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vinyl chloride, vinylidene chloride

CARCINOGENICITY OF VINYL CHLORIDE AND VINYLIDENE CHLORIDE

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Exposure of mice to 50, 250, or 1000 ppm of vinyl chloride (VC) in the air for 6 h/d, 5 d/wk, caused a high incidence of bronchioloalveolar adenoma, mammary gland tumors, and hemangiosarcoma. Mammary gland tumors occurred in the females and included ductular adenocarcinoma and squamous and anaplastic cell carcinomas with metastasis to the lung. Hemangiosarcoma occurred in the liver and, to a lesser extent, in various other organs. The incidence and severity of these tumors increased with the concentration of VC and the length of exposure. Malignant lymphoma involving various organs was observed in several mice. Rats were more resistant to the carcinogenic effects of VC. Exposure of rats to 250 or 1000 ppm of VC caused hemangiosarcoma in the liver. Many rats with hepatic hemangiosarcoma also developed hemangiosarcoma in the lung. Extrahepatic hemangiosarcoma also occasionally occurred in other organs. Exposure to 55 ppm of vinylidene chloride (VDC) caused hepatic hemangiosarcoma and probably bronchioloalveolar adenoma in mice. Hemangiosarcoma also occurred in the mesenteric lymph node or subcutaneous tissue in two rats exposed to 55 ppm of VDC.

INTRODUCTION

In 1971, the carcinogenic effect of vinyl chloride (VC) was first reported in animals (Viola et al., 1971). Male Ar/IRE rats exposed to 30,000 ppm of VC, 4 h/d, 5 d/wk, for 12 mo, developed epidermoid

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carcinomas, papillomas and mucoepidermoid carcinomas of the skin, adenocarcinomas of the lung, and osteochondromas of the metacarpal and metatarsal regions of the limbs. Hepatic angiosarcomas and other tumors were observed in rats exposed to 20,000, 500, or 50 ppm of VC (Caputo et al., 1974; Winell et al., 1976). Tumors of the skin and lung were also reported in rabbits exposed to 10,000 ppm (Caputo et al., 1974). Exposure to VC concentrations as low as 50 ppm, with the same exposure times, induced angiosarcomas in the liver and other tissues of Sprague-Dawley and Wistar rats, Swiss mice, and golden hamsters (Maltoni and Lefemine, 1975). In addition, other tumors were seen, including tumors of the zymbal glands and skin, nephroblastomas, hepatomas, and neuroblastomas in rats; tumors of the lung and skin in mice; and tumors of the skin and lymphomas in hamsters. These results were further confirmed, and dose-time and carcinogenic response relationships were established (Maltoni, 1977).

In 1974, four cases of hepatic angiosarcoma were reported in workers exposed to polyvinyl chloride (Creech and Johnson, 1974). The first case of hepatic angiosarcoma was reported in 1961 (Heath et al., 1975). Further cases of hepatic angiosarcoma and other hepatic diseases, notably portal fibrosis and portal hypertension, were identified among VC polymerization workers (Block, 1974; Falk et al., 1974; Lee and Harry, 1974; Makk et al., 1974; Byren and Holmberg, 1975; Lilis et al., 1975). Epidemiologic studies indicated that tumors at multiple sites developed in people working with VC and polyvinyl chloride (Monson and Peters, 1974; Tabershaw and Gaffey, 1974; Nicholson et al., 1975; Byren et al., 1976; Waxweiler et al., 1976). This report summarizes the carcinogenic effects of 50, 250, or 1000 ppm of VC in rats and mice and compares them with the effect of vinylidene chloride (VDC) at a concentration of 55 ppm.

METHODS

Inhalation Chambers and Air Supply

Five stainless steel cubical exposure chambers 3.5 m^3 in volume were used. VC or VDC was introduced through the top of the chamber. Each chamber contained a plenum, a diffusion plate, and two small squirrel cage fans (2.85 m^3/min) mounted on opposite sides of the top cone above the diffusion plate to ensure complete mixture of the gas with air. The outside air supply passed through a coarse filter; over coils for heating, cooling, and dehumidifying; and then through an absolute filter (99.97-99.99% retention of 0.3-μm particles) into the plenum of the chamber. Airflow rates were measured initially at the inlet side of the chamber with a Pitot tube connected to a magnahelix gauge, and later at the exhaust outlet with an orifice plate and a magnahelix gauge. The orifice plate was calibrated with an airflow transducer (Autotronics 100-SXX). These

measurements indicated airflow rates of about 12 chamber volumes (0.70 m^3/min) per hour.

