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DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

A thirty-day cooperative study designed to examine the pulmonary effects of vinyl chloride (VC) inhalation exposure in male CD-1 mice, Golden Syrian hamsters, and Sprague-Dawley rats was terminated after only 4 exposures due to unexpected early mortality in male mice. Animals were exposed to 0, 1000, or 5000 ppm VC (mice and hamsters only), 6 hours/day. By the fourth exposure, 4 of 30 mice exposed to 1000 ppm were dead and 2 more were moribund. There was no mortality in either of the other groups or species. VC-induced hepatic necrosis was determined to be the cause of death based upon viral serology, gross, and histopathologic examination.

A second study utilizing only five male CD-1 mice at each of the same exposure levels for 5 exposures produced similar results. Clinically, several mice exposed to either 1000 or 5000 ppm VC were lethargic and had distended abdomens. Gross necropsy examination disclosed ascites, a variable degree of subcutaneous edema, and liver lesions. The livers were generally swollen, congested, and had variable sized necrotic areas. Histopathological examination disclosed centrilobular to massive hepatic necrosis. Lesions considered secondary to the hepatic necrosis were noted involving the lymphoid and circulatory systems. Mice surviving the exposures and allowed to recover for up to 13 days had evidence of hepatic reparative and regenerative processes.

Generally, the hepatic lesions were more severe in mice exposed to 1000 ppm than in mice exposed to 5000 ppm VC. However, there was wide variability within each exposure group.

In contrast to mice, there were no significant lesions noted in either hamsters or rats.

The results of these two studies should be considered when evaluating results from experiments with vinyl chloride utilizing mice.

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VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

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Reviewed by:

M. J. McKenna

Final Report

February 3, 1981

R&S 114498

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SUMMARY

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The results of these two studies should be considered when evaluating results from experiments with vinyl chloride utilizing mice.

INTRODUCTION

As part of a cooperative study on the pulmonary effects of inhaled vinyl chloride (VC), male mice and hamsters were exposed to concentrations of 0, 1000, or 5000 ppm VC by the Inhalation Toxicology Section of the Toxicology Research Laboratory. To make efficient use of the inhalation chambers, male rats from a study on molecular mechanisms of carcinogenesis were included in the 0 and 1000 ppm exposure groups. The exposures were originally scheduled to last for 30 days but early mortality and other adverse effects were noted, necessitating the premature termination of the study after 4 exposures.

Because preliminary investigations indicated that the mortality was possibly due to unexpected acute hepatotoxic effects of VC at these exposure levels, a second small probe study, utilizing only male mice at each of the above exposure levels, was performed. This second study was designed to test the reproducibility of the previous observations and to obtain better necropsy specimens for histopathological characterization of acute lesions induced by VC inhalation.

This report will present the results of these two studies. Emphasis will be placed upon the histopathological description of the lesions.

MATERIALS AND METHODS

General Study Design

The initial study reported herein was a continuation of collaborative research pursued jointly with Dr. Yasunosuki Suzuki, Research Professor of Community Medicine, Mt. Sinai School of Medicine, New York, New York under a grant he received from the NIH. The grant was for investigation into the pulmonary effects of vinyl chloride exposure in mice and hamsters. This was a cooperative effort wherein the exposures to VC were to be conducted at the Dow Toxicology Research Laboratory and the animals were to be shipped alive to Dr. Suzuki at the completion of the exposure period for specimen collection, processing and evaluation. This study contained 30 male mice and 10 male hamsters at exposure levels of 0, 1000, or 5000 ppm VC. In addition, 10 male rats were included in the 0 and 1000 ppm exposure groups as part of a separate study on molecular mechanisms of carcinogenesis (Stott, 1979). For purposes of this report, this cooperative study will be termed Study A. This study was scheduled to last for 30 days but was terminated after 4 exposures.

The second study, hereafter termed Study B, consisted of 5 male mice at each of the three above exposure levels. This study was scheduled, and lasted, for 5 consecutive daily exposures.

Test Material

The vinyl chloride was supplied by The Dow Chemical Company and assayed for purity by gas chromatography by The Dow Chemical Company Analytical Laboratories, Midland, Michigan. The sample was found to be 99.9% pure (Cortes, 1979). Traces of methyl chloride and vinylidene chloride were found to be the only impurities.

Animals

The mice used in both Study A and Study B were male CD-1 mice, 5-6 weeks old (Charles River Breeding Laboratories, Inc., Portage, Michigan). Hamsters were 4-6 week old male Golden Syrian hamsters (Engle Laboratory Animals, Inc., Farmersburg, Indiana). The rats were male Sprague-Dawley rats weighing about 225 grams (Spartan Laboratories, Haslett, Michigan).

The animals were acclimated to the laboratory for at least 11 days prior to the initial exposure to VC. The animals were randomized from a single lot for each species (separate lots for mice in Study A and Study B) by use of a computer generated randomization procedure. The mice and rats were individually identified by a numbered metal ear tag. The hamsters were individually identified by cage and rack numbers. The animals were housed (and exposed) 1 animal/cage in stainless steel mesh cages. The animals were housed in rooms designed to maintain a controlled environment of humidity (40 to 60%), temperature (22±2°C), a 12 hour photocycle, and 12 air changes/hour. Food (Ralston Purina rodent chow) and water were available ad libitum except during exposure.

The animals in Study A were all weighed the day prior to the initial exposure to VC but subsequent weights were not taken. All animals were observed for signs of toxicity as well as changes in appearance and demeanor.

Chambers, Vapor Generation, and Analysis

The exposure chambers were 1 m^3 stainless steel and glass Rochester-type chambers. Airflow through the chambers was maintained at 150 liters per minute. The air supply system is designed to provide chamber temperatures of approximately 21°C and 50% relative humidity. The VC gas was metered through a calibrated flowmeter at a controlled rate into the main chamber air supply and further diluted to the desired concentration.

The analytical concentration of VC in each chamber was determined by infrared spectophotometry using a Miran I infrared spectrophotometer equipped with a variable pathlength gas cell. Analyses were performed at a wavelength of 10.9μ at least 10 times daily for each exposure chamber. Standards for the analyses were made by injecting a known volume of VC gas into a 100 liter Saran gas sampling bag filled with a known volume of air.

Exposure Regimen

The groups of animals as detailed in the general study design were exposed to 0 (control), 1000, or 5000 ppm VC for 6 hours per day. These exposures were to be performed 5 days/week. However, in Study A, the exposures were terminated after 4 consecutive days. In Study B, the animals were exposed for 5 consecutive days.

Microbiological Determinations

Blood samples were collected from anesthetized animals from Study A for serological determination of antibodies to several murine viruses commonly found in laboratory rodents. Samples from representative animals from each exposure level/species were taken 5 days after termination of the exposures. Additional (convalescent) serums were collected from mice 13 days after the termination of exposures. The serums were sent to Microbiological Associates, Bethesda, Maryland for their standard panel of murine virus antibody determinations. Included in this panel were pneumonia virus of mice, reovirus 3, encephalomyelitis virus, K virus, polyoma, minute virus, ectromelia, Sendai, mouse adenovirus, mouse hepatitis, lymphocytic choriomeningitis, and rodent corona.

