MRI REPORT

ADDITIONAL EVALUATION OF THE ENVIRONMENTAL TOXICANTS VINYL CHLORIDE (VC) AND VINYLIDENE CHLORIDE (VDC):

(1) Exposure of VC or VDC Followed by Observation for 12 Months in Rats and Mice

(2) The Effect of Disulfiram (DS) on VC Exposure in Mice

FINAL REPORT
January 1976 through December 1978

Contract No. NO1-ES-2-2084
(Continuation of NIH-NIEHS-72-2-2084)

MRI Project No. 3612-B

For

National Institute of Environmental Health Sciences
P.O. Box 12233
Research Triangle Park, NC 27709

Attn: Dr. Robert L. Dixon

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By

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PREFACE

This report was prepared at Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110, under Contract No. NO1-ES-2-2084 (Continuation of NIH-NIEHS-72-2084) with the National Institutes of Health, Department of Health, Education and Welfare, MRI Project No. 3612-B, "Research Capability for Environmental Toxicants." The research was sponsored by the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709. Drs. Robert L. Dixon and James S. Woods are the consecutive Project Officers.

This work was conducted in the Biological Sciences Division, under the directin of Dr. William B. House, between January 1976 and March 1978, and Dr. Harold M. Hubbard, between April 1978 and December 1978. Dr. Cheng-Chun Lee, Assistant/Associate/Deputy Director, Biological Sciences for Pharmacology and Toxicology, was the Principal Investigator. Dr. Joseph M. Winston, Associate Toxicologist, supervised the inhalation chamber operation with the consultation of Dr. William B. House. Necropsy, gross and microscopic examination of tissues were directed and performed by Dr. C. B. Hong, Senior Pathologist, with the assistance of Dr. Larry P. Thornburg, Assistant Professor in Veterinary Pathology, Department of Veterinary Pathology, College of Veterinary Medicine, University of Missouri, Columbia, Missouri, for the microscopic examination of tissues. Mrs. Ellen R. Ellis, Histologist, supervised the histology preparation with the technical assistance of Mr. Kerry L. Crabb and Mrs. Janet L. Kliethermes, and Mr. Ernesto Castillo, Assistant Biologist, supervised the necropsies with the technical assistance of Miss Judith Shifrin and Mr. Hung Hoong, under the direction of Dr. C. B. Hong. Dr. A. Monaem El-hawari and Dr. Robert D. Short, Jr., Senior Toxicologists performed the hepatic drug enzyme studies with the technical assistance of Miss Mary D. Sawyer. Mr. Jack H. Hagensen and Mrs. Karen J. Smith performed the analytical monitoring of chamber concentrations, and chamber and animal care operations.

This report was reviewed by the Quality Assurance Unit of Midwest Research Institute (Mr. Garfield K. Liu) and found to be acceptable according to Good Laboratory Practice Regulations (43 FR 60018, 21 CFR 58.185) for reporting of nonclinical laboratory study results. The raw data, tissues, slides and the final report are stored in our Archives.

Approved for:

MIDWEST RESEARCH INSTITUTE

Florence I. Metz

Florence I. Metz, Acting Director
Biological Sciences Division

January 1979

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(2) The Effect of Disulfiram (DS) on VC Exposure in Mice

FINAL REPORT

ABSTRACT

This report summarizes the results of (1) exposure of VC or VDC, 6 hours/day, 5 days/week, up to 10 months in rats and up to 6 months in mice, followed by observation for 12 months, and (2) DS on VC exposure in mice.

Exposure of rats of both sexes to 50, 250 or 1,000 ppm VC for 1 or 3 months followed by observation of 12 months did not cause any death during the observation period of 12 months following exposure. Exposure for 6 or 10 months increased the number of deaths and early terminations during the exposure and observation periods when compared with the respective control rats. Observation for 12 months after exposure of various levels of VC did not diminish the development of hemangiosarcoma in the liver and lung. In addition, exposure of 50, 250 or 1,000 ppm followed by the observation period caused neoplastic nodules and hepatocellular carcinoma in the liver and possibly malignant lymphoma involving various organs; and increased the incidence of mammary gland tumors, predominantly fibroadenoma and adenocarcinoma/carcinoma, in the females. The increase in deaths and early terminations of rats, and the incidence and severity of tumors, increased with increases in the level of VC and length of exposure.

Exposure of rats to 55 ppm VDC for 10 months, but not for 1, 3 or 6 months, increased the deaths and early terminations during the observation period after exposure. Exposure of VDC up to 10 months followed by the observation period did not cause or increase the incidence of any tumors.

Exposure of mice of both sexes to 50, 250 or 1,000 ppm VC for 1, 3 or 6 months followed by observation of 12 months increased the number of deaths and early terminations during the exposure and observation periods. Observation for 12 months after exposure of various levels of VC did not diminish the development of hepatic and extrahepatic hemangiosarcoma, and the increases in incidence of bronchioloalveolar tumor and metastatic adenocarcinoma, originated from the mammary gland, of the lung, and adenocarcinoma/carcinoma of the mammary gland in the females. As seen in rats, the increase in deaths and early terminations of mice, and the incidence and severity of tumors were proportional to the level of VC and length of exposure. In addition, the mice died or were terminated earlier in groups exposed to higher levels of VC and for longer durations.

Exposure of mice to 55 ppm VDC for 3 or 6 months, but not for 1 month, increased the deaths and early terminations during the observation period after exposure. Exposure of VDC up to 6 months followed by the observation period did not cause or increase the incidence of any tumors.

Simultaneous treatment of mice with DS exerted a partial protection against VC-induced carcinogenesis. Treatment of mice with DS during exposure to 250 or 1,000 ppm resulted in an increase in survival time, and appeared to reduce the incidence of VC-induced hepatic hemangiosarcoma in both sexes and VC-induced mammary gland tumors in females, but had no effect on the incidence of VC-induced bronchioloalveolar adenoma. Results of the hepatic cytochrome P-450 concentration and MFO activity studies suggest that DS may act by reducing the activation of VC to reactive metabolites via microsomal metabolism. However, it does not rule out that DS may exert its protective action by increasing the availability of free sulfydryl groups to combine with reactive VC metabolites or by a combination of both these two mechanisms.

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I. INTRODUCTION

Under Contract No. NO1-ES-2-2084 (NIH-NIEHS-2-2084) on "Research Capability for Environmental Toxicants," we have performed various studies on the toxic effects of the two commercially available dithiocarbamates, ferbam and thiram.[1] Following the completion of those studies, the inhalation toxicity of up to 12 months exposure to 50, 250 or 1,000 ppm of vinyl chloride (VC) or 55 ppm of vinylidene chloride (VDC) in mice and rate was studied[2] and reported.[3] Exposure of rats to 250 or 1,000 ppm of VC resulted in the occurrence of hemangiosarcomas of the liver, lung and other extrahepatic tissues starting in the 9th month. Exposure of mice to 50, 250 or 1,000 ppm of VC resulted in bronchioloalveolar adenomas, hepatic and extrahepatic hemangiosarcomas, and mammary gland tumors in females starting in the 2nd and 6th month. Exposure of rats and mice to 55 ppm of VDC resulted in a few extrahepatic or hepatic hemangiosarcomas. Additional studies in which rats and mice were exposed to VC or VDC for varying periods of time followed by a 12-month observation phase were carried out to evaluate the development of tumors during the post-exposure period. This report summarizes the results.

Disulfiram (DS) has been used clinically in the treatment of alcoholism because of its ability to sensitize humans to ethanol. This sensitization is a result of the inhibition by DS of aldehyde dehydrogenase, which causes the accumulation of acetaldehyde and a resulting acetaldehyde syndrome.[4] Among the other biochemical effects of DS is the inhibition of hepatic drug metabolism in the rat.[5,6] Disulfiram has also been demonstrated to inhibit the development of tumors in animals treated with polycyclic hydrocarbons or dimethylhydrazine.[7,8] In addition, recent work has shown that DS protected mice from the toxic effects of vinylidene chloride, a compound structurally related to VC.[9] The present study was also undertaken to determine the effect of DS on the carcinogenicity of VC in mice. In addition, hepatic drug metabolism was evaluated in mice exposed chronically to VC with and without DS treatment in order to assess one potential mechanism by which DS might affect VC-induced carcinogenesis.

II. MATERIAL AND METHODS

Material and methods employed in these studies are described below.

A. Methods of Exposure

Methods of exposure are essentially the same as described previously.[2/]

1. Chamber Design

Five stainless steel cubical type exposure chambers of 3.5 m^3 (123 ft^3) volume were used. Contaminant entered the air stream and was then mixed in a plenum at the top of each chamber. Each chamber contained a diffusion plate and two small squirrel-cage fans (100 cfm, 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.

2. Chamber Air Supply and Flow Rates

The air supply to the chambers was drawn from a stack on the roof of the building. Chamber air passed through a coarse filter and then over coils for heating, cooling, and dehumidifying. The air then passed through an absolute filter (99.97-99.99% retention of 0.3 μ particles) into the plenum of the chamber.

Air flow rates were measured by means of orifice plates installed on the chamber outlet exhause system. Air flow rates were then measured with an air flow transducer (Autotronics 100-SSX). The air flow rate was 26.4 ft^3/min (0.75 m^3/min) or approximately 13 air changes per hour.

3. Generation of VC and VDC Vapor

Vinyl chloride at a purity of 99.8% was obtained from Matheson Products. It is a gas at room temperature, and was metered with rotameters into the chamber air supply. VDC with a purity of 99% was obtained from the Aldrich Company. Its boiling point is 32°C (89.6°F) and was heated to 37°C (98.6°F) to generate the gas. All gas lines and the rotameter were heated to 40°C (104°F) to prevent condensation.

4. Chamber Atmosphere Monitoring

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°C, 65°C and 170°C, respectively.

5. Standards

VC standards at dilutions of 10, 50 and 100 ppm were obtained in lecture bottles from Supelco, Inc., Bellefonte, Pennsylvania. VC standard gas at a concentration of 1,000 ppm was obtained from Matheson Gas Products, Joliet, Illinois.

VDC standards were prepared by a serial dilution (weight/volume) of VDC in carbon tetrachloride. The desired final concentrations in a 4 μl injection were determined by the following procedure:

$$mg/\ell \quad (\mu g/ml) = \frac{ppm \times mw}{24,450}$$

With a molecular weight for VDC of 96.94, the final concentrations of 10, 50 and 100 ppm, the amount of VDC in a 1-ml sample from the chamber would be 0.04, 0.20 or 0.40 μg, respectively. For 1 μl of standard, these same amounts would be obtained from solutions of 40, 200 and 400 mg/liter; but since 4 μl of standard were injected, the final dilutions of VDC in carbon tetrachloride were 10, 50 and 100 mg/liter.

All liquids were injected at a volume of 4 μl and all gases at 1 ml. Standards at each point on the calibration curve were injected in triplicate.

6. Chamber Sampling

The chambers were sampled at least three times daily from a reference point in the center of each chamber; an automatic sampling system was used. The sampling system consisted of 0.25 in. teflon lines that were placed at the reference point in each chamber. The teflon lines ran through a series of sampling valves. The sample lines were purged with chamber atmosphere for several minutes before the anlaytical analysis was initiated. Samples were injected through twin 1 ml sampling loops which were allowed to reach equilibrium before the sample was injected. The sampling sequence was controlled by a Varian CDS-111® chromatography data system. The concentrations were determined by integration of peak areas by the CDS-111®. The integrator was calibrated each day with VC and VDC standards.

B. Safety

All chambers were operated with a slight negative pressure (0.1-0.2 in. water) to prevent escape of the test contaminants from the chambers

into the chamber room environment. Before being opened, the chambers were flushed with clean air for a minimum of 30 min. Room monitoring for VC and VDC was performed regularly by obtaining grab samples from the room environment with a gas-tight syringe, and the samples were analyzed by gas chromatography.

All air from the chambers traveled under negative pressure to a high-temperature incinerator outside the inhalation building prior to release to the surrounding environment. The burner of the incinerator operated at 1700 to 1800°F.

C. Animals

1. Species

Albino CD® rats and albino CD-1® mice (Charles River Breeding Laboratories, North Wilmington, Massachusetts), were used in these studies. All animals were approximately 2 months of age at the start of the inhalation studies. The animals were acclimatized for 1 to 2 weeks before inhalation exposures were begun.

2. Animal Housing

All animals lived in the stainless steel cages during exposure and outside of the chambers. Rats were housed two per cage and mice six to eight per cage. Powdered or block laboratory chow (Wayne Manufacturing Company) was provided at all times except during the 6-hr period of exposure each day. Water was available ad libitum.

The temperature in the chamber and in the room averaged 24 ± 1.3°C (75 ± 5°F). The relative humidity in the chambers ranged from 25 to 60%; in the room it also ranged from 25 to 60%, but was regulated at 45 ± 5% after the 6th month. A 12-hr light cycle was maintained at all times.

When out of the chambers, the animals were placed in hodling racks as described previously.[2/] A continuous supply of room air was drawn over the cages while in the holding racks. Provisions were made for covering the front of each compartment in the holding rack in the event of significant off-gassing from the animals. These procedures were found not to be necessary with VC or VDC.

