

Chronic Toxicity and Oncogenicity Study on Inhaled Vinylidene Chloride in Rats

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Chronic Toxicity and Oncogenicity Study on Inhaled Vinylidene Chloride in Rats. QUAST, J. F., MCKENNA, M. J., RAMPY, L. W., AND NORRIS, J. M. (1986). *Fundam. Appl. Toxicol.* 6, 105-144. Male and female Sprague-Dawley rats (Spartan substrain) were exposed to vinylidene chloride (VDC) by inhalation for 18 months to assess chronic toxicity and oncogenic potential of the subject test material. Interim sacrifices were performed at 1, 6, and 12 months. Rats were exposed to VDC concentrations of 10 and 40 ppm for 6 hr/day, 5 days/week for the first 5 weeks of the study. Based upon the absence of observable treatment-related effects among rats sacrificed after 1 month of exposure, the exposure concentrations were increased to 25 and 75 ppm VDC. Exposures were continued at these concentrations through the 18th month of the study after which the surviving animals were held until 24 months and then sacrificed. Cytogenetic evaluations were performed on a separate group of animals, four rats/sex, exposed to 0, 25, or 75 ppm VDC for 6 months. There were no exposure-related changes in the following parameters: mortality, appearance and demeanor, body weight data, clinical chemistry determinations, hematologic evaluations, urinalysis, or cytogenetic evaluation of bone marrow preparations. A target organ effect, characterized by hepatocellular fatty change in the midzonal region of the hepatic lobule which was minimal in severity, was observed in both male and female rats of both the 25- and 75-ppm exposure groups as early as the 6-month interim sacrifice. The midzonal fatty change was also observed at the 12-month sacrifice but no indication of progression of this lesion in either severity or incidence was apparent. During the last 6 months of the study, after exposures had been discontinued, this effect was no longer discernible; therefore this alteration was readily reversible. The incidences of several tumors and/or tumor types were statistically increased or decreased in VDC-exposed rats when compared to their respective control groups; none of these differences were judged to be attributable to VDC exposure. © 1986 Society of Toxicology.

Vinylidene chloride (1,1-dichloroethylene, $\text{CH}_2 = \text{CCl}_2$; VDC) is used extensively as a monomeric intermediate in the production of polymers, particularly in commercial resins and films for food packaging. Within the past decade there have been numerous studies reported on the evaluation of the chronic toxicity and/or oncogenicity of vinylidene chloride in laboratory animals exposed via oral, inhalation, and dermal routes and by subcutaneous injection. The liver is the primary target organ for toxicity of vinylidene chloride in rats, rabbits, dogs, and/or monkeys exposed via the oral or inhalation routes (Prendergast *et al.*,

1967; Gage, 1970; Rampy *et al.*, 1977; Quast *et al.*, 1983). In mice exposed via inhalation, the target organs are the liver and kidneys (Lee *et al.*, 1978; Maltoni, 1977). Laboratory investigations of the oncogenic potential of vinylidene chloride have been conducted on rats via inhalation and ingestion (Maltoni, 1977; Viola and Caputo, 1977; Lee *et al.*, 1978; NCI/NTP, 1982; Hong *et al.*, 1981; Quast *et al.*, 1983; Maltoni *et al.*, 1982), on hamsters via inhalation (Maltoni, 1977) and on mice via inhalation, ingestion, dermal application, and subcutaneous injection (Maltoni, 1977; Lee *et al.*, 1978; Van Duuren *et al.*, 1979; NCI/NTP, 1982; Hong *et al.*, 1981). Preliminary findings reported by Lee *et al.* (1978) suggesting an on-

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cogenic response in the rat were not confirmed in subsequent reporting (Hong *et al.*, 1981) or in studies conducted by the other investigators. Evidence of an oncogenic response to vinylidene chloride is limited to a preliminary report of kidney tumors of male Swiss mice exposed to 55 ppm, but not 10 ppm VDC in an inhalation study (Maltoni, 1977). The oncogenic response was associated with significant injury to the kidney tissue. Studies on the effect of vinylidene chloride on DNA synthesis and DNA repair in the mouse kidney suggest that the kidney tumors arose primarily as a result of the recurrent tissue injury (Reitz *et al.*, 1980).

The objectives of the present study were twofold. One objective was to assess the chronic toxicity of vinylidene chloride in the rat over an 18-month period and the reversibility of the target organ toxicity over a 6-month postexposure period. The other objective was to investigate the oncogenic potential of the chemical. This study represents the longest inhalation exposure duration used in a laboratory investigation on toxicity and oncogenicity of vinylidene chloride to date. Interim results of this study have been previously published (Rampy *et al.*, 1977).

METHODS

Experimental design. Male and female Sprague-Dawley rats (Spartan substrain),² 6 to 7 weeks of age, were assigned to treatment groups using a table of random numbers. The animals were housed, according to sex, two or three per cage. Food³ and tap water were available *ad libitum* other than during exposure periods. During nonexposure periods, the animals exposed to VDC vapors were maintained in a holding room which was controlled for temperature, humidity, and lighting cycle. The control animals were maintained in the holding room throughout the study.

Exposures were 6 hr/day, 5 days/week for 18 months with interim sacrifices at 1, 6, and 12 months. Animals remaining after 18 months of exposure were allowed to survive until 24 months, at which time they were sacrificed.

² Spartan Research Animals, Haslett, Mich.

³ Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

TABLE 1
NUMBERS OF RATS/SEX/EXPOSURE GROUP

Exposure group (ppm)	0	25	75
Chronic study	86	85	86
1-Month interim sacrifice ^a	4	4	4
6-Month interim sacrifice	5	5	5
12-Month interim sacrifice	5	5	5
6-Month cytogenetic evaluation	4	4	4
Total number rats on study	104	103	104

^a Exposure levels during first month of exposure were 0, 10, or 40 ppm.

Initially the exposure concentrations of VDC were 0, 10, and 40 ppm. A group of animals were sacrificed at 1 month for evaluation of possible toxic effects. Since no treatment-related effects were noted, the concentrations were changed to 0, 25, and 75 ppm on Day 43 of exposure. These concentrations were maintained throughout the remainder of the 18-month exposure portion of this study. A separate group of rats was exposed to 0, 25, or 75 ppm VDC for 6 months for cytogenetic evaluation of femoral bone marrow cells.

The numbers of animals used in the various portions of the study are given in Table 1.

Test material. Production-grade vinylidene chloride used in the study was supplied by The Dow Chemical Company. The material was 99% pure as determined by gas chromatography and was stabilized with hydroquinone monomethylether.

Exposure chambers, vapor generation, and analyses. Exposures were conducted in 3.7-m² stainless-steel chambers under dynamic airflow conditions. Vinylidene chloride vapor atmospheres were generated by metering the liquid test material into a glass vaporization flask at a controlled rate. The temperature of the flask was maintained at approximately 30°C. The concentrated VDC vapor was swept by tempered, filtered air into the exposure chamber at an airflow rate calculated to produce the desired concentration.

Analysis of the chamber atmosphere was normally performed 2-5 times during the 6-hr daily exposure period. Analyses were conducted by vapor phase infrared spectrometry. A Perkin-Elmer Model 12A infrared spectrophotometer was employed, a path length of 10 m and a wavelength of 12.6 μ m were used for the analysis. Chamber concentration was determined by interpolating from a standard curve using peak height measurements. Standards were prepared by injecting a known volume of vinylidene chloride into a bag made of Saran⁴ resin which was filled with a measured amount of filtered, compressed air.

⁴ Trademark of the Dow Chemical Co.

