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# FOLLOW-UP STUDY ON THE CARCINOGENICITY OF VINYL CHLORIDE AND VINYLIDENE CHLORIDE IN RATS AND MICE: TUMOR INCIDENCE AND MORTALITY SUBSEQUENT TO EXPOSURE

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*Carcinogenic and other toxic effects in rats and mice were examined during a 12-mo period following exposure to vinyl chloride (VC) or vinylidene chloride (VDC). Exposure of male and female mice to 50, 250, or 1000 ppm VC for 6 h/d, 5 d/wk, for 1, 3, or 6 mo resulted in increased numbers of deaths and increased moribundity at all dose levels during the exposure and postexposure periods, as compared with air-exposed controls. Similar observations were made with rats after 1, 3, 6, or 10 mo exposure to VC. Cumulative tumor incidence at various organ sites also increased in both species during the postexposure period in proportion to dose or duration of exposure at higher dose levels. However, except for mammary gland tumors in female mice, no significant increase in cumulative tumor incidence occurred in either species at 50 ppm VC or 25 ppm VDC, regardless of duration of exposure. These results suggest that exposure to vinyl halides at dose levels lower than those that elicit a significant increase in cancer incidence during the lifetime of the animal may, nonetheless, increase the risk of early death or moribundity from toxic pre- or subcarcinogenic effects. At dose levels higher than those consistent with the physiological defense or repair capabilities of the cell, ultimate tumor incidence becomes proportionate to length of exposure and may reflect the number of carcinogenic events elicited during the exposure period.*

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## INTRODUCTION

The carcinogenic effect of vinyl halides in laboratory animals (Viola et al., 1971; Maltoni, 1975, 1977; Vainio, 1978; Caputo et al., 1974) and in humans (Creech and Johnson, 1974; Bingham and Lane, 1980) is well known. In studies from these laboratories (Lee et al., 1978), mice and rats were exposed to vinyl chloride (VC) or vinylidene chloride (VDC) for various lengths of time up to 12 mo. Exposure of mice to 50, 250, or 1000 ppm VC resulted in the development of bronchioloalveolar adenomas, hepatic and extrahepatic hemangiosarcomas, and mammary gland tumors between 2 and 6 mo. Exposure of rats to 250 or 1000 ppm VC resulted in the development of hemangiosarcomas of the liver, lung, and other tissues during the 9th mo of the study period. Furthermore, exposure of mice and rats to 55 ppm VDC resulted in an increased incidence of hepatic and extrahepatic hemangiosarcomas during the exposure. Few studies, however, have been conducted to determine the cumulative incidence of carcinogenic effects during a postexposure period. Such studies are essential for the development of effective animal models to assess dose dependence and time-to-tumor variations in human responses to carcinogenic substances.

The work reported here was a sequel to the previous investigations (Lee et al., 1978); it was designed to evaluate the development and incidence of neoplastic changes and other effects during a 12-mo post-exposure follow-up period in rats and mice exposed to VC or VDC for various lengths of time.

## METHODS

### Materials

VC gas (99.8% pure) was obtained from Matheson Gas Products (East Rutherford, N.J.) and metered with rotameters inserted into the chamber air supply. VDC (99% pure) was obtained from Aldrich Chemical Co. (Milwaukee, Wis.) and was heated to 37°C to generate the vapor. The rotameter and the VDC supply lines were heated to 40°C to prevent condensation.

### Animals

Male and female albino CD-1 mice and CD rats (Charles River Breeding Laboratories, Wilmington, Mass.) acquired at 2 mo of age were used as described by Lee et al. (1978). Pulverized or block laboratory animal food (Wayne Lab Blox) and water were given *ad libitum* except during exposure, which was carried out between 9:00 a.m. and 3:00 p.m. on weekdays. Animal rooms were maintained at 24 ± 1.3°C, with 50 ± 10% relative humidity and a 12-h light cycle throughout the exposure and recovery periods.

### Exposure and Experimental Design

Eight to 28 mice of each sex and 4–16 rats of each sex were exposed to 50, 250, or 1000 ppm VC, 55 ppm VDC, or filtered air for 6 h/d, 5 d/wk. Inhalation chambers and chamber air monitoring have been described (Lee et al., 1978). Since the previous studies demonstrated that most mice exposed to 250 or 1000 ppm VC died between the 7th and 9th mo of exposure and most rats died between the 10th and 12th mo, those species in the present study were exposed for 1, 3, and 6 mo and 1, 3, 6, and 10 mo, respectively. At the end of the exposure period, all animals were removed from exposure chambers and maintained in their respective animal rooms for a 12-mo follow-up observation period. All animals were the same age (approximately 2 mo) at the initiation of exposure. Animals in each exposure group were maintained under control conditions before and after exposure periods. Controls were handled exactly as experimental animals with respect to daily transfers to inhalation chambers and other procedures. All animals were observed throughout the study for adverse signs. Food consumption was recorded weekly and body weight biweekly. Clinical laboratory tests and pathological studies were performed as described previously (Lee et al., 1977). When determined to be in a moribund condition, or at scheduled termination times, animals were sacrificed with ether and necropsied. Gross examination, especially for any abnormal growth or other lesions, was carefully performed on the entire animal, with special attention to mammary gland, lung, liver, spleen, kidney, and other tissues with pathological changes. These tissues were fixed, processed, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination. All external and internal tumors were examined and identified histologically.

