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Embryotoxicity and Fetotoxicity of Inhaled or Ingested Vinylidene Chloride in Rats and Rabbits[1,2]

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Embryotoxicity and Fetotoxicity of Inhaled or Ingested Vinylidene Chloride in Rats and Rabbits. MURRAY, F. J., NITSCHKE, K. D., RAMPY, L. W., AND SCHWETZ, B. A. (1979). *Toxicol. Appl. Pharmacol.* 49, 189–202. The teratogenic potential of inhaled or ingested vinylidene chloride was evaluated in Sprague–Dawley rats and New Zealand white rabbits. Both species were exposed to the test material by inhalation for 7 hr/day at concentrations of 20 (rats only), 80, or 160 ppm. For the ingestion study, rats were given drinking water containing 200 ppm vinylidene chloride. Rats were given vinylidene chloride from the 6th to the 15th days of gestation and rabbits on the 6th to the 18th days. A teratogenic effect was not seen in rats or rabbits inhaling concentrations of up to 160 ppm vinylidene chloride for 7 hr/day or in rats given drinking water containing 200 ppm vinylidene chloride. Toxicity to both the dams and their developing embryos was observed among the rats inhaling 80 or 160 ppm and among the rabbits inhaling 160 ppm. At exposure levels which caused little or no maternal toxicity (20 ppm in rats and 80 ppm in rabbits), there was no effect on embryonal or fetal development. Among the rats given drinking water containing 200 ppm VDC, there was no evidence of toxicity to the dams or their offspring.

Vinylidene chloride (1,1-dichloroethylene, VDC, $CH_2{=}CCl_2$) is used in the manufacture of various plastics, including films and coatings for food packaging. Reports on the acute toxicity of vinylidene chloride in laboratory animals have indicated moderate toxicity when given by inhalation or by gavage (Carpenter *et al.*, 1949; Rylova, 1953; Siegel *et al.*, 1971; Jenkins *et al.*, 1972; Jaeger *et al.*, 1973). Exposure by inhalation to 100 ppm vinylidene chloride for 6 weeks resulted in some weight loss in rabbits and monkeys, but not in rats, guinea pigs, or dogs (Prendergast, 1967). Rats exposed for up to 4 weeks to 500 ppm vinylidene chloride exhibited nasal irritation, decreased weight gain, and liver cell degeneration; at 200 ppm, the only sign of toxicity was slight nasal irritation (Gage, 1970).

Vinylidene chloride has been shown to be a weak mutagen in certain strains of bacteria (McCann *et al.*, 1975; Greim *et al.*, 1975; Bartsch *et al.*, 1975). This effect was seen in bacterial systems only under conditions of activation with microsomal enzymes, implicating a metabolite of vinylidene chloride rather than vinylidene chloride itself as the causative agent.

No information regarding the teratogenic potential of vinylidene chloride was found in

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the scientific literature. Thus, these studies were undertaken to evaluate the potential of inhaled or ingested vinylidene chloride to adversely affect embryonal and fetal development in rats and rabbits. For the inhalation studies, both rats and rabbits were exposed to vinylidene chloride at concentrations as large as 160 ppm. The Threshold Limit Value of vinylidene chloride is 10 ppm (ACGIH, 1978). In order to simulate exposure by ingestion, rats were given the test material in the drinking water. The concentration of vinylidene chloride given in the drinking water, 200 ppm, was the upper limit of solubility of the compound in water.

METHODS

Test material. Production grade vinylidene chloride[4] was used. Each sample of the test material had a minimum purity of 99.5%. That used for the inhalation studies contained 200–400 ppm monomethylether of hydroquinone (MEHQ) to inhibit polymerization. Since the vinylidene chloride used in various copolymers for food packaging applications is distilled to remove the inhibitor MEHQ, the test chemical added to the drinking water was distilled to obtain a residual concentration of 1–5 ppm MEHQ.

Animals. Adult Sprague–Dawley rats,[5] weighing approximately 250 g, and New Zealand white rabbits,[6] weighing 3.4–4.7 kg, were used. The day on which sperm were found in the vaginal smear of rats was considered Day 0 of pregnancy; in rabbits, the day of natural mating was designated Day 0 of pregnancy. Animals were housed individually in wire-bottom cages and fed commercial laboratory animal chow[7] and tap water *ad libitum*. Animals did not have access to food or water while in the exposure chamber. The food and water consumption of rats was measured at intervals of 3 days or less during the experimental period.

Experimental design. In the initial inhalation experiment, 30 bred rats and 18 bred rabbits were exposed to 160 ppm vinylidene chloride for 7 hr/day from Days 6 to 15 and 6 to 18 of gestation, respectively. Subsequently, an additional group of 30 bred rats and 22 bred rabbits was exposed to 80 ppm. Because some embryotoxicity was observed in rats exposed to 80 ppm, an additional group of 44 bred rats was exposed to 20 ppm. Groups of 20–47 bred rats and 16 bred rabbits were exposed in chambers to filtered room air to serve as negative controls; there was a concurrent control group for each level of exposure.

Pregnant animals were exposed in stainless-steel and glass Rochester-type chambers of 4.3-m^3 volume under dynamic airflow conditions. The chamber atmosphere was generated by metering vinylidene chloride at a known rate into a heated vaporization flask and then into the airstream being drawn into the exposure chamber. The concentration of vinylidene chloride in the chamber was analyzed continuously during exposure using a Miran I infrared spectrophotometer at a wavelength of 9.2 μm. The time-weighted mean analytical concentrations of vinylidene chloride in the chamber for the 20, 80, and 160 ppm exposures were 20 ± 1, 80 ± 5, and 158 ± 6 ppm (mean $\pm$ SD), respectively.

