

Developmental Toxicity of Ethylene Glycol Monopropyl Ether Acetate (EGPEA) in the Rat

by Walter J. Krasavage* and Gary V. Katz*

Pregnant rats were exposed by inhalation to vapor concentrations of 100, 200, 400 or 800 ppm of ethylene glycol monopropyl ether acetate on days 6 through 15 of gestation. Concentrations of 400 and 800 ppm reduced the feed intake, mean body weight and red blood cell counts. Mean corpuscular volume and mean corpuscular hemoglobin were increased. Clinical signs of toxicity included lethargy and red discolored urine in the dams exposed to 400 or 800 ppm. The incidence of resorptions was significantly increased and the mean fetal body weight was reduced in litters of dams exposed to 800 ppm. Reproductive indices were not affected. Examinations at cesarean section revealed no major external malformations. Internal soft tissue examinations revealed three fetuses with a cardiovascular defect consisting of a right-sided aortic arch. Two of these fetuses were from dams exposed to 800 ppm, while the third was from a control litter. Skeletal examinations revealed no major skeletal malformations, while minor rib anomalies were slightly increased in litters from dams exposed to 400 or 800 ppm of ethylene glycol monopropyl ether acetate. The incidence of common skeletal variants was slightly increased at 200 ppm.

Introduction

Several glycol ethers and their acetates have been shown to produce reproductive effects by various routes of exposure in a number of species of experimental animals. Ethylene glycol monomethyl ether produces testicular atrophy, fetotoxicity and teratogenic effects in rats, mice and rabbits (1-8). Ethylene glycol monoethyl ether has been reported to produce teratogenic and embryo/fetotoxic effects in rats and rabbits and testicular changes in rats and mice (1,7,9,11-13). Ethylene glycol monomethyl ether acetate and ethylene glycol monoethyl ether acetate produce testicular atrophy in mice (7), while ethylene glycol monoethyl ether acetate has also been reported to be teratogenic in rats and rabbits (8,14). Ethylene glycol monopropyl ether given orally in repeated doses of 1/2, 1/4, or 1/8 the acute oral LD₅₀ (LD₅₀ = 3089 mg/kg) over a 6-week period did not produce testicular effects in male rats, while its ester, ethylene glycol monopropyl ether acetate (LD₅₀ = 9450 mg/kg), given under the same conditions produced 80% mortality and testicular atrophy at 4400 mg/kg and testicular changes in 2 of 10 rats at 2200 mg/kg (1,10).

The objective of this study was to evaluate the potential embryo/fetotoxicity of ethylene glycol monopropyl ether acetate in pregnant rats exposed by inhalation during the period of organogenesis.

Materials and Methods

Test Compound

Ethylene glycol monopropyl ether acetate (EGPEA) was obtained from the Tennessee Eastman Company, Kingsport, TN. Pertinent physical-chemical properties are: structural formula, C₃H₇OCH₂CH₂OOCCH₃; molecular weight, 146.21; boiling point, 170-171°C; melting point, < -45°C; specific gravity, 0.93; vapor pressure, 0.5 mm Hg at 25°C; flash point: 142°F.

The purity of the batch tested was analyzed by gas chromatography and mass spectroscopy and found to be 99.6% pure. The main impurity was ethylene glycol monopropyl ether. Other possible impurities were acetic acid, propyl acetate, hexyl acetate, ethylene glycol monobutyl ether acetate and ethylene glycol monoethyl ether acetate (50-100 ppm).

Test Animals

Sexually mature male and female rats (COBS CD (SD)BR) from the Charles River Breeding Laboratories, Wilmington, MA, were acclimated to the laboratory for 2 weeks prior to mating. Males and females were housed 1:2 over a 9-day period to obtain 120 inseminated females. Insemination was verified by daily vaginal smears and the day sperm was found in the vagina was designated day 0 of gestation. The inseminated females were assigned to the treatment and control groups

*Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY 14650.

according to day 0 of gestation using a computer-generated stratified randomization scheme.

During nonexposure periods, the females were housed singly in suspended stainless steel wire bottom cages and Purina Rodent Laboratory Chow 5001 (ground) and tap water, delivered by an automatic watering system, were available *ad libitum*. Room temperature and relative humidity were controlled at $22 \pm 2^\circ\text{C}$ and $50 \pm 15\%$, respectively, and a 12-hr light-dark photocycle was maintained.