3. Animal Hygiene

All cages and feeders were washed and sanitized each week. The floors of the room, the holding racks, and the interior of the chambers were frequently washed with disinfectant. Animal caretakers wore gloves and surgical masks when handling animals.

It was found that when the cages were loaded into the chambers without drop-pans (so not to impair air flow) the animals on the bottom shelves were frequently drenched with urine and water. In order to alleviate this problem, the cages were loaded into the chambers with clean drop pans in place. Sampling studies showed that the vapor distribution was actually improved slightly. Drop pans were washed daily and a clean drop pan without bedding was inserted into each cage before loading into the chamber.

D. Experimental Protocol

1. Exposure to VC or VDC and Observed for 12 Months in Rats and Mice

a. Experimental Design

The experimental design originally called for mice and rats to be exposed to 50, 250 or 1,000 ppm VC or 55 ppm VDC 6 hr/day, 5 days/week for periods of 1, 3, 6 or 12 months, followed by 12 months of observation. However, the results of the initial study indicated that most mice exposed to 250 or 1,000 ppm VC died between the 7th and 9th months of exposure, and most rats exposed to these levels died between the 10th and 12th months of exposure.[2,3] Therefore, the experimental design was revised to include the following groups with indicated number of animals and length of exposure. However, this study was carried out simultaneously with the original study[2] whenever the cage and chamber spaces were available and the number in some groups was reduced due to limited space.

Rats

Exposure Groups	Length of Exposure	Number of Animals/ Exposure Group
Filtered air, 50, 250 or 1,000 ppm VC, or 55 ppm VDC	1 month	4 rats/sex/group
	3 months	8 rats/sex/group
	6 months	8 rats/sex/group
	10 months	16 rats/sex/group

Mice (number/sex/group)

		VC, ppm			VDC, ppm
Length of Exposure	Filtered Air	50	250	1,000	55
1 Month	16	16	16	16	8
3 Months	16	16	16	16	8
6 Months	28	12	12	12	12

b. Observations

Exposure for animals from the various groups was discontinued at the respective time. Thereafter, they were moved to separate animal quarters, placed in plastic cages, and observed for 12 months. Animal housing and hygiene were carefully maintained as previously described.

c. Necropsy and Histopathology

When moribund or at the end of the observation period, all animals were sacrificed for necropsy. Gross examination of all tissues, especially for the appearance of abnormal growths or other lesions, was performed. The lung, liver, spleen, kidney, mammary gland and any abnormal tissues were removed, fixed, processed, sectioned, and stained for microscopic examination.

2. Exposure to VC With and Without DS Treatment in Mice

a. Experimental Design

Male and female mice were used in these studies. The animals were housed, eight mice per cage, in stainless steel cages, and were allowed free access to feed and water except during the inhalation exposures when the feed was withheld.

Mice receiving control feed or feed containing 0.1% DS were exposed 6 hr/day, 5 days/week for 49 weeks, to filtered atmospheric air, 50, 250 or 1,000 ppm VC. Each exposure group was composed of four to eight mice of each sex. Feeding of the DS diet began 5 days prior to the first inhalation exposure and continued throughout the 49 weeks of exposure.

b. Necropsy and Histopathology

At the time of death or termination, the tissues of the mice were examined for abnormal growths and lesions. All tumors with adjacent normal tissues and all other tissues without tumors were fixed in 10% buffered formalin, processed, sectioned, and stained with hematoxylin and eosin for microscopic examination.

c. Liver Microsomes

The livers were removed, washed with ice-cold saline, then minced with scissors and homogenized with three volumes of 1.15% KCl containing 0.02 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 g for 20 min, and the post-mitochondrial fractions were centrifuged further for 60 min at 105,000 g. The microsomal pellets were resuspended, thoroughly mixed with the KCl-phosphate buffer, and recentrifuged for 60 min

at 105,000 g. The washed microsomal pellets were layered with KCl-phosphate buffer and stored in a frozen state. Overnight storage (≃14 hr) of microsomes in this manner did not result in significant losses of activity. Before use, the microsomes were suspended in the KCl-phosphate buffer mixture and adjusted to a protein concentration of 3 to 6 mg/ml. Microsomal protein content was determined[10] using bovine serum albumin as the reference protein.

d. Cytochromes P-450 and b_5

Spectral measurements were performed on the 100,000 g microsomal pellets using an ultraviolet spectrophotometer. Cytochrome P-450 and cytochrome b_5 concentrations were measured[11] using extension coefficients of 91 and 164 mM^{-1} cm^{-1}, respectively. The microsomes were diluted with 0.1 M phosphate buffer (pH 7.4) to yield 1 to 2 mg protein/ml.

e. Hepatic Mixed-Function Oxidase (MFO) Activities

Enzyme activities were determined under zero-order conditions regarding cofactor and substrate concentrations and linearity regarding enzyme concentrations and incubation times. Animline hydrozylase, ethylmorphine-N-demethylase and p-nitroanisole-O-demethylase were estimated using 3 mg of microsomal protein suspension. The incubation mixtures containing 5 mM $MgCl_2$, 0.43 mM NADP, 10 mM glucose-6-phosphate, 2 units of glucose-6-phosphate dehydrogenase, 50 mM Tris-HCl buffer, and 5 mM of each substrate. Incubations were carried out at 37°C in air for 10 to 15 min in a Dubnoff metabolic incubator. Aniline hydroxylase activity was determined by measuring the formation of p-nitrophenol at 420 nm.[12] Ethylmorphine-N-demethylase activity was measured by a modification of the procedure of Schenkman et al. (1967);[13] formaldehyde was trapped in semicarbozide (4.1 mM) and estimated according to the method of Nash.[14] The activity of p-nitroanisole-O-demethylase was determined by a modification of the method of Netter and Siedel.[15] The reactions were initiated by the addition of the microsomal protein.

f. Statistical Analyses

Survival and tumor incidence data were analyzed using the Fisher Exact Probability Test.[16] All other data were analyzed by Tukey's Omega Test.[17] A significance level of $p < 0.05$ was used in all cases.

III. RESULTS

The effects of exposure to VC or VDC followed by observation for 12 months were studied in rats and mice; the effects of DS on VC exposure were studied in mice. The results are described in the following sections.

A. Exposure to VC or VDC and Observation for 12 Months in Rats

1. General Observations and Number of Deaths

Rats were exposed to VC or VDC, 6 hr/day, 5 days/week, for periods of 1, 3, 6 or 10 months. During these periods, these rats were exposed for a total of 20, 61, 128, or 213 days, respectively. A number of rats died or were moribund and terminated during the exposure and observation periods. The number of deaths and early terminations depended upon the duration of initial exposure and level of VC. More rats died or were moribund and terminated in groups exposed to higher levels of VC and for longer durations. The clinical signs were rough hair coat, lethargy, and/or the appearance of external tumor masses, especially mammary tumors in the females. Some deaths occurred at night and autolysis took place in a few rats, especially during the weekends.

The number of deaths and early terminations and total number of rats exposed to VC or VDC followed by observation for 12 months are summarized in Table 1. There was no apparent difference in number of deaths and early terminations between the males and females of any group. Occasional deaths and early terminations occurred during the observation period in the control rats and rats of the various groups exposed to VC or VDC for 1 or 3 months. The number of deaths and early terminations increased in the rats during the observation period after being exposed for 6 months. Three of 16 control rats (19%) were terminated during the exposure and observation periods. The number of deaths and early terminations for the mice exposed to 50, 250 or 1,000 ppm VC was 4 (25%), 4 (25%), and 10 (63%), respectively. Only 1 of 16 rats exposed to 55 ppm VC was moribund and terminated during the observation period. In the rats exposed for 10 months, a total of 13 of 32 controls (41%) died or were terminated during the exposure and observation periods. The number of deaths and early terminations in the rats exposed to 50, 250 and 1,000 ppm VC was 17 of 26 (66%), 17 of 28 (61%), and 30 of 32 (94%), respectively, during the same periods. A total of 20 of 30 rats (67%) exposed to 55 ppm VDC died or were terminated during the exposure and observation periods. The distribution of deaths and early terminations spread throughout the observation period.

2. Tumors

The tumor occurrence in rats exposed to VC or VDC for 1, 3 or 6 months followed by observation for 12 months is summarized in Tables 2, 3 and 4, respectively. The tumor occurrence in rats exposed for 10 months followed by observation for 12 months is summarized in Tables 5 through 8.

a. Exposure for 1 Month

After exposure for 1 month followed by the observation period, no tumor was found in any of the control rats or rats exposed to 50 ppm VC (Table 2). Two females exposed to 250 ppm VC had fibroadenoma of the mammary gland; one female also had chromophobe-cell adenoma of the pituitary. Hepatocellular carcinoma occurred in one male exposed to 1,000 ppm VC and hepatic hemangiosarcoma occurred in one male exposed to 55 ppm VC.

b. Exposure for 3 Months

After exposure for 3 months followed by the observation period, epidermoid carcinoma of the lung occurred in one female control; cystadenoma of the ovary and chromophobe-cell adenoma of the pituitary occurred in another female control (Table 3). A few other tumors occurred in rats exposed to various levels of VC. In the lung, there was bronchioloalveolar tumor. In the liver, there were neoplastic nodules, hepatocellular carcinoma, or hemangioma. In the mammary gland, there were fibroadenoma, adenoma, adenocarcinoma, or carcinoma. Other occasional tumors included squamous-cell carcinoma of the skin and chromophobe-cell adenoma of the pituitary. In the rats exposed to 55 ppm VDC, one female had a fibroadenoma of the mammary gland.

c. Exposure for 6 Months

After exposure for 6 months followed by the observation period, similar types of tumors, seen in the previous group of rats exposed for 3 months, also occurred in these rats of the various groups including the controls (Table 4). Three rats exposed to 250 ppm VC had neoplastic nodules and/or hemangioma of the liver, and five rats exposed to 1,000 ppm VC had hepatocellular tumor, bile duct carcinoma, hepatic hemangioma or hemangiosarcoma. These hepatic tumors were not seen in the control or other treated groups. There was malignant lymphoma involving the lung, liver, spleen, and/or kidney of one male and one female rat exposed to 1,000 ppm VC. Other occasional tumors included papilloma, squamous-cell carcinoma or fibrosarcoma of the skin; bronchioloalveolar tumor; fibrosarcoma of the intestine; fibroadenoma or adenocarcinoma of the mammary gland; and chromophobe-cell adenoma of the pituitary. In these groups, a few rats, especially those exposed to 1,000 ppm VC with various tumors, died or were moribund and terminated during the observation period.

d. Exposure for 10 Months

After exposure for 10 months followed by the observation period, the tumor incidence and severity were greatly increased in various groups (Tables 5 through 8). In the control group (Table 5), 11 rats had tumors including fibroma of the skin in three males, hepatocellular tumor in one male, carcinosarcoma of the kidney in one male, adenoma or fibroadenoma of the mammary gland in five females and chromophobe-cell adenoma of the pituitary in one male and three females. Three of the 11 control rats with various tumors died or were terminated during the observation period.

In the group exposed to 50 ppm VC (Table 5), 15 rats had tumors similar to those of the control rats. However, incidence of the mammary gland tumors in the females, including fibroadenoma, adenocarcinoma, or fibrosarcoma, was higher than that seen in the control group. One female with fibrosarcoma in the mammary gland also had fibrosarcoma in the lung. Other occasional tumors included squamous-cell carcinoma or fibroma of the skin, hemangioma of the liver, Islet-cell adenoma of the pancreas, and chromophobe-cell of the pituitary. One male with fibroma and squamous-cell carcinoma of the skin died during the 31st week of exposure. In addition, nine of the remaining 15 rats with various tumors died or were moribund and terminated during the observation period.

In the group exposed to 250 ppm VC (Table 6), 21 rats had different types of tumors in various tissues. In the liver, the tumor incidence and severity were considerably increased over that seen in the group exposed to 50 ppm VC. The hepatic tumors included neoplastic nodules in six rats, hepatocellular in one rat, hemangioma in one rat, and hemangiosarcoma in four rats. Two females with hepatic hemangiosarcoma also had hemangiosarcoma of the lung. In the mammary gland, seven females had fibroadenoma. Other occasional tumors included squamous-cell carcinoma r fibroma of the skin, bronchioloalveolar tumor, adenocarcinoma or carcinoma of the kidney, malignant lymphoma of the spleen, and chromophobe-cell adenoma of the pituitary. One female rat with fibroadenoma of the mammary gland died during the 40th week of exposure and 10 other rats with various tumors died or were terminated during the observation period.

In the group exposed to 1,000 ppm VC (Table 7), 25 rats had different types of tumors in various tissues. In the liver, the tumor incidence and severity were further increased over those seen in the preceding group exposed to 250 ppm VC. The tumors included neoplastic nodules in one rat, hepatocellular carcinoma in six rats, hemangioma in one rat, and hemangiosarcoma in 12 rats. Six of the rats with hepatic hemangiosarcoma also had hemangiosarcoma of the lung. In addition, one other rat without hepatic hemangiosarcoma had hemangiosarcoma of the lung, and two rats had bronchioloalveolar tumor. Malignant lymphoma involving the lung, liver,

spleen, lymph nodes, and/or bone marrow occurred in two rats. Other occasional tumors included squamous-cell carcinoma or sebaceous gland adenocarcinoma of the skin, squamous-cell carcinoma of the nasal cavity, adenocarcinoma of the intestine, metastatic bronchioloalveolar carcinoma of the kidney, granulosal-cell tumor of the ovary, and chromophobe-cell adenoma of the pituitary. One rat with squamous-cell carcinoma of the skin died during the 40th week of exposure, and nearly all the remaining rats with various tumors died or were moribund and terminated during the exposure period.