Necropsy and Gross Pathology

All animals presented to necropsy alive were anesthetized with methoxyflurane and the animal was killed by decapitation. Immediately after

decapitation, the eyes were examined *in situ* by gently pressing a glass slide against the cornea and examining the eye under fluorescent light illumination.

A gross necropsy examination, both externally and internally, was performed with all alterations recorded. Representative portions of the following organs and tissues were preserved in 10%, neutral, phosphate-buffered formalin from each mouse:

- adipose tissue
- adrenal glands
- accessory sex glands
- aorta
- bone with bone marrow (thoracic vertebral column)
- brain
- epididymides
- esophagus
- eyes
- gallbladder
- heart
- kidneys
- lacrimal (Harderian) gland
- large intestine (cecum and colon)
- larynx
- liver
- lower jaw
- lungs
- lymph nodes (thoracic, mesenteric)
- mammary tissue
- pancreas
- peripheral nerve (sciatic)
- pituitary gland
- salivary gland (submandibular)
- skeletal muscle
- skin
- small intestine
- spinal cord (thoracic)
- spleen
- sternum
- stomach
- testes
- thymus
- thyroid (with parathyroid)
- tongue
- trachea
- urinary bladder
- all gross lesions

Only livers and kidneys were preserved from three of the hamsters and rats.

For Study A, two mice/exposure group were sacrificed three days after termination of exposures. Two days later three animals/species/exposure group were sacrificed. The remaining animals were held until 13 days after the termination of exposures, at which time they were sacrificed by decapitation following methoxyflurane anethesia. Because of the lack of further clinical signs of disease or mortality considered due to VC exposure, necropsies were not performed on these animals. However, the abdominal and thoracic viscera from all mice were exposed and examined and the livers from six mice were retained in formalin.

An external and internal necropsy examination was performed for all animals that died spontaneously during the course of the studies. This consisted of four mice exposed to 1000 ppm VC dying after the third or fourth exposure in Study A, one mouse exposed to 5000 ppm dying 13 days after termination of exposures in Study A, and one mouse exposed to 1000 ppm dying after 2 exposures in Study B. These animals were refrigerated from the time death was noted until necropsy. The tissues and organs listed above were retained in 10%, neutral, phosphate-buffered formalin.

Histological Preparations

Hematoxylin and eosin-stained sections of paraffin-embedded tissues were prepared by the standard operating procedures of the histology laboratory.

An extensive set of tissues, intended to include all the major soft tissue organs, was prepared from the six moribund or dead mice after 3 or 4 exposures in Study A.

The liver, gallbladder, thymus, and kidneys were prepared for histopathological examination from all mice from both studies except those mice from Study A terminated at 3 and 13 days following the final (fourth) exposure. From these mice, only the livers with gallbladders were prepared for histopathological examination. The four largest lobes of the liver (left lobe, right lobe and both portions of the medial lobe) were routinely prepared for histological examination.

The livers, gallbladders (hamsters), thymus, and kidneys were prepared for histopathological examination from all rats and hamsters necropsied.

For selected tissues, special-stained histological preparations (Giemsa stain, Von Kossa calcium stain, Dahl's calcium stain) were prepared by standard histopathology laboratory procedures (Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 1968).

RESULTS

Chamber Analysis

The daily mean analytical concentrations of VC were calculated from multiple daily chamber analyses. For Study A, these analytical concentrations were 968±33 ppm and 5137±165 ppm VC for the 1000 and 5000 ppm groups, respectively. For Study B, these values were 999±45 ppm and 5112±173 ppm VC.

Animal Observations

The morbidity and mortality data from these two studies are summarized in Table 1.

Study A: After 3 exposures, some of the mice exposed to either 1000 ppm or 5000 ppm VC were lethargic. Their coats were roughened and some had distended abdomens. On the morning following the third exposure (prior to the fourth exposure) 3 mice exposed to 1000 ppm were dead and 3 more were moribund. During the fourth exposure 1 of these moribund mice died. The hamsters and rats appeared normal. At this time, the exposures were terminated and all animals were held for further observation. The two moribund and four dead mice exposed to 1000 ppm VC were presented for necropsy examination. Three days after termination of the exposures, two more mice from each group were necropsied. Clinically, these animals were all active but one mouse exposed to 5000 ppm VC had a distended abdomen. Five days after termination of the exposures, three more mice and three rats and hamsters from each group were necropsied. These mice were active but two of the animals exposed to 5000 ppm VC had distended abdomens. The remaining animals were observed for 13 days. During this interval, clinical abnormalities were not noted; however, one mouse exposed to 5000 ppm VC was found dead thirteen days after the final exposure.

The rats and hamsters remained normal throughout the 2 week observation period. Three animals/exposure level/species were sacrificed five days after the fourth exposure.

Study B: The clinical observations of the mice in Study B were similar to those noted in Study A. One mouse exposed to 1000 ppm died after two exposures. Some mice exposed to 1000 ppm and 5000 ppm VC were lethargic and had roughened pelage after three exposures. Distended abdomens were noted after three or four exposures for 3 of the 4 remaining mice exposed to 1000 ppm and 2 of the 5 mice exposed to 5000 ppm VC.

Microbiological Determinations

To investigate the possible presence of viral infectious disease, serological examinations were performed on specimens collected from mice, rats, and hamsters from all exposure groups in Study A five days after the last (fourth) exposure. Convalescent titers from mice were analyzed on samples taken 13 days after the last exposure. The titers are presented in Table 2.

Antibodies to 12 different murine viruses were not detected in mice at any exposure level at either sampling time.

Hamsters and rats had antibodies to pneumonia virus of mice (PVM) and Sendai virus. The antibody level in rats to these viruses was low. In hamsters, antibody titers to Sendai virus were high, whereas those to PVM were low.

Gross Pathology

The gross lesions noted in Study A are presented in Tables 3 (mice), 4 (hamsters), and 5 (rats). The gross lesions noted in mice from Study B are presented in Table 6.

The only gross lesions considered to be directly related to the inhalation of vinyl chloride were noted in mice from both studies. Because of the irregular schedule of termination with the accompanying variable, but small, group size and also because the lesions noted were similar in both studies, the results of the gross pathologic examination will be presented without regard to study but with interpretation allowing for the temporal responses of the recovery period.

Externally, a low number of mice exposed to either 1000 or 5000 ppm VC developed distended abdomens. This was noted in animals presented alive to necropsy after 4 or 5 exposures and was still present in some animals 3 to 5 days after termination of exposure; however, this was not noted in animals dead or moribund after 2-4 exposures. One of the five animals exposed to 5000 ppm VC for 4 exposures and allowed a 5-day recovery period also had ventral subcutaneous edema noted externally. The animals dying spontaneously after 3 or 4 exposures to 1000 ppm VC had reddened nares considered to represent agonal congestion.

