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A Three-Generation Rat Reproductive Toxicity Study of Vinylidene Chloride in the Drinking Water[1,2]

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ABSTRACT

A Three-Generation Rat Reproductive Toxicity Study of Vinylidene Chloride in the Drinking Water. Nitschke, K.D., Smith, F.A., Quast, J.F., Norris, J.M. and Schwetz, B.A. (1983). *Fundam. Appl. Toxicol.* 3:75-79. A reproduction study was conducted with Sprague-Dawley rats to evaluate the effects of parental exposure through three generations to drinking water containing 0, 50, 100 or 200 ppm vinylidene chloride (VDC) on reproduction and the development of the resultant offspring. A low fertility rate was observed in three of the four groups including controls, for the f_0 generation rats. Remating the f_0 adults to produce the f_{1b} litters resulted in a lower fertility of dams ingesting 200 ppm VDC than of dams ingesting 0, 50 or 100 ppm. However, the fertility index of the f_0 dams ingesting 200 ppm VDC in the drinking water was higher for the f_{1b} litters than for the f_{1a} litters. No overall decrease in fertility was observed in subsequent matings to produce the f_2, f_{3a}, f_{3b} or f_{3c} litters. Although neonatal survival was decreased from concurrent control values in the f_2 and f_{3a} litters of dams ingesting VDC in the drinking water, the survival indices of the f_2 litters were within the range of control values for this strain of rat in this laboratory. The apparent effect seen in the f_{3a} litters was not repeated in subsequent matings of the same adults to produce either the f_{3b} or the f_{3c} litters. Consequently, the decreased survival observed in the f_{3a} was interpreted as being due to chance. Histopathologic examination of tissues of rats (f_1 and f_2 adults) exposed to vinylidene chloride in the drinking water in utero, during lactation, and post weaning revealed slight hepatocellular fatty change and an accentuated hepatic lobular pattern of a reversible nature in the adult rats. In conclusion, ingestion of drinking water containing vinylidene chloride at concentrations which caused mild, dose-related changes in the liver, did not affect the reproductive capacity of rats through three generations which produced six sets of litters.

INTRODUCTION

Vinylidene chloride (1,1-dichloroethylene, VDC) is an important material in the manufacture of various resins and films. Reports on the acute toxicity of vinylidene chloride in rats indicates moderate toxicity when administered by gavage (Jenkins, 1972).

Vinylidene chloride was not teratogenic when ingested by pregnant female rats at concentrations as high as 200 ppm in the drinking water (Murray, *et al*., 1979). However, no information was found in the literature on the effects of ingested vinylidene chloride upon reproduction in rats. Therefore, the purpose of the study presented herein was to evaluate the effects of ingested vinylidene chloride on the reproductive capacity of rats receiving the compound continuously throughout three generations. Rats were given the test material in the drinking water rather than in the diet because VDC was found to be stable in water for 24 hours but was rapidly lost due to its volatility when incorporated in the diet.

METHODS

Test material

Production grade vinylidene chloride[3] was used. The test material purity was 99.5% minimum as determined by gas chromatography. Since the vinylidene chloride used in various copolymers for food packaging applications is distilled to remove the inhibitor MEHQ, the test chemical was also distilled prior to addition to the drinking water.

Animals

Male and female Sprague-Dawley rats[4] were housed separately by sex except during mating periods in suspended wire-bottom cages (two per cage). The rats were allowed laboratory chow[5] and tap water *ad libitum*. Animals were housed in a room controlled for temperature (70°F), humidity (50%) and light cycle (12 hours light and 12 hours dark).

Experimental design

Throughout the three generations, the test animals were continuously given tap water containing 0, 50, 100 or 200 ppm VDC as the only drinking fluid. The maximum concentration of vinylidene chloride given in the drinking water, 200 ppm, was the upper limit of solubility of the compound in water. The f_0 rats were randomized into groups containing 15 male and 30 female (control group) or 10 male and 20 female rats (50, 100 or 200 ppm groups) at 6-7 weeks of age.