Clinical observations. Animals were observed daily during the exposure period for changes in appearance or demeanor and signs of eye or nasal irritation. Individual body weights were recorded weekly for the first month of exposure, biweekly during the second month of exposure, and a minimum of monthly thereafter. Body weights of animals fasted overnight were measured at the time of scheduled sacrifices. Records of mortality were maintained. Blood was collected for routine hematology from the tail vein of five animals/sex/exposure group shortly before the 6- and 12-month interim sacrifices and 10 animals/sex/exposure group at the terminal sacrifice. Hematology parameters included red blood cell count (RBC), hemoglobin concentration (Hgb), packed cell volume (PCV), and total and differential white cell count (WBC). Clinical chemistry determinations on serum of blood collected from the cervical vessel during the necropsy procedure at the interim sacrifices and on 5 animals/sex/exposure group at the terminal sacrifice included serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), and alkaline phosphatase (AP). Blood glucose was also determined at the 12-month interim sacrifice, repeated for confirmation

TABLE 2

SELECTED CUMULATIVE PERCENTAGE OF MORTALITY
DATA ON FEMALE RATS

Months on study	Exposure level (ppm)		
	0	25	75
No. of rats ^a :	84	86	84
6	1 (1) ^b	1 (1)	1 (1)
8	1 (1)	1 (1)	1 (1)
9	1 (1)	3 (4)	1 (1)
11	2 (2)	6 (7)	1 (1)
12	2 (2)	6 (7)	5 (6)
13	4 (5)	9 (11)	6 (7)
14	4 (5)	10 (12)	7 (8)
15	5 (6)	18 (21)*	13 (16)*
16	8 (10)	19 (22)*	16 (19)
17	9 (11)	19 (22)*	18 (21)*
18	16 (19)	29 (34)*	20 (24)
19	23 (27)	37 (43)*	29 (35)
20	30 (36)	49 (57)*	41 (49)
21	39 (46)	55 (64)*	51 (61)*
22	46 (55)	59 (69)*	54 (64)
23	56 (67)	65 (76)	61 (73)
24	64 (76)	72 (84)	68 (81)

* Excludes rats used for interim sacrifices and cytogenetic study.

^b Number dead (% dead).

* Statistically different from controls by Fischer's exact probability test, $\alpha = 0.05$.

TABLE 3

MEAN BODY WEIGHTS OF MALE AND FEMALE
RATS AT SELECTED TIMES

Months on study	Exposure level (ppm)		
	0	25	75
Males			
0	276	276	283*
1	399	386*	396
3	489	469*	488
6	562	529*	549
9	598	555*	565*
12	639	593*	586*
15	638	623	646
18	627	586*	618
21	607	582	563*
24	587	550	530
Females			
0	210	212	203*
1	259	259	264
3	294	301*	309*
6	333	343*	342*
9	340	348	348
12	368	377	363
15	386	391	402
18	396	419	426
21	443	412	449
24	427	463	439

* Statistically different from control mean by analysis of variance and Dunnett's test, $\alpha = 0.05$.

of a suggested treatment-related response at 18 months on blood obtained by orbital sinus puncture on 5 animals/sex/exposure group, and determined on all remaining animals at the terminal sacrifice.

Urinalyses. Urine samples were obtained from 10 animals/sex/exposure group shortly before the 6- and 12-month interim sacrifices and 10 animals/sex/exposure group at the terminal sacrifice. Urinalyses included determinations of specific gravity, pH, glucose, protein, ketones, occult blood, bilirubin and, at the terminal sacrifice only, urobilinogen. Specific gravity measurements were made using a T.S. Meter.⁵ All other measurements were made using Bililabstix.⁶

Cytogenetic analyses. Animals, four/sex/exposure group, scheduled for cytogenetic analysis were added to the study following the 1-month interim sacrifice. These rats, caged separately, were exposed to 0, 25, or 75 ppm

⁵ American Optical Co., Buffalo, N.Y.

⁶ Ames Co., Elkhart, Ind.

VDC for 6 months. At the end of the exposure period and 4 hr prior to sacrifice, the animals were injected intraperitoneally with 4 mg/kg colchicine. Femoral bone marrow cells obtained from each rat were processed using Hank's balanced salt solution, a 0.5% KCl solution, and a 10% acetic acid in methanol fixative (Legator *et al.*, 1969). The cells were scored for chromosomal aberrations according to established guidelines (Buckton and Evans, 1973).

Postmortem evaluations. A complete gross pathologic examination was performed on each rat regardless of whether the rat died during the study, was culled in moribund condition, or was submitted at the time of a scheduled sacrifice. Rats submitted alive were anesthetized with methoxyflurane and sacrificed by exsanguination. The following tissues were removed and placed in neutral buffered 10% Formalin: accessory sex glands, adipose tissue, adrenals, aorta, bone marrow (sternal), brain, epididymes,

esophagus, heart, intestines (large and small), kidneys, liver, lungs, lymph nodes (mesenteric, mediastinal), mammary gland, nasal turbinates, ovaries, oviduct, pancreas, parathyroid, peripheral nerve, pituitary gland, prostate, salivary glands, skeletal muscle, skin, spleen, spinal cord, sternum, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, and any gross lesion or mass. Eyes of rats which died or were culled from the study were preserved in Formalin. Eyes of a portion of the rats from the interim or terminal sacrifices were fixed in Zenker's fixative with the remainder in Formalin.

Statistical evaluation. Body weight data, clinical chemistry, hematology, urinalysis (specific gravity), and organ weight data were analyzed using analysis of variance and Dunnett's test (Steel and Torrie, 1960). Data for cumulative mortality, incidence of gross and microscopic pathological findings, and tumor incidence were analyzed

TABLE 4
SELECTED HEMATOLOGY PARAMETERS OF RATS AT 6-, 12-, AND 24-MONTH SACRIFICES

Month sacrifice	Exposure level (ppm)	No. of rats	PCV (%)	RBC ($\times 10^6/\text{mm}^3$)	Hgb (g/100 ml)	WBC ($\times 10^3/\text{mm}^3$)
Males						
6	0	5	53.7 (3.1)*	8.00 (0.49)	17.1 (0.8)	11.1 (1.2)
	25	5	53.2 (0.4)	7.46 (0.56)	17.4 (0.4)	10.3 (2.1)
	75	5	54.4 (0.5)	7.70 (0.66)	17.3 (0.3)	16.8 (13.1)
12	0	5	50.6 (1.7)	8.48 (0.44)	16.4 (0.6)	9.4 (1.8)
	25	5	50.6 (2.9)	8.46 (0.28)	17.1 (0.8)	7.7 (2.1)
	75	5	53.1 (0.7)	8.92 (0.32)	17.4 (0.5)	10.2 (2.6)
24	0	10	45.3 (8.1)	7.51 (1.1)	14.6 (2.1)	18.7 (7.2)
	25	10	43.4 (6.5)	7.09 (1.1)	14.4 (2.1)	17.3 (6.9)
	75	10	47.3 (5.3)	7.61 (0.67)	15.6 (1.4)	15.1 (4.8)
Females						
6	0	5	51.7 (3.4)	7.34 (0.48)	16.5 (0.5)	10.0 (2.2)
	25	5	49.7 (3.7)	6.65 (0.52)	16.2 (1.3)	8.0 (3.1)
	75	5	52.7 (3.3)	7.72 (0.90)	17.4 (0.8)	9.6 (1.4)
12	0	5	46.5 (1.5)	7.34 (0.22)	15.3 (0.4)	7.2 (1.5)
	25	5	48.3 (2.9)	7.74 (0.46)	15.8 (1.1)	4.0 (1.0)*
	75	5	46.4 (1.1)	7.84 (0.15)*	15.5 (0.3)	6.6 (1.3)
12*	0	5	46.9 (1.2)	7.32 (0.26)	15.2 (0.3)	7.5 (1.4)
	25	5	45.1 (2.8)	7.39 (0.60)	14.9 (1.1)	6.4 (1.2)
	75	5	45.8 (3.1)	7.46 (0.56)	15.0 (1.1)	9.6 (1.0)
24	0	10	39.0 (6.9)	6.04 (1.3)	12.7 (2.4)	13.2 (4.8)
	25	10	39.9 (8.5)	6.34 (1.41)	13.4 (2.9)	15.1 (6.8)
	75	10	41.3 (3.4)	6.66 (0.49)	13.8 (1.0)	14.3 (5.4)

* Mean $\pm$ (SD).

* Additional samples taken 3 days later.

* Statistically different from control mean by analysis of variance and Dunnett's test, $\alpha = 0.05$.

by the Fischer's exact test (Siegel, 1956). The level of significance chosen was $\alpha = 0.05$.

Clinical Observations

No eye or nasal irritation or changes in appearance and demeanor were observed in the animals during the study. Intercurrent murine pneumonia was observed among animals of both sexes during the last 6 months of the study.

Cumulative percentage of mortality data on male rats did not reveal increases as a result of exposure to VDC. Statistically significant non-dose-related mortality increases were ob-

RESULTS

Vapor Analyses

The mean chamber concentrations $\pm$ standard deviations during the first month were 9.5 ± 2.5 and 35.8 ± 6.2 ppm VDC. From months 2 to 18, the mean chamber concentrations $\pm$ standard deviations were 25.4 ± 4.6 and 72.6 ± 7.5 ppm VDC.