Exposure Conditions

On days 6 through 15 of gestation, the animals were housed in a suspended wire cage and exposed to vapors of ethylene glycol monopropyl ether acetate in 420-L stainless steel and glass inhalation chambers.

Vapors of ethylene glycol monopropyl ether acetate were generated by passing metered air over the surface of the liquid in a three-necked, round-bottomed flask. Temperature in the flask was slightly elevated (up to 40°C) to aid vaporization. The atmospheres within the chambers were sampled at least once per hour for concentration, temperature and relative humidity. Concentrations were quantitatively analyzed by using an infrared analyzer (Miran IA) which had been automated for sampling and analysis. Nominal concentrations were calculated daily based on the ratio of test material used (volumetric) to total volume of air. Airborne particulate counts were measured twice daily in each chamber by using a Royco five-channel particle analyzer. The animals were rotated daily within the chamber to minimize potential positional effects.

Experimental Design

Groups of 24 inseminated females were exposed to vapor concentrations of 100, 200, 400 or 800 ppm of ethylene glycol monopropyl ether acetate for 6 hr/day on days 6 through 15 of gestation. The exposure concentrations were selected based on data generated in a probe teratology study of ethylene glycol monopropyl ether acetate (15). A control group was exposed to filtered air under identical conditions.

Maternal body weights were recorded on days 0, 6, 9, 12, 16 and 19 of gestation, and individual feed consumption was measured on days 6, 9, 12, 16 and 19 of gestation. On days of exposure, all dams were observed for mortality and overt signs of toxicity prior to and subsequent to each exposure. Animals visible through the chamber windows were observed for signs of toxicity during exposure. On nonexposure days all females were observed twice daily for mortality and overt signs of toxicity, except on weekends and holidays, when they were observed for mortality only.

On day 20 of gestation, the dams were anesthetized by CO_2 inhalation, and the abdominal viscera were exposed by a midline incision through the abdominal wall. Ten dams, randomly selected from each group, were bled from the inferior vena cava for hematologic

determinations including total red blood cell and white blood cell counts, hemoglobin concentration, and hematocrit. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were calculated. All animals were fasted for 10 to 12 hr before bleeding. After exsanguination via the vena cava, the dams were weighed and the uterine horns were opened. The implantation sites were counted and categorized as viable or dead fetuses, early resorptions or late resorptions. A resorption was classified as "early" when no placenta or conceptus was distinguishable and as "late" when a placenta containing an embryo in the process of being resorbed (partially to fully formed) was present. The viable fetuses were removed from the placenta, blotted dry on absorbent paper, sexed, examined for gross external abnormalities, and weighed individually. The ovaries were removed, identified as left or right, and the corpora lutea of pregnancy were counted. Approximately one-half of the fetuses from each litter was fixed in Bouin's solution and examined for internal soft tissue anomalies using Wilson's free-hand razor blade technique (16). The other half of the fetuses was fixed in 95% ethanol, macerated with potassium hydroxide and stained with Alizarin Red S and examined for skeletal defects (17). The thoracic and abdominal viscera of the dams were examined *in situ* for gross abnormalities at the time of cesarean section.

Statistical Analyses

Test groups were compared to the control group at a level of significance of $p \leq 0.05$. Continuous data (e.g., maternal body weight, feed consumption, hematology) were analyzed using a one-way analysis of variance with significant F values further analyzed by Duncan's Multiple Range test. Homogeneity of variances was tested by Bartlett's test. Incidence data were compared by using chi-square contingency tables (2×5). Each test group was compared to the control group using Fisher's exact test (one-tailed) when chi square was significant. Resorptions per litter were analyzed by ANOVA after a Freeman-Tukey transformation. Both the proportion of fetuses affected and the number of litters involved were analyzed.

