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Teratological Assessment of Methanol and Ethanol at High Inhalation Levels in Rats

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Teratological Assessment of Methanol and Ethanol at High Inhalation Levels in Rats. NELSON, B. K., BRIGHTWELL, W. S., MACKENZIE, D. R., KHAN, A., BURG, J. R., WEIGEL, W. W., AND GOAD, P. T. (1985). *Fundam. Appl. Toxicol.* **5**, 727–736. Alcohols are widely used as industrial solvents. In spite of the fact that ethanol is a human teratogen, there has not been systematic investigation of the potential teratogenic effects of other alcohols, particularly using the inhalation route of exposure, as would be appropriate in assessing occupational and environmental types of experience. As part of a large teratological examination of industrial alcohols, methanol and ethanol were administered by inhalation to groups of approximately 15 pregnant Sprague–Dawley rats. Methanol was administered at 20,000 ppm (20ME), 10,000 ppm (10ME), 5000 ppm (5ME), and 0 ppm (MECO) for 7 hr/day on Days 1–19 of gestation (Days 7–15 for 20ME). Ethanol was administered at 20,000 ppm (20ET), 16,000 ppm (16ET), 10,000 ppm (10ET), and 0 ppm (ETCO) for 7 hr/day on Days 1–19 of gestation. Dams were sacrificed on Day 20 (sperm = Day 0). One-half of the fetuses were examined using the Wilson technique for visceral defects, and the other half were examined for skeletal defects. The highest concentration of methanol (20ME) produced slight maternal toxicity and a high incidence of congenital malformations ($p < 0.001$), predominantly extra or rudimentary cervical ribs and urinary or cardiovascular defects. Similar malformations were seen in the 10ME group, but the incidence was not significantly different from controls. No adverse effects were noted in the 5ME group. Dams in the 20ET group were narcotized by the end of exposure, and maternal weight gain and feed intake were decreased during the first week of exposure. The 16ET dams had slightly depressed weight gain ($p < 0.01$) during the first week of exposure, but there were no significant effects on feed consumption. There was no definite increase in malformations at any level of ethanol, although the incidence in the 20ET group was of borderline significance.

Alcohols are widely used as industrial solvents. Their toxicology has been thoroughly reviewed (von Oettingen, 1943), with a more recent review also available (Rowe and McCollister, 1982). As early as 1869, Richardson demonstrated that, in mature animals, the toxicity of aliphatic alcohols increases with molecular weight. However, there has not been sufficient research to ascertain if a similar relationship holds true for reproductive or teratogenic effects. Although chronic ingestion of large quantities of ethanol is widely known to be teratogenic in humans (e.g., Streissguth *et al.*, 1980), there has been relatively little investigation of other alcohols for teratogenicity, even in animals. The literature reports two studies, one completed and the other in progress. Daniel and Evans (1982) reported that tertiary butanol was more potent as a behavioral teratogen in mice than was ethanol. Mankes *et al.* (1983) indicated that they are investigating the teratogenic effects of substituted ethanols in rats. Thus there is recent interest in evaluating the teratogenic effects of other alcohols in experimental animals. As part of a large

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study to evaluate the teratogenic effects of industrial alcohols, concentrating on the structure–activity relationships of straight-chain alcohols, this report presents the results of an inhalation teratology study of methanol and ethanol in rats.

METHODS

Experimental animals: Housing conditions and procedures. Virgin female Sprague–Dawley rats (176–200 g) specified to be free of *Mycoplasma,* Sendai virus, and internal and external parasites (Charles River Breeding Laboratories, Wilmington, Mass.)[1] were acclimated to a 12-hr light/dark cycle and to a temperature of 24 ± 2°C after quarantine for 1–2 weeks. The humidity was not controlled, but was generally about 40% (range 20–60%). Breeder males over 300 g from the same source were housed individually under similar conditions in 32 × 41 × 18-cm suspended stainless-steel wire-mesh cages equipped with automatic water dispensers (Hoeltge Inc., Cincinnati, Ohio). Virgin females were housed three per cage in similar cages. Purina or a comparable-grade lab chow and tap water were available *ad libitum,* except when pregnant animals were in the exposure chambers.