### Statistical Analysis

A one-tailed Fisher exact probability test (Siegel, 1956) was used to compare tumor incidence between control and exposed groups at each dose level. No correction was made to ensure an overall significance level of 0.05 for the multiple simultaneous comparisons. The Armitage (1971) test for linear trends in proportions was used to test for a trend in the three treated groups. Under the assumption of a linear trend, this test determines whether the slope of the dose-response curve is different from zero (one-tailed) and whether a significant departure from linearity occurs (two-tailed).

## RESULTS

### Follow-up Studies with Mice

*Early Deaths or Terminations* A number of mice died or were terminated in a moribund condition during the exposure and follow-up

TABLE 1. Number of Deaths and Early Terminations and Total Number of Mice Exposed to Filtered Air (Control), VC, or VDC for 1, 3, or 6 mo followed by Recovery for 12 mo

| Month during follow-up period | VC (ppm) | | | | | | | | VDC (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 50 | | 250 | | 1000 | | 55 | |
| | M | F | M | F | M | F | M | F | M | F |
| **After exposure for 1 mo** | | | | | | | | | | |
| 0–3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3–6 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |
| 6–9 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 0 | 1 |
| 9–12 | 1[a] | 0 | 1 | 0 | 1 | 2 | 5 | 1 | 0 | 0 |
| Total | 1/16[b] | 1/16 | 3/16 | 1/16 | 2/16 | 3/16 | 8/16 | 2/16 | 0/8 | 1/8 |
| **After exposure for 3 mo** | | | | | | | | | | |
| During exposure | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | 0 |
| 0–3 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 3–6 | 0 | 0 | 0 | 1 | 1 | 4 | 6 | 0 | 0 | 0 |
| 6–9 | 1 | 1 | 1 | 3 | 3 | 2 | 0 | 4 | 2 | 1 |
| 9–12 | 1 | 1 | 2 | 1 | 4 | 3 | 0 | 3 | 1 | 0 |
| Total | 4/16 | 2/16 | 3/16 | 5/16 | 8/16 | 11/16 | 7/10 | 8/10 | 4/8 | 1/8 |
| **After exposure for 6 mo** | | | | | | | | | | |
| During exposure | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 4 | 0 | 0 |
| 0–3 | 2 | 0 | 1 | 2 | 3 | 3 | 3 | 6 | 1 | 1 |
| 3–6 | 1 | 1 | 0 | 2 | 3 | 4 | 7 | 2 | 1 | 1 |
| 6–9 | 2 | 0 | 1 | 4 | 4 | 0 | 0 | 0 | 1 | 0 |
| 9–12 | 1 | 4 | 5 | 0 | 1 | 0 | 1 | 0 | 3 | 3 |
| Total | 6/28 | 5/28 | 7/8 | 8/8 | 12/12 | 8/8 | 12/12 | 12/12 | 6/12 | 5/12 |

[a] Number of deaths and early terminations during the period.
[b] Number of deaths and early terminations over total number of mice studied.

periods (Table 1). Clinical signs included rough coat hair, lethargy, and the appearance of external tumor masses, particularly mammary tumors in females. There was no significant sex-related difference in number of deaths and early terminatiosn in any group, although substantially more males than females died after exposure to 1000 ppm VC for 1 mo. The number of deaths and early terminations rose with increased VC dose and increased length of exposure. After exposure for 6 mo, 11 of 56 (20%) control mice died or were terminated during the follow-up period. A total of 15 of 16 (94%) mice exposed to 50 ppm VC and all mice exposed to 250 or 1000 ppm VC died or were terminated during this period. Most deaths and early terminations among the mice exposed to 250 ppm occurred within 9 mo after exposure, and among the mice exposed to 1000 ppm within 6 mo after exposure. In addition, 2 of 20 (10%) mice exposed to 250 ppm and 5 of 24 (21%) mice exposed to 1000 ppm were

.posed to Filtered

| VDC (ppm) | |
|---|---|
| 55 | |
| M | F |
| 0 | 0 |
| 0 | 0 |
| 0 | 1 |
| 0 | 0 |
| 0/8 | 1/8 |
| 0 | 0 |
| 1 | 0 |
| 0 | 0 |
| 2 | 1 |
| 1 | 0 |
| 4/8 | 1/8 |
| 0 | 0 |
| 1 | 1 |
| 1 | 1 |
| 1 | 0 |
| 3 | 3 |
| 6/12 | 5/12 |

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terminated during the exposure period. A total of 11 of 24 (46%) mice exposed to 55 ppm VDC for 6 mo died or were terminated.

