The Effects of Maternally Inhaled Vinyl Chloride on Embryonal and Fetal Development in Mice, Rats, and Rabbits

J. A. JOHN, F. A. SMITH, B. K. J. LEONG, AND B. A. SCHWETZ

Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A. Midland, Michigan 48640

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The Effects of Maternally Inhaled Vinyl Chloride on Embryonal and Fetal Development in Mice, Rats, and Rabbits[1,2]

J. A. JOHN, F. A. SMITH, B. K. J. LEONG,[3] AND B. A. SCHWETZ

Toxicology Research Laboratory, Health and Environmental Research, Dow Chemical U.S.A. Midland, Michigan 48640

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The Effects of Maternally Inhaled Vinyl Chloride on Embryonal and Fetal Development in Mice, Rats, and Rabbits. JOHN, J. A., SMITH, F. A., LEONG, B. K. J., AND SCHWETZ, B. A. (1977). *Toxicol. Appl. Pharmacol.* **39**, 497–513. These studies evaluated the effects of inhaled vinyl chloride on mouse, rat, and rabbit embryonal and fetal development. Groups of pregnant CF-1 mice, Sprague–Dawley rats and New Zealand white rabbits were exposed to 500 ppm of vinyl chloride 7 hr daily during the period of major organogenesis. Subsequently, other groups of mice were similarly exposed to 50 ppm of vinyl chloride and rats and rabbits were exposed to 2500 ppm of vinyl chloride. While maternal toxicity was observed, vinyl chloride alone did not cause significant embryonal or fetal toxicity and was not teratogenic in any of the species at the concentrations tested. Maternal toxicity was more prominent among mice than among rats and rabbits. Simultaneous exposure of some of the pregnant animals to vinyl chloride by inhalation plus 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to vinyl chloride alone in the three species. The maternal toxicity was enhanced to an extent greater than the embryotoxicity.

Vinyl chloride is widely used in the preparation of polyvinyl chloride resin, as a copolymer in plastics, and, to a lesser extent, as a solvent and as a chemical intermediate. A report of the effect of single exposures of mice, rats, and guinea pigs to vinyl chloride by Mastromatteo *et al.* (1960) indicates that this compound has very low acute toxicity. Anesthesia is the primary significant effect of acute exposure to high concentrations (75,000–100,000 ppm). The effect of repeated exposure of laboratory animals to vinyl chloride has been reported by Torkelson *et al.* (1961). Repeated exposure for 6 months to 200 ppm resulted in histologic changes in the centrilobular area of the livers of rabbits, but not in rats, guinea pigs, or dogs. In a study reported by Viola *et al.* (1971), rats were exposed to 30,000 ppm of vinyl chloride vapor for 12 months. Findings on

[1] The majority of this study was supported by the companies sponsoring research on vinyl chloride and was administered by The Manufacturing Chemists Association.

[2] This work was reported, in part, at the 14th Annual Meeting of the Society of Toxicology, March 10, 1975, Williamsburg, Virginia.

[3] Present address: International Research and Development Corporation, Mattawan, Michigan.

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these rats were reported to include severe chronic hepatitis, interstitial pneumonia, as well as tumors of the skin, lungs, and bones. Maltoni and Lefemine (1974) reported the oncogenic effects of repeated exposure to inhaled vinyl chloride in rats and mice. Angiosarcomas, zymbal gland carcinomas, and nephroblastomas developed in rats exposed to concentrations of vinyl chloride ranging from 50–10,000 ppm, 4 hr/day, 5 days/week for 12 months and subsequently maintained and observed until death. Pulmonary adenomas, mammary carcinomas, and liver angiosarcomas were observed among mice exposed to the same range of concentrations for 7 months. Similarly, Keplinger *et al.* (1975) reported neoplasms in mice and the tentative diagnosis of tumors in hamsters and rats exposed to 50, 200, or 2500 ppm of vinyl chloride.

The carcinogenic potential of inhaled vinyl chloride has also been studied by exposing animals *in utero* (Maltoni, 1975), but observations to determine effects which are more in line with a classical teratologic study were not made. Reports on the embryotoxic potential of vinyl chloride in laboratory animals have not been found in the literature. Thus, the purpose of the studies described in this report was to assess the potential of inhaled vinyl chloride to have a deleterious effect on embryonal and fetal development in mice, rats, and rabbits.

Since previous studies in this laboratory suggested that the primary metabolic pathway for vinyl chloride is blocked by ethanol (Hefner *et al.*, 1975), it was considered possible that administration of ethanol in the drinking water of animals exposed to vinyl chloride might alter its metabolism in a manner which would enhance its toxic or teratogenic potential. To assess this possibility, some of the vinyl chloride-exposed animals were given 15% ethanol in their drinking water during the days of exposure to vinyl chloride. The teratogenic potential of 15% ethanol in the drinking water in mice, rats, and rabbits was previously studied in this laboratory and is summarized in Table 11 of this report (unpublished data, The Dow Chemical Co.).