For the ingestion study, 26 bred rats were given tap water containing 200 ppm vinylidene chloride from Days 6 to 15 of gestation. A group of 24 bred control rats was given tap water without vinylidene chloride. Water bottles were emptied and refilled daily from freshly prepared stock solutions. In an earlier study, vinylidene chloride was determined to be relatively stable in water for 24 hr.

Maternal and fetal observations. Animals were observed daily throughout the experimental period for signs of toxicity. The maternal body weights of rabbits were recorded on Days 6, 12, and 19 of gestation. The body weights of rats were recorded on Days 6, 10, and 16 of gestation. In addition, maternal body weight and the weight of the maternal liver were recorded at the time of Cesarean section (Day 21 in rats and Day 29 in rabbits).

The dams were killed with carbon dioxide, and the number and position of live, dead, and resorbed fetuses were noted. The uteri of nonpregnant animals were stained with a 10% solution of sodium sulfide and examined for evidence of early resorption sites (Kopf *et al.*, 1964); this procedure was conducted for the sole purpose of determining which dams conceived, not the incidence of resorptions. After being weighed, measured (crown–rump length), and the sexes noted, all fetuses were examined for external alterations and cleft palate. One-third of the fetuses from each litter, selected at random, was examined immediately for evidence of soft-tissue alterations by dissection under a stereomicroscope (Staples, 1974). The head of each rat fetus which was examined for soft-tissue alterations was placed in Bouin's solution and subsequently examined by the razor section technique of Wilson (1965). All fetuses were cleared with KOH, stained with alizarin red-S (Dawson, 1926), and examined for skeletal alterations.

[4] The Dow Chemical Company, Midland, Mich.

[5] Rats were obtained from Spartan Research Animals, Inc., Haslett, Mich.

[6] Rabbits were obtained from Langshaw's Rabbitry, Augusta, Mich.

[7] Ralston Purina Co., St. Louis, Mo.

Statistical evaluation. The incidence of pregnancy was analyzed by the Fisher exact probability test (Siegel, 1956). The Wilcoxon test as modified by Haseman and Hoel (1974) was used to evaluate the incidence of fetal alterations. Fetal body weights and body measurements, maternal body and liver weights, and food and water consumption data were analyzed by a one-way analysis of variance (Steel and Torrie, 1960). The level of significance reported was $p < 0.05$.

RESULTS

Rats

Maternal toxicity. No test dams died (Table 1). The incidence of pregnancy was not significantly altered by exposure to vinylidene chloride by either route of administration. Maternal weight gain on Days 6 through 9 of gestation was decreased significantly at 80 and 160 ppm (Table 1). Compared to the control dams, an increase in maternal weight gain was noted during the postexposure period among the dams exposed to 160 ppm. No significant effect on maternal body weight gain was discerned among the dams inhaling 20 ppm of vinylidene chloride or among those given the compound in the drinking water.

The amount of food consumed by the pregnant rats exposed to vinylidene chloride by inhalation was significantly decreased at 80 and 160 ppm but not at 20 ppm. Statistically significant increases in the amount of water consumed by the pregnant rats inhaling vinylidene chloride were observed at all concentrations. Inclusion of vinylidene chloride in the drinking water did not significantly affect the amount of food or liquid consumed by the dams. Dams given vinylidene chloride in their drinking water received an approximate daily dose of 40 mg vinylidene chloride/kg body wt/day.

Inhalation exposure of the dams to 160 ppm vinylidene chloride resulted in a significant increase in maternal liver weight relative to body weight, but the absolute liver weight was unaffected (Table 1). The maternal liver weight was not altered by exposure to the lower levels of inhaled vinylidene chloride or by ingestion of drinking water containing vinylidene chloride.

Embryo- and fetotoxicity. A statistically significant increase in the average number of corpora lutea per dam was discerned among the rats receiving vinylidene chloride in the drinking water but not among those exposed by inhalation (Table 2); since the number of corpora lutea is established before Day 6 of gestation, this increase was not a compound-related effect. No significant effect on the average number of implantations, live fetuses, or resorptions was discerned among litters of rats given vinylidene chloride by either route of administration. Exposure to vinylidene chloride by either route did not alter the fetal sex ratio or body weight. The mean fetal crown–rump length among the litters of rats receiving drinking water containing vinylidene chloride was significantly greater than that of the controls; this parameter was unaffected among the litters of rats exposed to the compound by inhalation.

The incidence of major malformations, considered individually or collectively, among the litters of rats given vinylidene chloride by inhalation or ingestion was not significantly different from that of the concurrent control litters (Table 3). Several minor skeletal alterations occurred significantly more often among the litters of rats exposed to 80 or 160 ppm vinylidene chloride by inhalation. At 160 ppm, these included delayed ossification of centra of cervical vertebrae, wavy ribs, and delayed ossification of bones of the skull. At 80 ppm, incidences of delayed ossification of skull bones and of wavy ribs were significantly increased. Since the amount of waviness varied between fetuses, wavy ribs were classified as either type I, those in which only a gentle curve in one or more ribs was observed, or type II, those in which one or more ribs had either a focal enlargement in width or a "U"-shaped bend (Figs. 1 and 2). Among the litters of rats exposed to 160 ppm of vinylidene chloride, the incidence of type II but not type I wavy ribs was significantly increased; in contrast, type I but not type II