VC-induced Tumors Hepatic hemangiosarcoma was seen in 1 of 120 (0.8%) control mice (Table 2). This type of tumor occurred during the recovery period in 2 of 80 (2.5%) mice exposed to 50 ppm VC. One such tumor occurred in a male exposed to VC for 1 mo and another in a female exposed for 6 mo. The incidence and severity of this tumor rose as the concentration and duration of VC exposure increased. The cumulative incidence was 13 of 84 (15%) or 18 of 76 (24%) in mice exposed to 250 or 1000 ppm, respectively. In addition, hemangiosarcoma occurred in the skin or peritoneum/mesentery of several mice exposed to VC. Hepatic hemangiosarcoma was also found in rats in the present study and in mice and rats in a previous study (Lee et al., 1978) after exposure to VC. This tumor was mostly multiple in site distribution and varied greatly in size. Microscopically, the tumor consisted of proliferating primitive endothelia, which formed wide cords, expanded outward, and replaced the hepatocytes with blood spaces of various sizes. Neoplastic cells with hyperchromatic nuclei were basophilic, very pleomorphic, and anaplastic. Hemangiosarcomas of the mesentery or subcutaneous tissue were slightly different. Their neoplastic endothelial cells were somewhat uniform, resembled fibroblasts, and were arranged in a loose compartment pattern filled with red blood cells, as was seen in liver. Rupture and hematoma formation from these tumors were seen more often from the mesentery than from the liver.

Bronchioloalveolar tumors were seen in the lungs of a number of control mice after exposure to filtered air for various periods followed by the recovery period (Table 2). The incidence and severity of these tumors were greater in mice exposed to increasing levels of VC and mice exposed for longer durations. The cumulative incidence was 16 of 120 (13%) in control mice and 18 of 80 (22%), 52 of 84 (62%), and 50 of 76 (66%) in mice exposed to 50, 250, and 1000 ppm, respectively.

Mammary gland adenocarcinoma/carcinoma occurred only in female mice. The cumulative incidence was 4 of 60 (7%) in female controls and 10 of 40 (25%), 13 of 40 (32%), and 6 of 38 (16%) in females exposed to the respective levels of VC. Metastatic adenocarcinoma originating from the mammary gland was also seen in lungs of mice exposed to VC, but not in lungs of controls. All six females exposed to 1000 ppm with mammary gland adenocarcinoma/carcinoma also had metastatic adenocarcinoma in the lung.

Bronchioloalveolar and mammary gland tumors were also found in rats in the present study and in mice previously (Lee et al., 1978). Microscopically, the bronchioloalveolar tumor was acinar or papillary growth. The tumor was not well delimited and resembled an adenomatous change of the alveolar epithelium. Neoplastic cells in the mammary gland adenocarcinoma were arranged in a variety of ways. Papillary projections and

TABLE 2. Tumor Incidence in Mice Exposed to Filtered Air (Control), VC, or VDC followed by Recovery for 12 mo

| Organ and tumor | VC (ppm) | | | | | | | | VDC (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 50 | | 250 | | 1000 | | 55 | |
| | M | F | M | F | M | F | M | F | M | F |
| **Exposure for 1 mo** | | | | | | | | | | |
| Liver: | | | | | | | | | | |
| Hemangiosarcoma | 0/16[a] | 0/16 | 1/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/8 | 0/8 |
| Hepatocellular tumor | 4/16 | 0/16 | 2/16 | 1/16 | 7/16 | 0/16 | 1/16 | 0/16 | 3/8 | 0/8 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 2/16 | 1/16 | 3/16 | 0/16 | 10/16 | 9/16 | 11/16 | 9/16 | 1/8 | 0/8 |
| Metastatic adenocarcinoma | 0/16 | 0/16 | 0/16 | 1/16 | 0/16 | 0/16 | 0/16 | 0/16 | 0/8 | 0/8 |
| Mammary gland: | | | | | | | | | | |
| Adenocarcinoma/carcinoma | 0/16 | 1/16 | 0/16 | 4/16 | 0/16 | 2/16 | 0/16 | 0/16 | 0/8 | 0/8 |
| **Exposure for 3 mo** | | | | | | | | | | |
| Liver: | | | | | | | | | | |
| Hemangiosarcoma | 0/16 | 0/16 | 0/16 | 0/16 | 1/16 | 3/16 | 1/10 | 4/10 | 0/8 | 0/8 |
| Hepatocellular tumor | 2/16 | 0/16 | 1/16 | 0/16 | 4/16 | 0/16 | 2/10 | 0/10 | 0/8 | 0/8 |
| Various organs: | | | | | | | | | | |
| Hemangiosarcoma | 0/16 | 0/16 | 0/16 | 1/16 | 2/16 | 2/16 | 0/10 | 1/10 | 1/8 | 0/8 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 2/16 | 0/16 | 7/16 | 5/16 | 11/16 | 10/16 | 9/10 | 7/10 | 2/8 | 1/8 |
| Metastatic adenocarcinoma | 0/16 | 0/16 | 0/16 | 2/16 | 0/16 | 1/16 | 0/10 | 1/10 | 0/8 | 0/8 |
| Mammary gland: | | | | | | | | | | |
| Adenocarcinoma/carcinoma | 0/16 | 0/16 | 0/16 | 4/16 | 0/16 | 6/16 | 0/10 | 2/10 | 0/8 | 0/8 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| [illegible] | 0/16 | 0/16 | 0/16 | 1/16 | 2/16 | 2/16 | 0/10 | 1/10 | 1/8 | 0/8 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 2/16 | 0/16 | 7/16 | 5/16 | 11/16 | 10/16 | 9/10 | 7/10 | 2/8 | 1/8 |
| Metastatic adenocarcinoma | 0/16 | 0/16 | 0/16 | 2/16 | 0/16 | 1/16 | 0/10 | 1/10 | 0/8 | 0/8 |
| Mammary gland: | | | | | | | | | | |
| Adenocarcinoma/carcinoma | 0/16 | 0/16 | 0/16 | 4/16 | 0/16 | 6/16 | 0/10 | 2/10 | 0/8 | 0/8 |