Results

Exposure Conditions

The analytical and nominal chamber concentrations, chamber temperature and relative humidity are shown in Table 1. The overall mean analytical concentrations (of all individual determinations) of 95, 203, 393 and 822 ppm were all within $\pm 5\%$ of the target concentrations of 100, 200, 400 and 800 ppm, respectively. The individual daily means (not shown) of each exposure level were within $\pm 15\%$ of their respective target concentrations. Except for the mean nominal concentration at

Table 1. Ethylene glycol monopropyl ether acetate inhalation teratology: summary of exposure conditions.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
Number of exposures	10	10	10	10	10
Analyzed concentration, ppm					
Overall mean \pm SD	0.0	95 \pm 8	203 \pm 11	393 \pm 22	822 \pm 44
Extremes of daily means	—	85–105	184–222	371–432	750–876
Nominal concentration \pm SD, ppm ^a	—	89 \pm 8	292 \pm 12	466 \pm 43	1232 \pm 205
Overall mean temperature \pm SD, °C	22 \pm 1	22 \pm 1	22 \pm 1	21 \pm 1	21 \pm 1
Overall mean relative humidity \pm SD, %	53 \pm 4	63 \pm 7	52 \pm 5	51 \pm 7	42 \pm 5

^aCalculated daily from volume of chemical used and total airflow.

Table 2. Ethylene glycol monopropyl ether acetate-inhalation teratology: maternal body weight.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
Mortality/pregnant female	0/21	0/21	0/20	0/23	0/21
Maternal weight (g) on various gestation days					
0	248 \pm 12 ^a	254 \pm 16	258 \pm 16	248 \pm 14	254 \pm 19
6	276 \pm 15	283 \pm 19	282 \pm 17	273 \pm 17	281 \pm 21
9	286 \pm 15	292 \pm 17	289 \pm 18	272 \pm 19*	254 \pm 23*
12	301 \pm 12	303 \pm 18	302 \pm 18	283 \pm 20*	274 \pm 22*
16	321 \pm 15	324 \pm 22	324 \pm 21	309 \pm 21*	299 \pm 27*
19	360 \pm 23	364 \pm 31	370 \pm 28	359 \pm 25	344 \pm 33

^aMean \pm 1 standard deviation.

*Statistically significant compared to control (ANOVA $p \leq 0.05$).

Table 3. Ethylene glycol monopropyl ether acetate-inhalation teratology: maternal feed consumption.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
Mortality/pregnant female	0/21	0/21	0/20	0/23	0/21
Maternal feed consumption during gestation days, g/rat/day ^a					
0–6	19.1 \pm 2.0 ^a	20.2 \pm 2.2	19.6 \pm 4.5	18.7 \pm 2.4	19.1 \pm 2.6
6–9	19.6 \pm 1.9	20.1 \pm 2.4	18.5 \pm 3.2	14.1 \pm 3.1*	5.7 \pm 3.5*
9–12	22.3 \pm 1.8	22.3 \pm 2.1	22.1 \pm 2.6	20.3 \pm 3.0*	17.2 \pm 3.1*
12–16	29.3 \pm 6.7	29.6 \pm 5.8	31.7 \pm 6.2	28.3 \pm 7.1	27.8 \pm 6.3
16–19	27.4 \pm 3.4	28.4 \pm 4.1	29.3 \pm 2.5	30.1 \pm 2.8*	31.7 \pm 4.0*

^aMean \pm 1 standard deviation.

*Statistically significant compared to control (ANOVA $p \leq 0.05$).

the lowest level which was slightly less than the analytical concentration, all mean nominal concentrations were slightly higher than their respective analytical concentrations. These differences probably reflect a loss of the compound in the generation system. The analytical data indicate that the chamber concentrations were representative of the chosen target concentrations.

Maternal Observations

Body Weight Response and Feed Consumption. Mean body weights of the dams exposed to 400 and 800 ppm of ethylene glycol monopropyl ether acetate were significantly ($p \leq 0.05$) lower than the control weights

on days 9, 12 and 16 of gestation (Table 2). During the first 3 days of exposure, 400 and 800 ppm produced a body weight loss which at the 800 ppm level was equal to 10.9% of the mean body weight of this group. During the following 3 days (9–12 of gestation) the 400 ppm animals gained significantly less weight than the controls, while the 800 ppm rats gained significantly more weight. Weight gain in these two groups was comparable to or greater than the controls between days 12 and 19 of gestation. Thus, 4 days after termination of exposure, the mean body weight of the 400 ppm animals was comparable to the controls and the mean body weight of the 800 ppm animals, though not statistically different, was slightly less (4.4%) than the control weight. Exposures to 100 and 200 ppm of

ethylene glycol monopropyl ether acetate had no effect on body weight gain.