Internally, some mice exposed to either 1000 or 5000 ppm VC had gross lesions suggesting toxicity to the liver, lymphoid system, and possibly cardiovascular system. In the affected mice, the livers were usually enlarged and had a variety of color changes. Frequently there was hepatic congestion that varied from generalized to a unique pattern of central or hilar congestion with pale or blanched margins. The hepatic lobular pattern was often accentuated, apparently due to congestion. Foci compatible with acute necrosis were noted. These varied from large foci up to several mm in size, occupying most of a liver lobe and varying in color from pale to red, to multiple pinpoint pale foci. These necrotic-appearing areas were generally larger and involved more of the liver in those mice dying or moribund after 2-4 exposures to 1000 ppm VC than in mice surviving the exposure periods.

Clear to yellow ascitic fluid and subcutaneous edema was also generally noted in the mice having liver lesions. The mice exposed to 1000 ppm VC had, generally, slightly more severe fluid accumulations than those exposed to 5000 ppm VC.

The animals dying spontaneously frequently had pulmonary lesions characterized by slight pulmonary edema and slight to moderate accumulations of serous fluid in the thoracic cavity. Since this was not noted in animals surviving the exposure period, it is believed that this represents agonal cardiovascular collapse.

A slight decrease in the size of the thymus was noted in all four mice surviving five exposures to 1000 ppm VC (Study B) and 3 of 5 surviving five exposures to 5000 ppm VC.

The other gross lesions presented in Tables 2 and 5 are considered to be either secondary non-specific lesions commonly noted in moribund animals, changes associated with the necropsy procedures, or spontaneous lesions frequently noted in mice of this age and strain. The one mouse found dead 13 days following termination of exposure to 5000 ppm VC was moderately autolytic but no other lesions were noted.

The gross observations noted in hamsters (Table 4) and rats (Table 5) from Study A were all considered to be artifacts induced by necropsy procedures or spontaneous lesions frequently noted for these species.

Histopathology

The histopathologic observations made in Study A are presented in Tables 7 (mice), 8 (hamsters), and 9 (rats). For the mice from Study B, the histopathologic observations are presented in Table 10.

The histopathologic examination of tissues from these animals confirmed the gross observations of liver, lymphoid, and possibly cardiovascular toxicity in mice. As was done in the section on gross pathology, the

histopathologic observations will be discussed by combining both studies. The lesions were similar in the two studies when allowance is made for the varying time period from termination of exposure until necropsy.

Mice dying spontaneously or moribund after 2-4 exposures to 1000 ppm VC were characterized by acute hepatic necrosis affecting a large portion of the liver. In the seven mice with this exposure history, about one-fourth of the liver area was necrotic in one mouse, between onefourth and one-half was necrotic in four mice, between one-half and three-fourths was necrotic in one mouse, and the remaining mouse had necrosis of over three-fourths of the liver as judged from histologic sections of the four major liver lobes. When the necrotic areas were small, they were centrilobular; however, they were frequently massive and involved confluent lobules with only a thin rim of viable hepatocytes around the portal triads. Frequently, minute basophilic intracellular granular structures were noted in swollen necrotic hepatocytes as well as viable hepatocytes at the margins of the necrotic areas. These were demonstrated to contain calcium by special staining techniques. There was minimal inflammatory reaction noted at the margin of the necrotic areas in these mice dying spontaneously. The few inflammatory cells present were predominantly macrophages. There were marked hepatic vascular changes noted in this group of mice. There was usually intense congestion within and at the margins of the necrotic areas. In the areas of viable hepatic parenchyma there was often severe dilatation of the centrilobular vein with compression of adjacent parenchyma along with focal areas of sinusoidal dilatation scattered throughout the hepatic lobule. These areas often appeared as vascular "lakes" among the parenchyma. The viable areas in the livers of these animals had centrilobular or perinecrotic hepatocytes generally characterized by swelling with vesicular or vacuolated cytoplasm. In some livers, these centrilobular or perinecrotic hepatocytes had increased cytoplasmic homogeneity and basophilia with occasional mitotic figures. A low number of small, eosinophilic, sinusoidal masses were frequently seen

among these cells. While this material could represent necrotic cellular debris, these masses were considered more likely to be microthromboses.

The livers of mice surviving 4 or 5 exposures to 1000 or 5000 ppm VC had marked histopathological variability. The four mice sacrificed immediately after completion of 5 exposures to 1000 ppm VC (Study B) had acute centrilobular hepatic necrosis. While this was qualitatively similar to that noted for those animals dying spontaneously at this exposure level, it was much less in degree -- being about one-tenth of the liver in three of these mice and one-fourth in the other one. These necrotic foci were often present in a subcapsular location. The reaction to the necrotic foci was similar to that described for those animals dying spontaneously but the vascular reaction was less dramatic and the inflammatory reaction included slight connective tissue proliferation. In centrilobular areas lacking necrosis, the hepatocytes often were enlarged with increased cytoplasmic homogeneity and basophilia and occasionally cytoplasmic vacuolization and mitotic figures. Hyaline sinusoidal masses consistent with microthrombi were seen in some of the centrilobular areas.

The five mice sacrificed immediately after completion of 5 exposures to 5000 ppm VC (Study B) had marked variation in histopathologic changes. Three of these mice had only microvesiculation of the centrilobular hepatocytes but lacked any significant amount of necrosis other than th scattered microscopic foci typically noted also in control animals. The other two animals were similar to those animals dying spontaneously in that they had large areas of acute necrosis (between one-fourth and one-half of the liver in one mouse and between one-half and three-fourths in the other). The inflammatory and vascular events roughly paralleled those noted in the mice dying spontaneously with comparable liver lesions.

With 3 to 5 days of recovery following completion of four exposures to vinyl chloride, there was again variability in the histopathologic changes in the liver. Three of five mice exposed to 1000 ppm VC and 2 of 5 mice exposed to 5000 ppm VC had changes of the centrilobular hepatocytes with either enlargement and cytoplasmic vesiculation or cytoplasmic homogeneity, basophilia, and occasional mitotic figures. In one mouse in each exposure level this reaction was more severe with marked cytoplasmic swelling and vesiculation, scattered necrotic individual hepatocytes and sinusoidal thrombi and dilatation. In one mouse exposed to 1000 ppm VC and 2 mice exposed to 5000 ppm VC, there were multifocal areas of centrilobular hepatic necrosis involving about one-tenth of the liver parenchyma. These were surrounded by a slight subacute inflammatory reaction in which fibroblasts were noted. In one mouse exposed to 5000 ppm VC, some multinucleate giant cells were noted in the reaction.

By 13 days after completion of the exposures, the livers of 4 of 6 mice in each exposure group were similar to controls or had only slight microvesiculation and enlargement of centrilobular hepatocytes. One mouse in the 5000 ppm VC exposure group had marked centrilobular hepatocyte enlargement with occasional mitotic figures and sinusoidal microthrombi. Two mice exposed to 1000 ppm VC and one mouse exposed to 5000 ppm VC had multifocal areas of fibrosis with mononuclear inflammatory cells and, characteristically, multinucleate giant cells containing a deeply basophilic structure. This was identified as calcified material by appropriate staining techniques. These fibrotic areas were centrilobular and often subcapsular. These livers also had slight periportal accumulations of mononuclear inflammatory cells and slight bile duct hyperplasia was noted in one mouse exposed to 1000 ppm VC. Foci of dilated sinusoids surrounded by thin fibrous connective tissue walls were noted throughout the liver, particularly subcapsularly.