For mating, virgin 200- to 300-g females were placed individually with breeder males. Each morning, the litter paper under each male's cage was examined for sperm plugs; if no plugs were detected, vaginal smears were taken. Females with sperm (Day 0 of gestation) were placed individually into 30 × 34 × 17-cm polycarbonate cages having autoclavable polyester filter covers. Bedding consisted of cleaned, heat-treated sawdust from a local supplier (Absorb-Dri, from Tasty Foods, Cincinnati, Ohio). Feed and water intake, along with maternal weights were measured weekly (i.e., on Gestation Days 0, 7, 14, and 20). Most females were also weighed each morning for the first week of exposure. From Gestation Day 1 to 19,[2] the females were transported from the animal quarters to the exposure chambers in their home-cage shoe boxes with filter tops in place. Females were placed into 13 × 25 × 18-cm compartments in stainless-steel wire-mesh caging within the exposure chambers. Controls were placed in similar caging within an adjacent exposure chamber for the same hours as the exposed animals. Exposures were conducted 7 hr/day, and the animals were left in the chambers for degassing for approximately ½ hr after vapor generation terminated. They were then removed and returned in their home cages to the animal quarters where the water bottles were replaced.

On Gestation Day 20, pregnant females were individually weighed and euthanized by CO_2 asphyxiation. The entire uterus (with ovaries attached) was removed, and the numbers of corpora lutea, resorptions (classified as to early, middle, or late), and live fetuses were counted. Fetuses were serially removed, examined for external malformations, blotted of excess fluids, weighed, and external sex was determined. One-half of the fetuses were randomly selected, placed into 80% ethanol, and subsequently eviscerated, macerated in 1.5% KOH, stained in alizarin red S and examined for skeletal malformations and variations. The other one-half of the fetuses were placed into Bouin's solution and subsequently examined for visceral malformations and variations using a razor blade cross-sectioning technique (Wilson, 1965).

Inhalation facility and procedures. The inhalation exposures were conducted in 0.5-m^3 Hinner-type exposure chambers (Charles Spengler and Associates, Cincinnati, Ohio). The vapor generation equipment was housed above the exposure chambers in glove boxes which were maintained under negative pressure to prevent any leakage of contaminants into the room. Reagent-grade methanol (Matheson, Coleman, and Bell Manufacturing Chemists, Cincinnati, Ohio) or reagent-grade (absolute-200 proof) ethanol (AAPER Alcohol and Chemical Co., Louisville, Ky.) was placed into a flask. A low-flow pump (RP model lab pump; Fluid Metering Inc., Oyster Bay, N.Y.) circulated liquid from the reservoir flask into a 10-ml syringe contained within the flask such that the syringe was constantly overflowing. Thus the syringe provided a constant head of chemical for a second pump (controlled by a micrometer adjustment) which injected the specified amount of liquid into a three-way valve which was attached to a Greensmith impinger. Heated compressed air was introduced through the second inlet of the three-way valve. Alcohol evaporation was controlled by regulating the preheating of compressed air. The impinger provided increased contact time between the air and the liquid to assure total evaporation. In generation of high concentrations, glass beads were also placed at the bottom of the impinger to further increase the heat transfer area between the alcohol and the compressed air. This vapor and air mixture was introduced into the chamber air flow prior to positioning of the orifice plate. The turbulence resulting from the pressure drop created by the orifice plate provided uniform mixing of the vapor and air before the mixture entered the chamber. Air flow through the chambers provided approximately one air change per minute.

The concentration within the chamber was monitored continuously by a Miran 1A general purpose infrared analyzer (Wilkes/Foxboro Analytical, South Norwalk,

[1] Mention of company or product names does not constitute endorsement by NIOSH.

[2] Following completion of the initial exposures to methanol (20,000 ppm), we changed the duration of exposure from Gestation Days 7–15 to Gestation Days 1–19 for the remainder of the study; the higher level of methanol was the only one in which exposures were conducted for the shorter duration.