METHODS

Animals and test material. Female CF-1 mice[4] weighing 25 to 30 g, Sprague–Dawley rats[5] weighing approximately 250 g, and New Zealand white rabbits[6] weighing 3.5 to 4.5 kg were used in this study. The day on which a vaginal plug was observed or the day on which sperm were seen in a vaginal smear was considered to be Day 0 of pregnancy for mice and rats, respectively. The day of mating was considered to be Day 0 for rabbits. Between daily exposures, animals were housed in wire-bottom cages in a room controlled for temperature, humidity, and light cycle. Commercial laboratory animal food[7] and water were available. Food consumption was measured at 3-day intervals for mice and rats and at 2-day intervals for rabbits.[8]

Exposure of bred animals was conducted in stainless steel chambers of 3.7 m^3 volume under dynamic airflow conditions. The atmosphere of vinyl chloride was generated by

[4] Mice were obtained from Carworth, Portage, Michigan.
[5] Rats were obtained from Spartan Research Animals, Inc., Haslett, Michigan.
[6] Rabbits were obtained from Langshaws Rabbitry, Augusta, Michigan.
[7] Ralston Purina Co., St. Louis, Missouri.
[8] Ethanol consumption was measured during a separate study in which animals were given only 15% ethanol in the drinking water; these results will be reported separately (unpublished data, The Dow Chemical Company).

diluting gaseous vinyl chloride with filtered room air at a rate calculated to give the desired concentration. Samples of inhibited vinyl chloride monomer (chloroethylene)[9] were used for the exposures. The actual concentration was measured with an infrared spectrophotometer (Perkin–Elmer 12A or Miran I) with a multipath gas cell.

Experimental design. In the initial experiment, groups of 30–40 bred mice, 20–35 bred rats, and 15–20 bred rabbits were exposed to 500 ppm of vinyl chloride for 7 hr daily on Days 6–15 (mice and rats) or 6–18 (rabbits) of gestation. Subsequently, additional groups of mice were exposed to 50 ppm of vinyl chloride. Additional groups of rats and rabbits were exposed to 2500 ppm of vinyl chloride. As summarized in Table 1, some of the animals which were exposed to vinyl chloride were also given 15% ethanol in their drinking water on Days 6–15 (mice and rats) or Days 6–18 (rabbits) of gestation.

TABLE 1

TERATOLOGIC STUDIES WITH VINYL CHLORIDE[a]

	Vinyl chloride (ppm)	Ethanol (%)
Mice	500	0
	500	15
	50	0
	50	15
Rats	2500	0
	2500	15
	500	0
Rabbits	2500	0
	2500	15
	500	0
Mice, rats, and rabbits	0	0

[a] Mice and rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice and rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Rabbits were exposed to vinyl chloride on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water on Days 6–18 of gestation.

Maternal and fetal observations. All animals were observed daily throughout pregnancy and maternal body weights were recorded on gestation Days 6, 12, 15, and 18 for mice and on Days 6, 10, 16, and 21 for rats. Maternal body weights for rabbits were recorded on Days 6, 12, 18, 22, and 29 of gestation. Pregnant mice and rats were sacrificed by carbon dioxide inhalation on Days 18 and 21 of gestation, respectively. Pregnant rabbits were sacrificed on Day 29 of gestation. The uterine horns were exteriorized through a midline incision in the abdominal wall and the number and position of live, dead, and resorbed fetuses were noted. After being weighed, measured (crown–rump length), and sexed (mice and rats), the fetuses were examined for external ano-

[9] Vinyl chloride was obtained from Matheson Gas Products, Joliet, Illinois.

malies. One-third of each litter was immediately examined for evidence of soft tissue anomalies by dissection under a low-power microscope. Rabbit fetuses were sexed on the basis of examination of internal genitalia. All fetuses were then eviscerated, preserved in alcohol, and subsequently cleared and stained with alizarin red-S (Dawson, 1926) for examination of skeletal anomalies.

Statistical evaluation. The Fisher exact probability test (Siegel, 1956) was used to evaluate the incidence of resorptions among litters. Maternal and fetal body weights and body measurements and maternal liver weights were analyzed statistically by an analysis of variance and the Dunnett test (Steel and Torrie, 1960). The incidence of fetal anomalies was analyzed by the Wilcoxon test as modified by Haseman and Hoel (1974).

Controls. The group of animals which was exposed only to vinyl chloride served as the control for those animals which were exposed to vinyl chloride in combination with 15% ethanol in the drinking water. The controls for animals exposed to vinyl chloride alone were exposed concurrently to filtered room air.

RESULTS

Maternal toxicity. Among mice exposed to 500 ppm of vinyl chloride by inhalation, there was a decrease in maternal weight gain and food consumption during gestation and in the absolute liver weight at the time of cesarean section compared to control values (Table 2). These effects were not observed among mice exposed to 50 ppm of vinyl chloride. Mice exposed to a combination of 500 ppm of vinyl chloride by inhalation and 15% ethanol in their drinking water also showed a decrease in weight gain during gestation and in liver weight (absolute and relative) on Day 18 of gestation. Ethanol in combination with 50 ppm of vinyl chloride also resulted in a decrease in maternal weight gain during gestation and a decrease in the absolute liver weight on Day 18 of gestation. Food consumption throughout gestation was decreased for mice exposed to both concentrations of vinyl chloride in combination with ethanol.