Generation of VC and VDC Vapor

VC gas (99.8% pure, Matheson Gas Products) was metered with rotameters into the chamber air supply. VDC (99% pure, Aldrich Co.) was heated to 37°C to generate the vapor. All VDC lines and the rotameter were heated to 40°C to prevent condensation.

Chamber Monitoring and Sampling

Chamber concentrations were monitored by using a gas chromatograph (Varian-2700) with a flame ionization detector. A 6 ft X 1/8 in stainless steel column packed with 0.4% Carbowax 1500 on Carbopak A was used with a nitrogen carrier flow rate of 80 ml/min. The injection, column, and detector temperatures were 135, 65, and 170°C, respectively. VC standards at dilutions of 10, 50, and 100 ppm were obtained in lecture bottles (Supelco, Inc., Bellefonte, Pennsylvania), and a 1000-ppm primary standard was obtained from Matheson Gas Products (Joliet, Illinois). VDC standards were prepared by serial dilution (w/v) of VDC in carbon tetrachloride.

Each chamber was fitted with 10 sampling ports on two sides. Polyethylene tubes were positioned through the ports at the center and near the periphery of the chamber. Samples were withdrawn with a syringe and introduced into the gas chromatograph. A valid sample could be withdrawn by pumping the syringe three times on the short sampling lines and five times on the long lines. All sampling was performed in triplicate. Distribution studies at all parts of the chamber were compared with a reference point in the center. The results indicated that average chamber concentrations were ±3% of the desired concentration and the reference point averages were 98.4–100.4% of the chamber averages. During the study, chambers were routinely sampled from the reference point three to four times a day.

An automatic sampling system was used later during the experiment. A polyfluoroethylene (Teflon, DuPont) line (¼-in diameter) connected each chamber to the automatic sampler. These lines were purged constantly. Samples were directed periodically to the gas chromatograph, where they were injected via a sampling valve with twin 1-ml sampling loops. The readout was processed by a Varian CDS 111 electronic integrator. The integrator was programmed to measure peak area and to calculate parts per million by an external standards program. A chart recorder connected to the integrator was occasionally used to visualize the chromatogram.

Experimental Design

Albino CD-1 mice and CD rats (Charles River) about 2 mo old were used. For each species, a total of 360 animals was divided into five groups,

each consisting of 36 males and 36 females. Each group of both species was exposed to 50, 250, or 1000 ppm of VC, 55 ppm of VDC, or uncontaminated air for 6 h/d, 5 d/wk. All animals were kept in the same stainless steel cages with wire bottoms both during exposure and when outside the chambers. Mice were housed six to eight to a cage and rats two to a cage. During exposure, the cages were constantly rotated throughout the study. Pulverized or block laboratory chow (Wayne Lab Blox) was provided except during exposure. Water was available *ad libitum*. A 12-h light cycle was maintained. The temperature in the chamber and in the room averaged 24 ± 1.3°C. The relative humidity ranged from 25 to 60% at the start of the experiment and was later regulated at 50 ± 10%.

Four animals of each species, sex, and exposure level were terminated for various laboratory tests and gross and histopathologic examinations at the end of 1, 2, 3, 6, and 9 mo; the surviving animals were terminated at the end of 12 mo.

Laboratory Evaluations

All animals were observed throughout the study for adverse signs. Food consumption was recorded weekly and body weight biweekly at a uniform time of day. Various clinical laboratory tests and specific studies were performed as described elsewhere (Lee et al., 1977). When moribund, or at termination, all animals were euthanized for necropsy after the collection of blood. Gross examination, especially for any appearance of abnormal growth or other lesions, was carefully performed on all tissues, including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and other tissues with pathological lesions. All tissues were fixed, processed, sectioned, and stained with hematoxylin and eosin (H & E) for microscopic examination. All external and internal tumors were carefully examined and identified histologically.

RESULTS

Chamber Concentrations

For VC, the average weekly concentrations in the 1000-ppm and 250-ppm chambers did not vary more than ±5% from the desired concentrations, except during the 3d wk, when the average concentrations were about 10% lower. The variations in the 50-ppm chamber were slightly greater during the 2d, 7th, and 8th wk. With a few exceptions, no sample varied more than 10% from the desired concentration. It was planned that the exposure concentration for VDC would be 50 ppm. However, the slightly higher concentration of 55 ppm was obtained and maintained throughout the experiment.