TABLE 1

MATERNAL TOXICITY AMONG RATS EXPOSED TO VINYLIDENE CHLORIDE

	Route of exposure[a] (ppm vinylidene chloride)							
	Inhalation						Drinking water	
	0	20	0	80	0	160	0	200
No. deaths/No. females	0/47	0/44	0/21	0/30	0/18	0/30	0/27	0/26
% (No.) Pregnant	85 (40)	91 (40)	95 (20)	97 (29)[b]	94 (17)	90 (27)[b]	89 (24)	100 (26)
No. additional pregnancies detected by stain	0	1	1	1	0	0	0	0
Body weight (g)[c] of dams on gestation Day 6	255±26	246±27	244±15	242±18	278±21	268±21	290±13	284±30
Body weight gain (g)[c] of dams on gestation Days								
6–9	15±12	12±12	20±8	11±4[d]	14±13	2±10[d]	19±7	16±18
10–15	37±17	44±14	43±12	41±18	36±7	44±14	39±6	40±14
16–20	80±18	80±12	75±15	72±16	64±10	80±11	72±16	72±20
Liver weight on gestation Day 21								
g Liver[c]	15.2±1.9	15.1±1.6	15.9±1.0	15.7±1.7	15.9±1.4	16.7±1.9	15.9±1.7	15.2±1.4
g Liver/kg body wt[c]	39.0±3.8	19.5±2.9	41.7±2.5	43.1±3.7	39.8±2.2	42.5±3.9[d]	37.0±3.9	37.0±3.4

[a] Rats were exposed to 20, 80, or 160 ppm vinylidene chloride for 7 hr/day or were given drinking water containing 200 ppm of the compound from Days 6 to 15 of gestation.

[b] Single dams at 80 and 160 ppm delivered a litter on Days 18 and 21 of gestation, respectively; due to the possibility of cannibalization, their litters were not examined for fetal alterations.

[c] Mean ± SD.

[d] Significantly different from the concurrent control value by a one-way analysis of variance, $p < 0.05$.

TABLE 2

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RATS EXPOSED TO VINYLIDENE CHLORIDE

	Route of exposure[a] (ppm vinylidene chloride)							
	Inhalation						Drinking water	
	0	20	0	80	0	160	0	200
No. of litters	40	40	20	28	17	26	24	26
Corpora lutea/dam[b]	13±2	14±3	12±2	12±2	14±2	13±2	12±2	14±3[c]
Implantation sites/dam[b]	12±3	12±2	12±2	11±3	13±2	12±2	11±3	12±4
Pregnancy wastage[b,d]	1±2	2±2	1±2	1±2	1±1	1±2	2±2	3±3
Live fetuses/litter[b]	12±3	12±2	12±2	10±4	12±2	11±2	10±3	11±4
Resorptions/litter[b,e]	0.2±0.5	0.4±0.7	0.4±0.9	0.8±2.4	0.4±0.7	0.5 ±0.7	0.3±0.5	0.4±0.8
Fetal sex ratio (M : F)	50 : 50	53 : 47	43 : 57	50 : 50	48 : 52	51 : 49	49 : 51	50 : 50
Fetal body weight (g)[f]	5.60±0.40	5.61±0.28	5.54±0.26	5.72±0.45	5.52±0.26	5.47±0.82	5.79±0.44	5.70±0.30
Fetal crown–rump length (mm)[f]	43.9±1.1	44.0±0.9	44.4±1.0	45.1±1.3	43.8±0.8	43.6±2.6	46.6±2.4	47.8±1.3[c]

[a] Rats were exposed to 20, 80, or 160 ppm vinylidene chloride for 7 hr/day or were given drinking water containing 200 ppm of the compound from Days 6 to 15 of gestation.

[b] Mean ± SD.

[c] Significantly different from the concurrent control value by a one-way analysis of variance, $p < 0.05$.

[d] No. of corpora lutea minus No. of implantations.

[e] Resorptions detected only by sodium sulfide staining were not included in these calculations.

[f] Mean of the litter means ± SD.

TABLE 3

INCIDENCE OF FETAL ALTERATIONS AMONG RATS EXPOSED TO VINYLIDENE CHLORIDE BY INHALATION OR BY INGESTION

	Route of exposure[a] (ppm vinylidene chloride)							
	Inhalation						Drinking water	
	0	20	0	80	0	160	0	200
	No. fetuses examined/No. litters examined							
External and skeletal examination	470/40	462/40	230/20	292/27	209/17	291/26	250/24	290/25
Soft tissue examination	162/40	158/40	77/20	103/27	70/17	100/26	86/24	100/25
Bones of the skull	308/39	304/39	153/20	189/26	139/17	191/26	164/23	190/24
	No. fetuses (litters) affected							
External examination								
Multiple defects[b]	1 (1)	0	0	0	1 (1)	0	0	0
Omphalocele	0	0	0	1 (1)	0	0	0	0
Narrow head, pointed snout	0	0	0	1 (1)	0	0	0	0
Soft tissue examination								
Diaphragmatic hernia	0	0	1 (1)	0	0	0	0	0
Ectopic ovaries (only)	1 (1)	0	0	0	0	0	0	0
Skeletal examination								
Missing one thoracic and one lumbar vertebrae	0	0	0	0	2 (1)	14 (4)	0	0
Extra thoracic vertebrae and pair of ribs	0	0	0	0	1 (1)	0	0	0
Vertebrae, delayed ossification of centra of cervical vertebrae[c]	34 (17)	58 (19)	24 (14)	47 (17)	30 (13)	107 (20)[d]	46 (16)	72 (18)
Wavy ribs[c]								
Type I[e]	5 (5)	10 (7)	0	9 (8)[d]	2 (1)	9 (5)	0	3 (2)
Type II[e]	4 (4)	3 (2)	0	2 (2)	1 (1)	13 (9)[d]	1 (1)	0
Total	9 (7)	13 (7)	0	11 (9)[d]	3 (1)	22 (10)[d]	1 (1)	3 (2)
Skull, delayed ossification[c]	42 (17)	36 (18)	13 (7)	38 (13)[d]	24 (8)	59 (20)[d]	19 (10)	32 (11)
Total malformed fetuses	2 (2)	0	1 (1)	2 (2)	4 (2)	14 (4)	0	0