Except for an apparent decrease in maternal weight gain in rats (Table 3) and a decrease in food consumption for rabbits (Table 4), no signs of toxicity were observed in the adult rats or rabbits during exposure to 500 ppm of vinyl chloride. The apparent decrease in maternal weight gain among rats exposed to 500 ppm is most likely due to the lower body weight of the control animals on Day 6 and, subsequently, a higher weight gain of these animals during the later days of gestation. Among rats exposed to 2500 ppm, both the absolute and relative liver weights were significantly increased on Day 21 of gestation, but maternal weight gain was no different from that of rats exposed to filtered room air. Maternal food consumption was, however, lower than among control rats in this group. The relative liver weight on Day 21 of gestation was significantly increased among rats exposed to 2500 ppm of vinyl chloride and 15% ethanol. Maternal weight gain for this group of rats was significantly decreased during gestation. Food consumption during the exposure period was further decreased among these rats which were exposed to vinyl chloride in combination with ethanol in the drinking water.

No effect on maternal weight gain, liver weight, or food consumption was observed among rabbits exposed to 2500 ppm of vinyl chloride by inhalation. A decrease in maternal weight gain was observed among rabbits during exposure to 2500 ppm of vinyl chloride in combination with 15% ethanol (Days 6–18 of gestation), but total

TABLE 2

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]			High concentration[b]		
	0 0	50 0	50 15	0 0	500 0	500 15
Number of dams	21	20	16	26	19	7
Body weight on gestation Day 6[c]	30 ± 2	31 ± 2	31 ± 2	29 ± 2	29 ± 3	30 ± 3
Weight gain during gestation Days 6–18[c]	16 ± 4	17 ± 6	11 ± 7[d]	20 ± 3	17 ± 4[e]	10 ± 7[d]
Liver weight on gestation Day 18[c]						
Absolute	2.75 ± 0.26	2.76 ± 0.40	2.37 ± 0.52[d]	2.75 ± 0.31	2.49 ± 0.29[e]	1.78 ± 0.36[d]
Relative[f]	59.5 ± 8.7	57.8 ± 4.5	56.6 ± 7.3	55.5 ± 5.5	54.4 ± 4.2	45.8 ± 5.1[d]
Food consumption during gestation						
Days 6–15[c]	6 ± 1	6 ± 1	4 ± 2[d]	6 ± 1	5 ± 1[e]	3 ± 1[d]

[a] Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation.

[b] Top row, vinyl chloride in drinking water (ppm); bottom row, percentage ethanol in drinking water.

[c] Grams, mean ± SD.

[d] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[e] Significantly different from control by an analysis of variance, $p < 0.05$.

[f] Milligrams of liver per gram of body weight.

TABLE 3

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0 0	500 0	0 0	2500 0	2500 15
Number of dams	28	31	19	16	16
Body weight on gestation Day 6[c]	258 ± 26	277 ± 20[d]	288 ± 27	274 ± 19	272 ± 14
Weight gain during gestation Days 6–21[c]	148 ± 11	125 ± 19[d]	127 ± 15	138 ± 23	120 ± 15[e]
Liver weight on gestation Day 21[c]					
Absolute	14.81 ± 1.79	15.00 ± 1.14	14.27 ± 1.38	15.55 ± 1.23[d]	16.52 ± 1.50
Relative[f]	36.5 ± 4.1	37.1 ± 2.6	34.4 ± 3.3	37.8 ± 2.6[d]	42.1 ± 2.4[e]
Food consumption during gestation					
Days 6–15[c]	21 ± 2	22 ± 2	22 ± 2	21 ± 2[d]	13 ± 2[e]

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation.

[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[c] Grams, mean ± SD.

[d] Significantly different from control by an analysis of variance, $p < 0.05$.

[e] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[f] Milligrams of liver per gram of body weight.

TABLE 4

MATERNAL WEIGHT GAIN, LIVER WEIGHTS, AND FOOD CONSUMPTION OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0 0	500 0	0 0	2500 0	2500 15
Number of dams	18	20	11	5	16
Body weight on gestation Day 6[c]	3.85 ± 0.23	3.82 ± 0.25	4.08 ± 0.21	4.39 ± 0.30[d]	4.02 ± 0.47
Weight gain during gestation Days 6–29[c]	0.05 ± 0.19	0.01 ± 0.19	0.06 ± 0.27	0.01 ± 0.13	−0.14 ± 0.42
Liver weight on gestation Day 29[c]					
Absolute	96 ± 19	89 ± 14	102 ± 16	122 ± 25	116 ± 30
Relative[e]	24.6 ± 3.6	23.2 ± 2.9	24.7 ± 2.7	27.7 ± 5.9	30.0 ± 6.3
Food consumption during gestation Days 6–18 (g)	98 ± 30	76 ± 29[d]	91 ± 36	89 ± 26	15 ± 9[f]

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water (15‰, v/v) on Days 6–18 of gestation.
[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[c] Kilograms, mean ± SD.
[d] Significantly different from control by an analysis of variance, $p < 0.05$.
[e] Grams of liver per kilogram of body weight.
[f] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

weight gain was not different from that among rabbits exposed to 2500 ppm of vinyl chloride alone (Table 4). Food consumption was significantly decreased among the rabbits exposed to 2500 ppm plus ethanol.