Mice

General condition. A few mice exposed to various levels of VC started to exhibit toxic signs including rough hair coat, lethargy, anorexia, and rapid weight loss during the 6th mo. Some mice died or were terminated before their imminent death. Thereafter, the general health of the remaining mice exposed to VC deteriorated. Abdominal distension and/or external tumor masses, especially mammary tumors in the females, occurred. All male and female mice exposed to 1000 ppm and all females exposed to 250 ppm died or were terminated during the 10th–12th mo. Of the mice exposed to 55 ppm of VDC, two males were terminated during the 9th mo and one female during the 10th mo. Most mice that died or were terminated ahead of schedule and many mice that were terminated on schedule at various times developed one or more types of tumors. In the control group, two males died during the 8th or 9th mo. One death resulted from injury from fighting; the other mouse was found dead with autolysis. No obvious mass was observed in any controls.

Gross lesions. Gross lesions were observed in several organs of some mice. In the lung there were raised, tan to grayish-white nodules of pinhead size to 0.5 cm or larger. In the liver there were moderate mottling and small dark hemorrhagic spots varying in size from petechiae to 1 cm in diameter, or dark nodular masses filled with blood or ruptures in several animals. Spleens were slightly to markedly enlarged. Subcutaneous masses occurred at various locations, varying in size from 1 to 3 cm or larger, and were moderately firm and grayish-white to dark in appearance.

Bronchioloalveolar adenoma. Bronchioloalveolar adenomas were found during the 2d mo in the mice exposed to 1000 or 250 ppm of VC and during the 3d mo in the mice exposed to 50 ppm. The adenoma was characterized by focal areas of acinar or papillary growth, forming small solitary nodules that were well demarcated but not encapsulated (Fig. 1). The incidence (Table 1) and severity of the tumor were in direct proportion to the level of VC and to the length of exposure. In more severe cases, the nodules were more numerous and increased in size by expanding and coalescing to cause consolidation of the affected lobes. There was no sex difference. Totals of 12, 22, and 48 mice exposed to 50, 250, and 1000 ppm of VC, respectively, developed bronchioloalveolar adenoma. This tumor was found in only one male control during the 9th mo. In the group exposed to 55 ppm of VDC, a few small nodules of bronchioloalveolar adenoma were found in one male during the 6th mo, two males during the 9th mo, and three males during the 12th mo.

Hemangiosarcoma. Hemangiosarcomas were found in the livers of mice exposed to 1000 or 250 ppm of VC starting the 6th mo. The hemangiosarcoma was characterized by moderate to severe proliferation of endothelial cells lining the sinusoids, dilation of the sinusoids, focal hemorrhage forming small to large cavernous blood spaces, invasion of the hepatic parenchyma with neoplastic cells, and mild to severe necrosis (Fig.

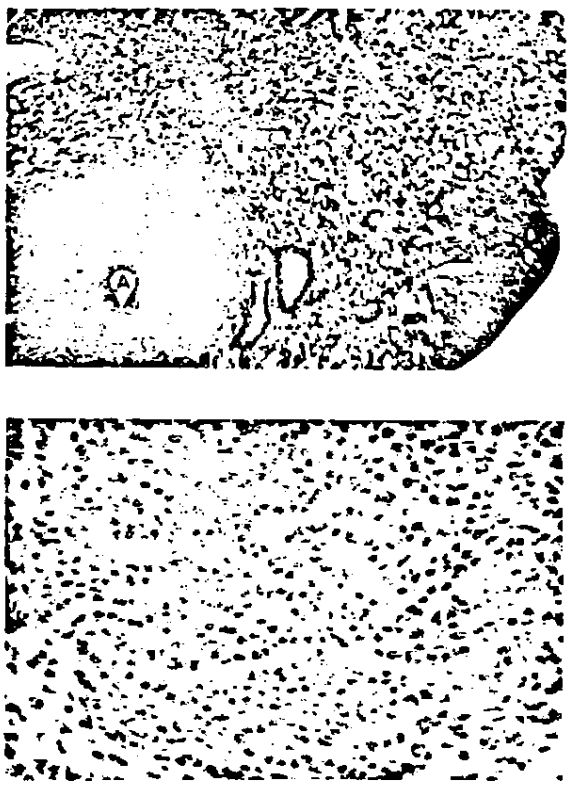

FIGURE 1. Bronchioloalveolar adenoma in mice exposed to VC. (Top) Two small nodules, one with a focus of metastatic squamous cell carcinoma (A) from the mammary gland, H&E, X 12. (Bottom) Higher magnification, showing papillary proliferation, H&E, X 80.