TABLE 5

CLINICAL CHEMISTRY VALUES AT 6, 12, 18, AND 24 MONTHS

Months on study	Exposure level (ppm)	No. of rats	SGPT (mU/ml)	BUN (mg/100 ml)	AP (mU/ml)	Glucose (mg/100 ml)
Males						
6	0	5	31.0 (7.0)*	17.6 (0.9)	109 (20.0)	
	25	5	27.0 (4.0)	22.2 (7.1)	124 (26.0)	
	75	5	30.0 (12.0)	16.6 (2.0)	114 (11.0)	
12	0	5	44.4 (4.4)	15.5 (3.0)	85.6 (12.7)	134.6 (9.6)
	25	5	47.4 (5.6)	18.5 (6.2)	104.6 (30.2)	124.8 (9.4)
	75	5	40.4 (5.5)	17.0 (5.6)	111.2 (26.8)	102.8 (7.0)*
18	0	5				92.0 (16.0)
	25	5				90.0 (6.0)
	75	5				96.0 (6.0)
24	0	13	34.0 (6.0)	29.0 (8.0)	94.0 (24.0)	103.0 (18.0)
	25	13	58.0 (63.0)	60.0 (67.0)	106.0 (33.0)	105.0 (26.0)
	75	8	40.0 (9.0)	32.0 (17.0)	99.0 (15.0)	93.0 (19.0)
Females						
6	0	5	28.0 (2.0)	26.3 (3.0)	108.0 (33.0)	
	25	5	29.0 (6.0)	26.2 (0.8)	119.0 (69.0)	
	75	5	22.0 (6.0)	22.2 (4.5)	80.0 (12.0)	
12	0	5	37.4 (5.8)	18.0 (5.5)	70.6 (16.6)	135.8 (8.6)
	25	5	37.4 (5.7)	14.9 (5.5)	65.6 (17.6)	137.0 (12.2)
	75	5	38.4 (9.9)	14.5 (1.7)	77.2 (17.1)	137.4 (14.6)
18	0	5				100 (9.0)
	25	5				94.0 (10.0)
	75	5				97.0 (7.0)
24	0	19	37 (12)	30 (30)	124 (95)	101 (23)
	25	11	37 (7)	22 (10)	94 (79)	89 (29)
	75	16	32 (8)	36 (54)	92 (55)	95 (20)

* Mean $\pm$ (SD).

* Significantly different from control mean by analysis of variance and Dunnett's test, $\alpha = 0.05$.

served among the female rats exposed to 25 ppm during the 15th through the 22nd months of the study. A trend toward increased cumulative percentage of mortality was noted in the female rats exposed to 75 ppm from the 14th through the 24th months of the study; the increases were statistically significant at months 15, 17, and 21 (Table 2).

The mean body weights for male rats exposed to 25 ppm vinylidene chloride were statistically lower than the control group for the first 13 months of exposure to VDC. Subsequently, mean body weights of this group remained lower than controls although they were not statistically decreased in a consistent fashion (Table 3). Male rats of the 75 ppm exposure group showed decreased mean body weights from 6th through the 12th month of the study. A general trend toward decreased mean body weight for animals of this exposure group when compared to controls was observed from the 17th through the 24th month of the study; however, statistically significant differences were only observed at the 17th- and 21st-month weighings.

The mean body weights of female rats exposed to either 25 or 75 ppm VDC were generally found to be statistically significantly greater than control means for the first 6 months of the study (Table 3). Subsequent to this period, no consistent effect on body weight could be noted which was attributed to exposure to VDC.

Hematology

The results of hematology determination performed at the scheduled sacrifices on male rats exposed to either 25 or 75 ppm vinylidene chloride revealed no effects which could be attributed to vinylidene chloride exposure (Table 4). Hematology data obtained on female rats exposed to 25 ppm VDC for 1 year indicated a decreased total white blood cell count when compared to the control mean (Table 4). Samples obtained at the same time interval for female rats exposed to 75 ppm indicated a statistically significant increase in

total red blood cell count when compared to control mean. To verify the repeatability of these observations, the same animals were sampled approximately 3 days following the scheduled 1-year hematology sampling; none of the aforementioned observations were noted. The observations were attributed to normal variation in these measurements and were not judged to be related to vinylidene chloride exposure.

Clinical Chemistry

Clinical chemistry determinations of serum glutamic pyruvic transaminase, blood urea

TABLE 6
CYTOGENETIC EVALUATION OF FEMORAL
BONE MARROW CELLS

Exposure group	No. of rats	Sex	Number of cytogenetic abnormalities/number of cells scored
Control	4	Male	0/34 0/23 0/40 0/38
Control	4	Female	0/0 0/50 0 0/40
25 ppm	4	Male	0/50 0/29 0/50
	4	Female	0/39 0/35 0/50 0/28
75 ppm	4	Male	0/50 0/50 0/50 0/50
75 ppm	4	Female	0/50 0/32 0/50 0/50

* One rat died spontaneously prior to the time of evaluation.

nitrogen, and alkaline phosphatase conducted on rats at the interim and terminal sacrifices were not statistically different than the values obtained on the control animals. Blood glucose determinations performed at the 12-month interim sacrifice were statistically significantly decreased only for the male rats exposed to 75 ppm VDC. Blood glucose determinations repeated at 18 months and at the terminal sacrifice were comparable to control values (Table 5).

Urinalysis

Urinalyses performed on randomly selected animals from the 25 or 75 ppm VDC exposure

groups at the 6- and 12-month interim sacrifices and at the terminal sacrifice did not reveal any consistent effects which were relatable to vinylidene chloride exposure.

Cytogenetic Evaluation

Cytogenetic evaluation of femoral bone marrow cells revealed no chromatid or chromosomal aberrations in either the control or the VDC-exposed groups of rats. The number of cells scored for each group of animals is given in Table 6. Although 50 good metaphase spreads were not found on the slides prepared for all the animals, sufficient representative spreads were found among some animals in each group.

TABLE 7

SELECTED ORGAN WEIGHTS IN MALE AND FEMALE RATS

Months on study	Exposure level (ppm)	No. of rats	Body weight (g)	Liver		Kidney	
				g	g/100 g	g	g/100 g
Males							
6	0	5	553 (42)*	15.22 (3.56)	2.73 (0.42)	3.77 (0.51)	0.68 (0.06)
	25	5	522 (29)	13.81 (1.86)	2.64 (0.22)	3.63 (0.36)	0.70 (0.03)
	75	5	520 (0.48)	13.23 (1.63)	2.54 (0.18)	3.57 (0.52)	0.69 (0.06)
12	0	5	660 (44)	20.57 (1.51)	3.13 (0.34)	4.39 (0.51)	0.67 (0.12)
	25	5	576 (48)*	15.28 (2.10)*	2.65 (0.23)	3.94 (0.39)	0.69 (0.06)
	75	5	563 (41)*	15.13 (2.06)*	2.69 (0.29)	4.04 (0.51)	0.72 (0.09)
24	0	13	527 (78)	18.28 (1.95)	3.50 (0.37)	5.85 (1.08)	1.14 (0.30)
	25	13	495 (85)	16.92 (2.94)	3.44 (0.41)	5.33 (0.93)	1.10 (0.24)
	75	8	492 (61)	16.96 (2.54)	3.44 (0.24)	5.41 (0.78)	1.11 (0.16)
Females							
6	0	5	327 (14)	7.25 (1.15)	2.22 (0.35)	2.03 (0.28)	0.62 (0.09)
	25	5	303 (50)	6.94 (1.27)	2.29 (0.20)	1.88 (0.31)	0.62 (0.05)
	75	5	327 (17)	7.47 (0.77)	2.28 (0.14)	2.06 (0.14)	0.63 (0.05)
12	0	5	329 (19)	7.45 (0.69)	2.26 (0.11)	2.09 (0.10)	0.63 (0.03)
	25	5	366 (26)	8.39 (1.51)	2.32 (0.55)	2.44 (0.26)*	0.67 (0.11)
	75	5	340 (38)	9.04 (0.80)	2.67 (0.21)	2.60 (0.18)*	0.77 (0.09)
24	0	21 ^b	402 (95)	13.47 (4.17)	3.37 (0.76)	2.94 (0.49)	0.77 (0.23)
	25	13 ^b	418 (109)	15.39 (3.77)	3.26 (0.51)	2.96 (0.63)	0.75 (0.24)
	75	16	404 (72)	13.99 (4.39)	3.47 (0.90)	3.10 (0.55)	0.80 (0.26)

* Mean $\pm$ (SD).

^b Four female rats from either the control or 25-ppm group were unidentified at the terminal sacrifice. For completeness of the data the organ weights of these were included, two in each of the two groups.

* Significantly different from control means by analysis of variance and Dunnett's test, $\alpha = 0.05$.