Observations made at the time of cesarean section. Observations made at the time of cesarean section of mice, rats, and rabbits are presented in Tables 5–7. Maternal deaths were observed among mice exposed to 500 ppm of vinyl chloride alone and in combination with ethanol (Table 5). There was an increase in the incidence of resorptions and the fetal body weights were lower than in controls for the 500-ppm vinyl chloride group. Litter size was also reduced. These effects were augmented among mice exposed to 500 ppm of vinyl chloride in combination with 15% ethanol. Two litters in this group were totally resorbed. In addition, the number of implantation sites per dam, fetal crown–rump length, and percentage pregnancy were significantly lower in the ethanol group as compared to mice receiving 500 ppm of vinyl chloride alone. Among mice exposed to 50 ppm, the fetal crown–rump length was significantly greater than among controls; a decrease, however, in fetal body weight and crown–rump length was observed in the 50-ppm vinyl chloride plus ethanol group. The incidence of resorptions was not significantly greater among mice treated with 50 ppm of vinyl chloride in combination with ethanol, although two litters were totally resorbed.

One maternal death was observed among rats exposed to 2500 ppm of vinyl chloride by inhalation (Table 6). There was no significant effect on litter size, the number of implantation sites per dam, or the incidence of resorptions among any of the exposed groups of rats. The pregnancy wastage was significantly lower among rats exposed to 500 ppm than among the control rats. Percentage pregnacy was unaffected among rats exposed to vinyl chloride alone or in combination with 15% ethanol in the drinking water. Fetal body weight and crown–rump length were significantly reduced among rats exposed to 2500 ppm of vinyl chloride in combination with ethanol. A significant reduction in fetal body weight was also observed among rats exposed to 500 ppm of vinyl chloride alone, but not among those exposed to 2500 ppm. Significant decreases in the number of corpora lutea per dam were observed among rats exposed to 500 ppm of vinyl chloride alone and among those exposed to 2500 ppm in combination with 15% ethanol. A significant increase, however, in the number of corpora lutea per dam was observed among rats exposed only to 2500 ppm of vinyl chloride. Since the number of corpora lutea is established prior to Day 6 of gestation, these differences are not considered to be a treatment-related effect, but rather a measure of the reproductive status of the animals prior to the beginning of the experiment.

Among rabbits, a significant increase in the incidence of resorptions was observed in the high concentration (2500 ppm) plus ethanol group, in which seven litters were totally resorbed (Table 7). Exposure of rabbits to either 500 or 2500 ppm of vinyl chloride alone did not alter the incidence of resorptions. A decrease in the number of live fetuses per litter was observed among rabbits exposed to 500 ppm of vinyl chloride alone, but not among those exposed to 2500 ppm of vinyl chloride alone or in combination with 15% ethanol in the drinking water. Since the decrease in litter size was associated with a decrease in the number of corpora lutea which is established prior to Day 6 of gestation, this effect is probably not due to exposure to vinyl chloride. Pregnancy wastage in rabbits was unaffected by exposure to vinyl chloride. No differences in fetal body weight or crown–rump length were observed in any of the exposed groups of rabbits.

TABLE 5

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration			High concentration		
	0 0	50 0	50 15	0 0	500 0	500 15
Number of litters	21	20	16	26	19	7
Implantation sites/dam[b]	12 ± 2	12 ± 4	11 ± 4	14 ± 2	13 ± 2	10 ± 6[c]
Live fetuses/litter[b]	10 ± 4	11 ± 4	10 ± 4	12 ± 2	11 ± 2[d]	8 ± 6[c]
Implantations resorbed (%)	15 (40/261)	8 (18/238)	11 (19/172)	7 (26/351)	13 (33/248)[e]	19 (13/69)
Litters with resorptions (%)	67 (14/21)	55 (11/20)	69 (11/16)	58 (15/26)	79 (15/19)	86 (6/7)
Litters totally resorbed	1	0	2	0	0	2
Resorptions/litters with resorptions	2.9 (40/14)	1.6 (18/11)	1.7 (19/11)	1.7 (26/15)	2.2 (33/15)	2.2 (13/6)
Sex ratio, M:F	50:50	50:50	48:52	54:46	52:48	64:36
Fetal body weight (g)[f]	1.00 ± 0.11	1.02 ± 0.10	0.84 ± 0.14[c]	1.07 ± 0.06	0.99 ± 0.11[d]	0.78 ± 0.15[c]
Fetal crown–rump length (mm)[f]	23.0 ± 1.9	24.2 ± 0.8[d]	22.4 ± 1.5[c]	23.7 ± 1.2	23.6 ± 1.0	21.2 ± 1.5[c]
Maternal deaths/treated dams (%)	0 (0/37)	0 (0/27)	0 (0/28)	0 (0/30)	17 (5/29)[e]	13 (4/30)
Percentage pregnancy[g]	57 (21/37)	74 (20/27)	57 (16/28)	88 (28/32)	72 (21/29)	31 (9/29)[h]

[a] Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high concentrations; top row, vinyl chloride in air (ppm), bottom row, percentage ethanol in drinking water.

[b] Mean ± SD.

[c] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[d] Significantly different from control by an analysis of variance, $p < 0.05$.