TABLE 1. Incidence of Bronchioloalveolar Adenoma in Male (M) and Female (F) Mice Exposed to VC or VDC

Exposure time (mo)	VC (ppm)								VDC (55 ppm)	
	0		50		250		1000			
	M	F	M	F	M	F	M	F	M	F
1–3	0/12[a]	0/12	1/12	0/12	2/12	0/12	3/12	3/12	0/12	0/12
4–6	0/4	0/4	2/5	0/5	1/4	4/7	6/6	4/5	1/4	0/4
7–9	1/6	0/4	2/7	4/14	5/11	8/15	13/15	19/19	2/6	0/4
10–12	0/4	0/16	3/5	0/3	2/2	0/0	0/0	0/0	3/13	0/15
Total	1/26	0/36	8/29	4/34	10/29	12/34	22/33	26/36	6/35	0/35

[a]Entries indicate no. of mice with bronchioloalveolar adenoma/no. of mice examined.

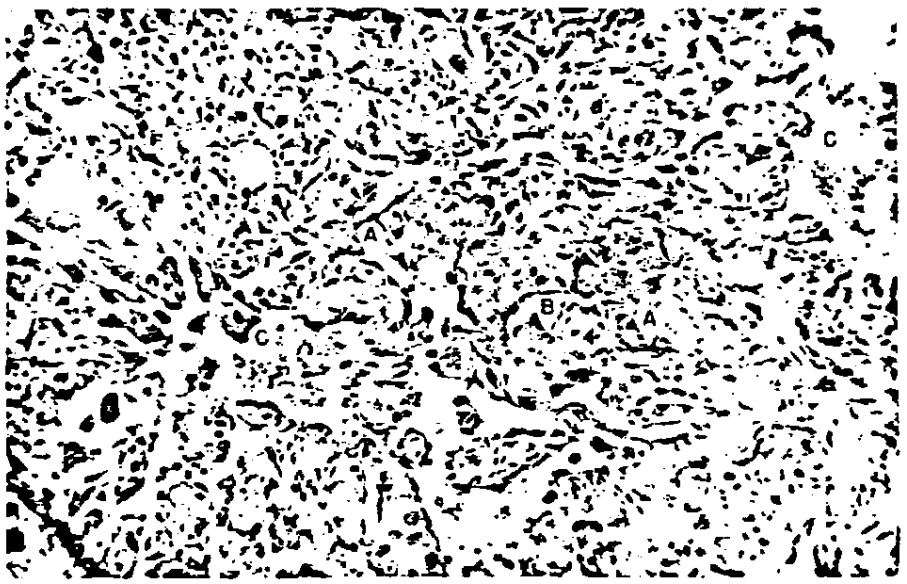

FIGURE 2. Hepatic hemangiosarcoma in mice exposed to VC, showing endothelial proliferation (A), invasion of hepatocyces (B), and hemorrhage (C) , H&E, X 52.

2), depending on the VC level and length of exposure. The incidence of hepatic hemangiosarcoma was also related to the VC level and to the length of exposure (Table 2). Totals of 3, 23, and 31 mice exposed to 50, 250, and 1000 ppm, respectively, developed hemangiosarcoma in the liver. Hepatic hemangiosarcoma appeared to occur more in females than in males exposed to 250 or 1000 ppm of VC. However, the differences between sexes were not statistically significant. Extrahepatic hemangiosarcomas occasionally occurred in mammary gland, heart, gastrointestinal tract, pancreas, kidney, epididymis, testis, mesenteric lymph nodes, and skeletal muscle. The incidence of extrahepatic hemangiosarcoma was not related to the VC level or to the length of exposure. Hemangiosarcoma was not found in any control mice. In the mice exposed to 55 ppm of VDC, hemangiosarcomas occurred in the livers of two males and one female. There were also hemangiomas in the mediastinum of one female exposed to 50 ppm of VC and in the connective tissue adjacent to the salivary gland of one male exposed to 1000 ppm.

Mammary tumors. Mammary gland tumors were observed in females exposed to various levels of VC starting in the 6th or 7th mo. The tumors were adenocarcinomas and squamous and anaplastic cell carcinomas (Fig. 3). The adenocarcinoma was characterized by proliferation of ductular epithelium with a marked anaplastic and squamous cell metaplasia; the

TABLE 2. Incidence of Hemangiosarcoma in Male (M) and Female (F) Mice Exposed to VC or VDC