Exposure to 400 and 800 ppm of EGPEA produced a significant ($p \leq 0.05$) reduction in feed intake between days 6 and 12 of gestation (Table 3). Feed intake was severely reduced between day 6 and 9 in the 800 ppm group, causing a severe loss of body weight in these dams. From day 12 to 19 of gestation, 400 and 800 ppm had no effect on feed consumption, and the intake of these two groups were comparable to or greater than the control intake during this time. Feed consumption was not affected by exposures of 100 or 200 ppm of EGPEA.

Clinical Signs. Significant clinical signs of toxicity during exposure were slight lethargy in the rats exposed to 800 ppm; seen also to a lesser degree in the 400 ppm animals. Red-discolored urine (positive for hemoglobin in a N-Multistix-C test) was found on the dropping trays of 16 of 24 animals exposed to 800 ppm and 1 of 24 animals exposed to 400 ppm after the first exposure. It was also seen in 2 of 24 animals after two exposures to

800 ppm. It was not seen after subsequent exposures nor was it seen at anytime in the controls or the animals exposed to 100 or 200 ppm.

Hematology. Exposure to 400 or 800 ppm EGPEA produced a statistically significant reduction in total red blood cells (Table 4). Mean corpuscular volume and mean corpuscular hemoglobin were increased at these concentrations; 800 ppm also increased the mean corpuscular hemoglobin concentration. The mean corpuscular volume was also slightly increased in the animals exposed to 200 ppm of EGPEA. However, none of the other hematologic parameters were affected by this level of exposure, thus this increase in MCV is not considered biologically significant. None of the exposure concentrations affected hemoglobin concentrations, hematocrit or the total white blood cell count.

Gross Pathology. At the time of cesarean section, one dam exposed to 800 ppm had an enlarged liver with rounded edges and the spleen was enlarged. These findings were probably compound related, but were seen only in one animal. Another dam, exposed to 100

Table 4. Ethylene glycol monopropyl ether acetate-inhalation teratology: maternal hematology.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
White blood cells/mm ³ × 10 ^{3a}	8.7 ± 1.6	9.3 ± 1.9	8.0 ± 1.4	8.2 ± 1.5	8.4 ± 1.8
Red blood cells/mm ³ × 10 ^{6a}	6.4 ± 0.8	6.1 ± 0.6	6.1 ± 1.0	5.2 ± 0.3*	5.2 ± 0.5*
Hemoglobin concentration, g/dL ^a	12.8 ± 1.4	12.2 ± 1.1	12.3 ± 1.8	11.7 ± 0.5	13.1 ± 1.2
Hematocrit, % ^a	39.3 ± 4.8	37.6 ± 3.8	38.2 ± 6.4	35.3 ± 1.9	38.0 ± 3.3
Mean corpuscular volume, μm ^{3a}	60.8 ± 1.1	61.1 ± 2.2	62.9 ± 1.5*	67.3 ± 2.2*	72.3 ± 1.6*
Mean corpuscular hemoglobin, pg ^a	19.9 ± 0.4	19.9 ± 0.7	20.3 ± 0.7	22.4 ± 0.9*	25.0 ± 1.2*
Mean corpuscular hemoglobin concentration, % ^a	32.6 ± 0.5	32.5 ± 0.9	32.4 ± 0.7	33.2 ± 0.6	34.5 ± 1.1*

^aMean ± 1 standard deviation ($n = 10$).

*Statistically significant compared to controls (ANOVA $p \leq 0.05$).

Table 5. Ethylene glycol monopropyl ether acetate inhalation teratology: summary of observations at time of cesarean sections.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
Number of females inseminated	24	24	24	24	24
Number of females pregnant (%)	21(88)	21(88)	20(83)	23(96)	21(88)
Number litters completely resorbed	0	0	0	0	1
Number litters with resorptions	13	6	12	11	16
Corpora lutea per dam ^a	14.9 ± 3.3	15.0 ± 3.8	16.1 ± 3.7	14.4 ± 2.4	15.4 ± 3.8
Implantations per dam ^a	12.4 ± 4.2	12.1 ± 4.5	13.4 ± 4.0	14.1 ± 2.8	14.4 ± 2.1
Viable fetuses per litter ^a	11.4 ± 4.2	11.5 ± 4.6	12.7 ± 3.8	13.5 ± 2.8	10.4 ± 4.8
Resorptions per litter ^a	0.95 ± 0.97	0.62 ± 1.2	0.70 ± 0.66	0.61 ± 0.72	4.00 ± 4.7*
Resorptions per litter with resorptions ^a	1.5 ± 0.8	2.2 ± 1.3	1.2 ± 0.4	1.3 ± 0.5	5.3 ± 4.7*
Dead fetuses per litter	0	0	0	0	0
Pre-implantation loss, % ^b	16.9	19.6	16.8	2.4	6.2
Post-implantation loss, % ^c	7.7	5.2	5.2	4.3	27.7
Sex ratio (M:F)	49:51	53:47	45:55	54:46	53:47
Fetal body weight, g ^a	3.37 ± 0.29	3.52 ± 0.31	3.19 ± 0.31	3.26 ± 0.22	2.57 ± 0.30