[e] Significantly different from control by the Fisher exact probability test, $p < 0.05$

[f] Mean of litters ± SD.

[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of Cesarean section.

[h] Significantly different from vinyl chloride alone by the Fisher exact probability test, $p < 0.05$.

TABLE 6

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0 0	500 0	0 0	2500 0	2500 15
Number of litters	28	31	19	16	16
Corpora lutea/dam[b]	15 ± 3	13 ± 2[c]	14 ± 2	15 ± 2[c]	14 ± 2[d]
Implantation sites/dam[b]	12 ± 2	13 ± 2	12 ± 2	14 ± 2	12 ± 2
Pregnancy wastage[b,e]	3 ± 2	0.4 ± 1[c]	1 ± 1	2 ± 1	2 ± 2
Live fetuses/litter[b]	12 ± 2	12 ± 2	12 ± 2	13 ± 2	12 ± 2
Implantations resorbed (%)	1 (4/342)	3 (11/398)	4 (9/238)	3 (6/220)	4 (7/195)
Litters with resorptions (%)	14 (4/28)	29 (9/31)	32 (6/19)	25 (4/16)	25 (4/16)
Litters totally resorbed	0	0	0	0	0
Resorptions/litters with resorptions	1.0 (4/4)	1.2 (11/9)	1.5 (9/6)	1.5 (6/4)	1.8 (7/4)
Sex ratio, M:F	52:48	50:50	49:51	53:47	51:49
Fetal body weight (g)[f]	5.67 ± 0.29	5.44 ± 0.38[c]	5.59 ± 0.27	5.62 ± 0.29	5.34 ± 0.32[d]
Fetal crown–rump length (mm)[f]	42.6 ± 1.2	43.6 ± 0.8[c]	43.6 ± 1.5	43.3 ± 1.1	42.4 ± 0.9[d]
Maternal deaths/treated dams (%)	0 (0/29)	0 (0/33)	0 (0/20)	6 (1/17)	0 (0/17)
Percentage pregnancy[g]	96 (28/29)	94 (31/33)	95 (19/20)	100 (17/17)	94 (16/17)

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Mean ± SD.

[c] Significantly different from control by an analysis of variance, $p < 0.05$.

[d] Significantly different from vinyl chloride alone by an analysis of variance, $p < 0.05$.

[e] The number of corpora lutea minus the number of implants.

[f] Mean of litters ± SD.

[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of Cesarean section.

TABLE 7

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0 0	500 0	0 0	2500 0	2500 15
Number of litters	18	19	11	5	16
Corpora lutea/dam[b]	9 ± 1	8 ± 1[c]	10 ± 2	10 ± 7	10 ± 2
Implantation sites/dam[b]	9 ± 1	8 ± 1[c]	8 ± 2	8 ± 4	9 ± 2
Pregnancy wastage[b,d]	0.4 ± 1	1 ± 1	2 ± 1	2 ± 3	1 ± 1
Live fetuses/litter[b]	8 ± 1	7 ± 2[c]	6 ± 3	6 ± 4	4 ± 4
Implantations resorbed (%)	6 (10/162)	9 (14/150)	22 (19/88)	24 (10/42)	53 (79/149)[e]
Litters with resorptions (%)	44 (8/18)	32 (6/19)	64 (7/11)	80 (4/5)	88 (14/16)
Litters totally resorbed	0	1	2	1	7
Resorptions/litters with resorptions	1.2 (10/8)	2.3 (14/6)	2.7 (19/7)	2.5 (10/4)	5.6 (79/14)
Sex ratio, M:F	53:47	50:50	61:39	50:50	43:57
Fetal body weight (g)[f]	35.23 ± 4.82	34.13 ± 4.17	36.46 ± 4.82	33.77 ± 4.48	32.48 ± 5.88
Fetal crown–rump length (mm)[f]	91.0 ± 4.2	92.6 ± 5.0	92.6 ± 4.7	87.1 ± 5.2	87.7 ± 6.3
Maternal deaths/treated dams (%)	0 (0/18)	0 (0/20)	0 (0/11)	14 (1/7)	16 (3/19)
Percentage pregnancy[g]	100 (18/18)	95 (19/20)	100 (11/11)	86 (6/7)	95 (18/19)

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6-18 of gestation. Some of the rabbits were given ethanol in their drinking water (15%, v/v) on Days 6-18 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Mean ± SD.

[c] Significantly different from control by an analysis of variance, $p < 0.05$.

[d] The number of corpora lutea minus the number of implants.

[e] Significantly different from vinyl chloride alone by the Fisher exact probability test, $p < 0.05$.

[f] Mean of litters ± SD

[g] Based on the presence of fetuses and/or resorption sites observed by gross examination at the time of cesarean section.