In the group exposed to 55 ppm VDC (Table 8), the tumor incidence and severity were similar to those seen in the control rats (Table 5). A total of nine rats had tumors including fibroma of the skin in three rats, fibroadenoma of the mammary gland in four rats, and chromophobe-cell adenoma of the pituitary in three rats. One rat with fibroma of the skin died during the 24th week of exposure, and six of the remaining eight rats with various tumors died or were terminated during the observation period.

3. Cumulative Tumor Incidence and Tumorigenesis

The cumulative tumor incidence in control rats and rats exposed to VC or VDC is summarized in Table 9. These rats were exposed to the filtered air, VC or VDC for 1, 3, 6 or 10 months followed by observation for 12 months. It must be pointed out that within each treatment group the life span of all rats was not the same.

a. In the Liver

In the liver, there were three major types of tumors in rats exposed to 250 or 1,000 ppm VC. Neoplastic nodules were seen in seven males and three females of a total of 68 rats (15%) exposed to 250 ppm, and in one male and one female of 72 rats (3%) exposed to 1,000 ppm. Hepatocellular carcinoma occurred in one male of a total of 72 control rats (1%), one male and one female of 68 rats (3%) exposed to 250 ppm, and in four males and three females of 72 rats (10%) exposed to 1,000 ppm. Hemangiosarcoma occurred in one male and four females of a total of 68 rats (7%) exposed to 250 ppm, and in five males and nine females of 72 rats (19%) exposed to 1,000 ppm. Exposure of 55 ppm VDC did not cause any of these tumors. Nevertheless, hemangiosarcoma was seen in the liver of one male exposed to VDC.

Hemangiosarcoma of the liver and lung was similarly reported in rats exposed to 250 or 1,000 ppm VC for 9 to 12 months without the observation period after exposure.[2,3] The characteristics of hepatic hemangiosarcoma are similar to those seen in mice described in the appropriate section. Classification of hepatocellular tumors in rats described by Squire and Levitt[18] was applied in this report. Microscopically, the neoplastic nodules were characterized by ground-glass areas of altered hepatocytes. The size of these nodules were larger than that of one liver lobule; their normal lobular architecture was distorted. They compressed

to the normal surrounding parenchyma, resulting in a sharp demarcating line. The cytoplasma of the altered hepatocytes were eosinophilic and vacuolated, and their nuclei were enlarged. Their sinusoids were dilated or obliterated. In comparing them to neoplastic nodules, hepatocellular carcinomas were larger and the cells were in a great diversity of differentiation. The tumors included a well-differentiated type and a moderately-differentiated type.

Other tumors in the liver of rats exposed to VC included hemangioma and bile duct carcinoma. Hemangioma occurred in one male, one and two females, and two males exposed to 50, 250 or 1,000 ppm VC, respectively. Bile duct carcinoma was seen in one male exposed to 1,000 ppm VC.

b. In the Lung

About half of the rats with hepatic hemangiosarcoma also had hemangiosarcoma in the lung. Hemangiosarcoma of the lung was seen in two females of a total of 68 rats (3%) exposed to 250 ppm VC, and in three males and four females of 72 rats (10%) exposed to 1,000 ppm VC. Grossly, there were multiple red patches on the surface of the lung and slightly firm in consistency. Their cut surface was wet and red. Microscopically, they were composed of the basophilic and immatured endothelia which encompassed varying amounts of RBC and fluid. In addition, bronchioloalveolar tumor occurred in one male and one female exposed to 250 ppm VC, and three males and one female exposed to 1,000 ppm VC. The microscopic feature of the bronchioloalveolar tumor in most rats was similar to that seen in mice. In a few cases, the tumor was not well delimited and resembled adenomatous change of alveolar epithelium. Fibrosarcoma, probably originated from the mammary gland, was seen in one female exposed to 50 ppm VC.

c. In the Mammary Gland

Mammary gland tumors observed only in the female rats and included fibroadenoma, adenoma, adenocarcinoma, carcinoma, and fibrosarcoma. Fibroadenoma was seen in five of 36 (14%) female controls, 11 of 36 (31%), nine of 32 (28%), and five of 36 (14%) females exposed to 50, 250 or 1,000 ppm VC, respectively, and in five of 36 (14%) females exposed to 55 ppm VDC. Adenoma occurred in one female control, one female exposed to 250 ppm VC, and two females exposed to 1,000 ppm VC. Adenocarcinoma/carcinoma occurred in one female control, four females exposed to 50 ppm VC, and one female exposed to 250 ppm VC. There was fibrosarcoma in one female exposed to 50 ppm VC.

Fibroadenoma was characterized by proliferation of both epithelial cells and connective tissues. There was a great variation in structure in terms of amount of connective tissue present. Adenomas were characterized by proliferation of gland epithelium arranged in a uniformly

pattern. The neoplastic cells in adenocarcinoma were arranged in a great variety of ways. Their acinar spaces were varying in size and shape. Papillary projection and cysts were seen in some cases. Carcinoma of ductular epithelium was most anaplastic and most malignant in this group of tumors. In most cases, the cells were basophilic, and arranged in cords or sheets. The stroma in this tumor was much more than in adenocarcinoma. Squamous differentiation was seen occasionally. A mixture of adenocarcinoma and carcinoma was noted in one rat.

d. Malignant Lymphoma

Malignant lymphoma was seen in three males and one female of a total of 72 rats (6%) exposed to 1,000 ppm VC. It involved the lung, liver, spleen, kidney, lymph nodes and/or bone marrow. Malignant lymphoma also occurred in the spleen of one male exposed to 250 ppm VC.

e. Other Organs

A number of other tumors occasionally occurred in the control and/or treated rats. They were spontaneous and were not related to any group. Chromophobe-cell adenoma of the pituitary occurred in one male and four female controls, two males and three females, two males and three females, and two males and two females exposed to 50, 250 and 1,000 ppm VC, respectively, and in four females exposed to 55 ppm VDC. Other occasional tumors included papilloma, squamous-cell carcinoma, sebaceous gland adenocarcinoma, fibroma and fibrosarcoma of the skin; squamous-cell carcinoma of the nasal cavity; adenosarcoma and fibrosarcoma of the intestine, Islet-cell adenoma of the pancreas; adenocarcinoma, carcinoma, carcinosarcoma and metastatic bronchioloaveolar carcinoma of the kidney; cystadenoma and granulosal-cell tumor of the ovary, mesothelioma of the epididymis, and pheochromocytoma of the adrenal gland.

4. Other Lesions

There were a number of spontaneous or incidental lesions in the tissues of control rats and rats exposed to various levels of VC or VDC followed by observation for 12 months. The lesions for rats exposed to VC or VDC for 1, 3, 6 or 10 months followed by the observation period are summarized in Tables 10, 11 through 13, 14 and 15, and 16 through 20, respectively. The lesions include inflammation or abcess of the skin; inflammatory cyst of the mammary gland; fibroplasia, myocarditis or calcification of the heart; pneumonia; fatty degeneration, focal inflammation, cystic changes, bile duct hyperplasia, cholangitis, telangiectasis, and/or necrosis of the liver; nephritis, calcification, or fat infiltration of the kidney; mineralization of the testes; prostatitis; cystic or fatty degeneration of the adrenal; and/or inflammation of the mesentery or peritonium. These lesions were inconsistent and occasionally seen in the control as well as in the treated rats. They are not related to VC or VDC.

B. Exposure to VC or VDC and Observation for 12 Months in Mice

1. General Observations and Number of Deaths

Mice were exposed to VC and VDC, 6 hr/day, 5 days/week, for periods of 1, 3 or 6 months. During these periods, these mice were exposed for a total of 21, 63, or 128 days, respectively. A number of mice died or were moribund and terminated during the exposure and observation periods. The number and time of deaths and early terminations depended upon the duration of initial exposure and level of VC. The clinical signs included rough hair coat, lethargy, and/or the appearance of external tumor masses, especially mammary tumors in the females. A number of mice died at night, and autolysis occurred in some mice, especially during the weekends.

The number of deaths and early terminations and total number of mice exposed to VC and VDC followed by observation for 12 months are summarized in Table 21. There was no apparent difference in number of deaths and early terminations between the males and females of any group. After exposure for 1 month, a total of two of 32 control mice (6%) were terminated during the observation period. The number of deaths and early terminations for the mice exposed to 50, 250 or 1,000 ppm VC was four (13%), five (16%), and 10 (31%), respectively. Only one of 16 mice (6%) exposed to 55 ppm VDC for 1 month was terminated during the observation period.

After exposure for 3 months, six of 32 control mice (19%) died or were terminated during the observation period. The number of deaths and early terminations for the mice exposed to 50, 250 or 1,000 ppm VC was eight of 32 (25%), 19 of 32 (59%), and 15 of 20 (75%), respectively. Five of 16 mice (31%) exposed to 55 ppm VDC died or were terminated during the observation period.

After exposure for 6 months, 11 of 56 control mice (20%) died or were moribund and terminated during the observation period. A total of 15 of 16 mice (94%) exposed to 50 ppm VC died or were terminated during this period. On the other hand, all the mice (100%) exposed to 250 or 1,000 ppm VC died or were terminated. Most of the deaths and early terminations in the mice exposed to 250 ppm occurred within 9 months after exposure and in the mice exposed to 1,000 ppm within 6 months after exposure. In addition, two of 20 mice (10%) exposed to 250 ppm and five of the 24 mice (21%) exposed to 1,000 ppm were terminated during the exposure period. A total of 11 of 24 mice (46%) exposed to 55 ppm VDC for 6 months died or were terminated during the observation period.

2. Tumors

The tumor occurrence in mice exposed to VC or VDC for 1 month followed by observation for 12 months is summarized in Tables 22 and 23, for 3 months in Tables 24 and 25, and for 6 months in Tables 26 and 27.

a. Exposure for 1 Month

After exposure for 1 month followed by the observation period, a few tumors occurred in eight of 32 control mice (Table 22). The tumors included bronchioloalveolar tumor of the lung in three mice and hepatocellular tumor of the liver in four mice. In addition, one mouse had an adenocarcinoma of the mammary gland and a hemangioma of the kidney; another mouse had a malignant lymphoma involving the kidney and lymph node. These tumors were also seen in mice exposed to various levels of VC or VDC (Tables 22 and 23). The incidence and severity of bronchioloalveolar tumor were greatly increased in mice exposed to 250 or 1,000 ppm VC. This tumor occurred in three of 32 mice exposed to 50 ppm, 19 of 32 mice exposed to 250 ppm, and 20 of 32 mice exposed to 1,000 ppm. In addition, adenocarcinoma/carcinoma of the mammary gland occurred in four of 16 females exposed to 50 ppm VC, two of 16 females exposed to 250 ppm VC, and none of 16 females exposed to 1,000 ppm VC. Hepatic hemangiosarcoma was seen in one male exposed to 50 ppm VC, and hemangioma in two females exposed to 1,000 ppm VC. Other occasional tumors included metastatic adenocarcinoma of the lung originating from the mammary gland, malignant lymphoma of the kidney and lymph node, or liver and spleen, and carcinoma of the kidney. In the 16 mice exposed to 55 ppm VDC, there were a few tumors including bronchioloalveolar tumor in one mouse, hepatocellular tumor in three mice, and renal carcinoma in one mouse.

b. Exposure for 3 Months

After exposure for 3 months followed by the observation period, bronchioloalveolar tumor of the lung and/or hepatocellular tumor of the liver were seen in three control mice (Table 24). The incidence and severity of tumors in several organs were greatly increased in mice exposed to all levels of VC (Tables 24 and 25). Bronchioloalveolar tumor of the lung occurred in 12 of 32, 21 of 32, and 16 of 20 mice exposed to 50, 250 or 1,000 ppm VC, respectively. Hepatic hemangiosarcoma was seen in four and five mice exposed to 250 or 1,000 ppm VC, respectively; adenocarcinoma/carcinoma of the mammary gland was seen in four, six or two females exposed to the respective levels of VC. Extrahepatic hemangiosarcoma also occurred in a few mice exposed to VC involving the mesentery/peritonium or skin. In addition, hepatic and extrahepatic hemangioma was seen in several mice exposed to VC. There were a few metastatic tumors in the lung. The metastatic tumors included adenocarcinoma from the mammary gland in three mice exposed to 50 or 250 ppm VC, and metastatic osteosarcoma in one mouse

exposed to 1,000 ppm VC. Other occasional tumors in mice exposed to VC for 3 months included squamous-cell carcinoma or fibrosarcoma of the skin, myxoma of the mammary gland, and malignant lymphoma involving the lung, kidney and lymph node. A number of mice exposed to various levels of VC with various tumors, especially those exposed to 250 or 1,000 ppm, died or were moribund and terminated during the observation period. In the 16 mice exposed to 55 ppm VDC (Table 25), bronchioloalveolar tumor of the lung occurred in three mice and hemangiosarcoma of the mesentery occurred in one mouse.