The thymus had histopathologic lesions that closely paralleled the degree of hepatic necrosis. Mice that were moribund or died spontaneously had loss of the thymic lymphoid cells that varied but was

usually moderate to marked in severity. These also had karyorrhectic nuclear fragments in the medulla of the thymic lobules. The thymuses of mice that survived the exposure period were frequently normal. When depletion of thymic lymphocytes was noted in surviving mice it was present in the mice having hepatic necrosis. The severity of thymic lymphoid depletion roughly paralleled the severity of hepatic necrosis. In the mice dying spontaneously, the other lymphoid tissues examined (spleen and lymph nodes) had decreased lymphoid cells similar in degree to that noted in the thymus.

Histopathologically, the kidneys of mice dying spontaneously frequently had tubules with sloughing epithelium, pyknotic nuclei, and hypereosinophilic cytoplasm. As these changes were not noted in properly fixed kidneys from sacrificed mice they were attributed to autolysis. Three of the five mice exposed to 5000 ppm VC (Study B) had slight to moderate cytoplasmic microvesiculation of the renal cortical tubules. All other histopathologic observations made on the kidneys of treated or control mice were considered to be spontaneous entities and not due to exposure to vinyl chloride.

The hearts of 3 of the 6 mice moribund or dead after 4 exposures to 1000 ppm VC (Study A) had isolated focal areas of minute basophilic structures within degenerating cardiac myocytes. This was shown to be calcified material by use of special histologic staining techniques.

Other organs examined from mice dying spontaneously (Study A) had occasional lesions. These were generally considered to be either secondary to the agonal condition of the mouse or spontaneous entities not due to VC exposure.

The histopathologic observations noted for the liver, gallbladder, kidneys, and thymus from hamsters (Table 8) and rats (Table 9) exposed to vinyl chloride were considered to be spontaneous lesions with no lesions ascribed to VC exposure.

Histologic examination of the liver and kidneys from the one mouse found dead 13 days after termination of exposure to 5000 ppm VC disclosed moderate autolytic changes. No lesions similar to those present in the affected mice were noted. Although a cause of death was not determined, it was felt that this mouse did not die from VC-induced hepatotoxicity and it was not included in the gross and histopathology tables (Tables 3 and 7).

DISCUSSION

The acute deaths in mice exposed to 1000 ppm VC were first suspected to be of infectious origin. Early results from histopathological examination combined with results of viral serology failed to support this presumption. Neither viral inclusion bodies nor bacteria were noted on histopathological examination. Mice failed to have titers to numerous viral pathogens, including those commonly associated with hepatic necrosis (ectromelia, mouse hepatitis virus, and reovirus). Although antibody titers to two viruses were present in both rats and hamsters, the titers were relatively low and the viruses both produce pneumonia rather than hepatic disease. These findings suggested that VC may be acutely toxic to the male mouse.

Although hindered by the low numbers of mice, the marked variation in severity of lesions, and the variable degree of autolytic change, the staggered necropsy times following termination of VC exposure allowed a morphologic assessment of the nature and progression of lesions in mice following short-term inhalation exposure to VC.

The major organ affected was the liver. The first histological changes appeared to be vesiculation, vacuolation and swelling of the centrilobular hepatocytes. This was noted in most of the animals exposed to either 1000 ppm or 5000 ppm VC. In a low proportion of animals this lesion was markedly more severe with marked centrilobular and midzonal cellular enlargement and vacuolization and compression of the sinusoids. In these areas small hyaline masses were frequently noted among the swollen cells. These masses could represent necrotic hepatocytes but are considered more likely to be microthromboses.

Following the initial cytoplasmic changes, the centrilobular and midzonal hepatocytes in some livers had homogeneous eosinophilic cytoplasm with changed staining affinity characterized by a slight basophilic tinge.

These cells were enlarged, sometimes markedly, and mitotic figures were often noted. As this change was more prominent in the animals sacrificed after several days of recovery, this was considered a regenerative response.

Next in the apparent progression of lesions was centrilobular necrosis. This was characteristically coagulation necrosis with only a very slight peripheral inflammatory reaction. The inflammatory reaction that ensued consisted primarily of macrophages and fibroblastic elements. The progression from the centrilobular hepatocyte swelling to necrosis was unclear. Frequently the perinecrotic hepatocytes were either vacuolated or contained eosinophilic intracytoplasmic masses assumed to be degenerated cystosol proteins. However, these changes in the hepatocytes adjacent to necrotic areas were of no greater severity than those present in cells in non-necrotic centrilobular areas. Thus, degree of vacuolar change alone did not appear to correlate with the necrosis.

Of interest are the minute basophilic structures noted in some of the necrotic hepatocytes and perinecrotic hepatocytes. These were demonstrated to contain calcium via the use of special histological stains. It is speculated that these structures represent mineralized mitochondria. Mitochrondrial calcification is commonly noted by agents primarily attacking the cell membrane (Trump and Arstila, 1975).

In all mice dying after 2-4 exposures to 1000 ppm VC, there was progression from multiple small centrilobular necrotic areas to massive confluent areas of necrosis. Most animals dying spontaneously had necrosis of about 50% of the hepatic area on the four liver sections examined. Survivors generally had necrosis of 25% or less of the liver area. With mice exposed to 5000 ppm VC, there were no deaths ascribed to VC and the amount of liver necrosis was generally less. However, in two mice exposed to 5000 ppm VC for 5 exposures (Study B), there was massive necrosis (up to 75%) and it is speculated that death may have been imminent had the animal not been sacrificed.

The role of the vascular system in the pathogenesis of the liver necrosis is uncertain. In the animals dying spontaneously there was dramatic congestion in and adjacent to the necrotic areas. There was frequently marked congestion of the central vein and focal areas of sinusoidal dilatation with compression of adjacent hepatocytes. There was slight to moderate pulmonary congestion and edema; however, other abdominal organs (spleen, kidneys, gastrointestinal tract) had little evidence of vascular congestion to suggest generalized heart failure. Although sinusoidal microthrombi were present, larger thrombi were not noted. Survivors frequently had small sinusoidal dilatations surrounded by fibrous connective tissue, but lacked other vascular changes. The dramatic vascular congestion noted in the livers of moribund or dying animals was considered to be a probable secondary reflection of the liver necrosis as well as terminal heart failure.

In a recent paper reviewing human hepatic angiosarcomas due to vinyl chloride and other causes, Popper et al. (1978) note a precursor stage characterized by hepatocellular proliferation along with sinusoidal dilatation and sinusoidal lining cell proliferation. Their early lesions are similar to those described in this report; however, their report does not note hepatic necrosis, but rather the progressive atrophy and disappearance of the hepatocytes with increasing proliferation of the sinusoidal lining cells leading to angiosarcoma.