[a] Rats were exposed to 20, 80, or 160 ppm vinylidene chloride for 7 hr/day or were given drinking water containing 200 ppm of the compound from Days 6 to 15 of gestation.

[b] Each of these fetuses had: short trunk, hypoplastic tail, ectopic ovaries, missing all lumbar and sacral vertebrae, and missing ribs.

[c] This alteration was considered to be a skeletal variant and was not included in the calculation of the total malformed fetuses.

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

[e] Type I, gentle wave; Type II, callous or "U"-shaped bend in rib.

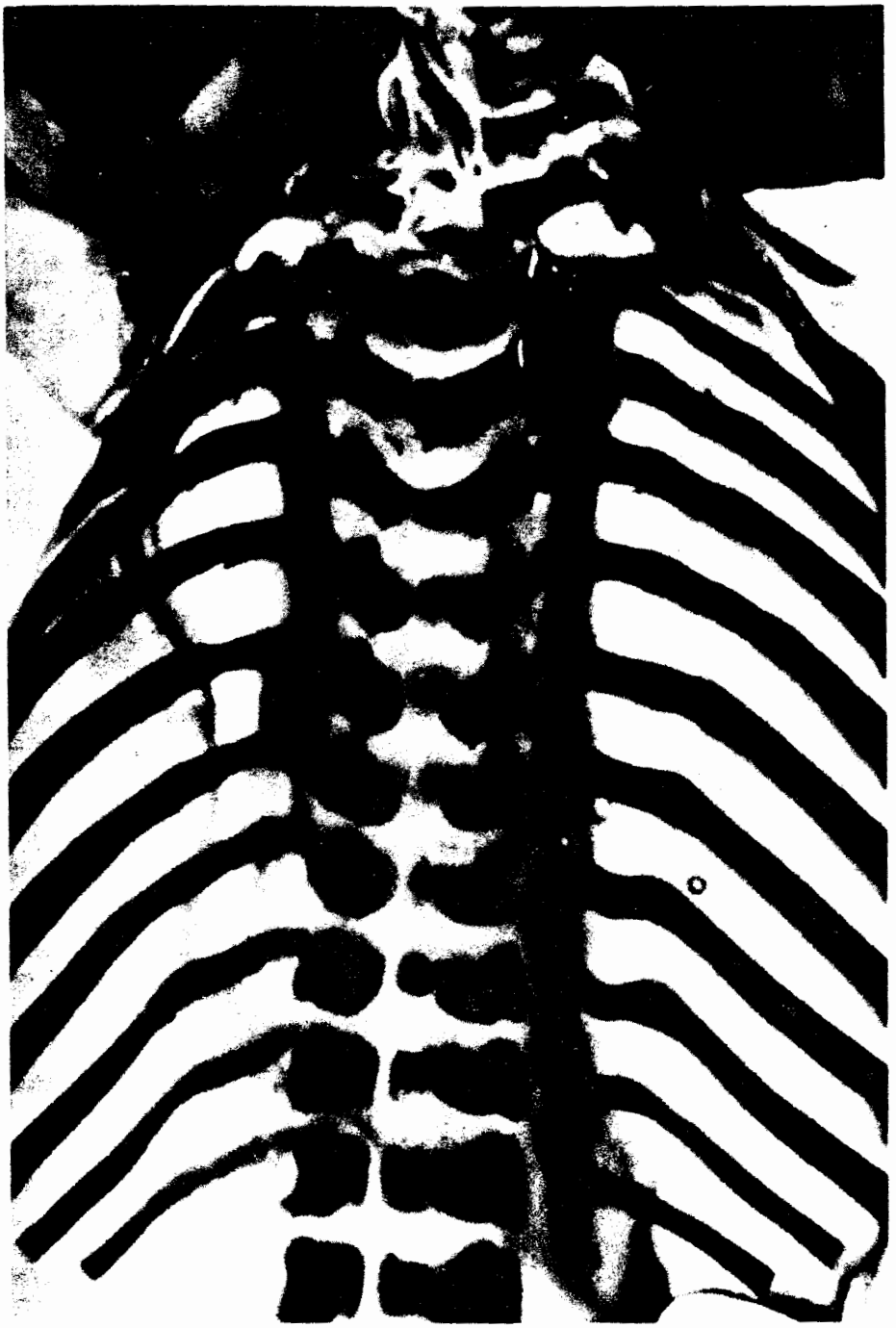

FIG. 1. Type I wavy ribs in a rat fetus from a dam exposed to 80 ppm of vinylidene chloride.