Conn.) which was calibrated within the range to be tested. The Miran 1A was connected to a stripchart recorder for continuous recording of the concentration throughout the day. On an hourly basis, the chamber concentration, chamber temperature, and room humidity were recorded on a daily observation sheet. At the end of each day, the stripchart was attached to the data sheet and the daily mean, range, and time-weighted average concentrations were calculated. At the conclusion of the study, these daily values were averaged for an overall study mean for each concentration. In addition, the infrared analyzer associated with the ethanol exposure chamber was interfaced with an Apple II+ computer which displayed and recorded 5-min means of the ethanol exposure concentration; these means were averaged each hour to give hourly and subsequent daily means, which were also used to calculate a study mean for each concentration.

Samples of the bulk chemical were analyzed by gas chromatography for purity. In addition, silica-gel (methanol) or charcoal-tube (ethanol) samples were collected from the chamber atmosphere for independent verification of chamber concentrations. Sampling times varied from 10 to 30 min in duration, and samples were collected at the rate of 5–10 samples/week. The samples were independently analyzed by NIOSH analytical methods (NIOSH, 1977a—No. 247 for methanol; NIOSH, 1977b—No. S-56 for ethanol, with slight modifications; the NIOSH Division of Physical Sciences and Engineering, Arthur D. Little, Inc., Cambridge, Mass., and Southern Research Institute, Birmingham, Ala., provided purity analyses of the bulk chemicals and analyzed the silica-gel and charcoal-tube samples).

Determination of blood levels. For determining blood levels of methanol and ethanol, three nonpregnant female rats were exposed for 1, 10, or 19 days along with the pregnant rats. Immediately upon the chamber concentration reaching near 0 (about 5 min after cessation of vapor generation), the rats were removed from the chamber and were euthanized by a CO_2 overdose. The abdomen was surgically opened and approximately 5 ml of blood was removed from the inferior vena cava and placed into Vacutainer tubes (having heparin or EDTA). These tubes were frozen at approximately 0°C until they were analyzed.

Blood samples from some methanol-exposed animals and one series of animals exposed to 16,000 ppm ethanol were analyzed by the gas-chromatographic head-space technique (Tolos, 1984). The remaining methanol blood samples were analyzed by direct injection of a protein-free filtrate prepared using 6% trichloroacetic acid. The separations were made on a 2-m Chromosorb 103 column at 80 to 120°C at 2°C per min with flame ionization detection. The remaining ethanol blood samples were analyzed using the Sigma Ethyl Alcohol Reagent Kit (No. 332-UV; Sigma, 1982).

Statistical analyses. For the maternal data, multivariate analysis (with baseline as a covariate) was used for weight comparisons across groups. The group differences in food and water intake were assessed using multivariate analysis of variance. A Kruskal–Wallis test was used for group comparisons of corpora lutea per animal.

For the fetal data, analysis of variance was used to compare fetal weights across groups × sex. Group comparisons of the variables including litter size, percentage alive/litter, percentage normal/litter, and percentage females/litter were made using the Kruskal–Wallis test. For the variables including skeletal malformations, skeletal variations, visceral malformations, visceral variations, external malformations, and nonnormal fetuses, the number of litters with one or more of the variables of interest was compared between groups using Fisher's exact test. The results of the tests were adjusted for multiple comparisons, when appropriate, using the Bonferroni technique.

RESULTS

The purity of methanol, as measured by a gas chromatograph equipped with a flame ionization detector, was 99.1%. The initial sample of ethanol was of 96.5% purity, with another 3–4% assumed to be water. However, analysis of a second sample did not reveal any water (detection limits = 0.5%). Since benzene is used in the dehydration process of ethanol, a third sample (of 99.3% purity) was analyzed for benzene, but none was detected (detection limits = 0.05%).

Throughout the exposures, concentrations of methanol and ethanol were stable and uniform. Target concentrations of methanol were 20,000 ppm (20ME), 10,000 ppm (10ME), 5000 ppm (5ME), and 0 ppm (MECO). Means from the infrared analyzer (and from silica-gel tubes) were 19,976 (18,571) ppm, 10,044 (8850) ppm, and 5047 (4717) ppm. For ethanol, target concentrations were 20,000 ppm (20ET), 16,000 ppm (16ET), 10,000 ppm (10ET), and 0 (ETCO) ppm. Means from the infrared analyzer (and from charcoal tubes) were 20,197 (18,362) ppm, 15,904 (12,975) ppm, and 10,013 (9748) ppm. The 5ME and the 20ET groups were added after the results from the other exposure groups were obtained; consequently, these two groups were compared with a third group of controls (EMCO). In most cases, the means from the infrared analyzer and