Incidence of anomalies. The incidence of anomalies among litters of mice, rats, and rabbits exposed to vinyl chloride by inhalation is indicated in Tables 8, 9, and 10, respectively. The incidence of gross anomalies observed by external examination of fetuses from mice was not significantly greater than among control litters (Table 8). One fetus among the litters of mice exposed to 500 ppm of vinyl chloride in combination

TABLE 8

INCIDENCE OF ANOMALIES AMONG LITTERS OF MICE EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration			High concentration		
	0 0	50 0	50 15	0 0	500 0	500 15
	[Number examined, fetuses (litters)]					
Gross anomalies	221 (20)	220 (20)	153 (14)	325 (26)	215 (19)	56 (5)
Soft tissue anomalies	74 (20)	75 (20)	50 (14)	107 (26)	73 (19)	19 (5)
Skeletal anomalies	221 (20)	220 (20)	153 (14)	325 (26)	215 (19)	56 (5)
Bones of the skull[b]	147 (20)	145 (19)	103 (14)	217 (26)	142 (19)	37 (5)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]					
Exencephaly	0	0	0	1 (8)	1 (10)	2 (20)
Anopthalmia	0	0	0	0	0	2 (20)
Cleft palate	1 (10)	1 (10)	2 (21)	0	1 (5)	4 (40)
Soft tissue anomalies						
Small thymus	0	0	4 (7)	0	0	0
Skeletal anomalies						
Sternebrae						
Unfused	3 (20)	3 (25)	13 (57)[c]	2 (19)	9 (42)[d]	34 (80)[c]
Delayed ossification	7 (50)	4 (35)	44 (100)[c]	1 (12)	6 (42)[d]	43 (100)[c]
No. 5 sternebra missing	0	0	3 (21)	0	1(10)	7 (40)[c]
Ribs						
Extra	4 (30)	5 (30)	0.6 (7)	3 (31)	3 (32)	14 (60)[e]
Spurs	4 (35)	5 (40)	2 (21)	4 (31)	3 (21)	14 (80)[c]
Vertebrae						
Forked atlas	0.4 (5)	1 (10)	4 (36)[c]	0	0	4 (20)
Missing cervical centra	0	0	0	0	1 (10)	38 (60)[c]
Delayed ossification of cervical arches	0	0	1 (14)	0	0	5 (40)[c]
Skull						
Delayed ossification	9 (35)	8 (37)	40 (100)[c]	13 (54)	30 (58)[d]	70 (100)[c]
Unfused occipital	0	0.7 (5)	24 (50)[c]	1 (12)	5 (21)	11 (20)

[a] Mice were exposed to vinyl chloride or filtered room air by inhaltion 7 hr daily on days 6–15 of gestation. Some of the mice were given ethanol in their drinking water (15% v v) on days 6–15 of gestation. Low and high concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Calculations of the incidence of anomalies of bones of the skull are based on approximately two-thirds of the total population of fetuses. The remainder of the fetuses are decapitated during the soft tissue examination. Among litters of mice which were exposed to 50 ppm of vinyl chloride, one litter consisted of only one fetus, which was decapitated during the soft tissue examination.

[c] Significantly different from vinyl chloride alone by the modified Wilcoxon test, $p < 0.05$.

[d] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

[e] $p = 0.057$, modified Wilcoxon test.

with 15% ethanol exhibited anopthalmia. The incidence of cleft palate in this group was slightly higher than that observed among mice receiving vinyl chloride alone.

No soft tissue anomalies occurred at an incidence significantly greater than control in mice. Two fetuses from one litter among mice exposed to 50 ppm of vinyl chloride plus ethanol exhibited a small thymus. No skeletal anomalies were observed at an incidence significantly greater than in controls among mice exposed to 50 ppm of vinyl chloride. Significant increases in the incidence of delayed ossification of sternebrae

(No. 5) and bones of the skull were observed among litters of mice exposed to 500 ppm of vinyl chloride. The incidence of unfused centers of ossification of sternebrae was also significantly higher in this group. Among mice exposed to vinyl chloride in combi-

TABLE 9

INCIDENCE OF ANOMALIES AMONG LITTERS OF RATS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration		High concentration		
	0 0	500 0	0 0	2500 0	2500 15
	[Number examined, fetuses (litters)]				
Gross anomalies	339 (28)	387 (31)	229 (19)	214 (16)	188 (16)
Soft tissue anomalies	113 (28)	129 (31)	76 (19)	73 (16)	63 (16)
Skeletal anomalies[b]	337 (28)	387 (31)	229 (19)	214 (16)	188 (16)
Bones of the skull[c]	225 (28)	259 (31)	153 (19)	141 (16)	125 (16)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]				
Omphalocele	0	1 (3)	0 4 (5)	0	0.5 (6)
Soft tissue anomalies					
Micropthalmia	0	0	0	0	2 (6)
Dilated ureter (unilateral or bilateral)	2 (7)	2 (6)	5 (10)	27 (50)[d]	5 (19)[e]
Small kidney	0	0	0	0	2 (6)
Skeletal anomalies					
Sternebrae					
Unfused	0	1(6)	3 (32)	0.5 (6)[d]	1 (12)
Ribs					
Spurs	1 (4)	9 (52)[d]	14 (68)	12 (69)	35 (69)[e]
Vertebrae					
Missing cervical centra	0.3 (4)	2 (16)	7 (53)	4 (50)	21 (81)[e]
Skull					
Delayed ossification	16 (61)	12 (61)	18 (58)	6 (31)[d]	3 (25)
Unfused	0	0	53 (90)	3 (12)[d]	2 (12)

[a] Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–15 of gestation. Some of the rats were given ethanol in their drinking water (15%, v/v) on Days 6–15 of gestation. Low and high Concentrations: top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[b] Among litters of the low concentration control rats, two fetuses were misplaced and were not available for examination for skeletal anomalies.