c. Exposure for 6 Months

After exposure for 6 months followed by the observation period, a number of tumors occurred in various tissues of 17 of the 56 control mice (Table 26). The percentage of control mice with tumors was not greater than that of the control mice in chamber for 1 or 3 months followed by the observation period. Most tumors occurred in the lung, liver, and mammary gland as seen in the control mice of the previous groups. Occasional tumors included fibrosarcoma of the skin, adenocarcinoma of the intestine, leiomyoma of the uterus, hemangioma of the spleen, and malignant lymphoma involving the liver, stomach and intestine. The incidence of the hepatic and extrahepatic hemangiosarcoma, bronchioloalveolar tumor of the lung and adenocarcinoma/carcinoma of the mammary gland in the females were increased in the mice exposed to 50, 250 or 1,000 ppm VC (Tables 26 and 27). Hepatic hemangiosarcoma occurred in one of 56 control mice, and one of 16, nine of 20, and 13 of 24 mice exposed to 50, 250 or 1,000 ppm VC, respectively. Hemangiosarcoma was also seen in the mesentery/peritonium or skin of a few mice exposed to VC. Bronchioloalveolar tumor of the lung occurred in 11 of 56 control mice, and 3 of 16, 12 of 20, and 14 of 24 mice exposed to the respective levels of VC. Adenocarcinoma/carcinoma of the mammary gland occurred in 3 of 28 female control mice, and 2 of 8, 5 of 8 and 4 of 12 females exposed to the respective levels of VC. Other occasional lesions in the mice exposed to 250 or 1,000 ppm VC included metastatic adenocarcinoma in the lung originating from the mammary gland, hepatocellular tumor of the liver, adenocarcinoma of the intestine, chromophobe-cell adenoma of the pituitary, and malignant lymphoma involving several organs. All the mice exposed to 50, 250 or 1,000 ppm with various tumors died or were terminated during the exposure and observation periods; whereas, only seven of the 17 control mice with tumors died or were terminated before the end of the observation period. A few tumors occurred in six of 24 mice exposed to 55 ppm VDC (Table 26); the incidence and type of tumors were not apparently different from those of the control mice in the chamber for 6 months.

3. Cumulative Tumor Incidence and Tumorigenesis

The cumulative tumor incidence in control mice and mice exposed to VC or VDC is summarized in Table 28. As for the rats, these mice were exposed to the filtered air, VC or VDC for 1, 3 or 6 months followed by observation for 12 months. The life spans of all mice within each treatment group were not the same.

a. In the Liver

Exposure of various levels of VC caused hemangiosarcoma of the liver. It occurred in two of 80 (2.5%), 13 of 84 (15%), and 18 of 76 (24%) mice exposed to 50, 250 or 1,000 ppm VC, respectively. This tumor was found in only one of 120 control mice and none in 56 mice exposed to 55 ppm VDC. Hepatic hemangiosarcoma was also found in rats of the present study after exposure to VC followed by observation for 12 months, and in mice and rats in a previous study[2,3] after exposure to VC without the observation period. This tumor was mostly multiple with random distribution among the lobes. Their size ranged from pin-point focus to a big bloody mass of several cm in diameter. Most large tumors ruptured with blood clot and/or bloody fluid filled the abdominal cavity. Many animals with this secondary internal bleeding had markedly enlarged spleen and a few of them also had subcutaneous edema at the lower part of body. Microscopically, the small tumors consisted of undifferentiated endothelia and some RBC which interspersed in the spaces encompassed by the neoplastic cells. The small neoplastic foci were seen in the early stage of tumor or in the peripheral zone of the large, advanced tumor. When the tumor became big, the proliferating immature endothelia formed a wide cord, expanded outwardly, and replaced the hepatocytes. With these cord growth, they anastomosed each other in an irregular pattern and formed spaces varying from a cleft to a large cavity which was full of RBC. These RBC-filled spaces in the center of the advanced tumor were large and those at the peripheral areas were small. The neoplastic cells were very pleomorphic and anaplastic. They were basophilic and their nuclei were hyperchromatic. Mitosis was not uncommon in this tumor. In some advanced tumors, there was cavernous structure lined by flat endothia mimic of cavernous hemangioma.

Extrahepatic hemangiosarcoma and hemangioma of the liver or other tissues were occasionally seen in various groups of mice, including hemangioma of the kidney in one control and of the spleen in another control mouse. There was also a lymphangioma in the liver of one female exposed to 55 ppm VDC. Hemangiosarcomas of the mesentery or subcutaneous tissue were slightly different from those in the liver. Their neoplastic endothelial cells were somewhat uniform, resembled the fibroblast, and arranged in a loose compartment pattern filled with RBC as seen in the liver. Rupture and hematoma formation from these tumors were seen more often from the mesentery than from the liver.

Hepatocellular tumors were seen in 11 of 120 control mice (9%), 5 of 80 (6%), 11 of 84 (13%), and 4 of 76 (5%) mice exposed to 50, 250 or 1,000 ppm VC, respectively, and in 4 of 56 mice (7%) exposed to 55 ppm VDC. This tumor appeared to be sex-related and occurred mostly in males. It was seen in only one female control and one female exposed to 50 ppm VC. Grossly, there were solitary or multiple round masses on the surface of the liver. They were paler and softer than the surrounding normal parenchyma. Microscopically, the cells were well-differentiated or slightly-undifferentiated, and arranged in a trabecula or solid pattern. They were mostly spherical nodules without much stroma present in the cell mass, or between neoplastic and normal tissues. The normal hepatic lobular pattern was obliterated. In a few animals, the sizes of neoplastic cells were larger than normal, and the cytoplasm was basophilic and vacuolated.

b. In the Lung

In the lung, bronchioloalveolar tumor occurred in 16 of 120 control mice (13%). The total incidence was increased in mice exposed to various levels of VC. The tumor occurred in 16 of 80 (20%), 52 of 84 (62%), and 50 of 76 (66%) mice exposed to 50, 250 or 1,000 ppm VC, respectively. A total of five of 56 mice (9%) exposed to 55 ppm VDC had this tumor. Bronchioloalveolar tumor of the lung was reported in mice exposed to VC without the observation period.[2,3]

There were a few occasional metastatic tumors in the lung of mice exposed to VC. These metastatic tumors were adenocarcinoma originating from the mammary gland. In addition, there was metastatic osteosarcoma in one female exposed to 1,000 ppm VC for 3 months, and metastatic squamous-cell carcinoma originating from the skin in one male exposed to 55 ppm VDC for 6 months.

c. In the Mammary Gland

Adenocarcinoma/carcinoma of the mammary gland, seen only in females, occurred in 4 of 60 control mice (7%), 10 of 40 (25%), 13 of 40 (33%), and 6 of 38 (16%) mice exposed to 50, 250 or 1,000 ppm VC, and none in 28 mice exposed to 55 ppm VDC. These tumors were also found in rats of the present study after exposure to VC and observed for 12 months, and in mice exposed to VC without the observation period.[2,3] Adenoma occurred in one female exposed to 250 ppm VC and myxoma in one female exposed to 50 ppm VC.

d. Other Organs

A number of other tumors occasionally occurred in various organs of these mice. Their occurrence was not related to any group. These tumors included malignant lymphoma involving various organs, chromophobe-cell adenoma of the pituitary, squamous-cell carcinoma and fibrosarcoma of the skin, adenocarcinoma of the intestine, carcinoma of the kidney, and lieomyoma of the uterus.

4. Other Lesions

Spontaneous and age-related lesions were seen in most mice from the control and treated groups. These lesions included inflammatory and degenerative changes. Amyloidosis occurred in most tissues of the older mice.

C. The Effects of DS on VC Exposure in Mice

Based on body weight and feed consumption data, it was estimated that the mice fed the 0.1% DS diet consumed approximately 100 to 150 mg/kg of DS daily. The mice receiving DS and exposed to VC appeared to be less lethargic and to have smoother hair coats than their counterparts exposed to VC without the DS diet.

1. Survival Time

At the conclusion of the 49-week study, DS significantly increased the number of survivors of both sexes of mice exposed to 250 ppm VC and in males exposed to 1,000 ppm VC (Table 29). The number of survivors in the control and 50 ppm VC exposed mice was not affected by the DS administration. In addition, the DS-treated mice exposed to 250 or 1,000 ppm VC had a longer mean survival time than mice not receiving DS. At 250 ppm VC, females survived 33 weeks compared to 48 weeks for DS-treated females. Males exposed to 1,000 ppm VC survived 27 weeks compared to 43 weeks for those receiving DS. Females exposed to 1,000 ppm VC survived 34 weeks compared to 43 weeks for DS-treated females. These differences in survival time would be much greater if the study had not been terminated at 49 weeks.

2. Tumor Incidence

The tumors found in the mice exposed to VC with or without DS treatment included bronchioloalveolar adenomas, hemangiosarcoma of the liver and/or mesentery, and mammary gland tumors in females. The tumor incidence is summarized in Table 30. DS treatment appeared to have no effect on the incidence of VC-induced bronchioloalveolar adenomas. Bronchioloalveolar adenomas occurred in 80 to 100% of the males and 50 to 100% of the females exposed to 250 or 1,000 ppm VC with or without DS. The incidence of this tumor in controls and 50 ppm VC exposed mice was much lower, ranging from 0 to 25%.

At both 250 and 1,000 ppm VC, there appears to be a trend toward a decreased incidence of hemangiosarcomas in both male and female mice treated with DS. At 250 ppm VC, 5 of 6 males and 2 of 5 females had hemangiosarcoma compared to 2 of 8 males and 1 of 8 females in the 250 ppm VC group receiving DS. At 1,000 ppm VC, 4 of 5 males and 3 of 5 females had hemangiosarcomas

compared to 3 of 7 males and none of 4 females exposed to 1,000 ppm VC and treated with DS. However, no statistical differences were demonstrated. One male and one female exposed to 50 ppm VC were found to have hemangiosarcomas, compared to none in the 50 ppm VC group treated with DS. No hemangiosarcomas were found in control mice or mice treated with DS alone.

There was also a trend toward reduction in the incidence of mammary gland tumors in female mice at all exposure levels of VC and treated with DS. The mammary gland tumors were composed of ductular adenocarcinoma, squamous-cell carcinoma, anaplastic-cell carcinoma, hemangiosarcoma or a combination of these tumor types. At 50 ppm VC, two of six females had mammary gland tumors compared to none of five in the DS treated females. At 250 ppm VC, two of eight females had tumors compared to one of eight in the females treated with DS. At 1,000 ppm VC, three of five females had mammary gland tumors compared to none of four in the females treated with DS. However, no statistical differences were demonstrated.

3. Hepatic Microsomal Composition and Function

The livers of mice surviving the 49-week study were removed and the microsomal fractions were examined for cytochrome P-450 and b_5 concentrations and for MFO activities. Alterations in hepatic microsomal composition and function in mice from chronic VC administration with and without DS treatment were compared. The results are summarized in Tables 31 and 32.

Treatment with DS alone resulted in a small increase in relative liver weights and a small decrease in microsomal protein concentrations compared to mice fed the control diet. A decrease in cytochrome P-450 concentration in DS treated mice was accompanied by reductions in the MFO activities, although the reductions in the enzyme activities were not parallel. Impairment of p-nitroanisole-O-demethylase and ethylmorphine-N-demethylase activities occurred to a lesser degree than the hydroxylation of aniline. Sex differences in the response to DS treatment were not apparent.

Exposure of mice to 50 or 250 ppm VC alone for 49 weeks resulted in a considerable increase in relative liver weights. Microsomal protein concentrations tended to decrease, but this was highly variable. Both cytochrome P-450 and b_5 were decreased in mice treated with 50 ppm VC, but these effects were not apparent in the animals exposed to 250 ppm. No significant alterations were observed in MFO activities of VC-treated mice, although ethylmorphine-N-demethylase and p-nitroanisole-O-demethylase activities tended to be higher.

The increase in relative liver weights of mice exposed to VC and fed DS was not as great as the increase seen in mice exposed to VC alone. A significant decrease in cytochrome P-450 was seen in the male mice fed DS

and exposed to 50 ppm VC. No significant changes were seen in cytochrome P-450 in DS-fed females exposed to 50 ppm VC or in males exposed to 250 ppm VC. The lower activity of aniline hydroxylase observed in mice treated with DS alone was also evident in DS-fed mice exposed to VC. The decreases in ethylmorphine-N-demethylase and p-nitroanisole-O-demethylase activities seen in DS-fed mice were not seen in DS-fed mice exposed to VC. In fact, ethylmorphine-N-demethylase activities were greater than those of mice fed either the control or DS diets alone.

IV. DISCUSSION AND CONCLUSION

A. Exposure to VC or VDC in Rats

We reported previously[2,3] that most rats exposed to 250 or 1,000 ppm VC died between the 10th and 12th months. These levels caused hemangiosarcoma of both the liver and lung starting the 9th month. Hemangiosarcoma also occurred in the mesenteric lymph node or subcutaneous tissue of two rats exposed to 55 ppm VDC for 12 months without the observation period.