Exposure time (mo)	VC (ppm) 0 M	0 F	50 M	50 F	250 M	250 F	1000 M	1000 F	VDC (55 ppm) M	VDC (55 ppm) F
					In liver					
6	0/16[a]	0/16	0/17	0/17	0/16	2/19	2/18	3/17	0/16	0/16
7–9	0/6	0/4	1/7	0/14	5/11	14/15	11/15	15/19	1/6	0/4
10–12	0/4	0/16	2/5	0/3	2/2	0/0	0/0	0/0	1/13	1/15
Total	0/26	0/36	3/29	0/34	7[b]/29	16[b]/34	13[b]/33	18/36	2/35	1/35
					In other organs					
6	0/16	0/16	0/17	0/17	0/16	0/19	0/18	2/17	0/16	0/16
7–9	0/6	0/4	1/7	0/14	1/11	3/15	0/15	7/19	0/6	0/4
10–12	0/4	0/16	4/5	1/3	1/2	0/0	0/0	0/0	0/13	0/15
Total	0/26	0/36	5[b]/29	1/34	2/29	3/34	0/33	9[b,c]/36	0/35	0/35

[a]Entries indicate no. of mice with hemangiosarcoma/no. of mice examined.
[b]Significantly different from incidence in control group by Fisher exact probability test (Siegel, 1956), $p < 0.05$.
[c]Significantly different from incidence in opposite sex exposed to same concentration by Fisher exact probability test (Siegel, 1956), $p < 0.05$.

squamous cell carcinoma was characterized by marked proliferation of stratified squamous epithelium, marked keratinization, marked purulent inflammation, and necrosis; and the anaplastic carcinoma was characterized by marked proliferation of undifferentiated cells in large sheets, irregular cords, and packets. These tumors occurred in 9, 3, and 13 females exposed to 50, 250, and 1000 ppm, respectively (Table 3). Most of these mice had a combination of the various tumors. Metastatic clusters of squamous and/or anaplastic cell carcinomas were also found adjacent to the pleura and/or in the lung of most of these females (Fig. 4). These primary and metastatic mammary gland tumors were more severe in the mice exposed to higher levels of VC and in the mice that died or were terminated at a later date. In the group exposed to 1000 or 250 ppm, the females developed these tumors earlier. Mammary gland tumors were not found in any control mice or mice exposed to 55 ppm of VDC.

Malignant lymphoma. During the 6th mo, a malignant lymphoma characterized by marked disseminated or diffused infiltration of lymphoreticular cells in the epicardium and myocardium and the perivascular and interstitial areas of the lung, liver, spleen, and kidney was found in one female mouse exposed to 50 ppm of VC. There was a loss of splenic architecture (Fig. 5). In addition, a malignant lymphoma characterized by a large mass of lymphoreticular cells and necrotic debris, infiltrating the

cervical tissue surrounding the trachea, blood vessels, and esophagus, was found in one male exposed to 1000 ppm. During the 9th mo, malignant lymphomas involving spleen, liver, lung, heart, subcutaneous tissue in the cervical area, and/or mammary gland were found in two females exposed to 250 ppm and one male and three females exposed to 1000 ppm. Malignant lymphoma was not found in any control mice or mice exposed to 55 ppm of VDC at any time.

Hepatoma and other tumors. A total of three mice exposed to 55 ppm of VDC developed hepatomas. This tumor was found in one male terminated during the 9th mo and in one male and one female terminated during the 12th mo. The hepatoma was characterized by marked proliferation of hepatocytes with loss of the lobular pattern, except in the male mouse terminated in the 12th mo. This mouse had only a tiny focus of the neoplastic cells. Hepatoma was not found in any control mice or mice

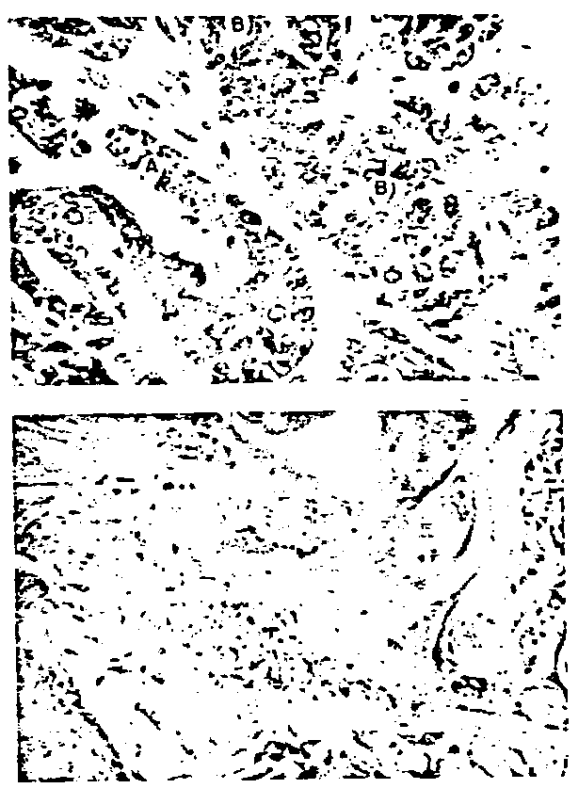

FIGURE 3. Mammary gland tumors in mice exposed to VC. (Top) Ductular adenocarcinoma (A) and anaplastic carcinoma (B), H&E, X 125. (Bottom) Squamous cell carcinoma, H&E, X 50.

