EPA 560/6-76-023

HEALTH AND ENVIRONMENTAL IMPACTS

TASK 1

VINYLIDENE CHLORIDE

OCTOBER 1976

FINAL REPORT

NMENTAL PROTECTION AGENCY

ICE OF TOXIC SUBSTANCES

WASHINGTON, D.C.

EPA-560/6-76-023

HEALTH AND ENVIRONMENTAL IMPACTS

Task 1

VINYLIDENE CHLORIDE

October 1976

Final Report

Contract 68-01-4116

EPA Project Officer

William Coniglio

Environmental Protection Agency
Office of Toxic Substances
Washington, D.C. 20460

NOTICE

This report has been reviewed by the Office of Toxic Substances, EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute indorsement or recommendation for use.

Table of Contents

Summary

Virtually no information is available on the absorption and metabolism of vinylidene chloride. Its oral and inhalation toxicity, however, indicates that it is readily absorbed from the gastrointestinal and respiratory tracts. Its fate following absorption has not yet been elucidated.

As indicated by the tabular summary, the metabolic effects of vinylidene chloride have received little study. It has been demonstrated, however, that vinylidene chloride affects the activity of several enzymes, notably hepatic glucose-6-phosphatase and serum alanine alpha-ketoglutarate transaminase. The extent of the effect on these enzymes is modified by prior or concurrent administration of enzyme inducing or inhibiting compounds such as phenobarbital and 3-methylcholanthrene.

The acute toxicity of vinylidene chloride has received considerable attention, but the results reported have been highly inconsistent. Four-hour inhalation LC_{50}'s for rats ranging from 600 ppm (27) to 15,000 ppm (25) have been reported. The lethal concentration has been found to vary greatly with the dietary condition (fed or fasted) and the hepatic glutathione content, which shows significant variations in a diurnal rhythm.

The pathological effects induced by vinylidene chloride are presented in Figures 14 and 15. With each effect is listed the lowest dosage level at which it was observed, the period of exposure, the period of observation (in parentheses), if longer than the period of exposure, and the bibliographic reference number. Information from reports which observed the effect at higher doses is listed in the footnotes.

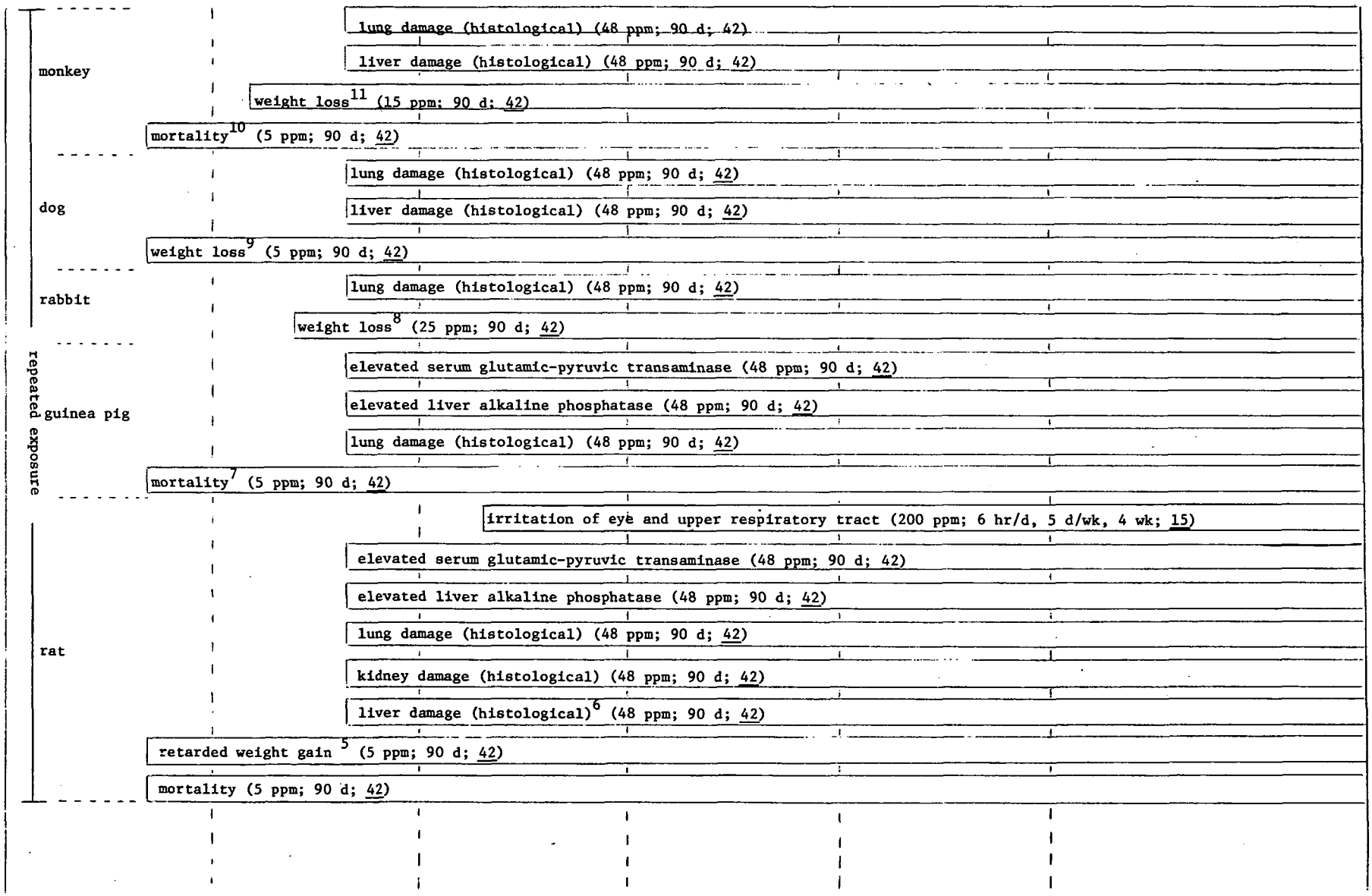
The minimum effective concentration of vinylidene chloride has not been clearly established. In 1971, the American Council of Government Industrial Hygienists (3) set the TLV at 10 ppm. In 1972, the Manufacturing Chemists Association (36) established a TLV of 5 mg/m^3 (approx. 1.25 ppm). However, Prendergast (42) observed toxic effects in animals exposed to vinylidene chloride at 101 mg/m^3 (26 ppm) for 90 days, and doubtful effects were observed at 20 mg/m^3 (5 ppm).