TABLE 8
NONTUMOROUS HISTOPATHOLOGIC LESIONS IN FEMALE RATS

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Liver							
Foci of eosinophilic ground-glass appearing cells	Control	0	0	0	15	10	25
	25 ppm	0	0	1	11	3	15 ^a
	75 ppm	0	0	2	4	10	16
Area of eosinophilic ground-glass appearing cells	Control	0	0	2	2	3	7
	25 ppm	0	0	0	4	1	5
	75 ppm	0	0	1	1	0	2
Foci of clear cell hepatocytes	Control	0	0	0	0	2	2
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Foci of basophilic hepatocytes	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Foci of mononuclear cells within the portal area or lobule	Control	0	0	6	14	7	27
	25 ppm	0	1	9	6	3	19
	75 ppm	1	0	4	4	4	13 ^a
Centrilobular degeneration	Control	1	0	1	10	0	12
	25 ppm	0	1	3	10	1	15
	75 ppm	0	0	3	13	0	16
Atrophy without fatty change	Control	0	0	1	22	2	25
	25 ppm	1	1	5	14	5	26
	75 ppm	0	0	1	19	8	28
Focal necrosis	Control	0	0	1	5	1	7
	25 ppm	0	1	2	3	0	6
	75 ppm	0	0	1	3	1	5

SL 066202

Focal vascular dilatation with hepatocellular alteration	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Multiloculated biliary cyst	Control	0	0	0	1	1	2
	25 ppm	0	0	0	3	2	5
	75 ppm	0	0	1	0	2	3
Fatty change—portal to midzonal regions most predominant	Control	1	0	0	1	0	2
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Fatty change—occasional individual hepatocytes	Control	0	0	1	2	2	5
	25 ppm	0	0	0	0	2	2
	75 ppm	0	1	1	0	0	2
Fatty change—moderate to severe, involves several lobes to varying degrees	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	1	0	0	1
Fatty change—some within hepatocytes of lobule—minimal	Control	0	0	0	0	2	2
	25 ppm	0	0	2	1	0	3
	75 ppm	0	0	1	0	1	2
Fatty change—focal area(s) or foci	Control	0	0	0	1	2	3
	25 ppm	0	0	1	0	1	2
	75 ppm	0	0	1	2	0	3
Fatty change—diffuse multifocal areas	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Fatty change—midzonal in location	Control	0	0	0	0	1	1
	25 ppm	0	0	6	0	0	6
	75 ppm	0	2	5	1	0	8*
Total number of animals with fatty change	Control	1	0	1	6	7	15
	25 ppm	0	0	9	1	3	13
	75 ppm	0	3	9	3	1	16

TABLE 8—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Focal biliary hyperplasia	Control	0	0	1	0	1	2
	25 ppm	0	0	2	1	0	3
	75 ppm	0	0	1	1	0	2
Extramedullary hematopoiesis	Control	0	0	6	10	9	25
	25 ppm	0	2	4	14	2	22
	75 ppm	1	2	4	17	4	28
Periportal hypertrophy	Control	0	0	6	15	7	28
	25 ppm	0	2	8	8	2	20
	75 ppm	0	2	6	9	1	18
Periportal fibrosis (biliary) and/or sclerosis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Biliary hyperplasia with or without fibrosis	Control	0	0	0	4	2	6
	25 ppm	0	0	0	3	0	3
	75 ppm	0	0	0	1	0	1
Sinusoidal vascular dilatation	Control	0	0	0	6	5	11
	25 ppm	0	0	0	3	0	3*
	75 ppm	0	0	1	2	5	8
Capsular sclerosis secondary to diaphragmatic hernia	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Hematocyst formation beneath the capsule	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1

Centrilobular atrophy	Control	1	0	10	16	13	40
	25 ppm	0	1	3	13	2	19*
	75 ppm	0	0	8	12	4	24*
Centrilobular necrosis	Control	0	0	1	8	0	9
	25 ppm	0	0	3	9	1	13
	75 ppm	0	0	0	10	0	10
Aggregates of hematogenous pigment containing material in cells	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Portal area cells with more eosinophilic staining affinity with or without swelling	Control	0	0	0	0	0	0
	25 ppm	0	2	1	1	0	4
	75 ppm	0	4	0	0	0	4
Respiratory system							
Trachea							
Focal subepithelial lymphoid infiltrate	Control	0	0	4	2	0	6
	25 ppm	0	0	0	1	0	1
	75 ppm	0	1	1	0	0	2
Chronic or acute tracheitis	Control	0	0	0	8	3	11
	25 ppm	0	0	2	15	4	21*
	75 ppm	0	0	1	22	3	26*
Exudative material in lumen	Control	1	0	0	0	0	1
	25 ppm	0	1	0	0	0	1
	75 ppm	0	0	0	0	0	0
Lungs							
Focal chronic interstitial pneumonitis	Control	0	0	1	3	0	4
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	3	0	3
Focal peribronchiolar mononuclear cell infiltrate	Control	0	0	11	42	15	68
	25 ppm	1	4	20	28	7	60
	75 ppm	1	4	14	29	12	60

TABLE 8—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Focal areas of alveolar histiocytosis with or without exudation of alveolar macrophages	Control	0	0	9	30	15	54
	25 ppm	0	0	13	21	5	39*
	75 ppm	0	1	6	21	9	37*
Acute suppurative pneumonia	Control	1	0	0	0	0	1
	25 ppm	0	1	0	0	0	1
	75 ppm	0	0	0	0	0	0
Alveolar mineralization	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Focal granulomatous inflammatory reaction in lung	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Cholesterol cleft formation in lung	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Pulmonary changes consistent with cardiac or renal failure; edema; interstitial thickening and fibrosis, inflammatory cell infiltrate and exudation of cells into alveoli, with or without atelectasis	Control	0	0	0	5	2	7
	25 ppm	0	0	0	3	0	3
	75 ppm	0	0	0	3	2	5
Focal areas of pleuritis	Control	1	0	0	0	0	1
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0

Chronic murine pneumonia	Control	0	0	1	6	2	9
	25 ppm	0	0	1	16	4	21*
	75 ppm	0	0	1	19	2	22*
Interstitial fibrosis	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Macrophages in lumen of alveoli containing hematogenous pigment	Control	0	0	0	0	1	1
	25 ppm	0	0	1	1	0	2
	75 ppm	0	0	0	4	2	6
Acute bronchitis	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Urinary system							
Kidney							
Minimal degree of chronic nephropathy	Control	1	0	9	36	8	54
	25 ppm	0	3	15	33	7	58
	75 ppm	1	4	8	32	5	50
Moderate degree of chronic nephropathy	Control	0	0	4	7	7	18
	25 ppm	0	0	2	9	2	13
	75 ppm	0	0	4	14	7	25
Severe degree of chronic nephropathy	Control	0	0	0	3	4	7
	25 ppm	0	0	0	1	2	3
	75 ppm	0	0	0	1	4	5
End-stage kidney	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	1	0	1
Focal mononuclear cell infiltrate	Control	0	0	4	2	0	6
	25 ppm	0	0	0	0	0	0
	75 ppm	0	2	2	0	0	4
Multifocal mononuclear cell infiltrate	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

TABLE 8—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Mineralized deposits within kidney tubules, focal	Control	0	0	7	23	6	36
	25 ppm	1	4	12	19	2	38
	75 ppm	0	0	5	17	6	28
Cortical cyst	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Renal tubular epithelial hyperplasia	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Acute nephritis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Acquired hydronephrosis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Chronic pyelitis	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Ischemic necrosis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0

* Significantly different from control data when analyzed using Fisher's exact probability test, $p < 0.05$.

Organ Weights

The only statistically significant changes in organ weights were observed at the 12-month interim sacrifice. Male rats exposed to either 25 or 75 ppm VDC had statistically significantly decreased absolute liver weights with a concomitant decrease in fasted body weights. Female rats had statistically significantly increased absolute kidney weights at 25 or 75 ppm VDC (Table 7). The biological significance of these observations is questionable since similar changes were not observed at 6 or 24 months.

Pathology

Gross and histologic examination of the respiratory tissues of male and female rats exposed to 25 or 75 ppm of VDC revealed a statistically increased incidence of lesions typical of chronic murine pneumonia, an infectious process due to *Mycoplasma pulmonis*. This disease was first recognized during the 13- to 18-month time interval and increased in incidence as the study progressed. The incidence of lesions of chronic murine pneumonia was much lower in the control groups. The physical separation of control and exposed rats in the holding room probably decreased the rate of spread of the infectious disease. In view of the fact that this infectious disease has previously been observed at a higher incidence in control groups from other studies, it was concluded that the chronic murine pneumonia was not the result of exposure to 25 or 75 ppm of VDC.