After 100 days of ingesting VDC in the water, the f_0 rats were mated to produce the f_{1a} litters. Ten days after the last litter was weaned, the f_0 rats were remated to produce the f_{1b} litters. The rats serving as parents for the f_2 and f_3 generation were randomly selected with the aid of a random number table from

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[3]The Dow Chemical Company, Freeport, TX.

[4]Rats were obtained from Spartan Research Animals, Inc., Haslett, MI.

[5]Purina Laboratory Chow, Ralston Purina Co., St. Louis, MO.

TABLE 1
Fertility Index (FI) of Rats Ingesting Vinylidene Chloride (VDC)

	ppm VDC in the drinking water							
	0		50		100		200	
Generation	FI[a]	%	FI	%	FI	%	FI	%
f_0 adults:								
f_{1a} litters	17/30	57	19/20[b]	95	13/20	65	9/20	45
f_{1b} litters	23/30	77	15/20	75	16/20	80	11/20	55
f_1 adults:								
f_2 litters	27/30	90	14/20	70	16/20	80	20/20	100
f_2 adults:								
f_{3a} litters	29/30	97	19/20	95	16/20	80	19/20	95
f_{3b} litters	32/36	89	22/26	85	19/23	83	21/24	88
f_{3c} litters	20/36	56	16/20	80	9/23	39	17/24	71

[a]Fertility Index (FI) = number of females delivering a litter / number of females placed with a male.

[b]Significantly different from the control value by Fisher's exact probability test, $p < 0.05$.

the f_{1b} and f_2 litters, respectively. The f_{1b} and f_2 rats were mated at approximately 110 days of age to produce the f_2 and f_{3a} litters, respectively. The f_2 rats were remated to produce the f_{3b} and f_{3c} litters, ten days after the last f_{3a} and f_{3b} litters were weaned, respectively.

Preparation of test solutions

Throughout the reproduction study, the water bottles were emptied and refilled daily from freshly prepared stock solutions of vinylidene chloride. The concentration of VDC present in the water decreased approximately 10% in a 24 hour period.

Mating procedure

For the initial mating of the f_0 rats, a male was placed with two females from the same group for a 15-day mating period (three estrus cycles). After weaning the f_{1a} litters, the f_0 rats were remated to produce the f_{1b} litters. Each female was placed with a male for three 10-day mating periods. The pan beneath the cage was examined each morning for the presence of a vaginal plug. If a vaginal plug was observed, the female was placed in a separate cage. The remating and altered mating procedure was undertaken because of the low fertility rate observed in the initial mating of the f_0 rats, including the controls. In the subsequent matings of the f_1 and f_2 rats, a female was placed with a male for two 6-day mating periods (different male for each period) separated by a 6-day resting period. The mating period for the f_2 and f_3 generations was shortened to six days because we found that the f_0 rats did not mate after six days of cohabitation.

After the mating period, the females were placed in individual cages containing ground corn cob litter for nesting. A screen floor was placed within each cage at the end of the first week of lactation.

Cross fostered litters

Pups from eight dams ingesting 200 ppm VDC in the drinking water from the f_{3b} litters were randomly reduced to 8 per litter on the day of birth and raised by dams ingesting only water. Pups from eight dams ingesting water from the f_{3b} litters were randomly reduced to eight per litter on the day of birth and raised by dams ingesting 200 ppm VDC.

Observations

Since the animals from the f_0 generation were a subgroup from a larger group of animals used for concurrent 90-day and 2-year toxicity studies of vinylidene chloride in the drinking water, no body weights and no food or water consumption data were obtained from the f_0 animals in this reproduction study. Body weights and food and water consumption data were obtained from the subgroup designated for the 90-day study (Quast, 1983). For the remaining generations, body weight and water consumption of male and female rats were recorded at least weekly until mating. After mating, the females were observed daily for signs of normal or abnormal parturition. The following records were maintained: date of parturition (Day 0), number of live and dead newborn, number of live pups on days, 1, 7, 14 and 21, litter weights on days 1, 7 and 14, and individual weanling weights on day 21. On days 1, 7, 14 and 21 each pup was examined for external anomalies, signs of toxicity and sex.