TABLE 3. Incidence of Mammary Gland Tumors and Metastasis in the Lung in Female Mice Exposed to VC

Exposure time (mo)	VC concentration (ppm)			
	0	50	250	1000
	Mammary gland tumors			
6	0/16[a]	0/17	1/19	3/17
7–9	0/4	7/14	2/15	10/19
10–12	0/16	2/3	0/0	0/0
Total	0/36	9/34	3/34	13/36
	Metastasis in the lung			
6	0/16	0/17	1/19	3/17
7–9	0/4	2/14	1/15	5/19
10–12	0/16	0/3	0/0	0/0
Total	0/36	2/34	2/34	8/36

[a]Entries indicate no. of mice with tumors or metastasis/no. of mice examined.

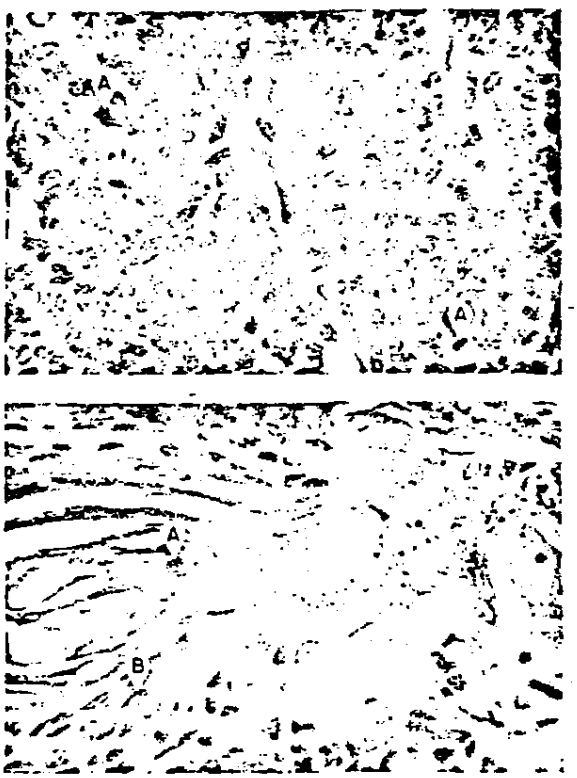

FIGURE 4. Metastatic mammary gland carcinomas in the lungs of mice exposed to VDC. (Top) Anaplastic carcinoma, mitotic figures (A), H&E, X 125. (Bottom) Squamous cell carcinoma, keratinization (A) and stratified squamous epithelium (B), H&E, X 125.

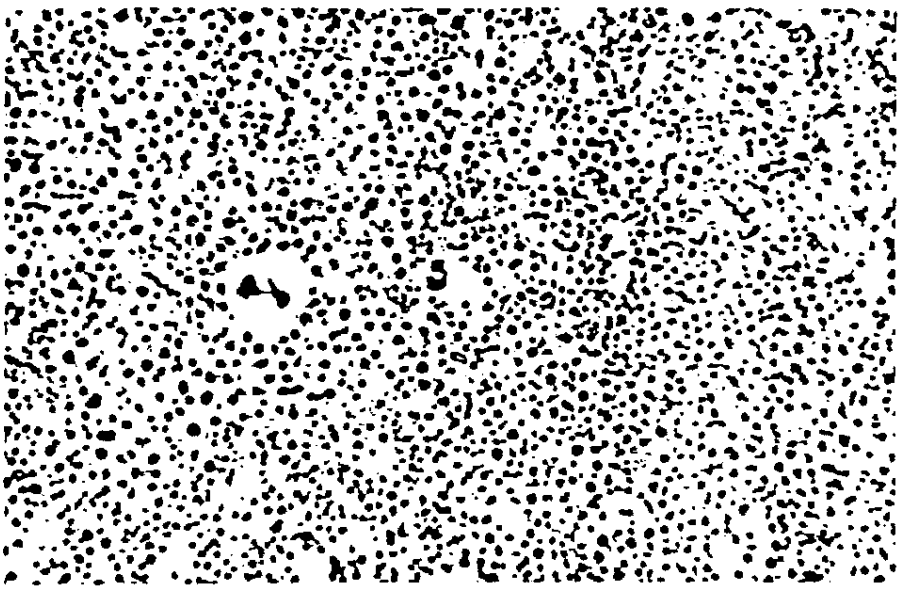

FIGURE 5. Malignant lymphoma in the spleen of mice exposed to VC, showing proliferation of lymphocytes and loss of splenic architecture, H&E, X 80.

exposed to any levels of VC. There were a hepatic cell carcinoma and a renal adenoma in one mouse each exposed to 50 or 1000 ppm of VC, and skin keratoacanthomas in two mice exposed to 55 ppm of VDC.

