THE EFFECTS OF MATERNALLY INHALED VINYL CHLORIDE ON EMBRYONAL AND FETAL DEVELOPMENT IN MICE, RATS AND RABBITS

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March 3, 1976

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Prepared for the companies sponsoring research on vinyl chloride administered by the Manufacturing Chemists Association.

ABSTRACT

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 vinyl chloride 7 hrs daily during the period of major organogenesis. Subsequently, other groups of mice were similarly exposed to 50 ppm vinyl chloride and rats and rabbits were exposed to 2500 ppm 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.

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INTRODUCTION

Vinyl chloride is widely used in the preparation of polyvinyl chloride resin and 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 six 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 vinyl chloride vapor for 12 months. Findings on 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 2,500 ppm 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 teratology 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 is being reported separately by Schwetz, et al., (1975), and is summarized in Table 11 of this report. The majority of this study was conducted under the auspices of the Manufacturing Chemists Association.

METHODS

Animals and Test Material. Female CF-1 mice (Carworth, Portage, Michigan), weighing 25 to 30 g, Sprague-Dawley rats (Spartan-substrain, Haslett, Michigan) weighing approximately 250 g and New Zealand white rabbits (Langshaws Rabbitry, Augusta, Michigan) 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 day zero of pregnancy for mice and rats, respectively. The day of hand mating was considered day zero 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 (Ralston Purina Company, St. Louis, Missouri) and water were available. Food consumption was measured at three day intervals for mice and rats and at two day intervals for rabbits.

Exposure of bred animals was conducted in stainless steel chambers of 3.7 cubic meter volume under dynamic conditions. The atmosphere of vinyl chloride was generated by diluting gaseous vinyl chloride with filtered room air at a rate calculated to give the desired concentration. Samples of inhibited vinyl chloride monomer (chloroethylene) obtained from Matheson Gas Products, Joliet, Illinois, were used for the exposures. The actual concentration was measured with an infrared spectrophotometer (Perkin Elmer 12A) with a multipath gas cell. In addition, the concentration in the chamber was continuously monitored using a recording combustion analyzer to assure the absence of significant deviations from the desired levels.

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 vinyl chloride for 7 hrs daily on days 6-15 (mice and rats) or 6-18 (rabbits) of gestation. Some of the mice were also given 15% ethanol in the drinking water. Subsequently, additional groups of mice were exposed to 50 ppm vinyl chloride. Some of these mice also received 15% ethanol in the drinking water. Additional groups of rats and rabbits were exposed to 2,500 ppm vinyl chloride. Some of these rats and rabbits also received 15% ethanol in the drinking water. The experimental design for each of the three species is summarized in Table 1.

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 day 18 and 21 of gestation, respectively. Pregnant rabbits were sacrificed on day 29 of gestation. The uterine horns were exteriorized through a mid-line 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 anomalies. 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 for 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 Dunnett's test

(Steel and Torrie, 1960). The incidence of fetal anomalies was analyzed by the Wilcoxon test as modified by Haseman and Hoel (1975).

Controls. In these studies the effect of inhaled vinyl chloride alone was compared to the effect of vinyl chloride in combination with ethanol in the drinking water. Thus, the group of animals which was exposed only to vinyl chloride served as the controls 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 in inhalation chambers.

RESULTS

Maternal Toxicity. Among mice exposed to 500 ppm vinyl chloride by inhalation, there was a decrease in maternal weight gain during gestation and in the absolute liver weight at the time of cesarean section compared to control values (Table 2). This effect was not observed among mice exposed to 50 ppm vinyl chloride. Mice exposed to a combination of 500 ppm vinyl chloride by inhalation and 15% ethanol in their drinking water also showed a decrease in weight gain and liver weight (absolute and relative) on day 18 of gestation. Ethanol in combination with 50 ppm vinyl chloride result d in a decrease in maternal weight gain throughout

gestation and a decrease in 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. Food consumption was decreased for mice exposed to 500 ppm vinyl chloride alone but not 50 ppm during days 6-15 of gestation.

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 vinyl chloride. The apparent decrease in maternal weight gain among rats exposed to 500 ppm vinyl chloride 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 2,500 ppm vinyl chloride, both the absolute and relative liver weight 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 2,500 ppm vinyl chloride and 15% ethanol. Maternal weight gain for this group of rats was significantly decreased during days 6-10

of gestation and food consumption was decreased during the exposure period.