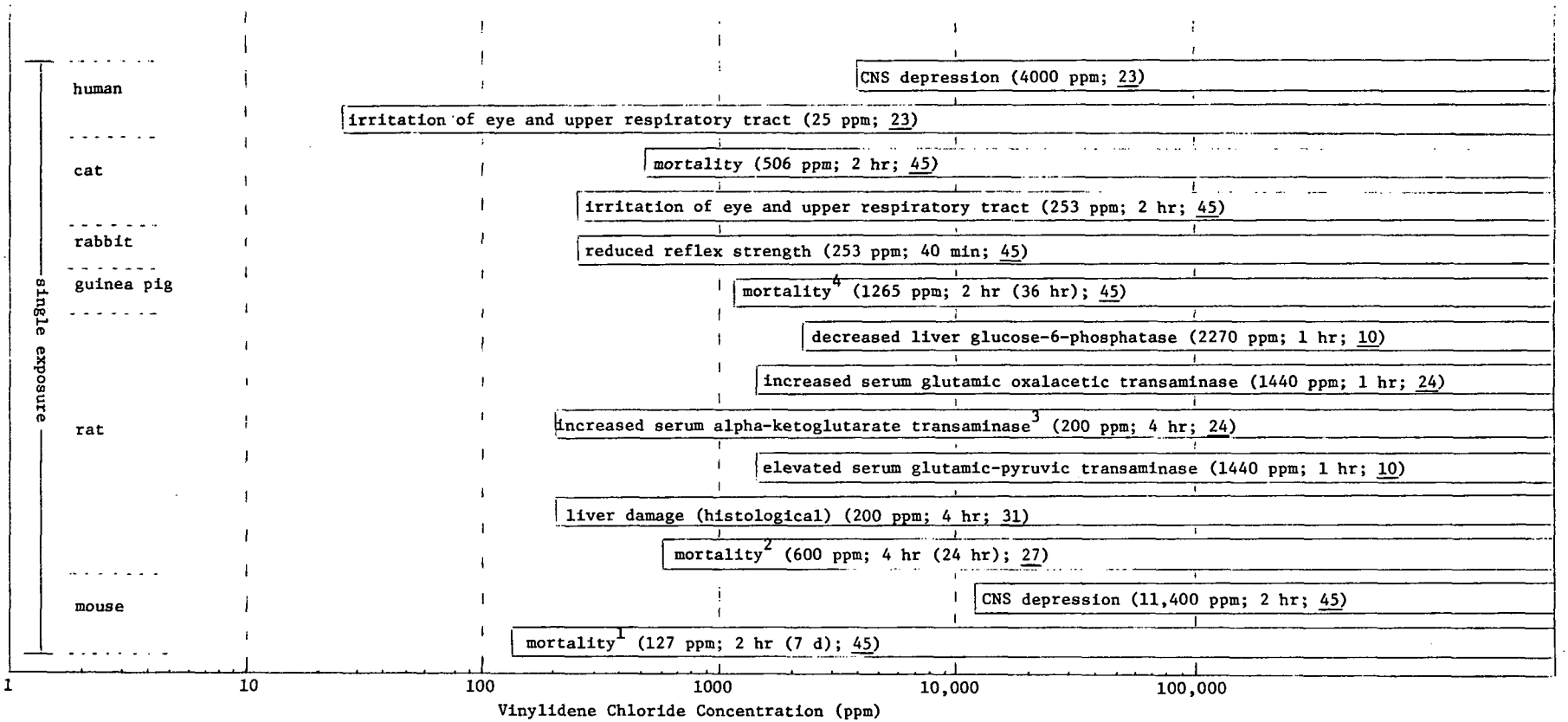
Several studies have shown vinylidene chloride to be mutagenic to S. typhimurium and E. coli. This effect, however, was attributed to a metabolite of vinylidene chloride.