Rats

General conditions. A number of rats had rough hair coat, lost muscular tone and weight, and were lethargic after 7 mo. Eight males and 13 females exposed to 1000 ppm of VC died or were terminated during the 8th–12th mo. Four males and 10 females exposed to 250 ppm died or were terminated during the same period. Two females exposed to 50 ppm died. No deaths occurred in the control group. One female rat exposed to 55 ppm of VDC was terminated during the 9th mo.

Hemangiosarcoma. All the rats that died or were terminated ahead of schedule and a number of the rats that were terminated on schedule during the 9th–12th mo developed hemangiosarcomas. During the 9th mo, hemangiosarcomas were found in the livers of two rats exposed to 250 ppm of VC and four rats exposed to 1000 ppm. By the end of the 12th mo, hepatic hemangiosarcomas were found in the livers of 12 and 21 rats exposed to 250 and 1000 ppm, respectively. Three of these rats exposed to 250 ppm and 13 of them exposed to 1000 ppm also had hemangiosarcomas in the lung (Fig. 6). As shown in Table 4, hemangiosarcomas in the liver occurred more in the females than in the males at 1000 ppm. Hemangiosarcomas also occurred in two rats (subcutaneous) exposed to 50

ppm of VC, two rats (omentum or mesentery) exposed to 250 ppm, one rat (omentum) exposed to 1000 ppm, and two rats (mesenteric lymph node or subcutaneous) exposed to 55 ppm of VDC. Hemangiosarcomas were not found in the liver, lungs, or any other organs of any control rats. There were also hemangiomas in the adrenal glands of two rats exposed to 1000 ppm of VC.

Other tumors. A few other tumors occasionally occurred in one or several rats. The tumors included a small nodule of bronchioloalveolar adenoma; reticuloendothelial cell carcinoma or hepatoma in the liver; ductular adenocarcinoma or fibroadenoma in the mammary gland of the female; malignant lymphoma in the spleen or other organs; adenoma in the kidney; squamous carcinoma, keratoacanthoma, or fibroma in the skin; adenocarcinoma in the sebaceous gland; and chromophobe cell

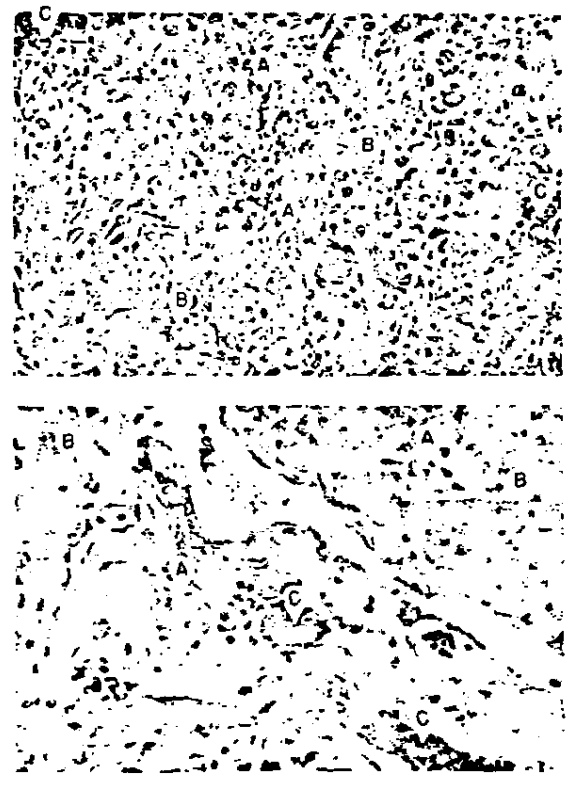

FIGURE 6. Hemangiosarcoma in rats exposed to VC. (Top) Liver, showing endothelial proliferation (A), invasion of hepatocytes (B), and hemorrhage (C), H&E, X 50. (Bottom) Lung, showing endothelial proliferation (A), invasion of alveoli (B), and hemorrhage (C), H&E, X 80.