In the present study, exposure of 50, 250 or 1,000 ppm VC for 1 or 3 months did not cause any death during the observation period of 12 months following exposure. Exposure for 6 or 10 months increased the number of deaths and early terminations during the exposure and observation periods when compared with the respective control rats. The increase in deaths and early terminations was proportional to the length of exposure and the level of VC.

After exposure of 50, 250 or 1,000 ppm VC for 1 or 3 months, the tumor incidence in rats examined at the end of the 12-month observation period including the tumor incidence in the rats terminated earlier was not apparently increased when compared with the spontaneous tumors seen in the respective control rats. Exposure for 6 months, 250 or 1,000 ppm VC caused various tumors in the liver. Exposure for 10 months, all three levels of VC caused a number of tumors in the lung, liver and mammary gland, and probably malignant lymphoma involving several organs.

In the liver, exposure of 250 or 1,000 ppm VC for 6 months caused three major types of tumors. Neoplastic nodules occurred in three of 16 rats exposed to 250 ppm (Table 4). The incidence was increased and occurred in six of 28 rats exposed to 250 ppm VC for 10 months, but not in rats exposed to 1,000 ppm VC (Table 6). Exposure of 1,000 ppm VC for 6 months caused hepatocellular carcinoma in one of 16 rats and hemangiosarcoma in two of 16 rats. The incidence and/or severity of these two tumors were increased in rats exposed to 250 and/or 1,000 ppm VC for 10 months. Exposure of VC might have also caused hemangioma in the liver. Hemangioma was seen in several rats exposed to 50, 250 or 1,000 ppm VC. Bile duct carcinoma occurred in one male exposed to 1,000 ppm VC for 6 months. It is not known whether or not this tumor is related to VC exposure.

About half the rats with hepatic hemangiosarcoma also had hemangiosarcoma of the lung. In addition to the hemangiosarcoma seen in the lungs of rats exposed to 250 or 1,000 ppm VC for 10 months, bronchioloalveolar tumors occurred in two rats exposed to 250 ppm VC, and in four rats exposed to 1,000 ppm VC after exposure for 3 months or longer. This tumor was not

seen in control or other treated groups. The increased incidence of this tumor in VC exposed rats might be related to the compound.

Exposure of various levels of VC increased the incidence of several tumors of the mammary gland in the female rats, predominently fibroadenoma and adenocarcinoma/carcinoma. These tumors were seen in seven of 36 control rats (19%) exposed to the filtered air. Their incidence was increased, and occurred in 16 of 36 rats (44%) exposed to 50 ppm VC. The incidence remained high and occurred in 11 of 32 rats (34%) exposed to 250 ppm VC. In rats exposed to 1,000 ppm VC, the incidence of the mammary gland tumors dropped and occurred in seven of 36 rats (19%), probably due to earlier deaths of this group of rats.

Exposure of 1,000 ppm VC caused malignant lymphoma in three male rats and one female rat that involved the lung, liver, spleen, kidney, lymph node, and/or bone marrow. Malignant lymphoma involving the spleen also occurred in one male rat exposed to 250 ppm VC.

A number of spontaneous tumors occasionally occurred in various organs of the control rats and the rats exposed to VC. They are not related to the exposure of VC.

Exposure of 55 ppm VDC for 10 months, but not for 1, 3 or 6 months, increased the deaths and early terminations of rats during the observation period after exposure. VDC did not increase the incidence of any spontaneous tumors or cause any other tumors.

B. Exposure to VC or VDC in Mice

We reported[2,3/] that most mice exposed to 250 or 1,000 ppm VC died starting the 7th month, and that these levels caused bronchioloalveolar tumor of the lung, hepatic hemangiosarcoma with occasional extrahepatic hemangiosarcoma involving various organs, tumors of the mammary gland in the females and possibly malignant lymphomas involving a number of organs. Exposure of 55 ppm VDC for 9 to 12 months without the observation period caused hepatic hemangiosarcomas in a few mice and probably bronchioloalveolar tumors.

In the present study, exposure of 50, 250 or 1,000 ppm VC for 1, 3 or 6 months increased the number of deaths and early terminations during the exposure and observation periods when compared with the respective control mice. The increase in, and time of, deaths and early terminations were proportional to the length of exposure and the level of VC. After exposure for 6 months, 94% of the mice exposed to 50 ppm and all the mice exposed to 250 or 1,000 ppm VC died or were terminated before the end

of the observation period, whereas only 20% of the control mice died or were terminated during the observation period. In addition, the mice died or were terminated earlier in groups exposed to higher levels of VC and for longer durations.

Exposure of VC caused hepatic and extrahepatic hemangiosarcomas. Hepatic hemangiosarcoma occurred in one of 32 mice exposed to 50 ppm VC for 1 month; and in four of 32 and five of 20 mice exposed to 250 or 1,000 ppm VC, respectively, for 3 months. After exposure for 6 months, the incidence was greatly increased. The tumor was seen in one of 16, nine of 20 and 13 of 24 mice exposed to the respective levels. The severity of this tumor was also increased in some mice exposed for 6 months. One of 56 control mice in the chamber with filtered air for 6 months developed hepatic hemangiosarcoma during the observation period. None of the mice from the other control groups had this tumor. Extrahepatic hemangiosarcoma was seen in several mice exposed to various levels of VC but not in any controls. Hepatic and extrahepatic hemangiomas were occasionally seen in various groups of mice including the controls. Hepatocellular tumors were seen in a number of control mice and mice exposed to VC or VDC. The incidence of these tumors was not apparently altered in the mice exposed to these compounds.

Exposure of 250 or 1,000 ppm VC for 1 month or exposure of 50, 250 or 1,000 ppm VC for 3 or 6 months increased the incidence of bronchiolo-alveolar tumor of the lung when the mice were kept for observation of 12 months. The cumulative incidence of this tumor in all mice was 16 of 120 control mice (13%), 19 of 80 (24%), 52 of 84 (62%), and 50 of 78 (64%) mice exposed to 50, 250 or 1,000 ppm VC, respectively. In addition, there were a few occasional metastatic adenocarcinoma in the lung originating from the mammary gland in mice exposed to various levels of VC.

In the mammary gland of the females, exposure of various levels of VC for 1 month or longer increased the incidence of adenocarcinoma/carcinoma. These tumors occurred in 4 of 60 female controls (7%), and 10 of 40 (25%), 13 of 40 (33%), and 6 of 38 (16%) females exposed to 50, 250 or 1,000 ppm VC, respectively. The lesser incidence of these tumors in the females exposed to 1,000 ppm was probably due to earlier death of this group of mice.

As for the rats, there were a number of spontaneous tumors in various organs of the control mice and mice exposed to VC. These tumors were occasionally seen, and are not related to the exposure of VC.

Exposure of 55 ppm VDC for 3 or 6 months, but not for 1 month, increased the deaths and early terminations of mice during the observation period after exposure. VDC did not increase the incidence of any spontaneous tumors or cause any other tumors.

C. Protective Effect of DS on VC Exposure in Mice

Exposure of mice to 50, 250 or 1,000 ppm VC was reported to cause a high incidence of bronchioloalveolar adenoma, mammary gland tumors and hemangiosarcoma.[2,3] In the present study, treatment with DS appeared to reduce the incidence of hemangiosarcoma of the liver in both sexes and mammary gland tumors in females. However, DS treatment had no apparent effect on the incidence of VC-induced bronchioloalveolar adenoma. Also, treatment of mice with DS during exposure to 250 or 1,000 ppm VC resulted in an increase in the survival time. These data suggest that DS exerts at least a partial protection against VC-induced carcinogenesis. Whether this protective effect of DS is an actual reduction in the occurrence of the VC-induced neoplasms or simply an increase in the latency period to tumor formation is not clear from the present study.

Treatment of mice with DS alone for a period of 49 weeks decreased the hepatic cytochrome P-450 concentration and reduced the MFO activities. These data are similar to previously reported studies in rats after single doses of DS[5] or 12 daily doses of DS.[6] Exposure to 50 ppm VC alone for 49 weeks increased the relative liver weight and decreased the cytochrome P-450 and b_5 concentrations without any apparent effect on the MFO activities. The effects on liver weight and cytochrome levels were not apparent in mice exposed to 250 ppm. Watanabe et al.[19] reported that exposure to 5,000 ppm VC for 7 weeks did not result in any changes in microsomal enzyme activity. Combination treatment of DS plus VC, in the present study, decreased the effects of VC on liver weight and cytochrome levels. The activity of aniline hydroxylase was decreased whereas the activities of ethylmorphine-N-demethylase and p-nitroanisole-O-demethylase were unaffected or slightly increased.

It has been reported that VC is metabolized, at least in part, by the hepatic MFO system to a reactive metabolite(s) which may covalently bind to cellular macromolecules and initiate the carcinogenic response to VC.[20] Repeated exposure to VC was also reported to increase the binding of reactive VC metabolites to hepatic macromolecules.[19] Our data show that DS inhibits the MFO system. This suggests that DS may act by reducing the activation of VC to reactive metabolites via microsomal metabolism. It has been also suggested that a potential pathway of VC detoxification may be the conjugation of reactive VC metabolites with nonprotein sulfhydryl groups.[21] Since DS is rapidly metabolized to compounds bearing free sulfhydryl groups,[22] it may exert its protective action by increasing the availability of free sulfhydryl groups to combine with reactive VC metabolites. Conceivably, DS may act by a combination of these two mechanisms.

V. PUBLICATIONS, PRESENTATIONS AND MANUSCRIPTS

A. Publications

Lee, C. C., J. C. Bhandari, J. M. Winston, W. B. House, P. J. Peters, R. L. Dixon, and J. S. Woods: Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride. Environ. Health Persp., 21:25-32 (1977).

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B. Presentations

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TABLE 1

THE 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 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS

Month During Observation Period	Control		VC, ppm 50		VC, ppm 250		VC, ppm 1,000		VDC, ppm 55	
	M	F	M	F	M	F	M	F	M	F
After exposure for 1 month										
6-9	-	-	-	-	-	-	-	-	1	-
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 months										
3-6	-	1[b]	-	-	-	-	1	-	-	-
6-9	-	-	-	-	-	-	-	-	-	-
9-12	-	3	-	2	-	-	-	-	-	-
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 months										
During exposure	1	-	-	-	-	-	1	-	-	-
3-6	1	-	1	-	-	-	1	-	-	-
6-9	-	1	-	-	-	1	3	3	-	-
9-12	-	-	3	-	2	1	-	2	1	-
Total	2/8	1/8	4/8	0/8	2/8	2/8	5/8	5/8	1/8	0/8
After exposure for 10 months										
During exposure	-	2	3	-	-	1	-	1	1	-
0-3	-	1	-	1	3	-	4	3	-	-
3-6	1	-	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	-	5
Total	6/16	7/16	6/10	11/16	11/16	6/12	15/16	15/16	11/14	9/16

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

TABLE 2

SUMMARY OF TUMORS IN RATS EXPOSED TO VC OR VDC FOR 1 MONTH FOLLOWED BY OBSERVATION FOR 12 MONTHS[a]

Treatment:	250 ppm VC		1,000 ppm VC	55 ppm VDC
Sex:	Female		Male	Male
Rat No.:	546	549	596	656
Weeks After Exposure:	52	52	52	52
Tumors[b]				
Liver				
Hepatocellular carcinoma			3	
Hemangiosarcoma				4
Mammary Gland				
Fibroadenoma	1	1		
Pituitary				
Chromophobe-cell adenoma		1		

a/ Includes only rats with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.

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TABLE 3

SUMMARY OF TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control		50 ppm VC				250 ppm VC						1,000 ppm VC						55 ppm VDC
Sex:	Female		Female				Male			Female			Male			Female			Female
Rat No.:	417	418	475	479	489	492	524	525	527	559	560	561	602	604	606	634	640	641	704
Tumors[b/] / Week After Exposure:	47	49	41	43	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
Skin																			
Squamous-cell carcinoma													2						
Lung																			
Bronchioloalveolar tumor									2							2			
Epidermoid carcinoma	2																		
Liver																			
Neoplastic nodules								1							1				
Hepatocellular carcinoma							2												
Hemangioma										1									
Epididymis																			
Mesothelioma														2					
Mammary Gland																			
Fibroadenoma				1	1											1			1
Adenoma												1					1	1	
Adenocarcinoma			2																
Carcinoma						2				2									
Ovary																			
Cystadenoma		1																	
Pituitary																			
Chromophobe-cell adenoma		1		1							1								

a/ Includes only rats with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.

TABLE 4

SUMMARY OF TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control		50 ppm VC					250 ppm VC				1,000 ppm VC									55 ppm VDC	
Sex:	Female		Male		Female			Male		Female		Male						Female			Male	Female
Rat No.:	399	423	436	454	494	497	504	533	538	555	560	612	592	589	599	608	611	632	633	643	674	712
Tumors[b/] Week After Exposure:	33	52	19	52	52	52	52	52	52	14	51	24[c/]	21	28	34	52	52	34	38	52	52	52
Skin																						
Papilloma											1											
Squamous-cell carcinoma			2									2										
Fibrosarcoma				2																		
Lung																						
Bronchiolo-alveolar tumor																2						
Liver																						
Neoplastic nodules								1	1		1											
Hepatocellular carcinoma																	2					
Bile duct carcinoma													2									
Hemangioma									1					1								
Hemangiosarcoma																		4	4			
Intestine																						
Fibrosarcoma																				3		
Mammary Gland																						
Fibroadenoma		1			1	1	1													1		
Adenocarcinoma	2																					
Pituitary																						
Chromophobe-cell adenoma										1												1
Malignant Lymphoma																						
Lung, liver, spleen and/or kidney															4					2		

a/ Includes only rats with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.

c/ During exposure.