Tumors have allegedly been induced in rats by inhalation of high concentrations of vinylidene chloride (53), but no reports to date have substantiated this allegation.

Very little information relevant to the epidemiology of vinylidene chloride has been reported. The reports made to date have involved cases where vinylidene chloride was only one of several, or many, chemicals to which the subjects were exposed. In no case was it possible to draw definite conclusions about vinylidene chloride.

Several studies have been performed, under simulated environmental conditions, to elucidate the environmental fate of vinylidene chloride. Possible mechanisms of reaction and reaction products have been suggested and an atmospheric half-life of 2.1 hours has been calculated. No information is available on the effects of vinylidene chloride on the environment/ecosystems, and the information available on monitoring and exposure levels is negligible.


repeated exposure
monkey
lung damage (histological) (48 ppm; 90 d; 42)
liver damage (histological) (48 ppm; 90 d; 42)
weight loss[11] (15 ppm; 90 d; 42)
mortality[10] (5 ppm; 90 d; 42)
dog
lung damage (histological) (48 ppm; 90 d; 42)
liver damage (histological) (48 ppm; 90 d; 42)
weight loss[9] (5 ppm; 90 d; 42)
rabbit
lung damage (histological) (48 ppm; 90 d; 42)
weight loss[8] (25 ppm; 90 d; 42)
guinea pig
elevated serum glutamic-pyruvic transaminase (48 ppm; 90 d; 42)
elevated liver alkaline phosphatase (48 ppm; 90 d; 42)
lung damage (histological) (48 ppm; 90 d; 42)
mortality[7] (5 ppm; 90 d; 42)
rat
irritation of eye and upper respiratory tract (200 ppm; 6 hr/d, 5 d/wk, 4 wk; 15)
elevated serum glutamic-pyruvic transaminase (48 ppm; 90 d; 42)
elevated liver alkaline phosphatase (48 ppm; 90 d; 42)
lung damage (histological) (48 ppm; 90 d; 42)
kidney damage (histological) (48 ppm; 90 d; 42)
liver damage (histological)[6] (48 ppm; 90 d; 42)
retarded weight gain[5] (5 ppm; 90 d; 42)
mortality (5 ppm; 90 d; 42)



1. 253 ppm; 2 hr (24 hr); 45
   3800 ppm; 2 hr (24 hr); 45
2. 4900 ppm; 4 hr (14 d); 47
   6150 ppm; 4 hr (14 d); 47
   15,000 ppm; 4 hr (24 hr); 27
   32,000 ppm; 4 hr (14 d); 11
3. 2000 ppm; 4 hr; 25
4. 2024 ppm; 2 hr (24 hr); 45
5. 15 ppm; 90 d; 42
   48 ppm; 90 d; 42
   500 ppm; 6 hr/d, 5 d/wk, 4 wk; 15
6. 500 ppm; 6 hr/d, 5 d/wk, 4 wk; 15
7. 15 ppm; 90 d; 42
   26 ppm; 90 d; 42
   48 ppm; 90 d; 42
8. 100 ppm; 8 hr/d, 5 d/wk; 6 wk; 42
9. 25 ppm; 90 d; 42
   48 ppm; 90 d; 42
10. 26 ppm; 90 d; 42
    48 ppm; 90 d; 42
11. 26 ppm; 90 d; 42
    48 ppm; 90 d; 42
    100 ppm; 8 hr/d, 5 d/wk, 6 wk; 42