Also prominently affected by short-term inhalation exposure to VC was the lymphoid system, particularly reflected by the degree of thymic lymphoid necrosis and atrophy. The degree of thymic lymphoid depletion generally paralleled the degree of liver necrosis but appeared to lag temporally slightly behind the liver necrosis. This was noted especially in survivors in which there were slight thymic changes in spite of liver necrosis. It is speculated that the lymphoid depletion is secondary to the liver necrosis, possibly due to elevated levels of adrenal corticosteroids produced in response to systemic stress due to the massive hepatic necrosis.

There were several changes suggestive of possible disturbed mineral metabolism in the mice with liver necrosis. In addition to the intracytoplasmic mineral noted in and adjacent to the necrotic liver areas, mineral deposits were noted in foci in the myocardium and larger bodies in necrotic areas of the thymus. It is speculated that the mineralization is dystrophic due to cellular injury rather than a reflection of calcium-phosphorous imbalance.

The kidneys had only slight changes considered to be insignificant in causing the death of these mice. The tubular changes noted in the mice dying spontaneously were regarded as artifacts due to autolysis. With sacrifice and prompt fixation, the only lesion attributed to VC exposure was slight to moderate microvesiculation of the cortical tubular epithelium.

Surprisingly, the acute hepatotoxic effect of VC in mice has apparently not been generally recognized. Early short-term inhalation experiments noted that 30 minute exposures to 300,000 ppm VC were lethal to mice and rats, but 100,000 ppm produced only narcosis (Mastromatteo et al. 1960). Prodan et al. (1975) calculated the LC_{50} for white mice as 11.75% VC in a ventilated chamber.

A dominant lethal test in CD-1 male mice (the same strain as that used in this report) exposed by inhalation to 3,000, 10,000, or 30,000 ppm VC for 5 daily 6-hour exposures was reported by Anderson et al. (1976). These doses were selected on the basis of their unreported probe toxicity studies which determined 30,000 ppm to be in the toxic range (the criteria of toxicity were not stated). In the published work, Anderson et al. had significant mortality (55%) at the 30,000 ppm exposure level and 5% and 10% mortality at the 10,000 ppm and 3,000 ppm exposure levels, respectively. The cause of death was not stated.

There have been several subchronic or chronic toxicity studies utilizing mice exposed to VC at levels close to those used in this report. Winell et al. (1976) exposed NMRI (albino) mice to 50 or 500 ppm VC. After 40 weeks the serum levels of lactate dehydrogenase and alkaline phosphatase were increased. Although these enzymes may indicate liver damage, they were apparently increased only after liver tumors were present. There is no indication of any acute deaths due to VC exposure in their report.

Carcinogenesis studies on VC in mice have been reported by Maltoni and Lefemine (1975), Keplinger et al. (1975), and Lee et al. (1977). In all these studies, the mouse was markedly more sensitive than the rat. Maltoni and Lefemine exposed Swiss mice 4 hours/day, 5 days/week for 30 weeks to levels of 50, 250, 500, 2500, 6000, or 10,000 ppm VC. Although no acute lethal effects of VC were presented, there appears to be slight excess mortality in male mice at the higher exposure levels as judged by the number of survivors at 16 weeks.

Keplinger et al. exposed CD-1 mice to 50, 200, or 2500 ppm VC for 7 hours/day, 5 days/week for 9 months. The interim data reported after 8 months of exposure indicated several mice with tumors but there was no indication of acute hepatoxicity.

The only published report noting acute hepatotoxicity in mice is that of Lee et al., in which CD-1 mice were exposed to 50, 250, or 1000 ppm VC for 6 hours/day, 5 days/week for 12 months. They report observations strikingly similar to those noted in this report. Two males and one female (from group size of 36/sex) were found dead between the third and ninth exposure to 1000 ppm VC. Histopathology disclosed acute toxic hepatitis characterized by diffuse coagulation necrosis beginning in the centrilobular areas and focal to marked congestion. They also reported renal tubular necrosis characterized by pyknosis and eosinophilic granulation of the cytoplasm. Their research protocol included the sacrifice of 4 mice/sex at the end of 1, 2, and 3 months. Review of

their progress report (No. 8 submitted to NIEHS) discloses no reported histological lesions for these 24 mice other than minor lesions also present in the control animals.

The studies reported herein confirm the acute hepatotoxicity of VC to CD-1 mice as first noted by Lee et al. and present a more detailed histopathological description of the associated lesions. Several noteworthy aspects of this response are presented including:

1. VC appears to be acutely hepatotoxic at these relatively low concentrations only in the mouse, particularly the CD-1 strain of mice.
2. There is a consistent association of greater severity of toxicity by virtually any of the parameters effected (mortality, gross lesions, or histologic lesions) in the lower exposure level of 1000 ppm VC as opposed to those in the higher exposure level (5000 ppm).
3. There is wide variability within a treatment group; i.e., up to 75% of the liver necrosis and death in some mice exposed to 1000 ppm VC while others survive with virtually undetectable histological lesions.

It is concluded that the observations reported herein need consideration when evaluating research involving vinyl chloride exposure of mice.

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QUALITY ASSURANCE STATEMENT

TITLE OF STUDY: VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES HET-K-1711-(29)

In compliance with Good Laboratory Practice Regulations, the study phases were inspected by the Quality Assurance Unit and the results of these inspections reported to Management and the Study Director on the dates listed below. The report accurately reflects the data generated in accordance with the regulations and standard operating procedures of the laboratory. All data and the reports are located at the submitting laboratory.

R&S 114523

Study Started:	30 Oct. 1979	Report Issued Date:	3 Feb. 1981
Dates of Inspection:	19 Oct. 1979	Date of Report:	19 Oct. 1979
	1 Nov. 1979		2 Nov. 1979
	31 Jan. 1980		5 Feb. 1980
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TABLE 1

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Morbidity and Mortality

Exposure Level (ppm)	0	1000	5000
STUDY A - 4 exposures			
Mice - Number Exposed	30	30	30
Number Died	0	4	1
(Number of exposures before death)		(3,3,3,4)	(4[a])
Number moribund or with distended abdomens	0	2	2
(Number of exposures)		(4,4)	(4,4[b])
Hamsters - Number Exposed	10	10	10
Number Died	0	0	0
Number Moribund	0	0	0
Rats - Number Exposed	10	10	0
Number Died	0	0	-
Number Moribund	0	0	-
STUDY B - 5 exposures			
Mice - Number Exposed	5	5	5
Number Died	0	1	0
(Number of exposures before death)		(2)	
Number with distended abdomens	0	3	2
(Noted at necropsy after 5 exposures)			

[a]This animal was found dead 13 days after exposures were terminated.

[b]These animals were noted with distended abdomens 3 and 5 days after the exposures were terminated.

- Indicates not applicable.

