation products). Thus it may be that high levels of 2-EEE would be teratogenic, though this remains as only speculation since we could not generate higher levels of 2-EEE as a vapor. Considering all of these results in conjunction with the previously reported teratogenic effects of 2-ethoxyethanol, it appears that longer chain glycol ethers are less embryotoxic than the short-chain glycol ethers; this would be expected due to the relatively lower lipid solubilities of the longer chain compounds (*19*). Further, based on our results with 2-ethoxyethyl acetate, it is likely that the acetates of other glycol ethers would have similar reproductive toxicity to their corresponding ethers.

Finally, the lack of teratogenic response of 2-methylaminoethanol was interesting and from a mechanistic or theoretical point of view, would merit follow up using a different route of exposure. At first glance, one might expect that its biotransformation would be similar to that of 2-ME. However, our results of no maternal or fetal toxicity at 150 ppm 2-MAE suggest that this may not be the case; since the amine is likely more lipid-soluble and less water-soluble than the methoxy portion, the absorption and excretion of the 2-MAE is likely quite different from that of 2-ME. Thus it would be of interest to see if a higher dose of 2-MAE would be teratogenic, though a route other than inhalation would be required, since the vapor concentration we used was near the saturation point.* This lack of teratogenicity at three times the concentration of a teratogenic level of its structurally similar glycol ether, points to a relatively strict structural requirement to produce teratogenic effects.

The present study does not elucidate the mechanism of action by which the solvents induce embryotoxicity. Nor did we determine biotransformation pathways, though it is likely that each glycol ether undergoes

* One purpose of this study was to select another glycol ether for inclusion in a behavioral teratology study. As exposure to 200 ppm 2-ethoxyethanol on gestation days 14–20 was previously reported to cause neonatal deaths (*6*), small groups of animals were also exposed to the chemicals in the present study on gestation days 14–20. Maternal animals reared their young (litters culled to four female and four male pups on the day of birth) to weaning, and the offspring were weighed weekly for 5 weeks. The results indicated that 100 ppm 2-ME (N = 6) delayed parturition by approximately 36 hr and no pups survived for 1 week; 50 ppm 2-HE (N = 5) produced little, if any, delay in parturition, and all pups survived. Exposure to 600 ppm EEAC (N = 3) delayed parturition by around 24 hr and produced 75% mortality in the offspring; 390 ppm (N = 3) delayed parturition by about 12 hr, but all offspring survived; neither 130 ppm EEAC (N = 4) nor 150 ppm BU (N = 4) produced any adverse effects in the offspring.

biotransformation via alcohol and aldehyde dehydrogenase, yielding its corresponding acetaldehyde and subsequently excreted primarily as the acetic acid derivative. We observed that embryotoxicity decreases as alkyl chain length increases, similar to observations with testicular atrophy (*7*). We found that short-chain glycol ethers and their corresponding esters are embryotoxic and teratogenic in rats. Since previous research found similar effects induced by methoxyethanol in mice via intubation (*3*) and ethoxyethanol in rabbits (*5*) via inhalation, and in rats via cutaneous exposure (*4*), teratogenic effects have been induced in three lower species of animals by common routes of human exposure. Such effects call for careful examination of human populations exposed to this class of solvents, particularly since these effects in animals were observed at concentrations which are at or below current permissible exposure limits.

NOTE: Since the time this paper was prepared, there has been a proliferation of research on the glycol ethers; we refer readers to the other paper in this issue for more recent research.

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