Figure 14. Effects Induced by Vinylidene Chloride-inhalation

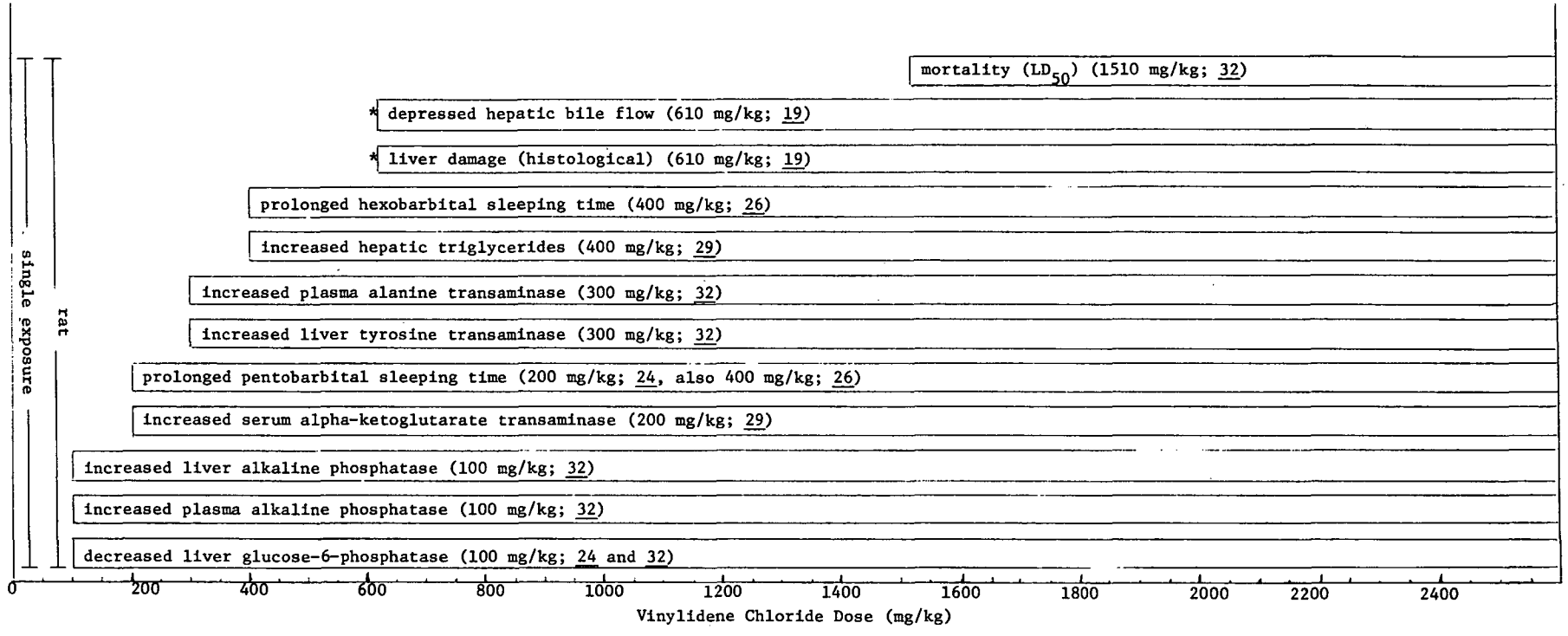


Figure 15. Effects induced by Vinylidene Chloride - p.o. and i.p.*

Tabular Summary

The following table is a graphic representation of the information available on the health and environmental impacts of vinylidene chloride. The information has been organized by the various subfields given in Appendix A. The route of administration of vinylidene chloride, the species of animal employed and the reference of each study are presented. In the case of human studies, the number of subjects, if it was specified, is included When one of these categories of information was not stated in the article, the letters n.s. are used to so indicate.

All studies reasonably applicable to a given subfield are included therein. Thus, while this table gives no indication of the quality of the information reported, it does give an excellent indication of the quantity of information available in each category.