As a result of the increased incidence of the more advanced lesions of chronic murine pneumonia noted in lungs of rats exposed to 25 or 75 ppm of VDC, there was a corresponding statistical decrease in the incidence rates for some of the minor pathologic observations in the lungs of rats at both exposure levels as compared to controls, presumably due to masking of the lesions by the pneumonia.

Advanced chronic renal disease was the primary cause of death or reason for culling VDC-exposed and control male rats from the study. Female rats frequently were culled from the study because of poor condition due to pressure necrosis, ulceration, and subsequent hemorrhage of subcutaneous tumors in the mammary gland region. The female Sprague-Dawley rat has a high spontaneous incidence of these tumors.

The incidence of histopathologic changes observed in the respiratory system, kidneys, and liver of control and VDC-exposed rats necropsied during the course of the study are present in Tables 8 and 9. Of the organs and tissues examined in this study, changes considered to be treatment related were limited to the liver. The liver changes observed on gross pathologic examination were characterized by a pale color with an accentuation of the lobular pattern. Treatment-related histopathologic finding, characterized as minimal midzonal hepatocellular fatty change, were found in both male and female rats at the 6- and 12-month interim sacrifices and in female rats culled or dying during the 18-month exposure period (Table 10). No unusual incidence of fatty change was observed in male rats culled or dying during this period or in male or female rats at the terminal sacrifice. Likewise, no unusual incidence of fatty change was observed among those animals submitted to necropsy during the postexposure period, months 18 to 24 (Table 10).

Tumor incidence data showed that there was no increase in any one tumor type in male rats or in the total numbers of tumors, benign and/or malignant, in the male or female rats exposed to VDC when compared with their respective controls (Tables 11 and 12). There was a statistical increase in the incidence of mammary adenocarcinomas in the females exposed to 25 ppm VDC but not at 75 ppm when compared with the controls (Table 13). The incidence of mammary gland adenocarcinoma in controls of this study were, however, at the low end of the range of the historical

TABLE 9
NONTUMOROUS HISTOPATHOLOGIC LESIONS IN MALE RATS

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Liver							
Accentuated hepatic lobular pattern due to centrilobular atrophy	Control	1	0	4	17	8	30
	25 ppm	1	0	5	21	7	34
	75 ppm	0	2	4	19	7	32
Generalized hepatic lobular atrophy	Control	1	0	6	15	1	23
	25 ppm	1	0	2	13	1	17
	75 ppm	0	1	3	9	0	13*
Accentuated hepatic lobular pattern due to centrilobular atrophy with hepatocellular vacuolization	Control	0	2	2	2	1	7
	25 ppm	0	0	2	3	0	5
	75 ppm	1	0	5	1	0	7
Foci of intralobular hepatocellular alteration	Control	0	0	0	1	6	7
	25 ppm	0	0	0	0	6	6
	75 ppm	0	0	0	1	3	4
Area of intralobular hepatocellular alteration	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Focal hepatic necrosis	Control	0	0	1	0	0	1
	25 ppm	0	0	1	2	0	3
	75 ppm	0	0	0	0	0	0
Multifocal hepatic necrosis	Control	0	0	2	2	0	4
	25 ppm	0	0	0	3	1	4
	75 ppm	0	0	0	1	1	2
Generalized centrilobular degeneration and/or necrosis	Control	0	2	4	5	0	11
	25 ppm	2	1	4	9	0	16

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(anoxic) with or without associated accentuation of the lobular pattern or associated fatty change	75 ppm	1	0	4	10	0	
Focal biliary hyperplasia	Control	0	0	2	8	10	20
	25 ppm	0	0	2	4	4	10*
	75 ppm	0	0	2	5	0	7*
Focal periportal fibrosis	Control	0	0	3	10	9	22
	25 ppm	0	0	1	7	5	13
	75 ppm	0	0	2	7	1	10*
Focal biliary cyst formation	Control	0	0	0	5	2	7
	25 ppm	0	0	0	3	2	5
	75 ppm	0	0	0	2	0	2
Focal mononuclear cell infiltrate	Control	0	4	15	36	13	68
	25 ppm	0	2	8	28	12	50*
	75 ppm	2	3	13	26	4	48*
Focal extramedullary hematopoiesis	Control	0	1	0	1	0	2
	25 ppm	0	0	1	4	0	5
	75 ppm	0	1	0	1	0	2
Focal vascular ectasia (dilatation)	Control	0	0	0	3	2	5
	25 ppm	0	0	0	2	0	2
	75 ppm	0	0	0	3	1	4
Minimal hepatocellular fatty change	Control	0	0	2	3	2	7
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	3	6	3	12
Moderate hepatocellular fatty change (centrilobular and/or midzonal)	Control	0	0	0	0	1	1
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	1	0	0	1
Severe hepatocellular fatty change	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Increased number of reticuloendothelial cells lining sinusoids (reticuloendothelial hyperplasia)	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1

TABLE 9—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Foci of cystic degeneration of hepatocytes	Control	0	0	0	0	2	2
	25 ppm	0	0	0	1	3	4
	75 ppm	0	0	0	0	2	2
Respiratory system							
Trachea							
Focal subepithelial lymphoid infiltrate	Control	0	0	5	6	0	11
	25 ppm	0	0	0	3	1	4*
	75 ppm	0	0	1	4	0	5
Chronic or acute tracheitis	Control	0	0	1	0	0	1
	25 ppm	2	0	4	16	2	24*
	75 ppm	1	0	3	15	2	21*
Lungs							
Chronic pulmonary lesions characterized by edema, interstitial thickening and fibrosis, inflammatory cell infiltrate and exudation of cells into the alveoli, with or without atelectasis (associated with advanced cardiac or renal lesions)	Control	0	1	3	19	0	23
	25 ppm	0	0	0	11	1	12*
	75 ppm	0	0	0	15	0	15
Focal alveolar histiocytosis	Control	2	2	8	21	10	43
	25 ppm	0	2	4	17	10	33
	75 ppm	0	3	3	14	3	23*
Peribronchial lymphoid cellular infiltrate	Control	0	5	17	27	13	62
	25 ppm	2	3	13	18	11	47*
	75 ppm	2	4	16	21	6	49*

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Focal interstitial pneumonitis	Control	1	0	0	2	4	6
	25 ppm	0	0	0	1	3	4
	75 ppm	0	0	0	4	1	5
Focal aggregates of macrophages containing hematogenous pigment within the alveoli	Control	0	0	0	1	5	6
	25 ppm	0	0	1	1	4	6
	75 ppm	0	0	0	0	1	1
Acute diffuse suppurative pneumonia	Control	1	0	0	0	0	1
	25 ppm	2	0	0	0	0	2
	75 ppm	2	0	1	0	0	3
Focal granulomatous inflammation	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Pleuritis	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Focal pulmonary abscess formation	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Focal mineralization of the intraalveolar septae	Control	0	0	5	9	0	14
	25 ppm	0	0	1	3	0	4*
	75 ppm	0	0	3	6	0	9
Chronic murine pneumonia	Control	0	0	0	0	0	0
	25 ppm	0	0	4	19	3	26*
	75 ppm	0	0	3	19	2	24*
Cholesterol cleft formation	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Urinary system							
Kidney							
Minimal degree of chronic nephropathy	Control	2	4	4	5	0	15
	25 ppm	0	2	7	7	1	17
	75 ppm	0	4	3	7	0	14

TABLE 9—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Moderate degree of chronic nephropathy	Control	0	1	7	9	6	23
	25 ppm	0	0	5	11	5	21
	75 ppm	0	0	6	16	5	27
Severe degree of chronic nephropathy	Control	0	0	1	19	7	27
	25 ppm	0	0	4	15	7	26
	75 ppm	0	0	4	20	3	27
End-stage kidney	Control	0	0	7	12	0	19
	25 ppm	0	1	2	14	0	17
	75 ppm	0	0	6	8	0	14
Mineralized deposits within kidney tubules, focal	Control	0	0	4	13	0	17
	25 ppm	0	0	1	9	0	10
	75 ppm	0	0	3	8	0	9
Focal renal cortical tubular hyperplasia	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Acquired hydronephrosis	Control	0	0	1	0	0	1
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Pyelonephritis	Control	0	0	1	1	0	2
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	0	0	0
Focal hyperplasia of the transitional epithelium	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

* Significantly different from control data when analyzed using Fisher's exact probability test, $p < 0.05$.