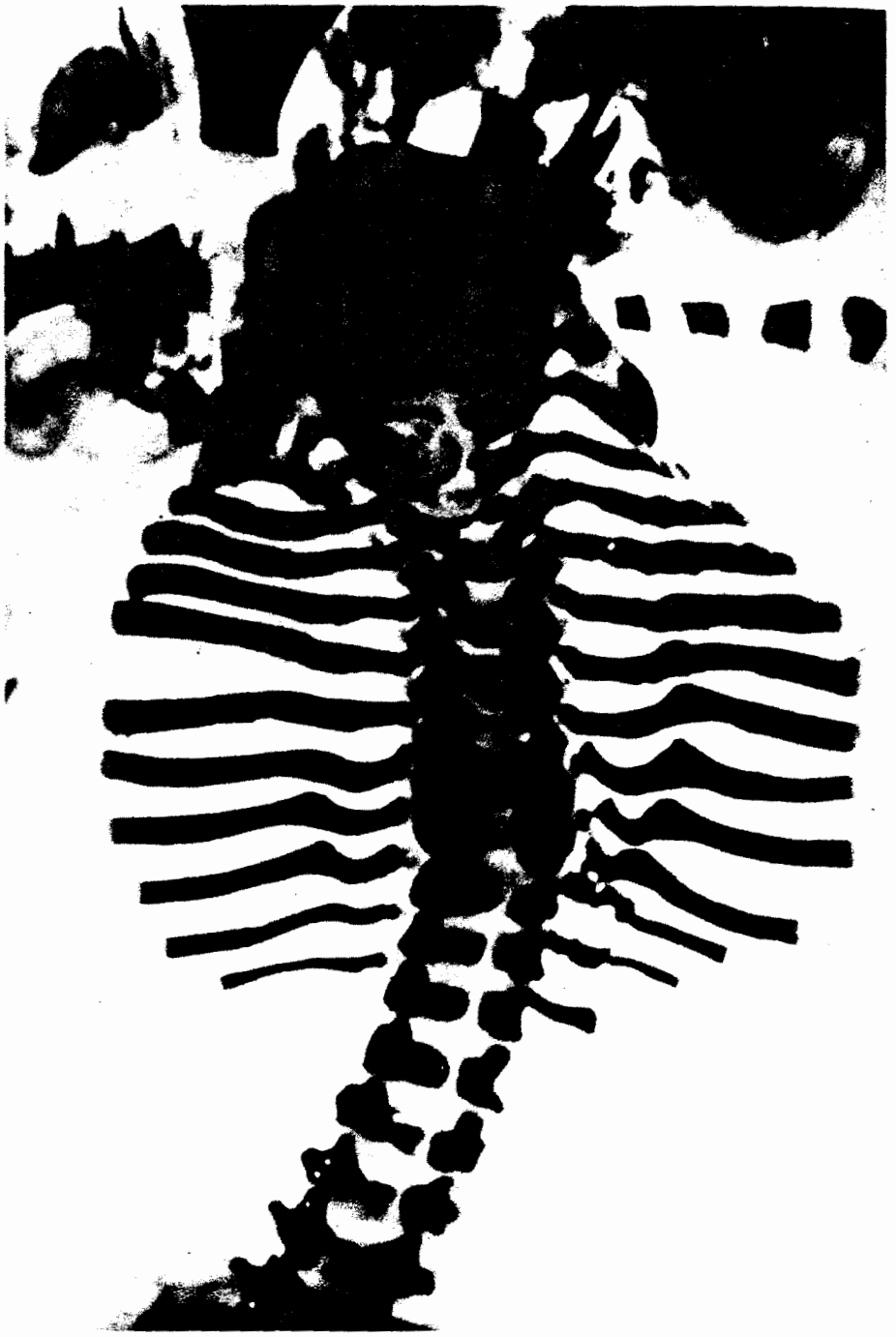

FIG. 2. Type II wavy ribs in a rat fetus from a dam exposed to 160 ppm of vinylidene chloride.

wavy ribs occurred significantly more often at 80 ppm. No skeletal alteration occurred at an increased incidence among the litters of rats inhaling 20 ppm vinylidene chloride or ingesting water containing 200 ppm vinylidene chloride.

Rabbits

Maternal toxicity. Two control dams, one exposed to 80 ppm, and two exposed to 160 ppm vinylidene chloride died (Table 4). All of these deaths were associated with pneumonia, enteritis, or perimetritis, and thus did not appear to be compound related. Fertility was not altered by exposure to either concentration of vinylidene chloride.

The body weight gain of dams exposed to 160 ppm vinylidene chloride was significantly decreased on Days 6–11 and 12–18 of gestation (during the period of exposure), and significantly increased on Days 19–28 (post-exposure) (Table 4). At the beginning of the experiment (Day 6 of gestation), dams exposed to 80 ppm weighed significantly less than the controls. The maternal weight gain of the 80 ppm-exposed dams was not significantly different from the control value during the period of exposure, but a significant increase was observed during the post-exposure period.

The liver weight of the dams exposed to 80 ppm was significantly increased on both an absolute and relative basis (Table 4). No effect on maternal liver weight was discerned among the dams exposed to 160 ppm.