No effect on maternal weight gain or liver weight was observed among rabbits exposed to 2,500 ppm vinyl chloride by inhalation. A decrease in maternal weight gain during days 6-12 and 12-18 of gestation was observed among rabbits exposed to 2,500 ppm vinyl chloride in combination with 15% ethanol (Table 4), but total weight gain in this group was not different from that among rabbits exposed to 2,500 ppm vinyl chloride alone. Food consumption was, however, significantly decreased among these rabbits exposed to 2,500 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 vinyl chloride alone and in combination with ethanol (Table 5). The percent pregnancy was reduced among mice exposed to 500 ppm vinyl chloride and ethanol. 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 vinyl chloride in combination with 15% ethanol. Two litters in this group were totally resorbed. In addition, the number of implantation sites per dam and

fetal crown-rump length were significantly lower in the ethanol group as compared to mice receiving 500 ppm vinyl chloride alone. Among mice exposed to 50 ppm vinyl chloride, the fetal crown-rump length was significantly greater than among controls; a decrease 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 vinyl chloride in combination with ethanol although 2 litters were totally resorbed.

One maternal death was observed among rats exposed to 2,500 ppm 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 vinyl chloride than among the control rats. Percent pregnancy 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 2,500 ppm vinyl chloride in combination with ethanol. A significant reduction in fetal body weight was also observed among rats exposed to 500 ppm vinyl chloride alone, but not among those exposed to 2,500 ppm. Significant decreases in the number of corpora lutea per dam were observed among rats exposed to 500 ppm vinyl chloride alone

and among those exposed to 2,500 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 2,500 ppm vinyl chloride. Since the number of corpora lutea is established prior to day 6 of gestation, these differences in the number of corpora lutea per dam among rats exposed to vinyl chloride is not considered 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 (2,500 ppm) plus ethanol group where 7 litters were totally resorbed (Table 7). Exposure of rabbits to either 500 or 2,500 ppm 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 vinyl chloride alone, but not among those exposed to 2,500 ppm vinyl chloride alone or in combination with 15% ethanol in the drinking water. This decrease in litter size was associated with a decrease in the number of corpora lutea and implantation sites in these does and therefore is probably not due to exposure to vinyl chloride. No differences in fetal body weight or crown-rump length were observed in any of the exposed groups of rabbits.

Incidence of Anomalies. The incidence of anomalies among litters of mice, rats and rabbits exposed to vinyl chloride by inhalation are 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 vinyl chloride in combination 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 vinyl chloride plus ethanol exhibited a small thymus. Significant increases in the incidence of delayed ossification of sternebrae (#5) and bones of the skull were observed among litters of mice exposed to 500 ppm vinyl chloride. The incidence of unfused centers of ossification of sternebrae was also significantly higher in this group. No skeletal anomalies were observed at an incidence significantly greater than in controls among mice exposed to 50 ppm vinyl chloride. Among mice exposed to vinyl chloride in combination with 15% ethanol, however, several skeletal anomalies occurred at an incidence significantly greater than among mice exposed to vinyl chloride

alone. In mice exposed to 500 ppm plus ethanol, increases in the incidence of delayed ossification of bones of the skull, sternebrae (#'s 2-6) and arches of the cervical vertebrae were observed. Significant increases in the incidence of lumbar spurs, missing centra of cervical vertebra and missing 5th sternebra were observed in this group as well. The incidence of extra ribs was slightly increased compared to the controls. Among litters of mice exposed to 50 ppm vinyl chloride plus ethanol, increases in the incidence of delayed ossification of bones of the skull and sternebrae (#'s 4-6) were again observed upon skeletal examination. The incidences of unfused occiptal, unfused sternebrae (#'s 5 and 6) and forked atlas were also significantly increased in the 50 ppm plus ethanol group.