Necropsy, organ weights and histopathology - weanlings

All weanlings which were not selected as future parents were necropsied. Body weights and liver and kidney weights

TABLE 2
Average Number of Days Between First Cohabitation and Parturition Among Rats Ingesting Vinylidene Chloride (VDC)

	ppm VDC in the drinking water			
Generation	0	50	100	200
f_0 adults:				
f_{1a} litters	28 ± 4[a]	27 ± 4	27 ± 3	28 ± 5
f_{1b} litters	26 ± 2	26 ± 4	33 ± 9[b]	26 ± 2
f_1 adults:				
f_2 litters	27 ± 3	26 ± 3	26 ± 2	26 ± 3
f_2 adults:				
f_{3a} litters	25 ± 2	23 ± 2	25 ± 2	26 ± 3
f_{3b} litters	25 ± 2	24 ± 2	25 ± 3	25 ± 3
f_{3c} litters	26 ± 3	25 ± 2	27 ± 4	25 ± 3

[a]Mean ± S.D.

[b]Significantly different from the control value by Dunnett's test, $p < 0.05$.

TABLE 3
Average Number of Pups per Litter at Birth Among Rats Ingesting Vinylidene Chloride (VDC)

Generation	ppm VDC in the drinking water			
	0	50	100	200
f_{1a}	10 ± 2[a]	9 ± 4	10 ± 3	11 ± 2
f_{1b}	8 ± 4	9 ± 3	9 ± 4	9 ± 3
f_2	9 ± 4	11 ± 3	12 ± 2	10 ± 3
f_{3a}	10 ± 4	11 ± 4	12 ± 3	11 ± 4
f_{3b}	12 ± 3	12 ± 4	11 ± 4	10 ± 5
f_{3c}	10 ± 3	11 ± 3	10 ± 5	9 ± 4

[a]Mean numbers of pups (live and dead) ± S.D.
No values were significantly different from control value by Dunnett's test, $p < 0.05$.

from two to seven 21-day old pups/sex/concentration level from the f_{1b}, f_2 and f_{3b} litters were recorded. Paraffin-embedded sections of the liver and kidneys from these animals were stained with hematoxylin and eosin and examined histologically.

Necropsy, organ weights and histopathology — adults

Rats found dead or moribund during the course of the study were necropsied. The f_1 and f_2 adults were also necropsied after the f_2 and f_{3c} litters, respectively, were weaned. Blood urea nitrogen (BUN) levels and serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (AP) activities were determined from 10 f_2 adult rats/sex/dose level. Weights of brain, heart, liver, kidneys, and testes were recorded from at least 10 male and 20 female f_1 and f_2 generation rats at each dose level. Paraffin-embedded sections of the liver and kidneys from at least 10 f_1 and f_2 adult rats/sex/dose level were stained with hematoxylin and eosin and examined by light microscopy. In addition, due to the advanced age of the f_2 rats, all grossly visible masses were examined histologically after similar preparation.

Statistical analysis

Statistical evaluation of the survival indices was made by the Wilcoxon Test as modified by Haseman and Hoel (1974). Fertility index, sex ratio and certain gross and histopathologic data were statistically analyzed by the Fisher Exact Probability Test (Siegel, 1956). Analysis of the neonatal and maternal body weights, number of days between observing a vaginal plug and parturition, and number of days between first cohabitation and parturition, food consumption and water consumption data was made by an analysis of variance and the means were compared with control values by Dunnett's test (Steel and Torrie, 1960). The level of significance reported in all cases was $p < 0.05$.

RESULTS

Concentration of VDC in the water

Analyses of the water solutions for VDC content were conducted 35 times during the test period. The mean (± S.D.) concentration of VDC in the drinking water, as determined by gas chromatographic analyses, was 68 ± 21 ppm, 99 ± 22 ppm and 206 ± 33 ppm for the respective nominal concentrations of 50, 100 and 200 ppm.

Clinical observations

Ingestion of water containing as high as 200 ppm VDC did not significantly alter the physical appearance of the f_0 animals. The body weight, water consumption and food consumption of the rats from the concurrent 90-day toxicity study were not significantly affected by the addition of vinylidene chloride to the drinking water (Quast, 1983). Likewise, the physical appearance, body weight and water consumption of the f_1, f_2 and f_3 rats was not affected by drinking water containing as high as 200 ppm VDC.