I. HEALTH IMPACT			
A. Toxicological Data			
1. Absorption, excretion, transport, and distribution	topical	mouse	38 Meyer 1959
	inhalation	rat	27 Jaeger 1974
	in vitro	rat liver	7 Bonse 1975
2. Metabolic effects			
3. Pharmacology	oral	rat	32 Jenkins 1972
	oral	rat	29 Jaeger 1973
	oral	rat	26 Jaeger 1973
	inhalation	rat	10 Carlson 1972
	inhalation	rat	27 Jaeger 1974
	inhalation	rat	48 Siletchnik 1974
	inhalation	rat	44 Reynolds 1975
	inhalation	rat	31 Jaeger 1975
	inhalation	rat	28 Jaeger 1975
	inhalation	rat	24 Jaeger 1975
	in vitro	rat liver	27 Jaeger 1974
4. Biochemical parameters	oral*	mouse	49 Sporn 1970
	oral*	rat	49 Sporn 1970
	oral	rat	32 Jenkins 1972
	oral	rat	29 Jaeger 1973
	oral	rat	26 Jaeger 1973
	oral	rat	30 Jaeger 1973
	i.p.	rat	19 Harms 1976
	inhalation	rat	42 Prendergast 1967
	inhalation	rat	10 Carlson 1972
	inhalation	rat	25 Jaeger 1973
	inhalation	rat	27 Jaeger 1974
	inhalation	rat	44 Reynolds 1975

* A copolymer of vinylidene chloride was used.

	inhalation	rat	31 Jaeger 1975
	inhalation	rat	28 Jaeger 1975
	inhalation	rat	24 Jaeger 1975
	inhalation	guinea pig	42 Prendergast 1967
	inhalation	rabbit	42 Prendergast 1967
	inhalation	dog	42 Prendergast 1967
	inhalation	monkey	42 Prendergast 1967
	in vitro	rat liver	27 Jaeger 1974
	in vitro	rat liver	7 Bonse 1975
	in vitro	rat spleen	46 Shmuter 1976
5. Acute, subacute, and chronic toxicity	oral*	rat	51 Wilson 1954
	oral	rat	32 Jenkins 1972
	oral	rat	30 Jaeger 1973
	oral*	dog	51 Wilson 1954
	i.v.*	rabbit	39 Miyasaki 1959
	topical	rabbit	45 Rylova 1953
	inhalation	mouse	45 Rylova 1953
	inhalation	rat	11 Carpenter 1949
	inhalation	rat	42 Prendergast 1967
	inhalation	rat	15 Gage 1970
	inhalation	rat	47 Siegel 1971
	inhalation	rat	10 Carlson 1972
	inhalation	rat	25 Jaeger 1973
	inhalation	rat	30 Jaeger 1973
	inhalation	rat	27 Jaeger 1974
	inhalation	rat	44 Reynolds 1975
	inhalation	rat	31 Jaeger 1975
	inhalation	guinea pig	45 Rylova 1953
	inhalation	guinea pig	42 Prendergast 1967
	inhalation	rabbit	45 Rylova 1953
	inhalation	rabbit	42 Prendergast 1967
	inhalation	cat	45 Rylova 1953
	inhalation	dog	42 Prendergast 1967

* A copolymer of vinylidene chloride was used.

	inhalation	monkey	42	Prendergast 1967
	inhalation	human	45	Rylova 1953
	inhalation	n.s.	23	Irish 1962
	in vitro*	HeLa cells	5	Bando 1973
6. Sensitization from repeated doses				
7. Teratogenicity and mutagenicity	in vitro	S. typhimurium	37	McCann 1975
	in vitro	E. coli K12	17	Greim 1975
	in vitro	S. typhimurium	6	Bartsch 1975
8. Carcinogenicity	oral	rat	22	Hushon 1976
	oral	dog	22	Hushon 1976
	s.c.*	rat	40	Oppenheimer 1955
	inhalation	rat	53	Anon. 1975
9. Dose-response relationships	oral	rat	32	Jenkins 1972
	inhalation	mouse	45	Rylova 1953
	inhalation	rat	11	Carpenter 1949
	inhalation	rat	47	Siegel 1971
	inhalation	rat	30	Jaeger 1973
	inhalation	rat	27	Jaeger 1974
	inhalation	rabbit	45	Rylova 1953
	inhalation	human	45	Rylova 1953
10. Behavioral effects				
11. Synergisms				
B. Epidemiological Data				
1. Occupational exposure studies	inhalation*	human(2)	8	Broser 1970
	inhalation	human(98)	33	Kramer 1972

* A copolymer of vinylidene chloride was used.