Exposure for 6 mo

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver: | | | | | | | | | | |
| Hemangiosarcoma | 0/28 | 1/28 | 0/8 | 1/8 | 7/12 | 2/8 | 5/12 | 8/12 | 0/12 | 0/12 |
| Hepatocellular tumor | 4/28 | 1/28 | 1/8 | 0/8 | 0/12 | 0/8 | 1/12 | 0/12 | 1/12 | 0/12 |
| Various organs: | | | | | | | | | | |
| Hemangiosarcoma | 0/28 | 0/28 | 1/8 | 0/8 | 0/12 | 2/8 | 0/12 | 1/12 | 0/12 | 0/12 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 4/28 | 7/28 | 2/8 | 1/8 | 8/12 | 4/8 | 7/12 | 7/12 | 1/12 | 0/12 |
| Metastatic adenocarcinoma | 0/28 | 0/28 | 0/8 | 1/8 | 0/12 | 1/8 | 0/12 | 1/12 | 0/12 | 0/12 |
| Mammary gland: | | | | | | | | | | |
| Adenocarcinoma/carcinoma | 0/28 | 3/28 | 0/8 | 2/8 | 0/12 | 5/8 | 0/12 | 4/12 | 0/12 | 0/12 |

Cumulative incidence

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver: | | | | | | | | | | |
| Hemangiosarcoma[b] | 0/60 | 1/60 | 1/40 | 1/40 | 8/44[d] | 5/40[c] | 6/38[d] | 12/38 | 0/28 | 0/28 |
| Hepatocellular tumor | 10/60 | 1/60 | 4/40 | 1/40 | 11/44 | 0/40 | 4/38 | 0/38 | 4/28 | 0/28 |
| Various organs: | | | | | | | | | | |
| Hemangiosarcoma | 0/60 | 0/60 | 1/40 | 1/40 | 2/44[d] | 4/40 | 0/38 | 2/38 | 1/28 | 0/28 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor[c] | 8/60 | 8/60 | 12/40 | 6/40 | 29/44[d] | 23/40 | 27/38[d] | 23/38 | 4/28 | 1/28 |
| Metastatic adenocarcinoma | 0/60 | 0/60 | 0/40 | 4/40[e] | 0/44 | 2/40 | 0/38 | 6/38[e] | 0/28 | 0/28 |
| Mammary gland: | | | | | | | | | | |
| Adenocarcinoma/carcinoma | 0/60 | 4/60 | 0/40 | 10/40[e] | 0/44 | 13/40[e] | 0/38 | 6/38 | 0/28 | 0/28 |

[a] Number of mice with tumors over total number of mice studied.
[b] Significant dose-related incidence (combined male and female) for VC.
[c] Significantly nonlinearity in dose-incidence curve (combined male and female) for VC.
[d] Combined incidence in male and female significantly different from controls for VC.
[e] Incidence in females significantly different from controls for VC.

cysts were seen in some cases. Carcinoma of ductular epithelium was mostly anaplastic and mostly malignant. In most cases, the cells were basophilic and arranged in cords of sheets with more stroma than in adenocarcinoma. Squamous differentiation was seen occasionally. A mixture of adenocarcinoma and carcinoma was occasionally noted.

**Other Tumors and Lesions** Numerous hepatocellular tumors were observed in control males as well as in males exposed to various levels of VC (Table 2). They were seen in only one female control and one female exposed to 50 ppm VC. Grossly, tumors on the surface of the liver were solitary or multiple round masses, paler and softer than the surrounding normal parenchyma. Microscopically, the cells were well-differentiated or slightly undifferentiated and were arranged in a trabecular or solid pattern. Several other tumors were occasionally observed in mice in the control or treated groups but were probably not related to VC. The lesions were inflamed and degenerative changes were observed. Amyloidosis was prevalent in many tissues of the older mice.

**Tumors and Lesions in VDC-exposed Mice** As shown in Table 2, hepatocellular tumors occurred in 4 of 28 (14%) male mice and bronchioloalveolar tumors in 5 of 56 (9%) male and female mice exposed to 55 ppm VDC. One male had hemangiosarcoma of the mesentery. However, these and other occasional tumors appeared to be age-related spontaneous lesions, not related to VDC exposure.