In rats, no gross anomalies occurred at an incidence significantly greater than among control animals (Table 9). Among litters of rats exposed to 2,500 ppm vinyl chloride, the incidences of unilateral and bilateral dilated ureter were significantly higher than among control litters. The incidence of dilated ureter was not affected by concomitant exposure to 2,500 ppm vinyl chloride and 15% ethanol. Petechial hemorrhage in the kidney was observed at a slightly higher incidence than control among litters of rats exposed to 500 ppm but not 2,500 ppm vinyl chloride. 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 vinyl chloride. No increases in the occurrence of skeletal anomalies were observed among litters of rats exposed to 2500 ppm vinyl chloride. The incidences of delayed ossification of bones of the skull and unfused centers of ossification of skull and sternebrae wer significantly decreased among litters of this group. The incidences of lumbar spurs and missing centers of the cervical vertebrae were, however, increased among litters of rats exposed to 2500 ppm 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, though a cleft palate was observed in one fetus among litters of rabbits exposed to 2,500 ppm vinyl chloride plus ethanol (Table 10). Dilated renal pelvis was also observed in two fetuses from one litter among rabbits exposed to 2,500 ppm plus ethanol. Two fetuses from this group also exhibited an enlarged right atrium of the heart. One fetus with 3 kidneys of normal size was observed among litters of rabbits exposed to 500 ppm vinyl chloride. Delayed ossification of the 5th and 6th sternebrae were the only skeletal anomalies observed in

rabbits at a significantly different incidence from controls. Among litters of rabbits exposed to 500 ppm vinyl chloride, the incidence of delayed ossification of the 5th sternebra was increased whereas that of the 6th sternebra was decreased. Delayed ossification of sternebrae did not occur at a higher incidence than control among litters of rabbits exposed to 2,500 ppm vinyl chloride alone or in combination with 15% ethanol in the drinking water.

DISCUSSION

The results of these studies indicate 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 mice during exposure or at the time of cesarean section. Among rats one maternal death and an increase in liver weight was observed at 2,500 ppm while only a slight decrease in maternal weight gain was observed at 500 ppm. Among rabbits, one maternal death was observed at 2,500 ppm and there was a decrease in food consumption at 500 ppm. In comparison, 500 ppm was quite maternally toxic to 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 was not consistently embryotoxic in the three species studied. A significant increase in the percent resorptions was observed among mice exposed to 500 ppm vinyl chloride, but not among rats or rabbits exposed to 500 ppm or 2500 ppm vinyl chloride. Some decreases in fetal body weight and crown-rump length were observed in rats and mice but not 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 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 variants; no 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 was lower than among animals exposed to vinyl chloride alone. The percent resorptions was slightly increased among mice and significantly increased among rabbits exposed to vinyl chloride in combination with 15% ethanol in the drinking water. Fetal body measurements were lower among litters of

mice and rats which received ethanol and vinyl chloride compared to vinyl chloride alone. Certain malformations were observed among litters of mice, rats and rabbits which also received ethanol but their incidence was not statistically different than control litters. 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 of the other two species. 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. Neither exposure to vinyl chloride alone or in combination with 15% ethanol in the drinking water caused a significant teratogenic response in mice, rats or rabbits.

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TABLE 1

TERATOLOGY STUDIES WITH VINYL CHLORIDE

	ppm Vinyl chloride[a]	Ethanol[a]
Mice	500	0
	500	15%
	50	0
	50	15%
Rats	2,500	0
	2,500	15%
	500	0
Rabbits	2,500	0
	2,500	15%
	500	0
Mice, Rats, Rabbits	0	0

[a]Mice and rats were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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.

TABLE 2

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

	Low Concentration			High Concentration		
ppm Vinyl chloride in air[a]	0	50	50	0	500	500
% Ethanol in drinking water[a]	0	0	15	0	0	15
Number of dams	21	20	16	26	19	7
			g, mean ± SD			
Body weight on gestation day 6	30±2	31±2	31±2	29±2	29±3	30±3
Weight gain during gestation						
days 6-12	3±3	4±2	0.4±4[d]	days 6-12 6±1	5±4	1±2[d]
12-15	6±3	5±1	4±2[d]	12-18 15±3	12±3[c]	8±6
15-18	7±2	7±4	7±3			
total	16±4	17±6	11±7[d]	total 20±3	17±4[c]	10±7[d]
Liver weight on gestation day 18						
absolute	2.75±0.26	2.76±0.40	2.37±0.52[d]	2.75±0.31	2.49±0.29[c]	1.78±0.36[d]
relative[b]	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	6±1	6±1	4±2[d]	6±1	5±1[c]	3±1[d]

[a]Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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]mg liver/g body weight.