^aMean ± 1 standard deviation.

^b% Pre-implantation loss = $\frac{(\text{number of corpora lutea} - \text{number of implantations}) \times 100}{\text{number of corpora lutea}}$

^c% Post-implantation loss = $\frac{(\text{number of implantations} - \text{number of viable implantations}) \times 100}{\text{Number of implantations}}$

*Statistically significant compared to controls (ANOVA $p \leq 0.05$).

ppm, had enlarged lymph nodes in the lower left inguinal region beneath the nipple of the mammary gland. This finding was noted only in one animal and was not seen at the higher concentrations thus it is not considered to be compound related.

Observations at the Time of Cesarean Sections. Signs of embryo/fetoletality and fetotoxicity noted in the animals exposed to 800 ppm of ethylene glycol monopropyl ether acetate included a significant increase in the number of resorptions per litter, indicative of an increased post-implantation loss, and a significant ($p \leq 0.05$) reduction in mean fetal body weight (Table 5). The lower exposure concentrations did not produce these effects. The rate of pregnancy, number of corpora lutea, implantation sites or viable fetuses per dam or the sex ratio of the fetuses were not affected by any of the exposure concentrations.

Fetal Observations

External and Internal Soft Tissue Examinations. Externally, two fetuses, one from each of two 800 ppm litters were edematous and had a stubby appearance (Table 6). Other external findings included a severely atrophied tail on one fetus from a dam exposed to 200 ppm, small hematomas on one fetus each in two litters exposed to 100 or 800 ppm and a hemorrhagic streak extending from between the eyes to the nares in one control fetus and in the occipital region of one fetus in the 100 ppm group. The eyelids of one fetus from a dam

exposed to 200 ppm appeared somewhat darker than normal. Except for the edema seen in the 800 ppm fetuses, none of the external findings are considered to be compound related.

Three of 635 fetuses examined for internal soft tissue anomalies had a major malformation of the cardiovascular system (Table 7). One fetus from a dam exposed to 800 ppm of EGPEA had a ringed aorta, and another fetus from a different litter had a right-sided aortic arch with a right-sided ductus arteriosus. One control fetus had a right-sided aortic arch with a left-sided ductus arteriosus. One fetus in an 800 ppm litter had dilated cardiac ventricles. This fetus was one of the two fetuses noted with edema and a stubby body. The dilation of the ventricles was probably related to the edematous condition. In addition, minor internal soft tissue anomalies including unilateral and/or bilateral hydronephrosis and hydroureter were seen in all treatment groups and the controls. Though not statistically significant, the occurrence of hydronephrosis was found most frequently in the 800 ppm fetuses.

Skeletal Examinations. No major skeletal malformations were seen in any of the exposed groups (Table 8). Minor skeletal anomalies included wavy and/or knobby ribs, fused, thickened or shortened (partially ossified) ribs and beginning sites of ossification (spurs), either unilateral or bilateral, adjacent to the seventh cervical vertebrae. Wavy and/or knobby ribs were found in all groups. Statistically, the proportion of fetuses with this defect was greater in the 800 ppm group

Table 6. Ethylene glycol monopropyl ether acetate-inhalation teratology: external fetal abnormalities.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
No. of fetuses (litters) examined	240(21)	241(21)	253(20)	310(23)	219(20)
External defects ^a					
Hematoma		2(2)	1		2(2)
Hemorrhagic streak					
Occipital area		1			
Between eyes to nares	1				
Eyelids dark in appearance			1		
Edematous with stubby appearance					2(2)
Tail: severe atrophy			1		

^aNumber of fetuses affected; litters in parentheses.