Embryo- and fetotoxicity. No statistically significant effect on the mean number of corpora lutea, implantations, or live fetuses was noted among the rabbits inhaling vinylidene chloride (Table 5). The average number of resorptions among the litters of rabbits exposed to 160 ppm vinylidene chloride was significantly increased compared to controls. Four dams that inhaled

TABLE 4

MATERNAL TOXICITY AMONG RABBITS EXPOSED TO VINYLIDENE CHLORIDE

	Vinylidene chloride[a] (ppm)			
	0	80	0	160
No. deaths/No. does	1/16	1/22	1/16	2/18
% (No.) Pregnant	100 (16)[b]	91 (20)[b]	88 (14)	94 (17)
No. additional pregnancies detected by stain	0	1	0	0
Body weight (kg)[c] of dams on gestation Day 6	4.33 ± 0.32	4.04 ± 0.34[d]	4.03 ± 0.27	4.07 ± 0.38
Body weight gain (kg)[c] of dams on gestation Days				
6–11	−0.05 ± 0.06	−0.03 ± 0.11	−0.02 ± 0.09	−0.17 ± 0.14[d]
12–18	0.06 ± 0.07	−0.02 ± 0.22	0.12 ± 0.08	−0.04 ± 0.14[d]
19–28	0.07 ± 0.10	0.23 ± 0.15[d]	0.07 ± 0.22	0.21 ± 0.13[d]
Liver weight on gestation Day 29				
g Liver[c]	99 ± 14	107 ± 12[d]	104 ± 16	109 ± 19
g Liver/kg body wt[c]	22.3 ± 2.9	25.3 ± 3.0[d]	24.8 ± 4.0	26.5 ± 3.6

[a] Rabbits were exposed to 80 or 160 ppm vinylidene chloride for 7 hr/day from Days 6 to 18 of gestation.
[b] One 80 ppm-exposed dam and one concurrent control dam delivered litters on Day 28 of gestation; their litters were not examined for fetal alterations.
[c] Mean ± SD.
[d] Significantly different from the concurrent control value by a one-way analysis of variance, $p < 0.05$.

TABLE 5

OBSERVATIONS MADE AT THE TIME OF CESAREAN SECTION OF RABBITS EXPOSED TO VINYLIDENE CHLORIDE

	Vinylidene chloride[a] (ppm)			
	0	80	0	160
No. of litters	14	18	13	15
Corpora lutea/dam[b]	9±2	9±2	9±2	8±3
Implantation sites/dam[b]	9±2	9±1	9±3	9±2
Pregnancy wastage[b,c]	0.1±0.4	0.3±0.8	1.0±1.8	0.6±0.8
Live fetuses/litter[b]	8±2	9±1	8±3	6±4
Resorptions/litter[b,d]	0.8±1.2	0.7±0.9	0.3±0.6	2.7±3.9[e]
Fetal sex ratio (M : F)	52 : 48	56 : 44	52 : 48	53 : 47
Fetal body weight (g)[f]	36.8±3.7	36.9±5.0	37.4±7.6	36.0±4.2
Fetal crown–rump length (mm)[f]	94.6±2.8	93.4±4.1	93.4±6.2	92.9±4.7

[a] Rabbits were exposed to 80 or 160 ppm vinylidene chloride for 7 hr/day from Days 6 to 18 of gestation.
[b] Mean ± SD.
[c] No. of corpora lutea minus No. of implantations.
[d] Resorptions detected only by sodium sulfide staining of the uterus were not included in these calculations.
[e] Significantly different from the control mean by a one-way analysis of variance, $p < 0.05$.
[f] Mean of litter means ± SD.

TABLE 6

INCIDENCE OF FETAL MALFORMATIONS AMONG LITTERS OF RABBITS EXPOSED TO VINYLIDENE CHLORIDE

	Vinylidene chloride[a] (ppm)			
	0	80	0	160
	No. fetuses/No. litters examined			
External and skeletal examination	116/14	155/18	111/13	91/12
Soft tissue examination	44/14	56/18	42/13	37/12
External examination	No. fetuses (litters) affected			
No malformation observed				
Soft tissue examination				
Dilated cerebral ventricles	0	1 (1)	0	0
Thinning of ventricular wall of heart	0	0	0	1 (1)
Skeletal examination				
Missing ribs	0	0	1 (1)	0
Extra vertebrae	0	1 (1)	1 (1)	0
Hemivertebrae and fused ribs	0	1 (1)	0	1 (1)
Total malformed fetuses	0	3 (3)	2 (2)	2 (2)

[a] Rabbits were exposed to 80 or 160 ppm vinylidene chloride for 7 hr/day from Days 6 to 15 of gestation. No value differed significantly from the control value by a modified Wilcoxon test, $p < 0.05$.

TABLE 7

SUMMARY—VINYLIDENE CHLORIDE TERATOLOGY STUDIES

	Vinylidene chloride (ppm)					
	Rats				Rabbits	
	Inhalation			Drinking water	Inhalation	
	20	80	160	200	80	160
Maternal toxicity						
Altered appearance	No	No	No	No	No	No
Body weight gain	—[a]	Dec	Dec	—	—	Dec
Food consumption	—	Dec	Dec	—	ND	ND
Water consumption	Inc	Inc	Inc	—	ND	ND
Liver weight						
Absolute	—	—	—	—	Inc	—
Relative	—	—	Inc	—	Inc	—
Embryo- and fetotoxicity						
Resorptions	—	—	—	—	—	Inc
Fetal body weight	—	—	—	—	—	—
Fetal crown–rump length	—	—	—	Inc	—	—
External alterations	—	—	—	—	—	—
Soft tissue alterations	—	—	—	—	—	Dec[b]
Skeletal alteration	—	Inc	Inc	—	—	Yes[c]
Major malformations	—	—	—	—	—	—

[a] (—), Not significantly different from the concurrent control value; Dec, decreased; Inc, increased; ND, no data.