TABLE 2

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Viral Antibody Titers From Mice, Hamsters, and Rats

Species	Exposure Level (ppm)	Time of Sample[a]	Antibody Titer to: PVM	Reo 3	GDV II	K	Polyoma	Minute	Extromelia	Sendai	Adeno	Hepatitis Mouse	LCM	Rodent Corona
Mouse	0	5	-	-	-	-	-	-	-	-	-	-	-	-
Mouse	1000	5	-	-	-	-	-	-	-	-	-	-	-	-
Mouse	5000	5	-	-	-	-	-	-	-	-	-	-	-	-
Mouse	0	13	-	-	-	-	-	-	-	-	-	-	-	ND
Mouse	1000	13	-	-	-	-	-	-	-	-	-	-	-	ND
Mouse	5000	13	-	-	-	-	-	-	-	-	-	-	-	ND
Hamster	0	5	-	-	-	-	-	-	-	≥80	-	-	-	-
Hamster	1000	5	40	-	-	-	-	-	-	≥80	-	-	-	-
Hamster	5000	5	20	-	-	-	-	-	-	40	-	-	-	-
Rat	0	5	40	-	-	-	-	-	-	10	-	-	-	-
Rat	1000	5	20	-	-	-	-	-	-	10	-	-	-	-

[a]Days following termination of exposures.
- Indicates no antibodies to the stated virus were present.
ND = Not Done

TABLE 3

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observation - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
EXTERNAL OBSERVATIONS						
No visible lesions	5	2	5	6	2	6
Distended abdomen	0	0	0	0	3	0
Subcutaneous edema	0	0	0	0	1	0
Reddened nares	0	4	0	0	0	0
Focal cannabalism	0	1	0	0	0	0
INTERNAL OBSERVATIONS						
No visible lesions	1	0	0	5	1	5
General						
Ventral subcutaneous edema - moderate to marked	0	0	0	0	2	0
Ascites - clear abdominal fluid - moderate to marked	0	0	0	0	2	0
Obese	0	1	0	0	0	0
Liver						
Generalized enlargement or swelling	0	5	1	0	0	0
Lobar or large areas of color change, suggesting necrosis (either pale or reddened)	0	2	0	0	1	1
Pinpoint pale foci, multiple, suggesting focal necrosis	0	0	0	1	1	0
Congestion or reddened	1	4	1	0	0	0
Accentuated reddening of hilar areas of multiple lobes	0	0	1	0	1	0
Pale lobar margins, focal or diffuse	0	2	1	0	0	0
Multiple small red foci	0	1	1	0	0	0
Increased lobular pattern	0	5	2	0	3	0
Generalized paleness	1	0	0	0	0	0

Data presented are number of mice having the stated observation.

[a] Grouped by days after final (fourth) exposure when necropsied.

[b] Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

TABLE 3 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observation - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Lungs						
Congested, diffuse	0	4	0	0	0	0
Edema - slight	0	1	0	0	1	0
Focal areas of emphysema	0	1	0	0	0	0
Multiple red foci consistent with aspirated blood	2	0	5	0	3	0
Thoracic Cavity						
Clear fluid - slight to moderate	0	6	1	0	0	0
Mesentery						
Edema	0	1	0	0	0	0
Stomach						
Gaseous distention	0	1	0	0	0	0
Congested or reddened	0	2	0	0	0	0
Small Intestine						
Congested or reddened	0	1	0	0	0	0
Decreased ingesta	0	2	0	0	0	0
Kidney						
Congested or reddened	1	0	0	0	0	0
Thymus						
Petechial hemorrhage	0	0	1	0	0	0

Data presented are number of mice having the stated observation.

[a]Grouped by days after final (fourth) exposure when necropsied.

[b]Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

TABLE 4

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observations - Hamsters[a]

Exposure Level	0	1000	5000
Number Examined	3	3	3
EXTERNAL OBSERVATIONS			
No visible lesions	3	3	2
Firm enlarged boney prominence on lower left rear leg	0	0	1
INTERNAL OBSERVATIONS			
No visible lesions	1	0	1
Lungs			
Multifocal red areas consistent with aspirated blood at necropsy	1	2	1
Focal, depressed, dark red area, isolated	1	0	0
Liver			
Slight accentuation of lobular pattern	0	2	1
Tibia			
Firm smooth boney mass, distal tibia; consistent with healed fracture	0	0	1

Data presented is number of hamsters with stated observation.

[a]Hamsters were necropsied 5 days after fourth exposure to vinyl chloride.

TABLE 5

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observations - Rats[a]

Exposure Level (ppm)	0	1000
Number Examined	3	3
EXTERNAL OBSERVATIONS		
No visible lesions	3	3
INTERNAL OBSERVATIONS		
No visible lesions	0	2
Thymus		
Multiple petechial hemorrhages	1	1
Lungs		
Multifocal red areas consistent with aspirated blood	2	0

Data presented is number of rats with stated observation.

[a]Rats were necropsied 5 days after the fourth exposure to vinyl chloride.

TABLE 6

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observations - Study B Mice

Exposure Level (ppm)	0	1000		5000
Necropsy Group	Final Termination[a]	2 Exposures	Final Termination	Final Termination
Number of Mice in Group	5	1[b]	4	5
EXTERNAL OBSERVATIONS				
No visible lesions	4	1	1	2
Corneal cloudiness, focal				
- unilateral	0	0	0	1
- bilateral	1	0	0	0
Distended abdomen	0	0	3	2
Small size	0	0	1	0
Clinically depressed	0	0	0	1
INTERNAL OBSERVATIONS				
No visible lesions	3	0	0	0
General				
Ventral subcutaneous edema				
- marked	0	0	1	0
- moderate	0	0	1	0
- slight	0	0	1	0
Ascites, clear abdominal fluid				
- marked	0	0	3	1
- moderate	0	0	0	1
Decreased abdominal fat	0	0	1	4
Liver				
Generalized enlargement	0	1	3	2
Lobar or large areas of color change (either pale or reddened)	0	0	2	2
Pinpoint pale foci, multiple	0	0	3	0
Accentuated reddening of hilar area of multiple lobes with pale lobar margins	0	1	0	2
Accentuated lobular pattern	0	1	2	2
Generalized paleness	0	0	2	0
Lungs				
Congested, consistent with hypostatic congestion	0	1	0	0
Pinpoint pink foci, few, indistinct	1	0	0	0
Multiple red foci consistent with aspirated blood	1	0	3	2
Stomach				
Submucosal edema, glandular portion - slight	0	0	1	0
Multifocal pinpoint dark brown foci, glandular mucosa	0	0	0	1
Small Intestine				
Decreased ingesta	0	1	0	0
Cecum				
Increased consistency of ingesta	0	1	0	0
Thymus				
Decreased size - slight	0	0	4	3
Vertebrae				
Prominent thoracic intervertebral discs	0	0	0	1

Data presented are number of animals having the stated observation.

[a] Necropsied after five exposures.

[b] Animal died following two exposures.