### Follow-up Studies with Rats

**Early Deaths and Terminations** As was the case with mice, a number of rats died or were terminated in a moribund condition during the exposure and recovery periods (Table 3). Clinical signs included rough coat hair, lethargy, and external tumor masses. There was no apparent sex-related difference in number of deaths and early terminations in any group. Occasional deaths and early terminations occurred during the recovery period in rats exposed to VC for 1 or 3 mo. Number of deaths and early terminations increased in rats exposed for 6 mo. In rats exposed for 10 mo, 13 of 32 (41%) controls died or were terminated during the exposure and recovery periods. Number of deaths and early terminations rose with increasing VC concentration. One rat exposed to 55 ppm VDC for 1 mo and one rat exposed for 6 mo became moribund and were terminated during the recovery period. A total of 20 of 30 (67%) rats exposed to 55 ppm VDC for 10 mo died or were terminated.

**VC-induced Tumors** Tumor incidence rates of rats following exposure to VC for 1 or 3 mo did not significantly differ from those of controls. Hence, only the tumor occurrence in the 12-mo period following 6 and 10 mo exposure is given (Table 4).

A number of hepatic tumors were found in rats exposed to VC. Squire and Levitt's classification of hepatocellular tumors in rats was used. Neoplastic nodules occurred in 10 of 68 (15%) rats exposed to 250 ppm

TABLE 3. Number of Deaths and Early Terminations and Total Number of Rats Exposed to Filtered Air (Control), VC, or VDC for 1, 3, 6, or 10 mo followed by Recovery for 12 mo

| Month during follow-up period | VC (ppm) | | | | | | | | VDC (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 50 | | 250 | | 1000 | | 55 | |
| | M | F | M | F | M | F | M | F | M | F |
| **After exposure for 1 mo** | | | | | | | | | | |
| 0–3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3–6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6–9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 9–12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0/4[a] | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 0/4 | 1/4 | 0/4 |
| **After exposure for 3 mo** | | | | | | | | | | |
| 0–3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3–6 | 0 | 1[b] | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 6–9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9–12 | 0 | 3 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 0/8 | 4/8 | 0/8 | 2/8 | 0/8 | 0/8 | 1/8 | 0/8 | 0/8 | 0/8 |
| **After exposure for 6 mo** | | | | | | | | | | |
| During exposure | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0–3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 3–6 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 6–9 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 3 | 0 | 0 |
| 9–12 | 0 | 0 | 3 | 0 | 2 | 1 | 0 | 2 | 1 | 0 |
| Total | 2/8 | 1/8 | 3/8 | 0/8 | 2/8 | 2/8 | 5/8 | 5/8 | 1/8 | 0/8 |
| **After exposure for 10 mo** | | | | | | | | | | |
| During exposure | 0 | 2 | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 0 |
| 0–3 | 0 | 1 | 0 | 1 | 3 | 1 | 4 | 3 | 0 | 0 |
| 3–6 | 1 | 0 | 1 | 1 | 1 | 2 | 4 | 3 | 3 | 3 |
| 6–9 | 3 | 1 | 1 | 4 | 5 | 2 | 5 | 3 | 7 | 1 |
| 9–10 | 2 | 3 | 1 | 5 | 2 | 1 | 2 | 5 | 0 | 5 |
| Total | 6/16 | 7/16 | 6/10 | 11/16 | 11/16 | 7/12 | 15/16 | 15/16 | 11/14 | 9/16 |

[a] Number of deaths and early terminations over total number of rats studied.
[b] Number of deaths and early terminations during the period.

for various lengths of time followed by a 12-mo recovery period and in 2 of 72 (3%) rats exposed to 1000 ppm. Hepatocellular carcinomas occurred in 2 of 68 (3%) and 7 of 72 (10%) rats exposed to 250 or 1000 ppm, respectively. Hepatic hemangiosarcomas occurred in 5 of 68 (7%) and 14 of 72 (19%) rats exposed to the respective levels of VC. These hepatic tumors were not seen in rats exposed to 50 ppm, and hepatocellular carcinoma was seen in only one control male rat. Microscopically, the neoplastic nodules were characterized by "ground-glass" areas of altered

TABLE 4. Tumor Incidence in Rats Exposed to Filtered Air (Control), VC, or VDC followed by Recovery for 12 mo