TABLE 4. Number of Male and Female Rats Exposed to VC or VDC that Developed Hemangiosarcoma

	VC (ppm)				VDC (ppm)
Sex	0	50	250	1000	55
			In the liver		
Male	0/35[a]	0/36	2/36	6/34	0/36
Female	0/35	0/36	10[b]/34	15[b,c]/36	0/35
			In the lungs		
Male	0/35	0/36	0/36	4/34	0/36
Female	0/35	0/36	3/34	9[b]/36	0/35
			In other organs		
Male	0/35	1/36	2/36	0/34	2/36
Female	0/35	1/36	0/34	1/36	0/35

[a]Entries indicate no. of rats with hemangiosarcoma/no. of rats examined.
[b]Significantly different from incidence in control group by Fisher exact probability test (Siegel, 1956), $p < 0.05$.
[c]Significantly different from incidence in opposite sex exposed to same concentration by Fisher exact probability test (Siegel, 1956), $p < 0.05$.

adenoma in the pituitary. These occasional tumors were not related to VC or VDC.

DISCUSSION AND CONCLUSIONS

Exposure to 50, 250, or 1000 ppm of VC, 6 h/d, 5 d/wk, was highly carcinogenic in mice. Bronchioloalveolar adenomas, mammary gland tumors, and hemangiosarcomas developed in these mice. The incidence and severity of these tumors were related to the level of VC and to the length of exposure. A few mice exposed to VC also developed malignant lymphomas. The total incidence of various tumors would probably be considerably higher if some of the mice had not been terminated early.

Bronchioloalveolar adenomas were observed starting in the 2d mo. Bronchioloalveolar adenoma, bronchiolar adenoma, or pulmonary adenomatosis has been reported to occur spontaneously in aging mice, mostly over 1 yr of age (Amaral-Mendes, 1969; Baillif and Jones, 1973; Deerberg et al., 1974). However, in the present study, large number of mice exposed to various levels of VC developed this tumor. In addition, the tumors were observed at a very early age; the incidence and severity were related to the VC level and the length of exposure. On the other hand, only a few small nodules of this tumor occurred at later times in

one control and several mice treated with 55 ppm of VDC. Its significance in VDC mice is questionable.

Hemangiosarcomas, primarily in the liver, were observed starting in the 6th mo, especially in the mice exposed to 250 or 1000 ppm of VC. Hepatic hemangiosarcomas also occurred in three mice exposed to 55 ppm of VDC. The severity of these tumors and the mammary gland tumors in females exposed to VC probably contributed to the deaths of most of the mice.

Mammary gland tumors were observed only in female mice starting in the 6th mo, and consisted of ductular adenocarcinoma and squamous and anaplastic cell carcinomas. The mammary gland tumor was the most complex. Observations of this tumor at several stages and sizes suggested that the tumor originated as ductular adenocarcinoma and then in a very early stage underwent an anaplastic and squamous cell metaplasia. The tumor at this stage appeared quite malignant and invasive. In many cases, there were metastases of the anaplastic and squamous cell carcinomas in the lung. In addition, the ductular or alveolar involvement seemed to be only minimal except in the early stages of some small tumors. This pattern is very different from that of spontaneously occurring mammary gland tumors in mice.

The incidence and severity of the mammary gland tumors appeared to be greater in mice exposed to higher levels of VC and in mice exposed for the longest periods of time. This may explain the higher incidence of mammary tumors in the group exposed to 50 ppm compared with the group exposed to 250 ppm. All females exposed to 250 ppm died by the end of the 9th mo, while many females exposed to 50 ppm survived beyond this time. This increased exposure time in the latter group may thus account for the greater number of tumors.

The significance of hepatomas in three mice as related to the exposure of VDC was considered questionable. Such tumors have been reported to occur spontaneously in small numbers at this age (Andervont, 1950; Percy and Jonas, 1971; Shen, 1974), even though they did not occur in any of the control animals. The other occasional tumors observed in mice were not related to exposure of VC or VDC.

Rats were more resistant to the carcinogenic effects of VC or VDC. Hepatic hemangiosarcomas were observed in rats exposed to 250 or 1000 ppm of VC starting in the 9th mo. In contrast to the mice, many of the rats with hepatic hemangiosarcomas also developed hemangiosarcomas in the lung. VC did not cause any other tumors in the rat. Two rats exposed to 55 ppm of VDC developed hemangiosarcomas in the mesenteric lymph node or subcutaneous tissue; these tumors were probably caused by VDC. The rats were also found to be more resistant than mice to the acute or other chronic effects of VC or VDC, as reported elsewhere (Lee et al., 1977).

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