TABLE 5

SUMMARY OF TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL) OR VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control											50 ppm VC										
Sex:	Male					Female						Male				Female						
Rat No:	362	370	372	374	369	401	402	408	409	412	415	466	433	444	456	470	499	474	472	476	477	478	498	484	486	500
Wk After Exposure:	31	52	52	52	52	46	46	52	52	52	52	31[c/]	15	52	52	13	29	34	37	47	47	47	50	52	52	52
Tumors[b/]																										
Skin																										
Squamous-cell carcinoma												2							2							
Fibroma	1	1			1							1														
Lung																										
Fibrosarcoma																		3								
Liver																										
Hepatocellular carcinoma				2																						
Hemangioma															1											
Pancreas																										
Islet-cell adenoma														2												
Kidney																										
Carcinosarcoma					1																					
Mammary Gland																										
Fibroadenoma						1	1	1			1										1	1	1	1	1	1
Adenoma									1																	
Adenocarcinoma																2	2									
Fibrosarcoma																		3								
Adrenal																										
Pheochromocytoma										1																
Pituitary																										
Chromophobe-cell adenoma			1						1	1	1		1	1						1	1					

a/ Includes only rats with tumors.
b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.
c/ During exposure.

TABLE 6

SUMMARY OF TUMORS IN RATS EXPOSED TO VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Tumors[b/]	Treatment:	250 ppm VC																			
	Sex:	Male										Female									
	Rat No.:	506	507	534	508	517	518	519	520	521	537	576	567	542	543	544	545	550	551	552	553	554
	Week After Exposure:	12	18	29	31	49	52	52	52	52	52	40[c/]	25	26	37	39	47	52	52	52	52	52
Skin																						
Squamous-cell carcinoma		2																				
Fibroma							1			1												
Lung																						
Bronchioloalveolar tumor																			2			
Hemangiosarcoma													4		3							
Liver																						
Neoplastic nodules			1	1		1					1				1		1					
Hepatocellular carcinoma																		2				
Hemangioma													1									
Hemangiosarcoma				2									3		2		2					1
Kidney																						
Adenocarcinoma								1											2			
Carcinoma															1							
Malignant lymphoma																						
Spleen								2														
Mammary Gland																						
Fibroadenoma												1		1		1		1	1	1	1	
Pituitary																						
Chromophobe-cell adenoma					1			1														

a/ Includes only rats with tumors.
b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.
c/ During exposure.

TABLE 7

SUMMARY OF TUMORS IN RATS EXPOSED TO VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	1,000 ppm VC																								
Sex:	Male												Female												
Rat No.:	577	579	580	583	578	582	585	587	590	591	607	598	647	615	613	616	617	588	618	619	621	622	623	624	631
Week After Exposure:	2	10	10	13	17	22	27	33	35	36	47	52	40[c/]	9	12	20	25	26	31	34	42	42	46	46	52
Tumors[b/]																									
Skin																									
Squamous-cell carcinoma				2									2												
Sebaceous gland adeno-carcinoma										2															
Lung																									
Bronchioloalveolar tumor					1	1																			
Hemangiosarcoma			4							2	4				4	4						4	4		
Nasal Cavity																									
Squamous-cell carcinoma																	2								
Liver																									
Neoplastic nodules																									1
Hepatocellular carcinoma				2				2		2							2			2			2		
Hemangioma	1																								
Hemangiosarcoma		4					2		1	2	4				4	4		4			2	2	2		4
Intestine																									
Adenocarcinoma																							2		
Kidney																									
Carcinoma																					2				
Metastatic bronchiolo-alveolar carcinoma					3																				
Ovary																									
Granulosal-cell tumor																								2	
Mammary Gland																									
Fibroadenoma																					1			1	1
Pituitary																									
Chromophobe-cell adenoma					1							1		1					1						
Malignant Lymphoma																									
Lung, liver, spleen, lymph nodes and/or bone marrow						2			2																

a/ Includes only rats with tumors.
b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.
c/ During exposure.

TABLE 8

SUMMARY OF TUMORS IN RATS EXPOSED TO VDC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	55 ppm VDC								
Sex:	Male			Female					
Rat No.:	684	676	677	687	688	689	690	691	692
Week After Exposure:	24[c/]	52	52	23	43	45	46	46	46
Tumors[b/]									
Skin									
Fibroma	1	1	1						
Mammary Gland									
Fibroadenoma				1			1	1	1
Pituitary									
Chromophobe-cell adenoma				1	1	1			

a/ Includes only rats with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.

c/ During exposure.

TABLE 9

CUMULATIVE TUMOR INCIDENCE IN RATS EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOLLOWED BY OBSERVATION FOR 12 MONTHS

Tumors	Control		50 ppm VC		250 ppm VC		1,000 ppm VC		55 ppm VDC	
	M	F	M	F	M	F	M	F	M	F
Liver										
Neoplastic nodules	0/36[a]	0/36	0/30	0/36	7/36	3/32	1/36	1/36	0/34	0/36
Hepatocellular carcinoma	1/36	0/36	0/30	0/36	1/36	1/32	4/36	3/36	0/34	0/36
Bile duct carcinoma	0/36	0/36	0/30	0/36	0/36	0/32	1/36	0/36	0/34	0/36
Hemangioma	0/36	0/36	1/30	0/36	1/36	2/32	2/36	0/36	0/34	0/36
Hemangiosarcoma	0/36	0/36	0/30	0/36	1/36	4/32	5/36	9/36	1/34	0/36
Lung										
Bronchioloalveolar tumor	0/36	0/36	0/30	0/36	1/36	1/32	3/36	1/36	0/34	0/36
Fibrosarcoma	0/36	0/36	0/30	1/36	0/36	0/32	0/36	0/36	0/34	0/36
Hemangiosarcoma	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
Adenoma	0/36	1/36	0/30	0/36	0/36	1/32	0/36	2/36	0/34	0/36
Adenocarcinoma/carcinoma	0/36	1/36	0/30	4/36	0/36	1/32	0/36	0/36	0/34	0/36
Fibrosarcoma	0/36	0/36	0/30	1/36	0/36	0/32	0/36	0/36	0/34	0/36
Malignant Lymphoma										
Lung, liver, spleen, kidney, lymph node and/or bone marrow	0/36	0/36	0/30	0/36	1/36	0/32	3/36	1/36	0/34	0/36
Pituitary										
Chromophobe-cell adenoma	1/36	4/36	2/30	3/36	2/36	3/32	2/36	2/36	0/34	4/36
Skin										
Papilloma	0/36	0/36	0/30	0/36	0/36	1/32	0/36	0/36	0/34	0/36
Squamous-cell carcinoma	0/36	0/36	2/30	1/36	1/36	0/32	3/36	1/36	0/34	0/36
Sebaceous gland adenocarcinoma	0/36	0/36	0/30	0/36	0/36	0/32	1/36	0/36	0/34	0/36
Fibroma	3/36	0/36	1/31	0/36	2/36	0/32	0/36	0/36	3/34	0/36
Fibrosarcoma	0/36	0/36	1/30	0/36	0/36	0/32	0/36	0/36	0/34	0/36
Nasal Cavity										
Squamous-cell carcinoma	0/36	0/36	0/30	0/36	0/36	0/32	0/36	1/36	0/34	0/36
Intestine										
Adenocarcinoma	0/36	0/36	0/30	0/36	0/36	0/32	0/36	1/36	0/34	0/36
Fibrosarcoma	0/36	0/36	0/30	0/36	0/36	0/32	0/36	0/36	1/34	0/36
Pancreas										
Islet-cell adenoma	0/36	0/36	1/30	0/36	0/36	0/32	0/36	0/36	0/34	0/36
Kidney										
Adenocarcinoma	0/36	0/36	0/30	0/36	0/36	1/32	0/36	0/36	0/34	0/36
Carcinoma	0/36	0/36	0/30	0/36	0/36	1/32	0/36	1/36	0/34	0/36
Carcinosarcoma	1/36	0/36	0/30	0/36	0/36	0/32	0/36	0/36	0/34	0/36
Metastatic bronchioloalveolar carcinoma	0/36	0/36	0/30	0/36	0/36	0/32	1/36	0/36	0/34	0/36
Ovary										
Cystadenoma	0/36	1/36	0/30	0/36	0/36	0/32	0/36	0/36	0/34	0/36
Granulosal-cell tumor	0/36	0/36	0/30	0/36	0/36	0/32	0/36	1/36	0/34	0/36
Epididymis										
Mesothelioma	0/36	0/36	0/30	0/36	0/36	0/32	1/36	0/36	0/34	0/36
Adrenal Gland										
Pheochromocytoma	0/36	1/36	0/36	0/36	0/36	0/32	0/36	0/36	0/34	0/36

a/ Number of rats with tumors/total number of rats studied.

TABLE 10

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOR 1 MONTH FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control				50 ppm VC				250 ppm VC			
Sex:	Male				Male			F	Male			
Rat No.:	365	366	367	368	435	439	440	481	512	513	514	515
Lesions[b/] Week After Exposure:	52	52	52	52	34	52	52	52	52	52	52	52
Skin												
Inflamation					2							
Lung												
Pneumonia					2		1					
Liver												
Fatty degeneration	1					1		1	1	2	2	3
Focal inflammation					1							
Kidney												
Nephritis	1	1	1	1						1		1

Treatment:	1,000 ppm VC						55 ppm VDC			
Sex:	Male			Female			Male			F
Rat No.:	593	595	596	627	628	629	656	657	659	694
Lesions[b/] Week After Exposure:	52	52	52	52	52	52	52	52	52	52
Heart										
Fibroplasia							1			
Liver										
Fatty degeneration	2	2		1	1	1			4	
Bile duct hyperplasia		1								
Kidney										
Nephritis	1	1	1				2	1		1
Testes										
Mineralization							3			

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 11

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL) OR VC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Lesions[b/]	Treatment:	Control								
	Sex:	Male							Female	
	Rat No.:	375	377	378	379	380	381	382	421	422
	Week After Exposure:	52	52	52	52	52	52	52	52	52
Heart										
Fibroplasia		1					1			
Liver										
Fatty degeneration		2	2			2	2	1		
Bile duct hyperplasia									2	1
Kidney										
Nephritis		1	2	1	2	1	2		1	

Lesions[b/]	Treatment:	50 ppm VC											
	Sex:	Male								Female			
	Rat No.:	446	447	448	449	450	451	452	453	479	488	490	492
	Week After Exposure:	52	52	52	52	52	52	52	52	43	52	52	52
Heart													
Fibroplasia		1											
Liver													
Fatty degeneration		2	4	1	1	1		1				1	
Cystic changes										1			
Kidney													
Nephritis						1	2		2		2		
Adrenal													
Cystic degeneration													1
Mesentery													
Inflammation					4								

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 12

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO VC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	250 ppm VC												
Sex:	Male								Female				
Rat No.:	522	523	524	525	526	527	528	529	556	557	559	562	563
Lesions[b/] Week After Exposure:	52	52	52	52	52	52	52	52	52	52	52	52	52
Heart													
Fibroplasia	1	1		2									
Liver													
Fatty degeneration	4	4	4	4	4	4	4	2		4	1	1	1
Necrosis									1				
Kidney													
Nephritis	2	1	2	1	1		1			1			1

Treatment:	1,000 ppm VC												
Sex:	Male							Female					
Rat No.:	600	601	602	603	604	605	606	634	635	636	637	640	641
Lesions[b/] Week After Exposure:	52	52	52	52	52	52	52	52	52	52	52	52	52
Liver													
Fatty degeneration	4	4	2	4	4	4	4	2	1	2	2	4	4
Kidney													
Nephritis		2		2	1		1					1	

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 13

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO VDC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a]

Lesions[b]	Treatment:	55 ppm VDC					
	Sex:	Male					F
	Rat No.:	666	667	668	670	671	707
	Week After Exposure:	52	52	52	52	52	52
Liver							
Fatty degeneration					4		
Kidney							
Nephritis		2	2	3	2	3	1

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

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TABLE 14

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL) OR VC FOR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control													
Sex:	Male							Female						
Rat No.:	396	364	384	385	386	391	392	399	423	425	428	429	722	723
Lesions[b/] Week After Exposure:	19[c/]	23	52	52	52	52	52	33	52	52	52	52	52	52
Heart														
Fibroplasia		2												
Liver														
Fatty degeneration	1		1	2	1	2	3	1	2	1	1	1		2
Bile duct hyperplasia						2								
Kidney														
Nephritis			2	1		1	3						3	

Treatment:	50 ppm VC									
Sex:	Male						Female			
Rat No.:	436	441	724	454	455	458	494	502	503	504
Lesions[b/] Week After Exposure:	19	42	48	52	52	52	52	52	52	52
Lung										
Pneumonia							1			
Liver										
Fatty degeneration			2		2			2		
Focal inflammation						2				
Kidney										
Nephritis	2	3		2	3				1	1

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

c/ During exposure.