[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, $p<0.05$.

TABLE 3

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

	Low Concentration		High Concentration		
ppm Vinyl chloride in air[a]	0	500	0	2,500	2,500
% Ethanol in drinking water[a]	0	0	0	0	15
Number of dams	28	31	19	16	16
			g, mean ± SD		
Body weight on gestation day 6	258±26	277±20[d]	288±27	274±19	272±14
Weight gain during gestation					
days 6-10[b]			13±10	17±6	-1±12[e]
10-16			37±11	40±11	45±14
16-21			77±15	82±16	77±13
total	148±11	125±19[d]	127±15	138±23	120±15[e]
Liver weight on gestation day 21					
absolute	14.81±1.79	15.00±1.14	14.27±1.38	15.55±1.23[d]	16.52±1.50
relative[c]	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 day 6-15	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 hrs 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]Interim values for maternal weight gain were not available for the rats exposed to 500 ppm vinyl chloride.

[c]mg liver/g body weight.

[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, $p<0.05$.

TABLE 4

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

	Low Concentration		High Concentration		
ppm Vinyl chloride in air[a]	0	500	0	2,500	2,500
% Ethanol in drinking water[a]	0	0	0	0	15
Number of dams	18	20	11	5	16
	kg, mean ± SD				
Body weight on gestation day 6	3.85±0.23	3.82±0.25	4.08±0.21	4.39±0.30[c]	4.02±0.47
Weight gain during gestation					
days 6-12	-0.06±0.11	-0.12±0.08	-0.11±0.17	-0.12±0.08	-0.41±0.17[d]
12-18	0.01±0.21	0.06±0.09	0.05±0.15	-0.03±0.07	-0.27±0.15[d]
18-22	0.08±0.26	0.02±0.08	0.07±0.18	0.03±0.05	0.10±0.33
22-29	0.03±0.16	0.03±0.15	0.05±0.17	0.13±0.04	0.42±0.36
Total	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					
absolute	96±19	89±14	102±16	122±25	116±30
relative[b]	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	98±30	76±29[c]	91±36	89±26	15±9[d]

[a]Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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]g liver/kg body weight.

[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, $p<0.05$.

TABLE 5

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

	Low Concentration			High Concentration		
ppm Vinyl chloride in air[a]	0	50	50	0	500	500
% Ethanol in drinking water[a]	0	0	15	0	0	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[e]
Live fetuses/litter[b]	10±4	11±4	10±4	12±2	11±2[d]	8±6[e]
%, Resorptions/implantations	15 (40/261)	8 (18/238)	11 (19/172)	7 (26/351)	13 (33/248)[f]	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[c]	1.00±0.11	1.02±0.10	0.84±0.14[e]	1.07±0.06	0.99±0.11[d]	0.78±0.15[e]
Fetal crown-rump length, mm[c]	23.0±1.9	24.2±0.8[d]	22.4±1.5[e]	23.7±1.2	23.6±1.0	21.2±1.5[e]
%, maternal deaths/ treated dams	0 (0/37)	0 (0/27)	0 (0/28)	0 (0/30)	17 (5/29)[f]	13 (4/38)
% Pregnancy[h]	57 (21/37)	74 (20/27)	57 (16/20)	88 (28/32)	72 (21/29)	31 (9/29)[g]

[a]Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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]Mean ± SD.

[c]Mean of litter means ± 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]Significantly different from control by the Fisher exact probability test, $p<0.05$.