TABLE 7

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Liver (number examined microscopically)	5	6	5	6	5	6
No visible lesions	0	0	0	0	0	0
Acute coagulation necrosis, - focal, centrilobular, about 10% of area of sections examined, often subcapsular	0	0	1	1	2	1
- focal, centrilobular to confluent, involving 15-25% of area of sections examined	0	1	0	0	0	0
- focal, centrilobular to confluent, involving 26-50% of area of sections examined	0	3	0	0	0	0
- focal, centrilobular to confluent, involving 51-75% of area of sections examined	0	1	0	0	0	0
- massive, large areas of liver, greater than 75% of area of sections examined	0	1	0	0	0	0
Minute, intracellular basophilic structures at margins of necrotic areas	0	5	0	0	0	0
Perinecrotic inflammatory cells, mixed, - very slight to slight	0	4	0	0	0	0
Perinecrotic subacute inflammatory and connective tissue cells - slight	0	0	1	2	2	2
Perinecrotic swollen, vacuolated hepatocytes	0	5	1	0	1	0
Centrilobular hepatocytes, microvesicular change - very slight to slight	2	0	2	3	0	4
- marked	0	0	1	0	1	1

Data presented are number of animals having the stated observation.

[a]Grouped by days after final (fourth) exposure when necropsied.

[b]Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

TABLE 7 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Gross Pathologic Observation - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Liver (Continued)						
Centrilobular and midzonal hepatocytes enlarged, homogeneous cytoplasm of slightly increased basophilia, occasional mitoses	1	2	2	1	3	2
Focal subcapsular hepatocyte degeneration with vacuolization and hyaline material	0	1	1	1	0	0
Multinucleate giant cells containing calcified material	0	0	0	2	1	1
Sinusoidal congestion and hemorrhage, in and near necrotic areas - moderate	0	0	0	1	1	0
- marked	0	6	0	0	0	0
Centrilobular vein congestion, marked, compression of adjacent hepatocytes	0	4	0	0	1	0
Vascular ectasia, apparently midzonal, with compression of adjacent hepatocytes	0	2	0	0	0	0
Focal telangiectasis	0	0	2	0	2	0
Small hyaline masses in sinusoids, margins of necrotic foci or in areas of swollen hepatocytes	0	1	2	1	1	2
Microvesicular change of periportal hepatocytes - slight	2	0	2	3	0	2
Multifocal microscopic foci of hepatocyte degeneration or necrosis with mononuclear inflammatory cell reaction						
- very slight or slight	4	0	2	3	3	2
- moderate	0	0	1	0	1	1
Periportal mononuclear inflammatory cells - slight	0	0	0	1	0	1
Bile duct hyperplasia - slight	0	0	0	1	0	0

Data presented are number of animals having the stated observation.

[a] Grouped by days after final (fourth) exposure when necropsied.

[b] Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

TABLE 7 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Gallbladder (number examined microscopically)	4	6	4	0	4	3
No visible lesions	3	6	3	-	3	3
Submucosal edema - slight	0	0	0	-	1	0
Submucosal inflammatory cells, mixed, focal - very slight	1	0	1	-	0	0
Kidney (number examined microscopically)	3	6	3	0	3	0
No visible lesions	2	0	1	-	1	-
Increased cytoplasmic granularity, cortical tubules - very slight to slight	0	2	0	-	0	-
Microvesiculation, cortical tubules - slight	0	0	1	-	0	-
Tubular eosinophilia or microvesiculation and nuclear pyknosis; consistent with autolytic change	0	4	0	-	0	-
Tubular casts, isolated - very slight	0	0	1	-	0	-
Cortical tubular cyst, isolated - very slight	0	1	1	-	0	-
Focus of basophilic tubules - very slight	0	0	1	-	0	-
Interstitial mononuclear inflammatory cells, focal - very slight	1	0	0	-	1	-
Pelvic dilatation - slight	0	0	0	-	1	-

Data presented are number of animals having the stated observation.

[a]Grouped by days after final (fourth) exposure when necropsied.

[b]Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

- Indicates not applicable.

TABLE 7 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Thymus (number examined microscopically)	3	5	3	0	4	0
No visible lesions	3	0	3	-	3	-
Atrophy, loss of cortical lymphocytes, with karyorrhectic nuclei						
- slight	0	1	0	-	0	-
- moderate	0	2	0	-	1	-
- marked	0	2	0	-	0	-
Karyorrhectic nuclei in medulla						
- slight	0	1	0	-	0	-
- moderate	0	2	0	-	0	-
- marked	0	2	0	-	0	-
Basophilic round bodies in cortex (mineralized debris)	0	1	0	-	0	-
Spleen (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	0	-	-	-	-
Lymphoid depletion - slight	-	3	-	-	-	-
- moderate	-	2	-	-	-	-
- marked	-	1	-	-	-	-
Extramedullary hemopoiesis - slight to moderate	-	4	-	-	-	-
Mesenteric Lymph Node (number examined microscopically)	0	2	0	0	0	0
No visible lesions	-	0	-	-	-	-
Lymphoid depletion - moderate	-	1	-	-	-	-
- marked	-	1	-	-	-	-

Data presented are number of animals having the stated observation.

[a]Grouped by days after final (fourth) exposure when necropsied.

[b]Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

- Indicates not applicable.

TABLE 7 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Thoracic Lymph Node (number examined microscopically)	0	1	0	0	0	0
No visible lesions	-	0	-	-	-	-
Acute subcapsular hemorrhage	-	1	-	-	-	-
Heart (number examined microscopically)	0	5	0	0	0	0
No visible lesions	-	2	-	-	-	-
Isolated focal areas of minute granular basophilia (mineralization) of myocyte cytoplasm, microscopic - very slight	-	3	-	-	-	-
Lungs (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	0	-	-	-	-
Edema - very slight to slight - perivascular and peribronchial	-	3	-	-	-	-
- alveolar	-	1	-	-	-	-
Alveolar macrophages, increased - very slight	-	2	-	-	-	-
Cecum (number examined microscopically)	0	1	0	0	0	0
No visible lesions	-	0	-	-	-	-
Submucosal edema, diffuse - moderate	-	1	-	-	-	-
Depletion of cells of lamina propria - very slight	-	1	-	-	-	-
Colon (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	5	-	-	-	-
Submucosal edema - slight	-	1	-	-	-	-

Data presented are number of animals having the stated observation.

[a]Grouped by days after final (fourth) exposure when necropsied.

[b]Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

- Indicates not applicable.

TABLE 7 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study A Mice

Exposure Level (ppm)	0	1000			5000	
Necropsy Group[a]	3 or 5 Days Recovery	Moribund or Dead after 3-4 Exposures[b]	3 or 5 Days Recovery	13 Days Recovery	3 or 5 Days Recovery	13 Days Recovery
Number of Mice in Group	5	6	5	6	5	6
Pancreas (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	5	-	-	-	-
Increased lobular separation, suggestive of edema	-	1	-	-	-	-
Testes (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	4	-	-	-	-
Scattered spermatid giant cells	-	2	-	-	-	-
Epididymis (number examined microscopically)	0	6	0	0	0	0
No visible lesions	-	4	-	-	-	-
Focal necrosis of mucosa - very slight	-	1	-	-	-	-
Decreased spermatozoa in lumen	-	1	-	-	-	-
For the following tissues, no visible lesions (number examined microscopically):						
Aorta	0	4	0	0	0	0
Brain	0	6	0	0	0	0
Coagulating Gland	0	3	0	0	0	0
Esophagus	0	5	0	0	0	0
Larynx	0	6	0	0	0	0
Parathyroid Gland	0	2	0	0	0	0
Peripheral Nerve (sciatic)	0	4	0	0	0	0
Pituitary Gland	0	4	0	0	0	0
Prostate	0	6	0	0	0	0
Salivary Glands	0	6	0	0	0	0
Seminal Vesicles	0	5	0	0	0	0
Skeletal Muscle	0	1	0	0	0	0
Small Intestine	0	6	0	0	0	0
Stomach	0	6	0	0	0	0
Thyroid Glands	0	6	0	0	0	0
Trachea	0	4	0	0	0	0
Urinary Bladder	0	5	0	0	0	0

Data presented are number of animals having the stated observation.