Fertility

The fertility of the f_0 rats was unusually low in the control rats and 2 of the 3 groups exposed to VDC for the f_{1a} litters (Table 1). When the f_0 adults were remated to produce f_{1b} litters, the fertility of dams ingesting 200 ppm VDC was lower than the fertility in dams ingesting 0, 50 or 100 ppm but higher than it was when these dams ingesting 200 ppm VDC were mated the first time to produce the f_{1a} litters. When the f_2 rats were mated to produce the f_{3c} offspring, a decreased fertility was observed in the control and 100 ppm groups; the age range for the f_2 rats at the time of mating for the f_{3c} litters was 276-298 days and the advanced age probably contributed to the decreased fertility.

No significant increase from the control values was observed in the mean number of days from copulation to delivery in any generation of rats ingesting as high as 200 ppm VDC. A significant increase in the average number of days from cohabitation to delivery was observed in the second mating of the f_0 rats ingesting 100 ppm VDC in the drinking water (Table 2); however, there was no effect on the mean number of days from cohabitation to delivery at the higher concentration of VDC in any generation.

TABLE 4
Postnatal Survival Index (PSI) Among Litters of Rats Ingesting Vinylidene Chloride (VDC)

Generation	ppm VDC in the Drinking Water							
	0		50		100		200	
	PSI[a]	%	PSI	%	PSI	%	PSI	%
f_{1a}	153/178	86	143/168	85	115/126	91	81/102	79
f_{1b}	155/190	82	122/140	87	101/140	72	65/87	75
f_2	234/252	93	121/149	81	148/192	77[b]	169/206	82[b]
f_{3a}	215/296	73	95/211	45[b]	74/192	38[b]	60/215	28[b]
f_{3b}	214/306	70	183/260	70	167/207	81	114/148	77
f_{3c}	193/205	94	145/171	85	86/91	94	117/152	77

[a]Postnatal Survival Index (PSI) is the number of liveborn pups surviving to 21 days of age/number of liveborn pups
[b]Significantly different from the control value by the Wilcoxon test as modified by Haseman and Hoel, $p < 0.05$.

TABLE 5
Postnatal Body Weight Among the Offspring of Rats Ingesting Vinylidene Chloride (VDC)

Generation	Day	ppm VDC in the Drinking Water			
		0	50	100	200
f_{1a}	1	7 ± 1[a]	7 ± 1	7 ± 1	7 ± 1
	7	15 ± 2	15 ± 2	15 ± 2	13 ± 3
	14	28 ± 4	27 ± 7	28 ± 3	25 ± 4
	21-M	42 ± 7	44 ± 8	42 ± 5	41 ± 6
	21-F	41 ± 7	40 ± 8	42 ± 6	36 ± 6
f_{1b}	1	7 ± 1	7 ± 1	7 ± 1	7 ± 1
	7	14 ± 3	14 ± 3	16 ± 2	14 ± 5
	14	29 ± 7	28 ± 5	30 ± 5	27 ± 7
	21-M	45 ± 8	45 ± 7	46 ± 9	42 ± 11
	21-F	43 ± 7	41 ± 7	43 ± 9	39 ± 8
f_2	1	8 ± 1	7 ± 1	7 ± 1	7 ± 1
	7	16 ± 3	14 ± 2[b]	14 ± 2[b]	14 ± 3
	14	28 ± 5	25 ± 4	23 ± 5[b]	26 ± 5
	21-M	42 ± 8	35 ± 6[b]	36 ± 6	38 ± 8
	21-F	40 ± 7	35 ± 5	34 ± 7	39 ± 8
f_{3a}	1	7 ± 1	7 ± 1	7 ± 1	6 ± 1
	7	14 ± 2	12 ± 3	12 ± 3	11 ± 2[b]
	14	25 ± 4	24 ± 5	22 ± 6	20 ± 4[b]
	21-M	36 ± 7	36 ± 9	35 ± 5	35 ± 4
	21-F	34 ± 6	32 ± 9	33 ± 7	30 ± 6
f_{3b}	1	7 ± 1	7 ± 1	8 ± 1	7 ± 1
	7	15 ± 4	14 ± 3	14 ± 3	13 ± 4
	14	26 ± 6	26 ± 5	25 ± 4	24 ± 6
	21-M	43 ± 10	37 ± 8	39 ± 8	38 ± 8
	21-F	42 ± 8	37 ± 8	38 ± 8	36 ± 8
f_{3c}	1	8 ± 1	7 ± 1	8 ± 2	7 ± 2
	7	16 ± 3	16 ± 3	17 ± 4	16 ± 4
	14	30 ± 5	29 ± 6	30 ± 6	30 ± 6
	21-M	49 ± 9	46 ± 10	47 ± 11	51 ± 9
	21-F	48 ± 9	45 ± 8	48 ± 14	45 ± 8