the silica gel or charcoal tubes were relatively close to the target concentration; hence, the target concentration is cited throughout this paper. The reason for the larger percentage difference between the infrared-analyzer results and the charcoal-tube results for 16,000-ppm ethanol is unclear. Between 15 and 30% of the total ethanol was found in the back-up sections of the charcoal tubes at all concentrations; consequently, some of the ethanol vapors may have dissipated from the tubes. Infrequent charcoal-tube samples were also collected from the control chamber (typically of approximately 6 hr duration), but no methanol or ethanol was detected in these samples.

Maternal Observations

Methanol was not severely toxic to the dams even at the highest concentration. The 20ME dams had slightly unsteady gait after the initial days of exposure, but feed intake, water consumption, and body weights were not significantly affected. No adverse effects were noted in the dams from 10ME or 5ME.

Ethanol was severely toxic to the dams at the highest concentration. The 20ET group was completely narcotized at the conclusion of exposures. In contrast, the 16ET and 10ET dams were not narcotized but (subjectively) appeared to be hyperactive after the exposures. Feed intake in the 20ET group was significantly ($p < 0.05$) lower than in their comparison control group during the first week of exposure ($\bar{x} \pm$ SD 86.2 $\pm$ 18.1 vs 121.4 $\pm$ 16.9 g), although body weights were not significantly lower than controls. No other treatment-related differences were observed in maternal body weight or feed and water consumption.

Blood alcohol levels are summarized in Table 1. After exposure to 20,000 ppm meth-

TABLE 1

BLOOD ALCOHOL LEVELS AFTER EXPOSURE OF NONPREGNANT RATS TO VARIOUS CONCENTRATIONS OF METHANOL AND ETHANOL FOR 7 hr/DAY[a]

	Days of exposure		
	1	10	19
Methanol (ppm)			
5,000	1.00 ± 0.21[b] (0.76–1.16)	2.17 ± 0.25[c] (1.90–2.39)	1.26 ± 0.39[c] (0.81–1.53)
10,000[b]	2.24 ± 0.20 (1.88–2.73)	1.84 ± 0.10 (1.42–2.17)	2.04 ± 0.09 (1.98–2.10)
20,000	8.65 ± 0.40[b] (8.34–9.26)	5.25 ± 0.65[c] (4.84–6.00)	
Ethanol (ppm)			
10,000[d]	0.031 ± 0.01 (0.022–.044)	0.017 ± 0.01 (<0.01–0.03)	0.027 ± 0.01 (<0.01–0.28)
16,000[b]	0.52 ± 0.06 (0.30–0.86)	0.53 ± 0.21 (0.28–0.67)	0.43 ± 0.20 (0.24–0.61)
16,000[d]	0.84 ± 0.24 (0.68–1.11)		0.42 ± 0.17 (0.33–0.61)
20,000[d]	1.93 ± 0.59 (1.36–2.54)		1.48 + 0.41 (0.90–1.84)

[a] $\bar{x} \pm$ SD (range) in mg/ml; most data represent means from three animals exposed for 7 hr/day for 1, 10, or 19 days.

[b] Analysis by head-space technique (see text for details).

[c] Analysis by direct gas-chromatographic injection (see text for details).

[d] Analysis by ethanol assay kit (see text for details).

anol, blood levels were just under 9 mg/ml; in contrast, exposure to 20,000 ppm ethanol resulted in only 2 mg/ml, a level more similar to that resulting from exposure to 10,000 ppm methanol. Although there were not consistent differences, some levels were lower after repeated exposures than after 1 day of exposure, probably reflecting the increased biotransformation of the alcohols after induction of the metabolizing enzymes.