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

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

TABLE 6

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

	Low Concentration		High Concentration		
ppm Vinyl chloride in air[a]	0	500	0	2,500	2,500
% Ethanol in drinking water[a]	0	0	0	0	15
Number of litters	28	31	19	16	16
Corpora lutea/dam[b]	15±3	13±2[d]	14±2	15±2[d]	14±2[e]
Implantation sites/dam[b]	12±2	13±2	12±2	14±2	12±2
Pregnancy Wastage[b]	3±2	0.4±1[d]	1±1	2±1	2±2
Live fetuses/litter[b]	12±2	12±2	12±2	13±2	12±2
%, Resorptions/implantations	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[c]	5.67±0.29	5.44±0.38[d]	5.59±0.27	5.62±0.29	5.34±0.32[e]
Fetal crown-rump length, mm[c]	42.6±1.2	43.6±0.8[d]	43.6±1.5	43.3±1.1	42.4±0.9[e]
%, maternal deaths/ treated dams	0 (0/29)	0 (0/33)	0 (0/20)	6 (1/17)	0 (0/17)
% Pregnancy[f]	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 hrs 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] Mean ± SD.

[c] Mean of litter means ± 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] 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

	Low Concentration		High Concentration		
ppm Vinyl chloride in air[a]	0	500	0	2,500	2,500
% Ethanol in drinking water[a]	0	0	0	0	15
Number of litters	18	19	11	5	16
Corpora lutea/dam[b]	9±1	8±1[d]	10±2	10±7	10±2
Implantation sites/dam[b]	9±1	8±1[d]	8±2	8±4	9±2
Pregnancy wastage[b]	0.4±1	1±1	2±1	2±3	1±1
Live fetuses/litter[b]	8±1	7±2[d]	6±3	6±4	4±4
%, Resorptions/implantations	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[c]	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[c]	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)
% Pregnancy[f]	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 hrs 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]Mean ± SD.

[c]Mean of litter means ± SD.

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

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

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

TABLE 8

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

ppm Vinyl chloride in air[a]		Low Concentration			High Concentration		
% Ethanol in drinking water[a]		0	50	50	0	500	500
		0	0	15	0	0	15
Number of fetuses		221	200	153	325	215	56
Number of litters		20	20	14	26	19	5
		Percent affected (number affected)					
Gross Anomalies							
Exencephaly	F[b]	0	0	0	1 (2)	1 (3)	2 (1)
	L	0	0	0	8 (2)	10 (2)	20 (1)
Ablepharia	F	0	0	0	1 (2)	0	0
	L	0	0	0	8 (2)	0	0
Anophthalmia	F	0	0	0	0	0	2 (1)
	L	0	0	0	0	0	20 (1)
Omphalocele	F	0.4 (1)	0	0	0	0	0
	L	5 (1)	0	0	0	0	0
Cleft palate	F	1 (2)	1 (2)	2 (3)	0	1 (2)	7 (4)
	L	10 (2)	10 (2)	21 (3)	0	5 (1)	40 (2)
Crooked tail	F	0.4 (1)	0.4(1)	0.6 (1)	1 (2)	1 (2)	0
	L	5 (1)	5 (1)	7 (1)	8 (2)	5 (1)	0
Short tail	F	0	0.4(1)	0	0	0	0
	L	0	5 (1)	0	0	0	0
Soft Tissue Anomalies							
Small thymus	F	0	0	4 (2)	0	0	0
	L	0	0	7 (1)	0	0	0
Hemorrhage, kidney	F	0	0	0	2 (2)	3 (2)	0
	L	0	0	0	8 (2)	10 (2)	0
Hemorrhage, liver	F	0	1 (1)	0	2 (2)	1 (1)	0
	L	0	5 (1)	0	4 (1)	5 (1)	0
Hemorrhage, thymus	F	0	5 (4)	0	0	0	0
	L	0	10 (2)	0	0	0	0
Liver hematoma	F	0	0	0	2 (2)	1 (1)	0
	L	0	0	0	8 (2)	5 (1)	0
Skeletal Anomalies							
Skull-delayed ossification	F	9 (13)	8 (2)	40 (41)	13 (28)	30 (42)	70 (26)
	L	35 (7)	37 (7)	100 (14)[d]	54 (14)	58 (11)[c]	100 (5)[d]
-unfused occipital	F	0	0.7(1)	24 (25)	1 (3)	5 (7)	11 (4)
	L	0	5 (1)	50 (7)[d]	12 (3)	21 (4)	20 (1)
Ribs - extra	F	4 (8)	5 (11)	0.6 (1)	3 (11)	3 (6)	14 (8)[c]
	L	30 (6)	30 (6)	7 (1)	31 (8)	32 (6)	60 (3)
- spurs	F	4 (8)	6 (12)	2 (3)	4 (12)	3 (6)	14 (8)
	L	35 (7)	40 (8)	21 (3)	31 (8)	21 (4)	80 (4)[d]
Vertebrae-forked atlas	F	0.4 (1)	1 (2)	4 (6)	0	0	4 (2)
	L	5 (1)	10 (2)	36 (5)[d]	0	0	20 (1)
- missing cervical centra	F	0	0	0	0	1 (2)	38 (21)
	L	0	0	0	0	10 (2)	60 (3)[d]
- delayed ossification of cervical arches	F	0	0	1 (2)	0	0	5 (3)
	L	0	0	14 (2)	0	0	40 (2)[d]
Sternum-unfused	F	3 (6)	3 (7)	13 (20)	2 (8)	9 (19)	34 (19)
	L	20 (4)	25 (5)	57 (8)[d]	19 (5)	42 (8)[c]	80 (4)[d]
- delayed ossification	F	7 (15)	4 (9)	44 (67)	1 (4)	6 (12)	43 (24)
	L	50 (10)	35(7)	100 (14)[d]	12 (3)	42 (8)[c]	100 (5)[d]
- no. 5 sternebra missing	F	0	0	3 (4)	0	1 (3)	7 (4)
	L	0	0	21 (3)	0	10 (2)	40 (2)[d]