TABLE 10
INCIDENCE OF MIDZONAL HEPATOCELLULAR FATTY CHANGE IN MALE AND FEMALE RATS

Time of examination	No. of animals with fatty change/no. of animals examined		
	Exposure level (ppm)		
	0	25	75
At scheduled sacrifice			
6-month interim	0/5 M 0/5 F	1/5 M 2/5 F	4/5 M 4/5 F
12-month interim	0/5 M 0/5 F	3/5 M 5/5 F	5/5 M 5/5 F
24-month terminal	1/13 M 1/19 F	0/13 M 0/11 F	0/8 M 0/16 F
At death or following culling	0/27 M	0/25 M	1/27 M
During 18-month exposure period	0/16 F	6/29 F	7/20 F
During 6-month postexposure period, but not including 24-month terminal sacrifice	0/46 M 0/49 F	1/47 M 0/46 F	0/51 M 1/48 F

experience for malignant mammary tumors in the Sprague-Dawley rat in this laboratory (Table 14).

There were a number of tumor types that were statistically decreased in incidence in the VDC-exposed groups when compared with the controls. Specifically, in male rats the incidence of pituitary adenomas was decreased at 25 and 75 ppm levels, and pancreatic islet cell adenomas and thyroid adenocarcinomas were decreased at the 75 ppm level. The incidence of pituitary adenomas was decreased in female rats exposed at 25 ppm.

The total incidence of histopathologically diagnosed primary tumors, benign and malignant, from the VDC-exposed groups was comparable to the concurrent control groups and historical control experience of this laboratory.

CONCLUSIONS

The findings from the initial 5-week phase of the chronic toxicity and oncogenicity study during which rats were exposed to 0, 10, or 40 ppm VDC, 6 hr/day, 5 days/week indicated that concentrations as high as 40 ppm did not

produce signs of toxicity in the target organ, the liver. The findings at the increased concentrations of 25 or 75 ppm indicated that on longer exposure both concentrations were effect levels for target organ toxicity. The liver effects, characterized by minimal hepatocellular fatty changes in the midzonal region of the hepatic lobule, were observed in both male and female rats of both the exposure groups as early as the 6-month interim sacrifice. Midzonal fatty changes were also observed at the 12-month sacrifice, but there was no indication of progression of this lesion in either severity or incidence. By the 18th month of the study the incidence of this change was no longer increased in male rats exposed to VDC when compared to controls. However, female rats of the 75-ppm exposure group had a significantly higher incidence of hepatic fatty change than did their controls and a trend toward an increased incidence (not statistically significant) was apparent in female rats of the 25-ppm group. During the last 6 months of the study, after exposures had been discontinued, the effect was no longer discernible indicating reversibility of the lesion.

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TABLE 11
HISTOPATHOLOGIC DIAGNOSIS AND NUMBER OF TUMORS IN MALE RATS

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Liver							
Undifferentiated sarcoma with invasion of lung and diaphragm	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic adrenal cortical carcinoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Respiratory system							
Lungs							
Metastatic adrenal cortical carcinoma	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Well-differentiated bronchial and/or alveolar carcinoma without metastasis	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic thyroid gland carcinoma	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Undifferentiated sarcoma with a pulmonary metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Metastatic mucinous cystadenocarcinoma	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0

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Urinary system							
Nephroblastoma	Control	0	1	0	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Transitional cell papilloma	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Gastrointestinal tract							
Stomach							
Metastatic adenocarcinoma of probable intestinal origin	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Small intestine							
Mucinous cystadenocarcinoma without metastasis	Control	0	0	0	0	1	1
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	2	1	3
Undifferentiated sarcoma with osseous metaplasia without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Mucinous cystadenocarcinoma with metastasis to lung	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Sclerosing adenocarcinoma of probable intestinal origin with metastasis to stomach	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Salivary gland							
Carcinoma, most probably salivary gland in origin without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	1	0	0	0	1
Pleomorphic sarcoma of probable salivary gland origin	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Pancreas							
Pancreatic acinar adenoma	Control	0	0	3	13	8	24
	25 ppm	0	0	2	3	1	6
	75 ppm	0	0	5	12	6	23

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TABLE II—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Pancreatic acinar adenocarcinoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	2	0	2
	75 ppm	0	0	0	0	0	0
Mixed exocrine and endocrine adenoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	0	0	0
Pancreatic islet cell adenoma	Control	0	0	1	3	8	12
	25 ppm	0	0	0	1	2	3
	75 ppm	0	0	1	3	1	5*
Reproductive system							
Testis							
Interstitial cell adenoma of the testis	Control	0	0	0	0	2	2
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	3	0	3
Mesothelioma of the tunica albuginea	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	2	3
Undifferentiated sarcoma without metastasis, most probable neurofibrosarcoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Nervous system							
Brain							
Astrocytoma	Control	0	0	0	1	0	1
	25 ppm	0	0	0	1	0	1
	75 ppm	0	1	0	0	0	1

Intracranial pleomorphic sarcoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	1	0	0	0	1
	75 ppm	0	0	0	0	0	0
Pituitary							
Pituitary adenoma	Control	0	0	3	16	4	23
	25 ppm	0	0	3	8	1	12*
	75 ppm	0	1	3	8	2	14*
Atypical adenoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Pituitary adenocarcinoma without invasion of brain	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Pituitary adenoma based on gross examination but microscopic section not available for evaluation	Control	0	0	0	0	0	0
	25 ppm	0	0	0	2	0	2
	75 ppm	0	0	0	0	0	0
Total number of animals with a pituitary tumor	Control	0	0	3	17	4	24
	25 ppm	0	0	3	11	1	15*
	75 ppm	0	1	3	8	2	14*
Spinal cord							
Neurofibrosarcoma within pelvic canal or ventral thoracic region without metastasis	Control	0	0	1	1	0	2
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Peripheral nerve							
Schwannoma in abdominal region— origin uncertain	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Adrenal gland							
Cortex							
Adenoma of adrenal cortex	Control	0	0	0	3	0	3
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Adrenal cortical carcinoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0

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TABLE 11—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Adrenal cortical carcinoma with metastasis to lung	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Adrenal cortical carcinoma with metastasis to liver	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Medulla							
Adrenal pheochromocytoma, unilateral	Control	0	0	3	14	5	22
	25 ppm	0	0	1	16	3	20
	75 ppm	0	0	4	12	7	23
Adrenal pheochromocytoma, bilateral	Control	0	0	0	8	3	11
	25 ppm	0	0	0	11	4	15
	75 ppm	0	0	0	7	1	8
Malignant pheochromocytoma with no apparent metastasis, unilateral	Control	0	0	0	2	1	3
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	2	0	2
Total number of animals with a pheochromocytoma	Control	0	0	3	24	9	36
	25 ppm	0	0	1	28	7	36
	75 ppm	0	0	4	21	9	34
Thyroid							
Thyroid adenoma	Control	0	0	0	4	5	9
	25 ppm	0	0	2	4	4	10
	75 ppm	0	0	1	5	1	7
Thyroid adenocarcinoma without metastasis	Control	0	0	1	5	1	7
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	0	1	1*

Thyroid carcinoma with metastasis to cervical lymph nodes and lung	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Total number of animals with a thyroid tumor	Control	0	0	1	9	7	17
	25 ppm	0	0	2	5	5	12
	75 ppm	0	0	1	5	2	8 ^a
Subcutaneous tissue							
Subcutaneous fibroma	Control	0	0	0	6/4	1/1	7/5 ^b
	25 ppm	0	1/1	1/1	4/3	0	6/5 ^b
	75 ppm	1/1	0	0	5/4	0	6/5 ^b
Subcutaneous fibrosarcoma without metastasis	Control	0	1/1	0	0	0	1/1 ^b
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1/1	0	1/1 ^b
Subcutaneous fibrosarcoma with metastasis to lung	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	1/1	0	0	1/1 ^b
Undifferentiated sarcoma with metastasis to lung	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1/1	0	1/1 ^b
Fibrolipoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	1/1	1/1 ^b
	75 ppm	0	0	0	0	0	0
Neurofibrosarcoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1/1	1/1 ^b
Mammary gland							
Fibroadenoma/adenofibroma	Control	0	0	0	0	0	0
	25 ppm	0	0	1/1	0	0	1/1 ^b
	75 ppm	0	0	1/1	1/1	0	2/2 ^b
Fibroma	Control	0	0	0	0	1/1	1/1 ^b
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