Fetal Observations

Exposure of pregnant rats to methanol had no effect on the numbers of corpora lutea or implantations or the percentage of dead or resorbed fetuses. However, at the two higher concentrations, methanol depressed fetal weights in a dose-related manner (Table 2). There was also a dose-related increase in the incidence of malformations after methanol exposure. There were nine fetuses (from four litters) with external malformations (three exencephaly plus six encephalocele) noted in the 20ME group. The numbers of litters with one or more skeletal malformations (14 of 15 litters, Table 3) and visceral malformations (10 of 15 litters, Table 4) were both significantly increased in the 20ME group. Overall, as may be seen in Table 5, there was a dose-dependent decrease in the frequency of normal fetuses (defined as no external, visceral, or skeletal malformations) with increasing methanol concentrations. The incidence of visceral variants (data not shown, but examples include dilated renal pelvis and position variation of the testes) did not vary among groups; however, there were more skeletal variants (data not shown, but examples include rudimentary thoracic ribs, decreased number of, or misaligned, sternebrae, and decreased number of metatarsals) in the 10ME (22% of the fetuses) and 20ME (69%) groups than the controls (12%), although the differences were not significant after correcting for multiple comparisons.

Ethanol did not significantly affect fetal weights of female pups, but it did depress the

TABLE 2

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RATS EXPOSED TO METHANOL AND ETHANOL[a]

	MECO	10ME	20ME	ETCO	10ET	16ET	EMCO	5ME	20ET
No. pregnant/No. bred	15/15	15/15	15/16	15/15	15/15	15/16	15/15	13/14	14/16
$\bar{x}$ Corpora lutea/dam ± SD	15 ± 1	17 ± 1	—[b]	15 ± 2	14 ± 1	16 ± 1	14 ± 3	16 ± 2	15 ± 2
$\bar{x}$ Implants/dam ± SD	15 ± 1	16 ± 1	14 ± 1	15 ± 2	14 ± 1	16 ± 1	14 ± 3	15 ± 1	14 ± 2
Percentage of implants resorbed	8	5	10	7	4	7	6	4	6
Sex ratio (F:M)	53:47	52:48	58:42	47:53	54:46	59:41	51:49	56:44	55:45
$\bar{x}$ Fetal weights ± SD (g)									
Female	3.15 ± 0.32	2.93 ± 0.26*	2.76 ± 0.47*	3.10 ± 0.36	3.10 ± 0.57	3.09 ± 0.43	2.99 ± 0.32	3.19 ± 0.24	2.94 ± 0.25
Male	3.34 ± 0.36	3.12 ± 0.30*	2.82 ± 0.56*	3.33 ± 0.31	3.26 ± 0.55	3.18 ± 0.39	3.16 ± 0.29	3.30 ± 0.24	3.06 ± 0.28

[a] Rats were exposed 7 hr/day throughout gestation. MECO = methanol controls; 10ME = 10,000 ppm methanol; 20ME = 20,000 ppm methanol (exposure was for Gestation Days 7–15); ETCO = ethanol controls; 10ET = 10,000 ppm ethanol; 16ET = 16,000 ppm ethanol; EMCO = ethanol, methanol controls; 5ME = 5000 ppm methanol; and 20ET = 20,000 ppm ethanol. (The EMCO group was added subsequent to our initial evaluation of methanol and ethanol; it was the comparison group for 5ME and 20ET.)

[b] Information not collected.

* Significantly different from appropriate control group at $p \leq 0.05$.

TABLE 3

SKELETAL MALFORMATIONS IN RATS AFTER PRENATAL EXPOSURE TO METHANOL AND ETHANOL[a]

	MECO	10ME	20ME	ETCO	10ET	16ET	EMCO	5ME	20ET
No. litters (fetuses) observed	15 (98)	15 (115)	15 (92)	15 (99)	15 (100)	15 (107)	15 (90)	13 (90)	14 (92)
No. litters (fetuses) affected									
Cranial	0	0			0	0	0	0	0
Abnormal exoccipital			2 (3)	0					
Abnormal zygomatic			1 (1)	0					
Abnormal nasal			0	1 (1)					
Shortened maxilla			0	1 (1)					
Split basisphenoid			0	1 (1)					
Vertebral	0				0	0	0	0	
Scoliosis		0	1 (1)	0					0
Decreased thoracic vert.		0	1 (1)	0					0
Fused thoracic centra		0	2 (2)	1 (1)					0
Decreased lumbar vert.		1 (1)	1 (1)	0					0
Fused lumbar centra		0	2 (2)	0					0
Fused cervical arches		0	0	1 (1)					0
Lordosis		0	0	0					1 (1)
Ribs	0				0		0		
Rudimentary cervical		2 (2)	12 (39)	1 (1)		2 (2)		0	2 (2)
Extra cervical		0	10 (35)	0		0		0	0
Wavy/fused		0	3 (3)	2 (2)		0		0	2 (3)
Missing		0	7 (7)	0		1 (1)		1 (1)	0
Total malformations, litters (fetuses)	0	2 (2)	14 (72)	4 (4)	0	2 (3)	0	1 (1)	4 (5)