[a] Grouped by days after final (fourth) exposure when necropsied.

[b] Includes 3 dead after three exposures and 1 dead and 2 moribund after four exposures.

- Indicates not applicable.

TABLE 8

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Hamsters[a]

Exposure Level (ppm)	0	1000	5000
Liver (number examined microscopically)	3	3	3
No visible lesions	0	0	0
Hepatocyte microvesicular change, centrilobular and midzonal - very slight to slight	2	0	2
Multifocal microscopic aggregates of mononuclear and reticuloendothelial cells - very slight	1	2	2
Isolated small focus of subcapsular coagulation necrosis with mixed inflammatory cell reaction	0	1	0
Periportal inflammatory cell accumulation, mixed type - slight	2	1	0
- moderate	1	1	1
Hemosiderin-laden macrophages, periportal - slight	1	0	0
Gallbladder (number examined microscopically)	1	2	3
No visible lesions	0	1	2
Submucosal mononuclear cells, primarily lymphoid, focal - slight	0	1	0
diffuse - very slight	0	0	1
Adventitial mononuclear cells - slight	1	0	0
Kidneys (number examined microscopically)	3	3	3
No visible lesions	3	3	2
Dilatation of multiple cortical tubules and epithelial vacuolization - very slight	0	0	1
Eosinophilic hyaline casts - very slight	0	0	1
Thymus (number examined microscopically)	3	3	3
No visible lesions	3	3	3

Data presented is number of hamsters with the stated observation.

[a]Hamsters necropsied 5 days after fourth consecutive exposure to vinyl chloride.

TABLE 9

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Rats[a]

Exposure Level (ppm)	0	1000
Liver (number examined microscopically)	3	3
No visible lesions	0	0
Microscopic mononuclear and reticulo-endothelial cell aggregates, multifocal, occasionally adjacent to isolated degenerated hepatocytes - slight	1	3
Periportal inflammatory cells, mixed type, - slight	2	3
- moderate	1	0
Kidney (number examined microscopically)	3	3
No visible lesions	0	1
Scattered tubules with eosinophilic casts - slight	1	1
Cortical interstitial mononuclear cell accumulation, focal - very slight	2	1
Focus of fibrosis and chronic inflammation - slight	1	0
Focus of mononuclear cells, primarily lymphoid, beneath pelvic epithelium - very slight	0	1
Isolated cortical focus of hypertrophied, basophilic tubules - very slight	1	0
Thymus (number examined microscopically)	3	3
No visible lesions	3	2
Petechial hemorrhage of medulla - very slight	0	1
Anterior Mediastinal Lymph Node (number examined microscopically)	0	1
Lymphoid hyperplasia and sinusoidal hemorrhage	-	1

Data presented is number of rats with stated observation.

[a]Rats were necropsied 5 days after the fourth consecutive exposure to vinyl chloride.

- Indicates not applicable.

TABLE 10

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study B Mice

Exposure Level (ppm)	0	1000		5000
Necropsy Group	Final Termination[a]	2 Exposures	Final Termination	Final Termination
Number of Mice in Group	5	1[b]	4	5
Liver (number examined microscopically)	5	1	4	5
No visible lesions	1	0	0	0
Acute coagulation necrosis, - focal, centrilobular, about 10% of area examined, often subcapsular	0	0	3	0
- focal, centrilobular to confluent, involving 25% of area of sections examined	0	0	1	0
- focal, centrilobular to confluent, involving 26-50% of area of sections examined	0	1	0	1
- focal, centrilobular to confluent, involving 51-75% of area of sections examined	0	0	0	1
Isolated focal necrotic area - slight	0	0	0	1
Minute, intracellular basophilic structures at margins of necrotic areas	0	1	0	2
Perinecrotic inflammatory cells, mixed - very slight to slight	0	0	0	1
Perinecrotic subacute inflammatory and connective tissue cells - slight	0	0	4	0
Perinecrotic swollen, vacuolated hepatocytes	0	1	1	1
Centrilobular hepatocytes, microvesicular change - very slight to slight	1	0	0	2
- marked	0	0	0	1
Centrilobular and midzonal hepatocytes enlarged, homogeneous cytoplasm of slightly increased basophilia, occasional mitoses	0	0	4	2
Focal subcapsular hepatocyte degeneration with vacuolation and hyaline material	0	0	1	0
Sinusoidal congestion and hemorrhage, in and near necrotic areas - moderate	0	0	1	0
- marked	0	1	0	2
Centrilobular vein congestion, compression of adjacent hepatocytes - marked	0	1	0	3
Focal telangiectasis	0	0	3	0
Small hyaline masses in sinusoids, margins of necrotic foci or in areas of swollen hepatocytes	0	0	2	0
Multifocal microscopic foci of hepatocyte degeneration or necrosis with mononuclear inflammatory cell reaction - very slight to slight	3	0	0	2
Gallbladder (number examined microscopically)	3	1	3	2
No visible lesions	3	1	3	2

Data presented are number of animals having the stated observation.

[a] Necropsied after five exposures.

[b] Animal died after two exposures.

TABLE 10 (Continued)

VINYL CHLORIDE: RESULTS OF TWO SHORT-TERM INHALATION STUDIES

Histopathologic Observations - Study B Mice

Exposure Level (ppm)	0	1000		5000
Necropsy Group	Final Termination[a]	2 Exposures	Final Termination	Final Termination
Number of Mice in Group	5	1[b]	4	5
Kidney (number examined microscopically)	5	1	4	5
No visible lesions	3	1	3	0
Enlarged cells, scattered, inner cortical tubules - slight	0	0	0	1
Microvesiculation, cortical tubules - slight to moderate	0	0	1	3
Focus of basophilic tubules - very slight	1	0	0	1
Interstitial mononuclear inflammatory cells, focal - very slight	1	0	0	0
Medullary casts, isolated, occasionally mineralized - slight	1	0	0	0
Thymus (number examined microscopically)	5	1	4	5
No visible lesions	5	0	2	3
Atrophy, loss of cortical lymphocytes, with karyorrhexic nuclei - slight	0	0	1	0
- marked	0	1	1	2
Basophilic round bodies in cortex (mineralized debris)	0	0	0	1
Thyroglossal duct cyst, isolated, microscopic	0	0	0	1

Data presented are number of animals having the stated observation.

[a] Necropsied after five exposures.

[b] Animal died after two exposures.

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