Table 7. Ethylene glycol monopropyl ether acetate inhalation teratology: internal soft tissue fetal abnormalities.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
No. of fetuses (litters) examined	121(21)	120(21)	127(20)	157(23)	110(20)
Soft tissue defects ^a					
Cardiovascular					
Right-sided aortic arch, right-sided ductus arteriosus					1
Right-sided aortic arch, left-sided ductus arteriosus	1				
Ringed aorta					1
Dilated cardiac ventricles					1 ^b
Renal					
Hydronephrosis	4(2)	2(2)	5(4)	1	9(5)
Hydroureter	4(1)	3(2)	1	2(2)	5(3)

^aNumber of fetuses affected; litters in parentheses.

^bOne of the fetuses with edema and a stubby body.

Table 8. Ethylene glycol monopropyl ether acetate inhalation teratology: summary of skeletal alterations.

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
No. of fetuses (litters) examined	119(21)	121(21)	126(20)	153(23)	108(19)
Major skeletal malformations ^a	0	0	0	0	0
Minor skeletal alterations: ribs ^a					
Spur at 7th cervical vertebra					7(2)
Only 12 pair present	1			3(1)	
Wavy/knobby ^b	5(4)	3(1)	2(2)	10(4)	17*(9)
Partial ossification	4(2)	1		4(2)	1
Fused				1	2(1)
Thickened					2(2)
Total rib defects	7(5)	4(2)	2(2)	12(5)	20(9)*
Alterations indicative of retarded development: skull bones					
Partial ossification					
Frontals	1				8(4)
Parietals	2(2)			4(2)	11(7)
Occipitals	1			4(2)	23(11)
Hyoid	5(4)	2(2)	3(3)	7(5)	19(8)
Presphenoid					18(9)
Nonossification					
Occipitals					2(2)
Hyoid	2(2)	1	12(9)	17(8)	42(16)
Presphenoid	1			1	26(12)
Total skull defects ^b	8(5)	3(3)	16(9)	24*(10)*	71*(16)*
Alterations indicative of retarded development: vertebrae					
Cervical arches					
Partial ossification (4th-7th)					2(1)
Thoracic centra					
Partial ossification	10(4)	6(3)	19(10)	20(7)	80(18) ^d
Nonossification			4(2)	1	56(17) ^d
Cleft (bilobed)	11(6)	22(9)	17(9)	21(11)	61(17)
Split	3(3)	5(3)	6(4)	2(2)	20(12)
Lumbar arches					
Partial ossification					1
Lumbar centra					
Partial ossification					3(2)
Fused					1
Cleft (bilobed)					12(9)
Split				1	4(2)
Extra lumbar vertebra		1			
Sacral arches and centra					
Partial ossification	1		1		20(9)
Nonossification					7(5)
Caudal					
None present	1		1		15(9)
Total vertebral defects ^b	18(8)	28(14)	34*(12)	40*(14)	98*(19)*
Alterations indicative of retarded development: sternbrae					
< 4 Present	6(4)		1		15(8)
Partial ossification	8(5)	4(4)	14(7)	12(8)	51(16)
Nonossification				4(3)	11(6)
Cleft (bilobed)	1			1	5(2)
Split			1		
Total sternbral defects ^b	13(7)	4(4)	15(8)	14(8)	56*(16)*
Alterations indicative of retarded development: pelvic girdle					
Partial ossification					
Pubis	1	1	2(1)	1	15(6)
Ilium					1
Ischium					3(2)
Nonossification					
Pubis			1		9(5)
Ilium			1		2(1)
Ischium			1		2(2)
Total pelvic girdle defects ^b	1	1	3(2)	1	26*(8)*
Alterations indicative of retarded development: fore and hind limbs					
Partial ossification					
Metacarpals	1				7(4)
Metatarsals					4(2)
Total limb defects ^b	1				8*(4)*

Table 8. (continued)

	Target concn				
	0	100 ppm	200 ppm	400 ppm	800 ppm
Common skeletal variants ^a					
Thoracolumbar ribs (14th)					
Rudimentary (< 1/2 13th rib) ^b	19(8)	24(14)	32*(14)*	48(18)*	71*(19)*
Extra (> 1/2 13th rib) ^b	3(2)	5(3)			19(10)*
Total skeletal alterations ^{b,c}	49(17)	53(20)	74*(19)	87*(19)	108*(19)

^aNumber of fetuses with specific alteration; number of litters involved in parentheses.