[a] Rats were exposed 7 hr/day throughout gestation. MECO = methanol controls; 10ME = 10,000 ppm methanol; 20ME = 20,000 ppm methanol (exposure was for gestation days 7–15); ETCO = ethanol controls; 10ET = 10,000 ppm ethanol; 16 ET = 16,000 ppm ethanol; EMCO = ethanol, methanol controls; 5ME = 5000 ppm methanol; and 20ET = 20,000 ppm ethanol. (The EMCO group was added subsequent to our initial evaluation of methanol and ethanol; it was the comparison group for 5ME and 20ET.)

TABLE 4

VISCERAL MALFORMATIONS IN RATS AFTER PRENATAL EXPOSURE TO METHANOL AND ETHANOL[a]

	MECO	10ME	20ME	ETCO	10ET	16ET	EMCO	5ME	20ET
No. litters (fetuses) observed	15 (107)	15 (107)	15 (96)	15 (107)	15 (106)	15 (114)	15 (99)	13 (90)	14 (97)
No. litters (fetuses) affected									
Cardiovascular	0			0	0		0	0	0
Right aortic arch		1 (1)	1 (2)			1 (1)			
Right ductus arteriosis		0	1 (1)			1 (1)			
Ventricular septal defect		1 (1)	3 (3)			0			
Missing innominate		0	2 (2)			0			
Abnormal subclavian		1 (1)	1 (1)			0			
Right azygos vein		0	1 (1)			0			
Aortic coarctation		1 (1)	0			0			
Urinary	0			0		0			
Hydroureter		1 (1)	3 (4)		0			0	0
Hydronephrosis		2 (2)	2 (4)		2 (2)			1 (2)	4 (4)
Ectopic kidney		0	1 (1)		0			0	0
Bladder hypoplasia		0	5 (10)		0			0	0
Bladder agenesis		0	1 (2)		0			0	0
Mis-shaped kidney		0	1 (1)		0			0	0
Eye	0				0	0	0	0	0
Microopthalmia		0	2 (2)	1 (1)					
Anopthalmia		1 (1)	0	1 (1)					
Abnormal/missing optic nerve		1 (1)	0	1 (1)					
Ablepharia		0	3 (3)	0					
Abnormal lens		0	2 (2)	0					
Brain	0			0	0	0	0	0	0
Hydrocephalus		2 (4)	2 (2)						
Exencephaly		0	3 (4)						
Encephalocele		0	2 (3)						
Total malformations, litters (fetuses)	0	2 (2)	7 (15)	1 (1)	2 (2)	1 (1)	0	1 (2)	4 (4)

[a] Rats were exposed 7 hr/day throughout gestation. MECO = methanol controls; 10ME = 10,000 ppm methanol; 20ME = 20,000 ppm methanol (exposure was for Gestation Days 7–15); ETCO = ethanol controls; 10ET = 10,000 ppm ethanol; 16ET = 16,000 ppm ethanol; EMCO = ethanol, methanol controls; 5ME = 5000 ppm methanol; and 20ET = 20,000 ppm ethanol. (The EMCO group was added subsequent to our initial evaluation of methanol and ethanol; it was the comparison group for 5ME and 20ET.)