| Organ and tumor | VC (ppm) | | | | | | | | VDC (ppm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 (control) | | 50 | | 250 | | 1000 | | 55 | |
| | M | F | M | F | M | F | M | F | M | F |
| **Exposure for 6 mo** | | | | | | | | | | |
| Liver: | | | | | | | | | | |
| Neoplastic nodules | 0/20[a] | 0/20 | 0/20 | 0/20 | 3/20 | 1/20 | 1/20 | 0/20 | 0/20 | 0/20 |
| Hepatocellular carcinoma | 0/20 | 0/20 | 0/20 | 0/20 | 1/20 | 0/20 | 2/20 | 0/20 | 0/20 | 0/20 |
| Hemangiosarcoma | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 2/20 | 1/20 | 0/20 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 1/20 | 1/20 | 0/20 | 0/20 |
| Mammary gland: | | | | | | | | | | |
| Fibroadenoma | 0/20 | 1/20 | 0/20 | 5/20 | 0/20 | 2/20 | 0/20 | 2/20 | 0/20 | 1/20 |
| Adenocarcinoma/carcinoma | 0/20 | 1/20 | 0/20 | 2/20 | 0/20 | 1/20 | 0/20 | 0/20 | 0/20 | 0/20 |
| Malignant lymphoma | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 0/20 | 1/20 | 1/20 | 0/20 | 0/20 |
| **Exposure for 10 mo** | | | | | | | | | | |
| Liver: | | | | | | | | | | |
| Neoplastic nodules | 0/16 | 0/16 | 0/10 | 0/16 | 4/16 | 2/12 | 0/16 | 1/16 | 0/14 | 0/16 |
| Hepatocellular carcinoma | 1/16 | 0/16 | 0/10 | 0/16 | 0/16 | 1/12 | 3/16 | 3/16 | 0/14 | 0/16 |
| Hemangiosarcoma | 0/16 | 0/16 | 0/10 | 0/16 | 1/16 | 4/12 | 5/16 | 7/16 | 0/14 | 0/16 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor | 0/16 | 0/16 | 0/10 | 0/16 | 0/16 | 1/12 | 2/16 | 0/16 | 0/14 | 0/16 |
| Hemangiosarcoma | 0/16 | 0/16 | 0/10 | 0/16 | 0/16 | 2/12 | 3/16 | 4/16 | 0/14 | 0/16 |
| Mammary gland: | | | | | | | | | | |
| Fibroadenoma | 0/16 | 4/16 | 0/10 | 6/16 | 0/16 | 7/12 | 0/16 | 3/16 | 0/14 | 4/16 |
| Adenocarcinoma/carcinoma | 0/16 | 0/16 | 0/10 | 2/16 | 0/16 | 0/12 | 0/16 | 0/16 | 0/14 | 0/16 |
| Malignant lymphoma | 0/16 | 0/16 | 0/10 | 0/16 | 1/16 | 0/12 | 2/16 | 0/16 | 0/14 | 0/16 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hemangiosarcoma | 0/16 | 0/16 | 0/10 | 0/16 | 0/16 | 2/12 | 3/16 | 4/16 | 0/14 | 0/16 |
| Mammary gland: | | | | | | | | | | |
| Fibroadenoma | 0/16 | 4/16 | 0/10 | 6/16 | 0/16 | 7/12 | 0/16 | 3/16 | 0/14 | 4/16 |
| Adenocarcinoma/carcinoma | 0/16 | 0/16 | 0/10 | 2/16 | 0/16 | 0/12 | 0/16 | 0/16 | 0/14 | 0/16 |
| Malignant lymphoma | 0/16 | 0/16 | 0/10 | 0/16 | 1/16 | 0/12 | 2/16 | 0/16 | 0/14 | 0/16 |

Cumulative Incidence

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver: | | | | | | | | | | |
| Neoplastic nodules[b] | 0/36 | 0/36 | 0/30 | 0/36 | 7/36[c] | 3/32 | 1/36 | 1/36 | 0/34 | 0/36 |
| Hepatocellular carcinoma[d] | 1/36 | 0/36 | 0/30 | 0/36 | 1/36 | 1/32 | 4/36[c] | 3/36 | 0/34 | 0/36 |
| Hemangiosarcoma[d] | 0/36 | 0/36 | 0/30 | 0/36 | 1/36[c] | 4/32 | 5/36[c] | 9/36 | 1/34 | 0/36 |
| Lung: | | | | | | | | | | |
| Bronchioloalveolar tumor[d] | 0/36 | 0/36 | 0/30 | 0/36 | 1/36 | 1/32 | 3/36 | 1/36 | 0/34 | 0/36 |
| Hemangiosarcoma[d] | 0/36 | 0/36 | 0/30 | 0/36 | 0/36 | 2/32 | 3/36 | 4/36 | 0/34 | 0/36 |
| Mammary gland: | | | | | | | | | | |
| Fibroadenoma | 0/36 | 5/36 | 0/30 | 11/36 | 0/36 | 9/32 | 0/36 | 5/36 | 0/34 | 5/36 |
| Adenocarcinoma/carcinoma | 0/36 | 1/36 | 0/30 | 4/36 | 0/36 | 1/32 | 0/36 | 0/36 | 0/34 | 0/36 |
| Malignant lymphoma[d] | 0/36 | 0/36 | 0/30 | 0/36 | 1/36 | 0/32 | 3/36 | 1/36 | 0/34 | 0/36 |

[a] Number of rats with tumors over total number of rats studied.
[b] Significant nonlinearity in dose-incidence curve (combined male and female) for VC.
[c] Combined incidence in male and female significantly different from controls for VC.
[d] Significant dose-related incidence (combined male and female) for VC.

hepatocytes. Each nodule was larger than one liver lobule; their normal lobular architecture was distorted. They compressed to the normal surrounding parenchyma, resulting in a sharp demarcation line. The altered hepatocytes had an eosinophilic and vacuolated cytoplasm and enlarged nuclei. The sinusoids were dilated or obliterated. The hepatocellular carcinomas were larger and the cells were in different stages of differentiation. The tumors included a well-differentiated type and a moderately differentiated type with glandular formation (Fig. 1).