[c] Calculations of the incidence of anomalies of bones of the skull are based on approximately two-thirds of the total population of fetuses. The remainder of the fetuses are decapitated during the soft tissue examination.

[d] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

[e] Significantly different from vinyl chloride alone by the modified Wilcoxon test, $p < 0.05$.

nation with 15% ethanol, several skeletal anomalies occurred at an incidence significantly greater than among mice exposed to vinyl chloride alone. Among litters of mice exposed to 50 ppm of vinyl chloride plus ethanol, increases in the incidence of delayed ossification of bones of the skull and sternebrae (Nos. 4–6) were observed upon skeletal examination. The incidences of unfused occipital, unfused sternebrae (Nos. 5 and 6),

and forked atlas were also significantly increased in the 50-ppm plus ethanol group. Among litters of mice exposed to 500 ppm plus ethanol, increases in the incidence of delayed ossification of bones of the skull, sternebrae (Nos. 2–6), and arches of the cervical vertebrae were observed. Significant increases in the incidence of lumbar spurs, missing centra of the cervical vertebrae, unfused sternebrae, and missing fifth sternebrae were observed in this group as well. The incidence of extra ribs was slightly, but not significantly, increased compared to the controls.

In rats, no gross anomalies occurred at an incidence significantly greater than among control animals (Table 9). Among litters of rats exposed to 2500 ppm, the incidence of

TABLE 10

INCIDENCE OF ANOMALIES AMONG LITTERS OF RABBITS EXPOSED TO VINYL CHLORIDE BY INHALATION[a]

	Low concentration[b]		High concentration[b]		
	0	500	0	2500	2500
	0	0	0	0	0
	[Number examined, fetuses (litters)]				
Gross anomalies	152 (18)	136 (18)	69 (9)	32 (4)	70 (9)
Soft tissue anomalies	50 (18)	47 (18)	24 (9)	10 (4)	25 (9)
Skeletal anomalies	152 (18)	136 (18)	69 (9)	32 (4)	70 (9)
Gross anomalies	[Fetuses affected (%) (litters affected, %)]				
Cleft palate	0	0	0	0	1 (11)
Soft tissue anomalies					
Dilated renal pelvis	0	0	0	0	8 (11)
Dilated cerebral ventricle	0	0	0	10 (25)	0
Enlarged right atrium of heart	0	0	0	0	8 (11)
Skeletal anomalies					
Sternebrae					
Delayed ossification No. 5	28 (77)	38 (94)[c]	20 (44)	16 (75)	24 (67)

[a] Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hr daily on Days 6–18 of gestation. Some of the rabbits were given ethanol in their drinking water (15%, v/v) on Days 6–18 of gestation.

[b] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.

[c] Significantly different from control by the modified Wilcoxon test, $p < 0.05$.

dilated ureter was significantly higher than among control litters. The incidence of dilated ureter was significantly lower, however, among litters of rats exposed to 2500 ppm of vinyl chloride in combination with 15% ethanol. Among litters of rats exposed to vinyl chloride, only minor skeletal variations were observed at an incidence higher than that of controls. The incidence of lumbar spurs was increased among litters exposed to 500 ppm. No increases in the occurrence of skeletal anomalies were observed among litters of rats exposed to 2500 ppm. The incidences of delayed ossification of bones of the skull and unfused centers of ossification of skull and sternebrae were significantly decreased among litters of this group. The incidences of lumbar spurs and missing centra of the cervical vertebrae were, however, significantly increased among

litters of rats exposed to 2500 ppm of vinyl chloride in combination with 15% ethanol in the drinking water.

In rabbits, no gross anomalies were observed at an incidence greater than among control litters, although a cleft palate was observed in one fetus among litters of rabbits exposed to 2500 ppm of vinyl chloride plus ethanol (Table 10). Dilated renal pelvis was also observed in two fetuses from one litter among rabbits exposed to 2500 ppm plus ethanol. Two fetuses from this group also exhibited an enlarged right atrium of the heart. Among litters of rabbits exposed to 500 ppm, the incidence of delayed ossification of the fifth sternebra was significantly higher than that of control litters. Delayed ossification of sternebrae did not occur at a significantly higher incidence than control among litters of rabbits exposed to 2500 ppm of vinyl chloride alone or in combination with 15% ethanol in the drinking water.

DISCUSSION

The results of these studies indcate that exposure of pregnant mice, rats, or rabbits to vinyl chloride by inhalation at concentrations sufficiently high to cause maternal toxicity was not teratogenic in any of the three species. The responses of mice, rats, and rabbits are summarized in Table 11. Less maternal toxicity was observed among rats and rabbits than among mice during exposure or at the time of cesarean section. Among rats, one maternal death and an increase in liver weight were observed at 2500 ppm and a decrease in maternal weight gain was observed at 500 ppm. Among rabbits, one maternal death was observed at 2500 ppm and there was a decrease in food consumption at 500 ppm. In comparison, 500 ppm was quite toxic to pregnant mice, as evidenced by the significantly decreased weight gain and food consumption and by the occurrence of a number of maternal deaths.