[a]Mean of litter means ± S.D. (g)
[b]Significantly different from the control value by Dunnett's test, $p < 0.05$.

Litter size and survival

The average number of pups per litter (live and dead) for any group ingesting VDC in the drinking water was not significantly different from the respective control value in any generation (Table 3). No significant decrease in the percentage of pups alive at birth was observed in any group ingesting VDC. The postnatal survival index among the groups ingesting VDC (the percentage of liveborn pups surviving until 21 days of age) was not significantly different from control values in the f_{1a} and f_{1b} litters (Table 4). The postnatal survival index for the f_2 litters ingesting 50, 100 or 200 ppm VDC in the drinking water was significantly decreased from the concurrent control group. The decreased postnatal survival in the f_2 generation (Table 4) was most likely due to the larger litter size at birth (Table 3). The postnatal survival index for the f_{3a} litters was significantly decreased in a dose related manner in dams given VDC. Subsequent breeding of the same f_2 dams to produce the f_{3b} and f_{3c} litters did not show a significant decrease in postnatal survival from the concurrent control groups. Similarly, the postnatal survival index for the two sets of cross fostered litters (0 and 200 ppm VDC in the drinking water) were comparable to each other and the f_{3b} control litters (data not shown).

Postnatal body weight

The average body weight of the f_{1a} or f_{1b} pups from dams ingesting vinylidene chloride in the water was not significantly different from that of the control pups (Table 5). Significant decreases in postnatal body weight were observed in the f_2 litters from dams ingesting 50 or 100 ppm VDC in the drinking water. However, the decreased body weight in these litters relative to controls was most likely due to the increased number of pups/litter at birth (Table 3) from the dams ingesting 50 or 100 ppm VDC. The postnatal body weights of pups in the f_2 litters from dams ingesting 200 ppm VDC in the drinking water were not significantly different from control litters. The average body weight of the f_{3a} pups from dams ingesting 200 ppm vinylidene chloride was occasionally significantly decreased from that of the control pups. No effect on postnatal body weight was detected in the f_{3a} pups from dams ingesting 50 or 100 ppm VDC or from the f_{3b} or f_{3c} litters ingesting 50, 100 or 200 ppm VDC in the water.

The body weights of the dams ingesting VDC were not significantly affected during the period of lactation.

Gross pathology, organ weights and histopathology — weanlings

A necropsy was performed on the 21-day old pups that were not randomly selected as parents for the next generation. No gross lesions due to the ingestion of VDC in the drinking water were observed in the pups. Nor was any effect observed in the absolute or relative liver or kidney weights of 21-day old pups from dams ingesting VDC. Light microscopic examination of

the liver and kidneys of the weanlings revealed no histopathologic alterations considered to be related to the ingestion of VDC in the drinking water.

Gross pathology, organ weights and histopathology — adults

Gross examination at necropsy of the f_1 and f_3 adults revealed no pathologic alterations considered to be related to the ingestion of vinylidene chloride in the drinking water. Upon gross examination, the f_2 male rats ingesting 200 ppm VDC in the drinking water had an increased incidence and severity of chronic renal disease which was accompanied by a loss of body fat (intraabdominal deposits). Chronic renal disease occurs spontaneously at a high incidence among the male rats of this strain hence its relation to VDC is uncertain. Gross examination of the liver of the f_2 female rats ingesting 100 or 200 ppm VDC in the drinking water revealed a pale appearance, small pale foci and an accentuated lobular pattern.