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TABLE II—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	3	5	19	46	13	86
	25 ppm	4	3	18	47	13	85
	75 ppm	4	4	19	51	8	86
Integument							
Sebaceous squamous cell carcinoma of the external auditory canal without metastasis (Zymbal gland carcinoma)	Control	0	0	1	1	0	2
	25 ppm	0	0	0	2	1	3
	75 ppm	0	0	0	1	0	1
Squamous cell carcinoma of the orbit of the eye without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Undifferentiated sarcoma of probable neural origin without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Unclassified malignant epithelial neoplasm of the nose	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Squamous papilloma with keratinization	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	1
	75 ppm	0	0	0	0	0	0
Musculoskeletal system							
Osteogenic sarcoma of the rear limb without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Hard palate—squamous cell carcinoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1

Lymphoreticular system							
Lymphosarcomas and leukemias							
Generalized leukemia with multiple tissue metastasis	Control	0	0	0	2	0	2
	25 ppm	0	0	1	1	1	3
	75 ppm	0	0	0	2	0	2
Lymphosarcoma of the small intestine without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Lymphosarcoma of the large intestine without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Thymus							
Thymoma	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Other lymph nodes							
Metastatic thyroid gland carcinoma to cervical lymph nodes	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Total number of primary tumors in the male rats as previously listed	Control	0	2	14	86	43	145
	25 ppm	0	2	12	66	21	101
	75 ppm	1	3	16	71	24	115

* Significantly different from control data when analyzed using Fisher's exact probability test, $p < 0.05$.

^b Number of tumors/number of animals with tumors.

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TABLE 12
HISTOPATHOLOGIC DIAGNOSIS AND NUMBER OF TUMORS IN FEMALE RATS

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Liver							
Cholangioma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	0	0	0
Metastatic adenocarcinoma of uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Respiratory system							
Lung							
Metastatic leiomyosarcoma of the uterocervix region	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic adenocarcinoma of uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic adenocarcinoma of mammary gland region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic osteogenic sarcoma of the forelimb	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Metastatic carcinoma of the thyroid	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Metastatic cortical carcinoma of the adrenal gland	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	0	0	0

Urinary system

Kidney

Metastatic adenocarcinoma of the
uterocervix region

Control	0	0	0	1	0	1
25 ppm	0	0	0	1	0	1
75 ppm	0	0	0	0	0	0
Renal cortical carcinoma without metastasis—well differentiated	Control	0	0	0	0	0
	25 ppm	0	0	0	0	0
	75 ppm	0	0	0	1	0

Cardiovascular system

Heart

Undifferentiated endocardial sarcoma
without metastasis

Control	0	0	0	0	0	0
25 ppm	0	0	0	1	0	1
75 ppm	0	0	0	0	0	0

Blood vessels

Metastatic leiomyosarcoma of the
uterocervix region—to several blood
vessels

Control	0	0	1	0	0	1
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	0	0	0

Gastrointestinal tract

Stomach

Leiomyosarcoma with multiple
intraabdominal metastasis

Control	0	0	0	0	0	0
25 ppm	0	0	0	1	0	1
75 ppm	0	0	0	0	0	0

Small intestine

Mucinous cystadenocarcinoma without
metastasis

Control	0	0	0	1	0	1
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	0	0	0

Metastatic leiomyosarcoma of the
uterocervix region

Control	0	0	1	0	0	1
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	0	0	0

Metastatic adenocarcinoma of probable
uterine origin

Control	0	0	0	0	0	0
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	1	0	1

Large intestine

Metastatic adenocarcinoma of uterocervix
origin

Control	0	0	0	1	0	1
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	0	0	0

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TABLE 12—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Salivary gland							
Carcinoma most probably salivary gland in origin without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	1	0	0	1
Reproductive system							
Ovary							
Granulosa cell tumor without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Ovarian adenocarcinoma of sclerosing type with multiple tissue metastases	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Ovarian hemangioma	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Metastatic adenocarcinoma of uterocervix origin	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Uterus							
Endometrial polyp formation	Control	0	0	1	9	3	13
	25 ppm	0	1	2	2	5	10
	75 ppm	0	0	1	4	5	10
Endometrial polyp based on gross examination only	Control	0	0	0	0	1	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Stromal sarcoma of uterocervix origin without metastasis	Control	0	1	0	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

Undifferentiated sarcoma of uterocervix origin without metastases	Control	0	0	2	0	0	2
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Leiomyosarcoma of uterocervix region with metastasis to lung, small intestine and blood vessels	Control	0	0	1	0	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Adenocarcinoma of uterocervix origin with multiple tissue metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Endometrial carcinoma of the uterus without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Adenocarcinoma of the uterus without metastasis	Control	0	0	0	1	2	3
	25 ppm	0	0	0	2	0	2
	75 ppm	0	0	0	0	0	0
Leiomyoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Cervix—fibroma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	0	1	1
Scirrhus adenocarcinoma, origin undetermined, most probable ovary and uterine horn with widespread abdominal metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Carcinoma of uterocervix with metastasis to abdominal viscera and one kidney	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Nervous system							
Brain							
Astrocytoma	Control	0	0	0	1	1	2
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Oligodendroglioma	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

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TABLE 12—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Metastatic lymphoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Pituitary							
Pituitary adenoma	Control	0	0	2	20	15	37
	25 ppm	0	0	5	13	6	24 ^a
	75 ppm	0	0	2	13	11	26
Pituitary adenocarcinoma without invasion of brain	Control	0	0	0	1	0	1
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	0	0	0
Pituitary adenocarcinoma with invasion of brain	Control	0	0	0	3	0	3
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Adenoma based on gross examination only	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	2	0	2
Total number of animals with a pituitary tumor	Control	0	0	2	24	15	41
	25 ppm	0	0	5	15	8	28 ^a
	75 ppm	0	0	2	15	11	28
Thyroid							
Thyroid adenoma	Control	0	0	0	11	3	14
	25 ppm	0	0	1	9	3	13
	75 ppm	0	0	2	7	7	16
Thyroid adenocarcinoma without metastasis	Control	0	0	0	1	5	6
	25 ppm	0	0	0	0	0	0 ^a
	75 ppm	0	0	0	1	0	1

Thyroid carcinoma with pulmonary metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Total number of animals with a thyroid tumor	Control	0	0	0	12	8	20
	25 ppm	0	0	1	10	3	14
	75 ppm	0	0	2	8	7	17
Parathyroid							
Parathyroid adenoma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Adrenal gland							
Cortex							
Adenoma of adrenal cortex	Control	0	0	1	1	1	3
	25 ppm	0	0	0	1	1	2
	75 ppm	0	0	0	0	0	0
Adrenal cortical carcinoma without metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Adrenal cortical carcinoma with metastasis to lung	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	0	0	0
Metastatic adenocarcinoma of uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Medulla							
Adrenal pheochromocytoma, unilateral	Control	0	0	0	2	2	4
	25 ppm	0	0	1	3	0	4
	75 ppm	0	0	0	2	1	3
Adrenal pheochromocytoma, bilateral	Control	0	0	0	5	1	6
	25 ppm	0	0	0	0	1	1
	75 ppm	0	0	0	0	2	2
Total number of animals with a pheochromocytoma	Control	0	0	0	7	3	10
	25 ppm	0	0	1	3	1	5
	75 ppm	0	0	0	2	3	5

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TABLE 12—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Ganglioneuroma—unilateral	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Pancreas Pancreatic islet cell adenoma	Control	0	0	0	5	1	6
	25 ppm	0	0	0	0	2	2
	75 ppm	0	0	1	0	0	1
Metastatic adenocarcinoma of the uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Subcutaneous tissue Subcutaneous fibroma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1/1	0	1/1 ^b
Subcutaneous fibrosarcoma without metastasis	Control	0	0	0	1	1/1	1/1 ^b
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Mammary gland Fibroadenoma/adenofibroma	Control	0	0	17/10	78/39	37/15	132/64 ^b
	25 ppm	0	2/2	40/20	76/38	16/8	134/68 ^b
	75 ppm	1/1	6/4	17/14	100/40	33/15	157/74 ^b
Fibroma	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1/1	1/1	2/2 ^b
Adenocarcinoma with pulmonary metastasis	Control	0	0	0	1/1	0	1/1 ^b
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0