^b1st, 2nd centra most frequently affected.

^cNumber of fetuses (litters) with at least one of the above listed skeletal alterations.

^dThese data were analyzed statistically.

*Statistically different from controls $p \leq (0.05)$ chi-square table and Fisher's exact test (one-tailed).

compared to the controls; however, the number of litters involved, though on the borderline of significance ($p = 0.058$), was not different from the controls. Fused ribs were seen in one litter each at the 400 and 800 ppm levels, while thickened ribs were seen in two litters of the 800 ppm level. Neither of these defects were seen in the control fetuses. The frequency of partially ossified ribs seen in the treated groups was equal to or less than in the controls. Cervical spurs were seen only at the 800 ppm level and were found in seven fetuses from two litters.

Other skeletal defects noted were those indicative of delayed ossification and common skeletal variants, i.e., partial and/or nonossification of the bones of the skull, sternbrae, vertebral centra, metacarpals, metatarsals, and the presence of rudimentary and/or extra ribs. Statistically significant effects on the process of ossification of the skull bones, vertebrae (particularly the thoracic centra), sternbrae, bones of the pelvic girdle and the metacarpals and metatarsals were produced by 800 ppm of EGPEA. A statistically significant proportion of the fetuses exposed to 400 ppm had decreased ossification of the skull bones and thoracic vertebral centra. Effects on the thoracic vertebral centra were also seen in the 200 ppm group; however, the number of litters involved at this level and at the 400 ppm level was not statistically different from the controls.

Exposure to 200, 400 and 800 ppm produced a significant increase in rudimentary ribs, while 800 ppm significantly increased the incidence of extra 14th ribs.

Discussion

Exposure of pregnant rats to 400 and 800 ppm of ethylene glycol monopropyl ether acetate on days 6 through 15 of gestation produced maternal toxicity, which was not seen in dams exposed to 100 and 200 ppm of EGPEA. At 800 ppm, EGPEA produced significant fetolethality (increased incidence of resorptions) and a significant reduction of mean fetal body weight.

No major external or skeletal malformations were found in fetuses from dams exposed to concentrations of EGPEA as high as 800 ppm. Internal soft tissue examinations revealed two fetuses, each from different

dams, exposed to 800 ppm of EGPEA and one control fetus with persistent right aortic arches. Cardiovascular anomalies have been reported to occur spontaneously in this strain of rat, albeit at a very low rate (18). The occurrence of this malformation in a control fetus in this study and the significant maternal toxicity produced by 800 ppm of EGPEA indicate that this finding is probably not a compound-related effect. Minor soft tissue anomalies were slightly increased in the 800 ppm group only; while minor skeletal anomalies were significantly increased by 800 ppm EGPEA and slightly, but not statistically, increased by 400 ppm. Thus, 800 ppm of ethylene glycol monopropyl ether acetate was a no-observed effect level for major skeletal malformations, while minor skeletal anomalies were seen at a very low incidence in the 400 ppm group.

Other skeletal changes were those indicative of delayed ossification and the skeletal variants commonly seen in teratology studies. These consisted of partial or nonossification of: bones of the skull, vertebral centra, sternbrae, bones of the pelvic girdle, and the occurrence of 14th rudimentary and/or extra thoracolumbar ribs. The incidences of all of these alterations were significantly increased by 800 ppm of EGPEA. Delayed ossification of the skull bones was still slightly increased at 400 ppm, while common variants such as delayed ossification of the first and second thoracic centra and the occurrence of 14th rudimentary ribs were slightly increased at 200 ppm.

Conclusions

Vapor concentration of ethylene glycol monopropyl ether acetate as high as 800 ppm did not produce teratogenic effects in the rat. Concentrations of 800 and 400 ppm, which were overtly toxic to the maternal animal, produced minor embryo/fetotoxicity indicative of retarded development, while 200 ppm of EGPEA did not significantly affect development, but did slightly increase the incidence of common skeletal variants. EGPEA does appear to have an effect on fetal development, but only at high doses which are overtly toxic to the mother. Thus, if the exposure level of EGPEA is kept below the levels toxic to the adult, the conceptus should not be affected.

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