No organ weight changes considered to be related to the ingestion of VDC in the drinking water were observed in the f_1 adults; elevated relative liver weights of the female rats ingesting 200 ppm VDC were observed in the f_2 generation. An increase in SGPT activity (25% above control mean) was observed in female f_2 rats ingesting 200 ppm VDC in the water and may have been due to ingestion of VDC. No changes occurred in values for BUN or AP in f_2 rats ingesting as much as 200 ppm VDC in the water.

Chronic renal disease, which occurs spontaneously at a high incidence in males of this strain, was observed histologically at a higher incidence among the male f_1 rats ingesting 200 ppm VDC in the drinking water. Light microscopic examination of the liver and kidneys revealed an accentuated hepatic lobular pattern and a minimal degree of hepatocellular fatty change in the f_1 rats ingesting 100 or 200 ppm VDC in the water. Similar hepatocellular fatty changes were observed in each group of f_2 rats ingesting vinylidene chloride in the drinking water. Due to the advanced age of the f_2 adults, 384-406 days old, all grossly visible masses were also examined by light microscopy but no effect due to ingestion of water containing VDC was observed.

DISCUSSION

This study evaluated the potential of vinylidene chloride to interfere with the reproductive capacity of rats ingesting the compound in drinking water during three successive generations which produced six sets of litters. Fluctuations in reproductive performance were observed but were not relatable to ingestion of VDC in the drinking water. For example, at the two highest concentrations (200 and 100 ppm), and in the controls, fertility was slightly decreased in the f_0 generation relative to historic controls. An improvement in reproductive performance was observed in subsequent generations.

The survival indices of neonates during lactation were decreased in a non-dose response manner in the f_2 generation and in a dose related manner in the f_{3a} generation when compared to concurrent controls. Survival in the f_{3b} litters of dams ingesting 100 or 200 ppm VDC was higher than in concurrent controls. The mean and standard deviation of the neonatal survival from all control litters in this study and from historical background data is 83 ± 10% and 83 ± 8%, respectively. Except for the f_{3a} set of litters, mean values for neonatal survival for the 50, 100, and 200 ppm groups was 80, 83, 78%, respectively; these values fall within the limits of historical control data. In an effort to determine whether or not the decreased neonatal survival in the f_{3a} litters was due to causes other than chance, the f_2 adults were remated to produce the f_{3b} generation and several of the litters were cross-fostered, i.e., the litters from dams ingesting 200 ppm vinylidene chloride were raised by dams ingesting only water and vice versa. No decrease in litter size was observed in either of the cross-fostered sets of litters or in the litters raised by their own mothers. In fact, survival in the litters of dams ingesting 100 or 200 ppm VDC was higher than concurrent controls. This would indicate that there was no treatment-related effect on the neonates, the dams, or the neonates and dams combined. In addition, a third mating of the f_2 adults to produce the f_{3c} offspring did not reveal any significant effect on neonatal survival which was attributable to treatment. Consequently, the decreased survival observed in the f_{3a} generation was interpreted as being due to chance.

At necropsy and in the subsequent histopathologic examination, the adult f_1 rats ingesting 100 or 200 ppm VDC and the adult f_2 rats ingesting 50, 100 or 200 ppm VDC exhibited a significant increase in the incidence of slight hepatocellular fatty change and an accentuated hepatic lobular pattern. Similar hepatocellular fatty changes and accentuated lobular patterns were observed in rats inhaling 25 or 75 ppm VDC for 18 months (personal communication, J.F. Quast); these changes were found to be no longer discernible within 6 months after the exposure to VDC had terminated. Therefore, this alteration was considered to be readily reversible. No fatty changes were observed in the livers from f_1, f_2 and f_3 generation weanlings examined histopathologically at 21 days of age.

In conclusion, ingestion of vinylidene chloride in the drinking water by rats at concentrations which cause mild, dose-related changes in the liver did not affect the reproductive capacity through three successive generations which produced six sets of litters.

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