[b] Decreased incidence of minor satellite vessels off innominate artery.

[c] Decreased incidence of delayed ossification of the fifth sternebra and increased incidence of 13 pairs of ribs.

160 ppm had pregnancies in which all or most of the implantations were resorbed; they lost an average of approximately 450 g body wt during Days 6–17 of gestation. Among the litters of dams exposed to 80 ppm vinylidene chloride, the incidence of resorptions was unaffected. Exposure to either concentration of vinylidene chloride had no significant effect on fetal body weight, crown–rump length, or sex ratio.

The incidence of major malformations was not significantly affected by exposure to vinylidene chloride (Table 6). A significant increase in the incidence of 13 pairs of ribs and a decrease in the incidence of delayed ossification of the fifth sternebrae (neither considered to be terata) was observed among the litters of rabbits inhaling 160 ppm vinylidene chloride. At 80 ppm, no skeletal variant was observed at an incidence significantly different from that of the controls.

DISCUSSION

The results of these studies, summarized in Table 7, indicate that vinylidene chloride was not teratogenic in rats or rabbits inhaling the compound for 7 hr/day at concentrations sufficiently high to produce maternal toxicity. In rats, inhalation of 80 or 160 ppm vinylidene chloride produced significant maternal effects including decreased weight gain, decreased food consumption, increased water consumption, and increased liver weight (160 ppm only). At 20 ppm, the only effect noted among the dams was a minor increase in the amount of water consumed. In comparison, pregnant rabbits inhaling vinylidene chloride gained

less weight at 160 ppm but not at 80 ppm. A statistically significant increase in liver weight was noted among the rabbits exposed to 80 ppm vinylidene chloride; because a similar effect was not discerned at 160 ppm, this increase was not interpreted to be compound related.

No single major malformation occurred among the litters of vinylidene chloride-exposed rats or rabbits at an incidence significantly different from that seen in the control litters. In addition, the incidence of major malformations, when considered collectively, was not significantly altered by vinylidene chloride in either species. The few major malformations which were observed among the vinylidene chloride-exposed litters have been noted to occur among control litters in past studies.

Although a teratogenic effect was not observed, some evidence of embryotoxicity and fetotoxicity was noted in both rats and rabbits. In rats, exposure to inhaled vinylidene chloride resulted in a significantly increased incidence of several skeletal alterations at 80 and 160 ppm, concentrations which were toxic to the mother. These alterations included wavy ribs and delayed ossification of various bones. At 20 ppm, a concentration which caused little evidence of maternal toxicity, no adverse effect on embryonal or fetal development was discerned.

Rabbits exposed to 160 ppm vinylidene chloride had a marked increase in the incidence of resorptions. The occurrence of resorptions in each litter correlated well with the degree of weight loss observed in the individual dams. Thus, it is not clear whether the increased incidence of resorptions was due directly to the compound or indirectly to maternal toxicity. Exposure of rabbits to 160 ppm vinylidene chloride also resulted in a significant change in the incidence of several minor skeletal variations, including an increased incidence of 13 pairs of ribs (the normal number is 12 or 13) and a decreased incidence of delayed ossification of the fifth sternebra. At 80 ppm, there was no evidence of toxicity to the dams or their developing offspring.

The severity of the wavy ribs seen in rats in this study increased in a dose-related manner. At 160 ppm, most of the fetuses with wavy ribs exhibited a focal enlargement in width or a "U"-shaped bend in the rib, whereas at 80 ppm a gentle wave in the rib was seen more often. The increased occurrence of wavy ribs in rats is regarded to be a manifestation of embryotoxicity often occurring concurrently with maternal toxicity (personal communication, J. G. Wilson).[8] Wavy ribs have been associated with several compounds including insulin (Lichtenstein *et al.*, 1951), clomiphene, and other basic phenolic ethers (Diener and Hsu, 1967) and hexachlorophene (Kimmel *et al.*, 1972).

The consumption by rats of drinking water containing 200 ppm of vinylidene chloride resulted in fewer adverse effects (dam and offspring) than did exposure to either 80 or 160 ppm of the test material by inhalation. The average daily dose of vinylidene chloride received by the rats given the compound in drinking water was calculated to be approximately 40 mg/kg/day. Estimates of the total amount of vinylidene chloride metabolized in rats indicate that a single administration of 40 mg/kg vinylidene chloride by gavage is approximately equivalent to a single 7-hr inhalation exposure to 120 ppm of the compound (personal communication, M. J. McKenna).[9] Thus, the sensitivity of the rat to vinylidene chloride was apparently greater when vinylidene chloride was inhaled than when it was ingested.

Diurnal variation in the metabolism of vinylidene chloride may have been responsible for the apparent route-dependent difference in the sensitivity to vinylidene chloride. A major pathway for the detoxification of vinylidene chloride is conjugation of a reactive metabolite with glutathione

[8] Childrens Hospital Research Foundation, Elland and Bethesda Avenues, Cincinnati, Ohio.

[9] Toxicology Research Laboratory, The Dow Chemical Company, Midland, Mich.

(McKenna *et al.*, 1977). Levels of glutathione are known to undergo diurnal variation (Jaeger *et al.*, 1973; Watanabe *et al.*, 1976), rising markedly during the late evening and early morning hours (the period during which the rat normally eats and drinks). Therefore, it is hypothesized that the available levels of glutathione may have been sufficient to prevent toxicity in rats given water containing VDC, whereas in the inhalation study, toxicity may have resulted due to the lowered availability of glutathione during the exposure period (approximately 8:30 AM–3:30 PM).