Exposure to vinyl chloride alone was not consistently embryotoxic in the three species studied. Among mice, at 500 ppm, the incidence of resorptions was significantly higher (13%) than among the concurrent controls (7%). Since the incidence of resorptions among the mice which served as controls for the 50-ppm group was 15% and the incidence among groups of control mice from recent studies in our laboratory was 10% (193/1895), the apparent increase at 500 ppm was probably due to the unusually low incidence of resorptions among their concurrent control group. Resorptions among exposed rats and rabbits occurred at a frequency comparable to controls. Some decreases in fetal body weight and crown–rump length were observed in rats and mice, but not in rabbits. A teratogenic response to maternally inhaled vinyl chloride was not observed in mice, rats, or rabbits. With the exception of unilateral and bilateral dilated ureter among litters of rats exposed to 2500 ppm of vinyl chloride, no external or soft tissue anomalies were observed at an incidence significantly higher than control in any of the three species. Examination of the skeletons revealed only minor skeletal variations; no major skeletal malformations were found at an incidence significantly greater than in the control groups.

Ingestion of 15% ethanol in the drinking water enhanced some of the effects of inhaled vinyl chloride. In each of the three species tested, maternal weight gain and food consumption were lower than among animals exposed to vinyl chloride alone. The percentage resorptions was slightly but not significantly increased among mice exposed

TABLE II

SUMMARY VINYL CHLORIDE TERATOLOGIC STUDIES

	Mice[a]					Rats[a]				Rabbits[a]			
	50		500		0	500	2500		0	500	2500		0
	0	15	0	15	15	0	0	15	15	0	0	15	15
Gestation days of treatment	6-15		6-15		6-15	6-15	6-15		6-15	6-18	6-18		6-18
Maternal deaths	No	No	Yes	Yes	No	No	Yes	No	No	No	Yes	Yes	No
Percentage pregnancy	-[b]	-	-	Dec	-		—	-	--	--	-	--	--
Number of litters examined	20	16	19	7	21	31	16	16	19	19	5	16	14
Maternal weight gain	-	Dec	Dec	Dec	-	Dec	-	Dec	--	--	--	-	--
Maternal food consumption	-	Dec	Dec	Dec	Dec	-	Dec	Dec	Dec	Dec	--	Dec	Dec
Maternal liver weight													
Absolute	--	Dec	Dec	Dec	—	--	Inc	—	--	--	--	--	--
Relative	--	-	--	Dec	--	--	Inc	Inc	Inc	--	--	--	—
Implantation sites/dam	--	--	--	Dec	--	--	--	--	—	Dec	-	-	—
Percentage resorptions	--		Inc[c]	-	--	--	--	--	—	--	--	Inc	--
Litters totally resorbed	0/20	2/16	0/19	2/7	0/21	0/31	0/16	0/16	0/19	1/19	1/5	7/16	2/14
Litter size	-		Dec	Dec	—	-	-	--	--	Dec	--	--	--
Fetal body weight	--	Dec	Dec	Dec	Dec	Dec	-	Dec	Dec	-	--	--	—
Fetal crown-rump length	Inc	Dec	-	Dec	Dec	Inc	--	Dec	--	-	-	-	-
External anomalies	-	--	—		—	—	--	—	-	--		—	—
Visceral anomalies		-	-	--	—	-	Inc[d]	—	-			-	--
Skeletal anomalies	-	Inc	-	Inc	Inc	-	—	—	Inc		--	—	—

[a] Top row, vinyl chloride in air (ppm); bottom row, percentage ethanol in drinking water.
[b] —, No change; Dec, decrease; Inc, increase as compared to control values.
[c] This apparent increase was due to a lower than normal incidence of resorptions among the control group; see Discussion for details.
[d] Dilated ureter.

to vinyl chloride in combination with 15% ethanol in the drinking water. A statistically significant increase in the percentage of resorptions was observed among rabbits exposed to vinyl chloride plus ethanol. Fetal body measurements were lower among litters of mice and rats which received ethanol and vinyl chloride compared to vinyl chloride alone. The combination of ethanol and vinyl chloride did not cause a teratogenic response in the three species tested, although higher incidences of some skeletal variations were observed among litters of mice and rats. Certain malformations were observed among litters of mice, rats, and rabbits which also received ethanol, but their incidence was not statistically different than among litters of animals exposed to vinyl chloride alone. The effect of simultaneous ingestion of ethanol on the disposition of vinyl chloride in these animals seemed to enhance maternal toxicity to an extent greater than embryotoxicity.

In summary, the results of these studies indicate that exposure of pregnant mice, rats, and rabbits to vinyl chloride by inhalation was not teratogenic at the concentrations tested. Mice were more susceptible to the toxic effects of vinyl chloride than either rats or rabbits. Simultaneous exposure to vinyl chloride by inhalation and 15% ethanol in the drinking water resulted in toxic effects greater than those associated with exposure to vinyl chloride alone in the three species. Exposure to vinyl chloride alone or in combination with 15% ethanol in the drinking water did not cause a significant teratogenic response in mice, rats, or rabbits.

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