Adenocarcinoma without metastasis	Control	0	0	0	1/1	0	1/1 ^b
	25 ppm	0	1/1	2/2	1/1	4/3	8/7 ^{a,b}
	75 ppm	0	0	2/2	0	2/2	4/4 ^b
Leiomyosarcoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1/1	0	1/1 ^b
	75 ppm	0	0	0	0	0	0
Carcinoma of the mammary gland	Control	0	0	0	1/1	1/1 ^b	
	25 ppm	0	0	0	0	0	
	75 ppm	0	0	0	0	0	
Adenofibroma based on gross examination only	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1/1	0	1/1 ^b
Musculoskeletal system							
Metastatic adenocarcinoma of the uterocervix region to the mandible	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Osteogenic sarcoma of the forelimb with metastasis to the lung	Control	0	0	0	0	0	0
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	1	1
Lymphoreticular system							
Mediastinal lymphoid tissue							
Metastatic adenocarcinoma of the uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Mesenteric lymphoid tissue							
Metastatic adenocarcinoma of uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Spleen							
Metastatic adenocarcinoma of uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Lymphosarcoma and leukemias							
Generalized lymphosarcoma with multiple tissue metastasis	Control	0	0	1	0	0	1
	25 ppm	0	0	0	2	0	2
	75 ppm	0	0	0	0	0	0

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TABLE 12—Continued

		0-6 Months	7-12 Months	13-18 Months	19-24 Months	Terminal kill	Cumulative results
Number of rats necropsied during the time period indicated	Control	1	1	14	49	19	84
	25 ppm	1	5	23	46	11	86
	75 ppm	1	4	15	48	16	84
Monocytic myelogenous leukemia with multiple tissue metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Malignant lymphoma, primarily of mesenteric lymph nodes with intestinal and brain metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	0	1	0	1
	75 ppm	0	0	0	0	0	0
Eye							
Retobulbar fibrosarcoma without metastasis	Control	0	0	0	0	0	0
	25 ppm	0	0	1	0	0	1
	75 ppm	0	0	0	0	0	0
Adipose tissue							
Metastatic adenocarcinoma of the uterocervix region	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	1	0	1
Miscellaneous							
Undifferentiated mesenchymal sarcoma of the mediastinal region with extension into the surrounding mediastinal tissue and vessels but without distant metastasis	Control	0	0	0	1	0	1
	25 ppm	0	0	0	0	0	0
	75 ppm	0	0	0	0	0	0
Total number of primary tumors in the female rats as previously listed	Control	0	1	25	149	73	248
	25 ppm	0	4	54	119	43	220
	75 ppm	1	6	26	137	65	235

* Significantly different from control data when analyzed using Fisher's exact probability test, $p < 0.05$.

* Number of tumors/number of animals with tumors.

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Concentrations of 25 or 75 ppm did not produce exposure-related effects on hematology, urinalyses, clinical chemistry, or cytogenetic parameters. Slight increases in cumulative mortality were observed in the VDC-exposed female rats throughout the latter portion of the study. There was, however, no indication of a dose-response relationship. The higher incidence of murine pneumonia observed among the animals in both exposure groups as the study progressed was considered to have contributed to this increased mortality. Similarly, body weight decreases observed among the male and female rats were neither consistent or dose related, but may have been associated with the murine pneumonia. The only statistical changes in organ weight measurements occurred at the 12-month interim sacrifice; a decrease in mean absolute liver weights and concomitant decreased body weights were observed in male rats exposed to 25 or 75 ppm. Comparable effects were not observed in the animals at 1, 6, or 24 months. At the 12-month interim sacrifice, female rats at both concentrations had significantly increased mean absolute kidney weights which were not accompanied by any gross or histopathologic observations. Kidney weight changes were not observed in the female rats at 1, 6, or 24 months. No treatment-related histopathology was observed either at the interim or terminal sacrifices in the kidneys of rats on this study.

TABLE 13

INCIDENCE OF MAMMARY TUMORS IN FEMALE RATS

	Exposure level		
	Controls	25 ppm	75 ppm
No. of rats	84	86	84
Mammary gland adenocarcinoma	2/2*	8/7*	4/4

* Number of tumors/number of animals with tumors.

* Significantly different from controls by Fisher's exact probability test, $\alpha = 0.05$.

TABLE 14

HISTORICAL INCIDENCES OF MALIGNANT MAMMARY TUMORS IN CONTROL FEMALE SPRAGUE-DAWLEY RATS

Study	Incidence
1	1/85
2	6/88
3	8/86
4	4/80
5	13/92
6	3/84
7	6/80
8	9/100
9	13/86

Although the incidences of several tumors and/or tumor types were found to be statistically increased or decreased in VDC-exposed rats compared to controls, none of these statistically significant differences were judged to be attributable to VDC exposure. The majority of pathologic alterations observed in this study were associated with intercurrent disease (murine pneumonia), and commonly found geriatric lesions in this strain of rat, or both. The total incidence of histologically diagnosed primary tumors (benign and malignant) was similar in control and VDC-exposed groups of both sexes. In addition, the tumor incidence data for both control and treated rats in this study was comparable to historical control data for the Sprague-Dawley rat (Spartan substrain) used by this laboratory for several studies of similar design and duration.

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REFERENCES

- BUCKTON, K. E., AND EVANS, H. J. (1973). *Methods for the Analysis of Human Chromosome Aberrations*. World Health Organization, Q. Corp., Albany, N.Y.
- GAGE, J. C. (1970). The subacute inhalation toxicity of 109 industrial chemicals. *Brit. J. Ind. Med.* 27, 1-18.
- HONG, C. B., WINSTON, J. M., THORNBURG, L. P., LEE, C. C., AND WOODS, J. S. (1981). Follow-up study on carcinogenicity of vinyl chloride and vinylidene chloride in rats and mice. Tumor incidence and mortality sub-

- sequent to exposure. *J. Toxicol. Environ. Health* 7, 909-924.
- LEE, C. C., BHANDARI, J. C., WINSTON, J. M., AND HOUSE, W. B. (1978). Carcinogenicity of vinyl chloride and vinylidene chloride. *J. Toxicol. Environ. Health* 4, 15-30.
- LEGATOR, M. L., PALMER, K. A., GREEN, L., AND PETERSEN, K. W. (1969). Cytogenetic studies in rats of cyclohexylamine, a metabolite of cyclamate. *Science (Washington, D.C.)* 165, 1139-1140.
- MALTONI, C. (1977). Recent findings on the carcinogenicity of chlorinated olefins. *Environ. Health Persp.* 21, 1-5.
- MALTONI, C., CLIBERTI, A., AND CARRETTI, D. (1982). Experimental contributions in identifying brain potential carcinogens in the Petrochemical Industry. *Ann. N.Y. Acad. Sci.* 381, 216-249.
- NCI/NTP (National Cancer Institute/National Toxicology Program) (1982). NTP technical report on the carcinogenesis bioassay of vinylidene chloride in F344/N rats and B6C3F1/mice (Gavage Study). NPT No. 80-82, NIH Pub. No. 82-1784.
- PRENDERGAST, J. A., JONES, R. A., JENKINS, L. J., AND SIEGEL, J. (1967). Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. *Toxicol. Appl. Pharmacol.* 10, 270-289.
- QUAST, J. F., HUMISTON, C. G., WADE, C. E., BALLARD, J., BEYER, J. E., SCHWETZ, R. W., AND NORRIS, J. M. (1983). A chronic toxicity and oncogenicity study in rats and subchronic toxicity study in dogs on ingested vinylidene chloride. *Fundam. Appl. Toxicol.* 3, 55-62.
- RAMPY, L. W., QUAST, J. F., HUMISTON, C. G., BALMER, M. F., AND SCHWETZ, B. A. (1977). Interim results of two-year toxicological studies in rats of vinylidene chloride incorporated in the drinking water or administered by repeated inhalation. *Environ. Health Perspect.* 21, 33-43.
- REITZ, R. H., WATANABE, P. G., MCKENNA, M. J., QUAST, J. F., AND GEHRING, P. J. (1980). Effects of vinylidene chloride on DNA synthesis and DNA repair in the rat and mouse: A comparative study with dimethyl nitrosamine. *Toxicol. Appl. Pharmacol.* 52, 357-370.
- SIEGEL, S. (1956). *Non-parametric Statistics for the Behavioral Sciences*. McGraw-Hill, New York.
- STEEL, R. G. D., AND TORRIE, H. H. (1960). *Principles and Practices of Statistics*. McGraw-Hill, New York.
- VAN DUUREN, B. L., GOLDSCHMIDT, B. M., LOEWENGERT, G., SMITH, A. C., MELCHIEENNE, S., SEIDMAN, L., AND ROTH, D. (1979). Carcinogenicity of halogenated olefinic and aliphatic hydrocarbons in mice. *J. Natl. Cancer Inst.* 63, 1433-1439.
- VIOLA, P. L., AND CAPUTO, A. (1977). Carcinogenicity studies on vinylidene chloride. *Environ. Health Perspect.* 21, 45-47.