TABLE 15

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO VC OR VDC FOR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Lesions[b/]	Treatment:	250 ppm VC									
	Sex:	Male							Female		
	Rat No.:	725	531	532	533	538	539	540	555	565	570
	Wk After Exposure:	50	52	52	52	52	52	52	34	52	52
Mammary Gland											
Inflammatory cyst										1	
Heart											
Fibroplasia											1
Liver											
Fatty degeneration		3	4	4	4	4	4	4	3	1	
Kidney											
Nephritis		3	2	1	3	1	3	2			3

Lesions[b/]	Treatment:	1,000 ppm VC							55 ppm VDC							
	Sex:	Male					Female		Male					Female		
	Rat No.:	612	592	608	609	611	644	724	672	673	674	675	680	709	720	721
	Wk After Exposure:	24[c/]	21	52	52	52	52	52	52	52	52	52	52	52	52	52
Heart																
Fibroplasia								1								
Calcification			3													
Liver																
Fatty degeneration				3	2	4		2	1			1		1	1	4
Kidney																
Nephritis		2		2	1	2	1			2	1	3			1	1
Calcification			3													
Fat infiltration													2			

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

c/ During exposure.

TABLE 16

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO FILTERED AIR (CONTROL) FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

		Males											
Lesions[b/]	Rat No.:	361	362	387	369	383	370	371	372	373	374	388	389	390
	Week After Exposure:	31	31	36	41	47	52	52	52	52	52	52	52	52
Heart														
Fibroplasia			1		2		1		1	1		1	1	1
Liver														
Fatty degeneration		4	4	1	2	3			1	1			4	1
Focal inflammation										1				
Cholangitis					2									
Telangiectasis								1						
Kidney														
Nephritis		1	3		1	1	2	2	1	1	2	2	3	2
Prostate														
Inflammation										2				
Mesentary														
Inflammation													2	

		Females										
Lesions[b/]	Rat No.:	397	400	401	402	416	408	409	410	411	412	413
	Week After Exposure:	12	35	46	46	52	52	52	52	52	52	52
Heart												
Fibroplasia									1	1		
Liver												
Fatty degeneration		1		1	2		1	2	1		2	1
Focal inflammation						1						
Kidney												
Nephritis					2		1	1	2			
Adrenal												
Fatty degeneration							1					
Peritoneum												
Inflammation			1									

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 17

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO 50 PPM VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Lesions[b/]	Males								
Rat No.:	466	464	433	434	437	442	443	444	456
Week After Exposure:	31[c/]	35[c/]	15	28	45	52	52	52	52
Heart									
Fibroplasia					1				
Lung									
Pneumonia				3	3				
Liver									
Fatty degeneration	1	4				4	4	2	
Kidney									
Nephritis		1		2			3	2	1

Lesions[b/]	Females										
Rat No.:	469	471	499	474	472	473	477	485	486	487	500
Week After Exposure:	16	27	29	34	37	42	47	52	52	52	52
Heart											
Fibroplasia				1					1	1	
Lung											
Pneumonia	2			3		3					
Liver											
Fatty degeneration	2	3		2	1		2	1	4		2
Telangiectasis			3								
Kidney											
Nephritis		1						1	2	2	1

a/ Includes only rats with lesions.
b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.
c/ During exposure.

TABLE 18

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO 250 PPM VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a]

	Males															
Rat No.:	506	510	507	535	509	534	508	511	516	517	518	519	520	521	536	537
Week After Exposure:	12	12	18	27	29	29	31	34	49	49	52	52	52	52	52	52
Lesions[b]																
Heart																
Fibroplasia		2			2	1			2	1	1					3
Lung																
Pneumonia				3	2			2								
Liver																
Fatty degeneration	2		1	3			2		1	1	1	1			4	3
Cholangitis				3										1		
Telangiectasis											2			1		
Kidney																
Nephritis			1	1		2				2		3	3	1	3	3

	Females		
Rat No.:	567	544	550
Lesions[b] Week After Exposure:	25	39	52
Heart			
Fibroplasia			1
Liver			
Telangiectasis	2	3	2

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 19

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO 1,000 PPM VC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Lesions[b/]	Males								
Rat No.:	579	583	581	586	587	590	591	610	598
Week After Exposure:	10	13	19	31	33	35	36	43	52
Heart									
Fibroplasia	1	1	2						
Lung									
Pneumonia	2								
Liver									
Fatty degeneration				3					2
Kidney									
Nephritis	3	2	3		1	2	1	2	2

Lesions[b/]	Females			
Rat No.:	613	620	624	646
Week After Exposure:	12	37	46	52
Liver				
Fatty degeneration		3	4	
Kidney				
Nephritis	1			3

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

TABLE 20

SUMMARY OF LESIONS OTHER THAN TUMORS IN RATS EXPOSED TO 55 PPM VDC FOR 10 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Lesions[b/]		Males											
	Rat No.:	684	649	651	652	653	654	655	660	661	662	676	677
	Week After Exposure:	24[c/]	14	22	42	47	47	47	49	51	52	52	52
Heart													
Fibroplasia			1		3		2		1	2		3	
Myocarditis						3							
Liver													
Fatty degeneration			3	3			1					1	
Bile duct hyperplasia					2								
Kidney													
Nephritis		1		1	3	3	3	2		2	2	3	3

Lesions[b/]		Females									
	Rat No.:	686	687	714	688	689	691	692	699	716	717
	Week After Exposure:	17	23	30	43	45	46	46	52	52	52
Skin											
Abscess				1							
Heart											
Fibroplasia		1									
Lung											
Pneumonia				3							
Liver											
Fatty degeneration			1		3	3	3	1	1		
Kidney											
Nephritis										3	2

a/ Includes only rats with lesions.

b/ Severity of lesions: 1 - minimal, 2 - moderate, 3 - severe, and 4 - very severe.

c/ During exposure.

TABLE 21

THE NUMBER OF DEATHS AND EARLY TERMINATIONS AND TOTAL NUMBER OF MICE EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOR 1, 3, OR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS

Month During Observation Period	Control		VC, ppm 50		250		1,000		VDC, ppm 55	
	M	F	M	F	M	F	M	F	M	F
After exposure for 1 month										
3-6	-	-	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 months										
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 months										
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/total number of mice studied.

TABLE 22

SUMMARY OF TUMORS IN MICE EXPOSED TO FILTERED AIR (CONTROL) OR VC FOR 1 MONTH FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control								50 ppm VC									
Sex:	Male						Female		Male					Female				
Mouse No.:	371	377	378	379	382	386	748	419	441	442	443	451	452	475	484	492	493	496
Tumors[b/] Week After Exposure:	45	52	52	52	52	52	32	52	52	52	52	52	52	24	52	52	52	52
Mammary Gland																		
Adenocarcinoma/carcinoma							4								3	2	2	2
Lung																		
Bronchioloalveolar tumor				2	2			1	1	1	1							
Metastatic adenocarcinoma																		X
Liver																		
Hepatocellular tumor		1	1		1	2					1	2				1		
Hemangiosarcoma													4					
Kidney																		
Hemangioma							1											
Malignant Lymphoma																		
Kidney and lymph node	3																	

Treatment:	250 ppm VC																							
Sex:	Male													Female										
Mouse No.:	520	521	522	523	524	525	526	527	528	529	530	531	532	546	554	556	557	558	560	562	563	564	567	799
Tumors[b/] Week After Exposure:	52	52	52	52	52	52	52	52	52	52	52	52	52	26	44	50	52	52	52	52	52	52	52	52
Mammary Gland																								
Adenocarcinoma														2									3	
Lung																								
Bronchioloalveolar tumor	1		1	1	2	2	2	1			4	1	2		1	2	1	1	1	1	2	3		2
Liver																								
Hepatocellular tumor	1	1			3	1	1		2	1														
Malignant Lymphoma																								
Liver and spleen															2									

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated; X - present.

TABLE 23

SUMMARY OF TUMORS IN MICE EXPOSED TO VC OR VDC FOR 1 MONTH FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Tumors[b/]	Treatment:	1,000 ppm VC																		
	Sex:	Male										Female								
	Mouse No.:	587	590	595	596	600	601	603	604	605	606	607	622	624	625	626	627	628	629	630	631
	Week After Exposure:	27	33	47	48	51	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
Lung																					
Bronchioloalveolar tumor		1	2	2	2	1	2	2	1	2	1	1	1	1	2	3	1	1	2	1	2
Liver																					
Hepatocellular tumor							2														
Hemangioma																1	1				

Tumors[b/]	Treatment:	55 ppm VDC		
	Sex:	Male		
	Mouse No.:	661	664	666
	Week After Exposure:	52	52	52
Lung				
Bronchioloalveolar tumor		1		
Liver				
Hepatocellular tumor		1	1	1
Kidney				
Carcinoma			3	

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated.

TABLE 24

SUMMARY OF TUMORS IN MICE EXPOSED TO FILTERED AIR (CONTROL) OR VC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control			50 ppm VC															
Sex:	Male			Male							Female								
Mouse No.:	730	732	733	440	460	462	464	465	475	477	478	480	481	497	501	779	780	781	783
Tumors[b/] Week After Exposure:	52	52	52	38	52	52	52	52	52	52	28	35	36	41	52	52	52	52	52
Skin																			
Squamous-cell carcinoma												2							
Mammary Gland																			
Adenocarcinoma/carcinoma													4	3	2		4		
Myxoma																			1
Lung																			
Bronchioloalveolar tumor	2	2		1	1	1	1	1	1	1		1		1	1	1		1	
Metastatic adenocarcinoma													X	X					
Liver																			
Hepatocellular tumor	2		2							2									
Hemangioma								1							1	1			
Spleen																			
Hemangioma								1											
Lymph Node																			
Hemangioma													1						
Mesentery-Peritonium																			
Hemangioma															1				
Hemangiosarcoma											2								
Malignant Lymphoma																			
Lung, kidney and lymph node									3										

Treatment:	250 ppm VC																					
Sex:	Male												Female									
Mouse No.:	516	517	519	533	534	535	786	787	788	791	792	793	547	552	550	551	553	800	802	803	804	805	806
Tumors[b/] Week After Exposure:	32	32	38	40	41	49	51	52	52	52	52	52	14	22	26	26	31	43	52	52	52	52	52
Skin																							
Fibrosarcoma							2																
Hemangiosarcoma														2		2							
Mammary Gland																							
Adenocarcinoma/carcinoma													4		2	4		3				2	2
Lung																							
Bronchioloalveolar tumor	2	1	2	1	2	3	2		3	3	2	2	1		1	1	1	2	1	1	2	1	1
Metastatic adenocarcinoma															X								
Liver																							
Hepatocellular tumor								2	2	2	2												
Hemangiosarcoma								2										2			3		2
Mesentery-Peritonium																							
Hemangiosarcoma			2	2																			

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated; X - present.

TABLE 25

SUMMARY OF TUMORS IN MICE EXPOSED TO VC OR VDC FOR 3 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	1,000 ppm VC																		
Sex:	Male									Female									
Mouse No.:	608	592	594	597	598	599	609	811	812	555	620	621	631	634	635	821	823	824
Tumors[b/] / Week After Exposure:	19	31	33	35	35	35	52	52	52	34	38	38	39	41	41	43	52	52
Skin																		
Hemangioma																		1
Hemangiosarcoma											1							
Mammary Gland																		
Adenocarcinoma/carcinoma															2	2		
Lung																		
Bronchioloalveolar tumor	3	2	2	1	1	2	2	2	3	1	1	2		2	1		1	1
Metastatic osteosarcoma																X		
Liver																		
Hepatocellular tumor							2		1									
Hemangioma																		1
Hemangiosarcoma		3									2		2	2	2			

Treatment:	55 ppm VDC			
Sex:	Male			F
Mouse No.:	828	670	831	706
Tumors[b/] / Week After Exposure:	36	43	52	52
Lung				
Bronchioloalveolar tumor		2	1	1
Mesentery				
Hemangiosarcoma	2			

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated; X - present.

TABLE 26

SUMMARY OF TUMORS IN MICE EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Treatment:	Control																
Sex:	Male								Female								
Mouse No.:	370	364	374	366	392	723	724	837	405	406	421	423	403	424	426	764	768
Tumors[b/] / Week After Exposure:	18	28	46	52	52	52	52	52	45	45	49	51	52	52	52	52	52
Skin																	
Fibrosarcoma			2														
Mammary Gland																	
Adenocarcinoma/carcinoma									3			3					2
Lung																	
Bronchioloalveolar tumor	2		1			1		2			1	1	1	1	1	1	1
Liver																	
Hepatocellular	1			1	1			2			1						
Hemangiosarcoma											2						
Intestine																	
Adenocarcinoma							2										
Uterus																	
Leiomyoma										1			1				
Spleen																	
Hemangioma										1							
Malignant Lymphoma																	
Liver, stomach and intestine		3															

Treatment:	50 ppm VC									55 ppm VDC					F
Sex:	Male					Female				Male					
Mouse No.:	438	439	773	434	435	474	473	477	500	649	657	825	833	834	701
Tumors[b/] / Week After Exposure:	44	45	47	48	49	21	22	30	32	11	39	41	52	52	51
Skin															
Hemangioma		1													
Squamous-cell carcinoma										2					
Mammary Gland															
Adenocarcinoma/carcinoma							3		4						
Lung															
Bronchioloalveolar tumor		1		1		1							1		
Metastatic tumor									X	X					
Liver															
Hepatocellular tumor					2							2			
Hemangiosarcoma						2									
Lymphangioma														1	
Kidney															
Carcinoma											2				
Mesentery-Peritonium															
Hemangioma								1							
Hemangiosarcoma	4														
Malignant Lymphoma															
Heart, liver, kidney, spleen, lymph node, and/or skin			4												2

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated; X - present.