[a] Mice were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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] F=fetuses; L=litters.

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

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

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

TABLE 9

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

ppm Vinyl chloride in air[a]		Low Concentration		High Concentration		
% Ethanol in drinking water[a]		0 0	500 0	0 0	2,500 0	2,500 15
Number of fetuses		339	387	229	214	188
Number of litters		28	31	19	16	16
		Percent Affected (number affected)				
Gross Anomalies						
Omphalocele	F[b]	0	1 (2)	0.4 (1)	0	0.5 (1)
	L	0	3 (1)	5 (1)	0	6 (1)
Tail-less	F	0	0	1 (3)	0	0.5 (1)
	L	0	0	5 (1)	0	6 (1)
Runt	F	0	0	0.4 (1)	0	0
	L	0	0	5 (1)	0	0
Soft Tissue Anomalies						
Microphthalmia	F	0	0	0	0	2 (1)
	L	0	0	0	0	6 (1)
Dilated ureter						
- unilateral	F	2 (3)	1 (1)	4 (3)	15 (11)	1 (2)
	L	11 (3)	3 (1)	10 (2)	44 (7)[c]	12 (2)
- bilateral	F	0	1 (1)	3 (2)	12 (9)	2 (1)
	L	0	3 (1)	5 (1)	38 (6)[c]	6 (1)
Small kidney	F	0	0	0	0	2 (1)
	L	0	0	0	0	6 (1)
Petechial hemorrhage, kidney	F	0	10 (13)	0	0	0
	L	0	13 (4)	0	0	0
Hemorrhage, liver	F	0	0	0	1 (1)	0
	L	0	0	0	6 (1)	0
Hemorrhage, kidney	F	0	1 (1)	0	0	0
	L	0	3 (1)	0	0	0
Short trunk; ectopic ovaries	F	0	0	1 (1)	0	0
	L	0	0	5 (1)	0	0
Skeletal Anomalies						
Skull - delayed ossification	F	16 (35)	12 (31)	18 (28)	6 (9)	3 (4)
	L	61 (17)	61 (19)	58 (11)	31 (5)[c]	25 (4)
- unfused	F	0	0	53 (81)	1 (4)	2 (2)
	L	0	0	90 (17)	12 (2)[c]	12 (2)
Ribs - shortened	F	0	0	1 (2)	0	0
	L	0	0	5 (1)	0	0
- crooked	F	0	0	0.4 (1)	0	1 (2)
	L	0	0	5 (1)	0	6 (1)
- missing	F	0	0	2 (4)	0.5(1)	2 (4)
	L	0	0	10 (2)	6 (1)	19 (3)
- extra	F	0	0	0.4 (1)	0.5(1)	4 (7)
	L	0	0	5 (1)	6 (1)	19 (3)
- spurs	F	1 (2)	9 (34)	14 (33)	12 (25)	35 (66)
	L	4 (1)	52 (16)[c]	68 (13)	69 (11)	69 (11)[d]
Vertebrae - missing	F	0	0	1 (3)	0	2 (3)
	L	0	0	5 (1)	0	12 (2)
- missing cervical centra	F	0.3 (1)	2 (7)	7 (16)	4 (9)	21 (40)
	L	4 (1)	16 (5)	53 (10)	50 (8)	81 (13)[d]
Sternum - delayed ossification	F	1 (5)	2 (4)	9 (21)	9 (19)	11 (21)
	L	11 (3)	19 (6)	68 (13)	44 (7)	56 (9)
- unfused	F	0	1 (2)	3 (8)	0.5(1)	1 (2)
	L	0	6 (2)	32 (6)	6 (1)[c]	12 (2)
- fused	F	0	0	0.4 (1)	0	0.5 (1)
	L	0	0	5 (1)	0	6 (1)
- missing	F	0	0.2 (1)	2 (4)	0	0
	L	0	3 (1)	16 (3)	0	0
Other - missing fibula	F	0	0	0.4 (1)	0	0
	L	0	0	5 (1)	0	0
- missing scapula	F	0	0	0	0	0.5 (1)
	L	0	0	0	0	6 (1)