In these studies, adult rats appeared to be slightly more sensitive than adult rabbits to the toxicity of inhaled vinylidene chloride. However, the susceptibility of the developing embryo to vinylidene chloride appeared to be comparable in the two species since embryotoxicity was observed only at levels of exposure which caused maternal toxicity.

In conclusion, vinylidene chloride was not teratogenic in rats or rabbits inhaling up to 160 ppm of the compound for 7 hr/day or in rats given water containing 200 ppm of the compound. Some evidence of embryotoxicity or fetotoxicity was observed in both rats and rabbits exposed to vinylidene chloride by inhalation; these effects were associated with maternal toxicity. At concentrations of vinylidene chloride which had little or no effect on the dam, no adverse effect on embryonal or fetal development was discerned in either species.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists) (1978). *Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1978.*

BARTSCH, H., MALAVEILLE, C., MONTESANO, R., AND TOMATIS, L. (1975). Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in *Salmonella typhimurium*. *Nature* **255**, (*London*) 641–643.

CARPENTER, C. P., SMYTH, H. F., JR., AND POZZANI, U. C. (1949). The assay of acute vapor toxicity, and the grading and interpretation of results on ninety-six chemical compounds. *J. Ind. Hyg. Toxicol.* **31**, 343–346.

DAWSON, A. B. (1926). A note on the staining of the skeleton of cleared specimens with alizarin red-S. *Stain Technol.* **1**, 123–124.

DIENER, R. M., AND HSU, B. Y. D. (1967). Effects of certain basic phenolic ethers on the rat fetus. *Toxicol. Appl. Pharmacol.* **10**, 565–576.

GAGE, J. C. (1970). Subacute inhalation toxicity of 109 industrial chemicals. *Brit. J. Ind. Med.* **27**(1), 1–18.

GREIM, H., BONSE, G., RADWAN, Z., REICHERT, D., AND HENSCHLER, D. (1975). Mutagenicity *in vitro* and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. *Biochem. Pharmacol.* **24**, 2013–2017.

HASEMAN, J. K., AND HOEL, D. G. (1974). Table of Gehan's generalized Wilcoxon test with fixed point sensoring. *J. Statist. Comput. Simul.* **3**, 117–135.

JAEGER, R. J., CONOLLY, R. B., AND MURPHY, S. D. (1973). Diurnal variation of hepatic glutathione concentration and its correlation with 1,1-dichloroethylene inhalation toxicity in rats. *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 465–471.

JENKINS, L. J., JR., TRABULUS, M. J., AND MURPHY, S. D. (1972). Biochemical effects of 1,1-dichloroethylene in rats. Comparison with carbon tetrachloride and 1,2-dichloroethylene. *Toxicol. Appl. Pharmacol.* **23**, 501–510.

KIMMEL, C. A., MOORE, W., AND STARA, J. F. (1972). Hexachlorophene teratogenicity in rats. *Lancet* **2**, 765.

KOPF, R., LORENZ, D., AND SALEWSKI, E. (1964). Der Einflub Thalidomid auf die Fertiletät von Ratten im Generationsversuch über zwei Generationen. *Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmacol.* **247**, 121–135.

LICHTENSTEIN, H., GUEST, G. M., AND WARKANY, J. (1951). Abnormalities in offspring of white rats given protamin zinc insulin during pregnancy. *Proc. Soc. Exp. Biol. Med.* **78**, 398–402.

MCCANN, J., CHOI, E., YAMASAKI, E., AND AMES, B. N. (1975). Detection of carcinogens in the salmonella microsome test: Assay of 300 chemicals. *Proc. Nat. Acad. Sci. USA* **72**, 5135–5139.

MCKENNA, M. J., WATANABE, P. G., AND GEHRING, P. J. (1977). The pharmacokinetics of vinylidene chloride in the rat. *Environ. Health Perspectives* **21**, 99–105.

PRENDERGAST, J. A., JONES, R. A., JENKINS, L. J., JR., AND SIEGEL, J. (1967). Effects on experimental animals of long-term inhalation of trichloroethylene carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluromethane, and 1,1-dichloroethylene. *Toxicol. Appl. Pharmacol.* **10**, 270–289.

RYLOVA, M. L. (1953). Toxicity of 1,1-ethylene dichloride. *Farmakol. Toksikol.* (*Moscow*) **16**, 47–50.

SIEGEL, J., JONES, R. A., COON, R. A., AND LYON, J. P. (1971). Effects on experimental animals of acute, repeated and continuous inhalation exposures to dichloroacetylene mixtures. *Toxicol. Appl. Pharmacol.* **18**, 168–174.

SIEGEL, S. (1956). *Non-Parametric Statistics for the Behavioral Sciences.* McGraw–Hill, New York.

STAPLES, R. E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology* **9**, A–37.

STEEL, R. G. D., and TORRIE, H. H. (1960). *Principles and Procedures of Statistics.* McGraw–Hill, New York.

WATANABE, P. G., HEFNER, R. E., JR., AND GEHRING, P. J. (1976). Vinyl chloride-induced depression of hepatic nonprotein sulfhydryl content and effects on bromosulphalein (BSP) clearance in rats. *Toxicology* **6**, 1–8.

WILSON, J. G. (1965). *Teratology: Principles and Techniques*, pp. 262–277. Univ. of Chicago Press, Chicago.