The characteristics of hepatic hemangiosarcoma in rats were similar to those in mice, and approximately half of these rats had pulmonary hemangiosarcoma (Table 4) as well, possibly representing metastasis from the liver. Grossly, this condition was manifested as multiple red patches, slightly firm in consistency, on the surface of the lung. Microscopically, the patches were composed of basophilic and primitive endothelial cells, which encompassed varying amounts of red blood cells and fluid (Fig. 2). Bronchioloalveolar tumors and malignant lymphoma also occurred in some of the rats exposed to 250 or 1000 ppm VC (Table 4). Microscopically, the bronchioloalveolar tumors were similar to those in mice. Malignant lymphoma involving multiple organs, including lung, liver, spleen, kidney, lymph nodes, and bone marrow, was also seen.

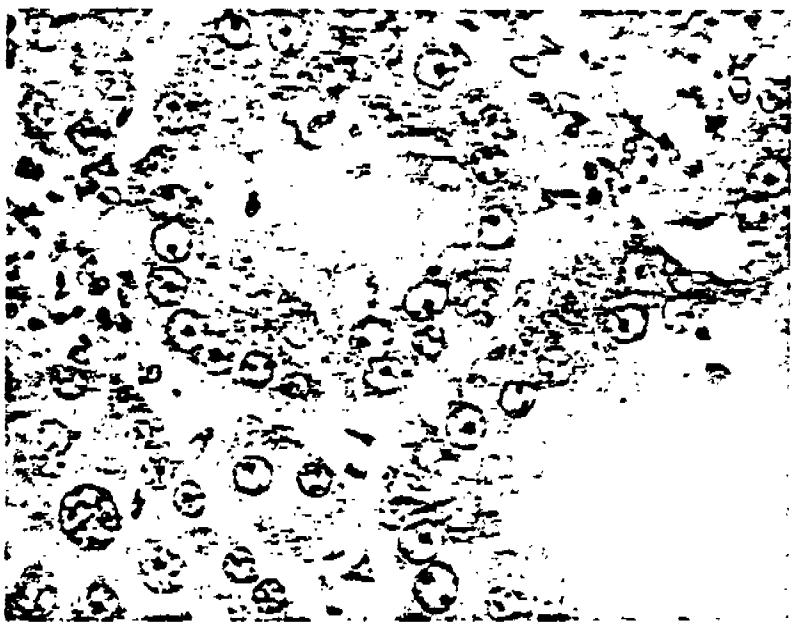

FIGURE 1. Photomicrograph of hepatocellular carcinoma from rat exposed to 1000 ppm VC. Note the glandular arrangement, vesiculated and polyploid nucleus of hepatocytes. H&E. ×750.

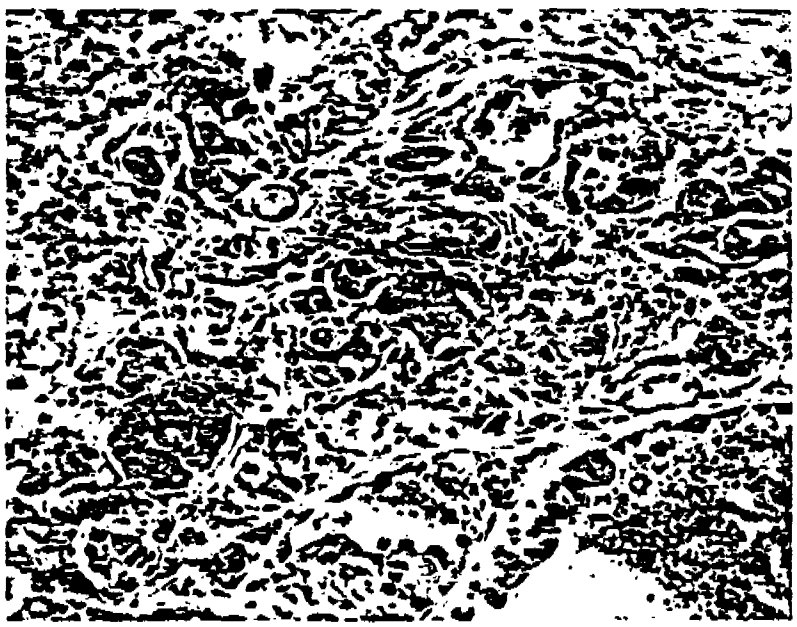

FIGURE 2. Photomicrograph of pulmonary hemangiosarcoma from rat exposed to 1000 ppm VC. Note the proliferation of immature endothelial cells with neovascularization. H&E, ×350.