[a]Rats were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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]F-fetuses; L-litters.

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

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

TABLE 10

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

ppm Vinyl chloride in air[a]		Low Concentration		High Concentration		
% Ethanol in drinking water[a]		0 0	500 0	0 0	2,500 0	2,500 15
Number of fetuses		152	136	69	32	70
Number of litters		18	18	9	4	9
		Percent affected (number affected)				
Gross Anomalies						
Cleft palate	F[b]	0	0	0	0	1 (1)
	L	0	0	0	0	11 (1)
Exencephaly	F	1 (1)	0	0	0	0
	L	6 (1)	0	0	0	0
Crooked tail	F	0	1 (1)	0	0	0
	L	0	6 (1)	0	0	0
Soft Tissue Anomalies						
3 kidneys	F	0	2 (1)	0	0	0
	L	0	6 (1)	0	0	0
Dilated renal pelvis	F	0	0	0	0	8 (2)
	L	0	0	0	0	11 (1)
Dilated cerebral ventricle	F	0	0	0	10 (1)	0
	L	0	0	0	25 (1)	0
Enlarged right atrium	F	0	0	0	0	8 (2)
	L	0	0	0	0	11 (1)
2 left sub-clavian arteries	F	0	0	0	0	4 (1)
	L	0	0	0	0	11 (1)
Skeletal Anomalies						
Sternum - delayed ossification #5	F	28 (43)	38 (52)[c]	20 (14)	16 (5)	24 (17)
	L	77 (14)	94 (17)	44 (4)	75 (3)	67 (6)
- delayed ossification #6	F	22 (33)	11 (15)[c]	10 (7)	9 (3)	9 (6)
	L	72 (13)	50 (9)	44 (4)	75 (3)	22 (2)

[a]Rabbits were exposed to vinyl chloride or filtered room air by inhalation 7 hrs 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]F=fetuses; L=litters

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

TABLE 11

SUMMARY - VINYL CHLORIDE TERATOLOGY STUDIES

Vinyl chloride, 7 hr/day	Mice					Rats				Rabbits			
	50 ppm		500 ppm		0	500 ppm	2500 ppm		0	500 ppm	2500 ppm		0
Gestation days of treatment	6-15		6-15		6-15	6-15	6-15		6-15	6-18	6-18		6-18
15% Ethanol in drinking water	no	yes	no	yes	yes	no	no	yes	yes	no	no	yes	yes
Maternal deaths	no	no	yes	yes	no	no	yes	no	no	no	yes	yes	no
% Pregnancy	--	--	--	dec.	--	--	--	--	--	--	--	--	--
No. litters examined	20	14	19	5	21	31	16	16	19	18	4	9	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.	--	--	--
% Resorptions	--	--	inc.	--	--	--	--	--	--	--	--	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.[b]	--	--	--	--	--	--
Skeletal anomalies	--	--	--	inc.	inc.	--	--	--	inc.	--	--	--	--

[a] -- = no change; dec. = decrease; inc. = increase.

[b] Dilated ureter.