TABLE 27

SUMMARY OF TUMORS IN MICE EXPOSED TO VC FOR 6 MONTHS FOLLOWED BY OBSERVATION FOR 12 MONTHS[a/]

Tumors[b/]	Treatment: 250 ppm VC																
	Sex: Male										Female						
	House No.: 505	506	507	508	509	512	513	514	515	518	568	541	801	542	543	545	548
	Week After Exposure 12	12	13	16	20	28	28	12	32	40	27[c/]	5	9	10	14	20	25
Skin																	
Hemangiosarcoma																	2
Mammary Gland																	
Adenoma												1					
Adenocarcinoma/carcinoma											1		2		2	1	3
Lung																	
Bronchiolonlveolar tumor	1	1	1	2	1	1	1	2			1				2	1	1
Metastatic adenocarcinoma													X				
Liver																	
Hemangiosarcoma	2	4	4		4	2	2		3						2	3	
Intestine																	
Adenocarcinoma								2									
Mesentery-Peritonium																	
Hemangiosarcoma																3	2
Pituitary																	
Chromophobe-cell adenoma														1			
Malignant Lymphoma																	
Lung, liver, kidney, spleen and lymph node										4							

Tumors[b/]	Treatment: 1,000 ppm VC																
	Sex: Male							Female									
	House No.: 580	581	582	584	586	588	583	645	643	613	614	615	616	617	618	619	822
	Week After Treatment: 14	14	17	21	22	21	40	24[c/]	25[c/]	5	6	9	9	10	13	14	18
Skin																	
Hemangioma																	1
Hemangiosarcoma											4						
Mammary Gland																	
Adenocarcinoma/carcinoma										2			2		2	2	
Lung																	
Bronchiolonlveolar tumor	1	1	2	1	1	2	3		2	1	1	1		1	2		1
Metastatic adenocarcinoma																X	
Liver																	
Hepatocellular tumor				1													
Hemangiosarcoma	2	2	2	3	2			2	3	2		2	2	2	2	2	

a/ Includes only mice with tumors.

b/ Tumor classification: 1 - benign; 2 - malignant, well-differentiated; 3 - malignant, moderately differentiated; 4 - malignant, poorly differentiated; X - present.

c/ During exposure.

TABLE 28

CUMULATIVE TUMOR INCIDENCE IN MICE EXPOSED TO FILTERED AIR (CONTROL), VC OR VDC FOLLOWED BY OBSERVATION FOR 12 MONTHS

Tumors	Control		50 ppm VC		250 ppm VC		1,000 ppm VC		55 ppm VDC	
	M	F	M	F	M	F	M	F	M	F
Liver										
Hepatocellular tumor	10/60[a/]	1/60	4/40	1/40	11/44	0/40	4/38	0/38	4/28	0/28
Hemangioma	0/60	0/60	1/40	2/40	0/44	0/40	0/38	3/38	0/28	0/28
Hemangiosarcoma	0/60	1/60	1/40	1/40	8/44	5/40	6/38	12/38	0/28	0/28
Lymphangioma	0/60	0/60	0/40	0/40	0/44	0/40	0/38	0/38	0/28	1/28
Lung										
Bronchiolcalveolar tumor	8/60	8/60	12/40	6/40	29/44	23/40	27/38	23/38	4/28	1/28
Metastatic tumors	0/60	0/60	0/40	4/40	0/44	2/40	0/38	2/38	1/28	0/28
Mammary Gland										
Adenoma	0/60	0/60	0/40	0/40	0/44	1/40	0/38	0/38	0/28	0/38
Adenocarcinoma/carcinoma	0/60	4/60	0/40	10/40	0/44	13/40	0/38	6/38	0/28	0/28
Myxoma	0/60	0/60	0/40	1/40	0/44	0/40	0/38	0/38	0/28	0/28
Malignant Lymphoma										
Heart, lung, liver, spleen, kidney, lymph node, stomach, intestine and/or skin	2/60	0/60	2/40	0/40	1/44	1/40	0/38	0/38	0/28	1/28
Pituitary										
Chromophobe-cell adenoma	0/60	0/60	0/40	0/40	0/44	1/40	0/38	0/38	0/28	0/28
Skin										
Squamous-cell carcinoma	0/60	0/60	0/40	1/40	0/44	0/40	0/38	0/38	1/28	0/28
Fibrosarcoma	1/60	0/60	0/40	0/40	1/44	0/40	0/38	0/38	0/28	0/28
Hemangioma	0/60	0/60	1/40	0/40	0/44	0/40	0/38	0/38	0/28	0/28
Hemangiosarcoma	0/60	0/60	0/40	0/40	0/44	3/40	0/38	2/38	0/28	0/28
Intestine										
Adenocarcinoma	1/60	0/60	0/40	0/40	1/44	0/40	0/38	0/38	0/28	0/28
Kidney										
Carcinoma	0/60	0/60	0/40	0/40	0/44	0/40	0/38	0/38	1/28	0/28
Hemangioma	0/60	1/60	0/40	0/40	0/44	0/40	0/38	0/38	0/28	0/28
Uterus										
Lieomyoma	0/60	2/60	0/40	0/40	0/44	0/40	0/38	0/38	0/28	0/28
Spleen										
Hemangioma	0/60	1/60	1/40	0/40	0/44	0/40	0/38	0/38	0/28	0/28
Lymph Node										
Hemangioma	0/60	0/60	0/40	1/40	0/44	0/40	0/38	0/38	0/28	0/28
Mesentery-Peritonium										
Hemangioma	0/60	0/60	0/40	2/40	0/44	0/40	0/38	0/38	0/28	0/28
Hemangiosarcoma	0/60	0/60	1/40	1/40	2/44	2/40	0/38	0/38	1/28	0/28

a/ Number of mice with tumors/total number of mice studied.

TABLE 29

SURVIVAL AND MORTALITY OF MICE EXPOSED TO VINYL CHLORIDE WITH OR WITHOUT DISULFIRAM FOR 49 WEEKS

VC (ppm)	Diet	Survival After 49 Weeks		Mean Week of Death or Termination[a/]	
		Male	Female	Male	Female
0	-DS	6/8 (75)[b/]	8/8 (100)	47	49
	+DS	8/8 (100)	6/8 (75)	49	45
50	-DS	6/8 (75)	5/8 (62)	43	46
	+DS	3/7 (43)	5/8 (62)	44	43
250	-DS	3/8 (38)	0/8 (0)	42	33
	+DS	8/8 (100)[c/]	7/8 (88)[c/]	49	48
1,000	-DS	0/8 (0)	0/8 (0)	27	34
	+DS	6/8 (75)[c/]	3/7 (43)	43	43

a/ Study terminated after 49 weeks.
b/ Number of survivors/ number of exposed (% survivors).
c/ Significantly different from corresponding VC exposure without DS.

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TABLE 30

SUMMARY OF TUMORS IN MICE EXPOSED TO VINYL CHLORIDE WITH OR WITHOUT DISULFIRAM

Tumor	Diet	Vinyl Chloride (ppm) 0 Male	0 Female	50 Male	50 Female	250 Male	250 Female	1,000 Male	1,000 Female
Bronchioloalveolar adenoma	-DS	0/4 (0)[a]	0/8 (0)	1/6 (17)	0/6 (0)	6/6 (100)	4/5 (80)	4/5 (80)	5/5 (100)
	+DS	1/8 (12)	0/6 (0)	1/4 (25)	1/5 (20)	7/8 (88)	4/8 (50)	7/7 (100)	4/4 (100)
Hemangiosarcoma (liver and/or mesentery)	-DS	0/4 (0)	0/8 (0)	1/6 (17)	1/6 (17)	5/6 (83)	2/5 (40)	4/5 (80)	3/5 (60)
	+DS	0/8 (0)	0/6 (0)	0/4 (0)	0/5 (0)	2/8 (25)	0/8 (0)	3/7 (43)	1/4 (25)
Mammary gland[b]	-DS	0/4 (0)	0/8 (0)	0/6 (0)	2/6 (33)	0/6 (0)	2/5 (40)	0/5 (0)	3/5 (60)
	+DS	0/8 (0)	0/6 (0)	0/4 (0)	0/5 (0)	0/8 (0)	1/8 (12)	0/7 (0)	0/4 (0)

a/ Number of mice with tumor/number of mice examined (% with tumor).

b/ Tumors composed of ductular adenocarcinoma, squamous-cell carcinoma, anaplastic-cell carcinoma and/or hemangiosarcoma.

TABLE II

EFFECT OF DISULFIRAM (DS) AND VINYL CHLORIDE (VC) ON THE COMPOSITION AND ENZYME ACTIVITY OF THE HEPATIC SMOOTH ENDOPLASMIC RETICULUM IN MALE MICE

	Control Diet			DS Diet		
	VC (ppm)			VC (ppm)		
	0	50	250	0	50	250
Liver Weight (% body weight)	4.7 ± 0.2[a/]	6.6 ± 0.2[b/]	6.5 ± 0.2[b/]	5.4 ± 0.2	5.9 ± 0.6[b/]	5.5 ± 0.2
Microsomal protein (mg/g liver)	19.0 ± 0.9	14.7 ± 1.6	13.8 ± 1.9	14.5 ± 0.7	11.2 ± 0.1	12.9 ± 1.6
Cytochrome P-450 (nmole/mg protein)	1.17 ± 0.03	0.90 ± 0.06[b/]	1.14 ± 0.07	0.87 ± 0.02[b/]	0.86 ± 0.05[b/]	1.02 ± 0.03
Cytochrome b_5 (nmole/mg protein)	0.43 ± 0.01	0.35 ± 0.01[b/]	0.40 ± 0.04	0.45 ± 0.01	0.38 ± 0.02	0.48 ± 0.02
Aniline hydroxylase (nmole PHP/mg protein/min)	1.08 ± 0.01	0.94 ± 0.02	0.97 ± 0.10	0.29 ± 0.05[b/]	0.37 ± 0.06[b,c/]	0.49 ± 0.10[b,c/]
Ethylmorphine-N-demethylase (nmole HCHO/mg protein/min)	8.6 ± 0.2	9.1 ± 0.8	9.6 ± 0.6	5.1 ± 0.6[b/]	11.6 ± 0.3[b,c,d/]	10.6 ± 0.7[b,d/]
p-Nitroanisole-O-demethylase (nmole PNP/mg protein/min)	5.3 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	3.4 ± 0.2[b/]	5.5 ± 0.1[d/]	5.9 ± 0.5[d/]

a/ All values indicate mean ± S.E. of two to six determinations.
b/ Significantly different from mice receiving control diet alone, $p < 0.05$.
c/ Significantly different from mice not fed DS and exposed to same concentration of VC, $p < 0.05$.
d/ Significantly different from mice receiving DS diet alone, $p < 0.05$.

TABLE 32

EFFECT OF DISULFIRAM (DS) AND VINYL CHLORIDE (VC) ON THE COMPOSITION AND ENZYME ACTIVITY OF THE HEPATIC SMOOTH ENDOPLASMIC RETICULUM IN FEMALE MICE

	Control Diet		DS Diet	
	VC (ppm)		VC (ppm)	
	0	50	0	50
Liver weight (% body weight)	4.8 ± 0.1[a/]	5.8 ± 0.1[b/]	5.0 ± 0.2	5.3 ± 0.2
Microsomal protein (mg/g liver)	16.0 ± 0.5	15.6 ± 1.7	14.9 ± 0.5	13.8 ± 0.5
Cytochrome P-450 (nmole/mg protein)	1.07 ± 0.03	0.90 ± 0.11	0.81 ± 0.02[b/]	0.94 ± 0.07
Cytochrome b_5 (nmole/mg protein)	0.44 ± 0.01	0.36 ± 0.01[b/]	0.38 ± 0.01	0.44 ± 0.02[c/]
Aniline hydroxylase (nmole PHP/mg protein/min)	1.15 ± 0.05	1.09 ± 0.05	0.38 ± 0.02[b/]	0.66 ± 0.05[b,c,d/]
Ethylmorphine-N-demethylase (nmole HCHO/mg protein/min)	8.4 ± 0.2	11.8 ± 2.0	3.9 ± 0.1[b/]	11.9 ± 0.3[b,d/]
p-Nitroanisole-O-demethylase (nmole PNP/mg protein/min)	5.4 ± 0.2	6.5 ± 0.6	3.5 ± 0.3[b/]	6.1 ± 0.3[d/]

a/ All values indicate mean ± S.E. of two to six determinations.
b/ Significantly different from mice receiving control diet alone, $p < 0.05$.
c/ Significantly different from mice not fed DS and exposed to same concentration of VC, $p < 0.05$.
d/ Significantly different from mice receiving DS diet alone, $p < 0.05$.