TABLE 5

SUMMARY OF MALFORMATIONS AND VARIATIONS IN RATS AFTER PRENATAL EXPOSURE TO METHANOL AND ETHANOL[a]

	MECO	10ME	20ME	ETCO	10ET	16ET	EMCO	5ME	20ET
No. litters (fetuses) examined	15 (205)	15 (222)	15 (188)	15 (206)	15 (206)	15 (221)	15 (189)	13 (180)	14 (189)
No. litters/% fetuses with skeletal malformations	0	2/2	14*/79	3/3	0	2/3	0	1/1	4/5
No. litters/% fetuses with visceral malformations	0	5/8	10*/29	1/1	2/2	1/1	0	1/3	4/5
No. litters/% fetuses with skeletal variations	8/12	11/24	14/69	13/30	15/38	14/45	14/42	12/27	14/47
No. litters/% fetuses with visceral variations	11/21	13/26	12/26	10/16	7/10	7/15	6/8	7/11	8/9
No. litters with abnormal fetuses[b]	0	7	14	4	2	2	0	2	7
% of Litters with abnormal fetuses[b]	0	47	93*	27	13	13	0	15	50
% of Normal fetuses	100	96	46*	98	99	98	99	98	95

[a] Rats were exposed 7 hr/day throughout gestation. MECO = methanol controls; 10ME = 10,000 ppm methanol; 20ME = 20,000 ppm methanol (exposure was for Gestation Days 7–15), ETCO = ethanol controls; 10ET = 10,000 ppm ethanol; 16ET = 16,000 ppm ethanol; EMCO = ethanol; methanol controls; 5ME = 5000 ppm methanol; and 20ET = 20,000 ppm ethanol. (The EMCO group was added subsequent to our initial evaluation of methanol and ethanol; it was the comparison group for 5ME and 20ET.)

[b] Having skeletal or visceral malformations.

* Significantly different from the appropriate control group $p < 0.05$.

weight of males in both 16ET and 20ET groups relative to their control groups (Table 2). There was no significant difference in the incidence of skeletal (Table 3) or visceral (Table 4) malformations or of visceral or skeletal variations, although the 20ET group had seven litters with abnormal fetuses as opposed to a high number of four litters in one of the control groups.

DISCUSSION

In this teratological evaluation of methanol and ethanol administered by inhalation to rats, methanol was teratogenic at high concentrations, but ethanol was not. Methanol induced a dose-related decrease in fetal weights and increase in malformations; it was definitely teratogenic at 20,000 ppm, possibly teratogenic (i.e., some notable differences from controls, but not consistent statistically significant differences) at 10,000 ppm, but not teratogenic at 5000 ppm. In contrast, ethanol at even maternally toxic levels (20,000 ppm) was only possibly teratogenic (defined above), and lower concentrations were not teratogenic.

The majority of recent research with methanol has involved its ingestion (e.g., Roe, 1982). However, methanol is toxic by inhalation and can be absorbed in toxic amounts through the skin. We found that methanol administered by inhalation at extremely high concentrations was teratogenic to rats. However, our findings that methanol is not teratogenic to rats at 5000 ppm lends support to the adequacy of the present permissible exposure limit (PEL) as being sufficiently protective of the developing organism; that is, a concentration of methanol 100 times the current PEL was not teratogenic to rats.

Of course the literature is replete with studies on ethanol (Abel, 1981). A multitude of reports related to fetal alcohol effects in both humans (Abel, 1982a) and experimental animals (Abel, 1982b) are available. However, no studies reported have utilized inhalation as the method of exposure. Our results indicate that the developing embryo (at least in rats) is not particularly sensitive to the toxic effects of ethanol; even at concentrations with which the maternal animal was narcotized, there was not clear evidence of teratogenic effects. In fact, examining the types of defects observed in the ethanol groups, one sees malformations that are not unusual in control animals, thus decreasing the probability that defects were induced by ethanol. The blood ethanol levels we observed are within the range observed clinically in alcoholics, but are substantially lower than the peak blood concentrations observed after oral administration (Abel, 1980). Levels similar to those in our study (achieved by administration of approximately 6–26% ethanol-derived calories in liquid diets) were teratogenic in mice (Chernoff, 1977). However, our studies lend support to the tenet that rats do not serve as particularly good models for the malformations induced by ethanol.

In summary, our research has demonstrated for the first time that methanol administered at high concentrations by inhalation to rats is teratogenic, but that 5000 ppm is a no-effect level in this test system. Further, 10,000–20,000 ppm ethanol administered via inhalation is not teratogenic in rats.

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