Other Tumors and Lesions Mammary gland tumors, predominantly fibroadenomas, were observed only in female rats (Table 4). The cumulative incidence of fibroadenomas increased in females exposed to 50 or 250 ppm but not in females exposed to 1000 ppm. Fibroadenoma was characterized by proliferation of epithelial cells with various amounts of connective tissue. Adenocarcinoma/carcinoma was also seen in one female control and several females exposed to 50 or 250 ppm. Chromophobe adenoma of the pituitary gland occurred in some control rats as well as rats exposed to VC. Several other tumors and incidental lesions, as specified in Table 4, were occasionally seen in the control and treated rats. These were considered spontaneous and unrelated to VC exposure.

Tumors and Lesions in VDC-exposed Rats As shown in Table 4, hepatic hemangiosarcoma was observed in one male and mammary gland fibroadenoma in five females exposed to 55 ppm VDC. These and other occasional tumors appeared to be age-related spontaneous lesions, unrelated to VDC exposure.

## DISCUSSION

The purpose of this study was to investigate the development of neoplastic changes in various organ systems over a 1-yr period following

exposure to VC and VDC for various lengths of time. Organ systems such as liver, lung, and mammary gland, in which VC and VDC had been shown in previous studies to produce carcinogenic changes, were of particular interest with respect to tumor development subsequent to exposure. The results indicate that the cumulative incidence of tumors at various organ sites and the number of associated deaths and occurrence of moribundity during the postexposure period increase in proportion to either the dose or the duration of exposure at high dose levels.

The relation of dose or exposure to tumor incidence from VC has been investigated in previous studies (Lee et al., 1978; Caputo et al., 1974; Maltoni and Lefemine, 1974; Winell et al., 1976). Those studies indicated a direct relation between dose level and incidence of tumors of various organ systems both during and after the exposure period. Maltoni (1977), for example, in experiments with rats exposed to 50–30,000 ppm VC for 1 yr, found that the cumulative incidence of hepatic tumors at the end of the exposure period and 18 mo after cessation of exposure increased with dose. This may reflect a decrease in the capacity of carcinogen-exposed cells to repair or resist the formation of lesions by carcinogenic agents such as VC or its reactive metabolites at higher dose levels. In this respect, Gehring et al. (1976) found that, in rat liver cells, physiological defense mechanisms are capable of preventing the induction of carcinogenic lesions by reactive metabolites of VC at dose levels as low as 50 ppm, but not at higher dose levels. Support for such a mechanism is also found in the experiments of Maltoni (1975), who observed successively shorter latency periods for tumor induction in animals exposed to increasing VC doses. These findings have been interpreted (Gehring et al., 1976) as supporting a threshold of carcinogenicity for VC with respect to the dose required to induce tumors in liver and possibly other organ systems. The results reported here are consistent with those observations inasmuch as cumulative tumor incidence for essentially all organ sites in animals exposed to 50 ppm VC or 55 ppm VDC was similar to that in controls (except for mammary gland tumors in female mice). Only at higher dose levels of VC did significant increases in cumulative incidence occur.

Another interesting finding of the present study is that cumulative tumor incidence during the period after VC exposure increased with duration of exposure, independent of dose level. Observations consistent with these findings have been made in studies with other types of carcinogenic chemicals. Topping et al. (1979), for example, showed that 7,12-dimethylbenz[*a*]anthracene (DMBA) produced focal lesions in trachea that did not progress to advanced malignancies until long after cessation of exposure. Similarly, Teebor and Becker (1971) showed that the incidence of hepatic tumors subsequent to exposure to *N*-2-fluorenylacetamide was influenced by the duration of initial exposure. Observations of this nature have also been made with regard to tumors of the skin (Stenback, 1978) and the uterine cervix (Reagan, 1964) in relation to exposure to various

carcinogens. These findings led to the theory (Topping et al., 1979) that numerous focal lesions that may eventually become malignant tumors are induced during exposure to chemical carcinogens, but many such lesions persist for long periods after exposure before progressing to invasive neoplasms. The results reported here are consistent with this theory inasmuch as cumulative tumor incidence in most tissues continued to increase with time after VC exposure. Since tumor incidence would be expected to reflect the number of lesions elicited during the direct exposure to VC, it is reasonable that more potentially malignant lesions are produced as exposure duration increases.

Finally, these results may have important implications with respect to the assessment of risk of cancer or other forms of toxicity resulting from exposure of humans to VC and perhaps other carcinogens. Since epidemiologic studies have not yet provided sufficient information to assess dose dependence and time-to-tumor variations with respect to vinyl halide-induced carcinogenesis in humans, animal models currently represent the best approach to such assessments. Although many critical problems underlying the assumptions of current animal extrapolation models (Whittemore, 1980; Purchase, 1980) remain to be resolved, models such as those of Cornfield (1977) and Guess and Crump (1976) incorporate physiological, biochemical, and pharmacological information into interspecies risk extrapolation procedures. The similarities observed here between the toxic and tumorigenic responses of rats and mice suggest that these findings may be applicable in such model systems for assessing long-term cancer risks in other species. These results may thus contribute to a clearer understanding of the risks of vinyl halide-induced cancer or nonmalignant toxic effects both during and after human exposure to these agents.

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