		inhalation	human(4)	52 Anon. 1974
2.	Environmental incidents, poisonings, and case histories	topical	human(1)	42 Osbourn 1973
3.	Clinical and subclinical manifestations	inhalation	human(98)	33 Kramer 1972
4.	Statistical or risk-composition studies			
5.	Other controlled studies			
II.	ENVIRONMENTAL IMPACT			
A.	Environmental Fate			
1.	Chemical and biochemical reactions in the environment			13 Dilling 1976 14 Dilling 1975 16 Gay 1976 20 Heicklen 1975
2.	Transport in soils, aquatic systems and biota			
B.	Environmental/Ecosystem Effects			
1.	Fish and other aquatic organisms			
2.	Birds			
3.	Mammals of economic importance			
4.	Other terrestrial organisms			
5.	Atmosphere and/or climate			
6.	Manmade structures			

III. Monitoring Data and Exposure Levels

A. Human exposure profile	inhalation	4	Anderson 1963
	inhalation	2	Altman 1966
	inhalation	22	Hushon 1976
B. Exposure of other organisms			
C. Monitoring data			

Introduction

Vinylidene chloride (1,1-dichloroethylene; 1,1-dichloroethene; 1,1-DCE) was first reported, as a "strange new fluid," in 1838, but was regarded only as a laboratory curiosity until the late 1920's when its tendency toward polymerization was discovered. It began to find extensive commercial use in the 1940's, and its use has continued to grow to the extent that an estimated 265 million pounds were produced in the U.S. in 1974.

The bulk of the vinylidene chloride produced in this country is used in two applications: (1) the production of methyl chloroform; and (2) the formation, with other monomers, of a polymer. Because of its good barrier properties, the polymer is used to fabricate packaging films and is applied as a coating to other packaging materials. The polymer is also used in the production of flame-resistant fibers and is applied as a coating to impart flame resistance to textiles, carpets, non-wovens and paper.

Human exposure and environmental contamination are inevitable results of the widespread use of vinylidene chloride. It is important, therefore, that the risks and liabilities involved are fully understood. To this end, a number of investigations into the health and environmental impacts of vinylidene chloride have been performed over the past thirty years. It is the objective of this report to compile and review those investigations and, specifically, to identify any aspects of human or environmental toxicology of vinylidene chloride of which the current knowledge is inadequate.

Scope

Research Request No. 1 of EPA Contract No. 68-01-4116 authorized Tracor Jitco to (1) search the domestic and foreign literature for information on health and environmental impacts of vinylidene chloride, and (2) prepare a final report consisting, at a minimum, of a summary of the information derived from the literature search, with appropriate bibliographic references. An outline of factors to be identified was provided by the EPA, and is reproduced as Appendix A.

Information Sources

The secondary journals and on-line data bases employed in the literature search on vinylidene chloride are presented in Appendix B. In addition to those information sources, a large number of standard handbooks and desk references were searched. Furthermore, the bibliography of each article selected was scanned for relevant citations.

Review of the Literature

The earliest reference to vinylidene chloride toxicity appeared in a 1945 review of the toxicology of plastics, in which A.G. Cranch stated, with no supporting evidence, that vinylidene chloride is "essentially inert physiologically" (12).

In 1945, Reinhardt reported, without details, that experimental studies on laboratory animals indicated that the vapor toxicity of vinylidine chloride is of the same order as that of ethylene dichloride (43).

In a study reported in 1949, groups of six male or female Sherman albino rats, weighing 100-150 g. each, were used by Carpenter, et al., to measure the acute vapor toxicity of 96 chemical compounds. Each group of animals was exposed to a given concentration of a chemical for a period of 4 hours and then observed for 14 days. Chemical concentrations were increased by a factor of 2 until a concentration was reached which killed 2,3 or 4 of 6 rats within the observation period. Autopsies were performed on all of the rats to assure that they did not die of extraneous infection. The concentration of vinylidene chloride which was found to kill 2-4 of 6 rats was 32,000 ppm (11).

In an article published in 1953, M.L. Rylova reported on studies on the toxicity of technical grade vinylidene chloride that had been performed using white mice, guinea pigs, rabbits, cats and humans

Acute toxicity was tested by exposure of mice for two hours to vinylidene chloride vapors in a concentration of 0.5 to 45 mg/l. A narcotic effect, indicated by a lateral position of 2 out of 12 mice, was observed only at the highest concentration. Exposure at the lowest concentration was lethal for 6 out of 24 mice, death occurring during the first week after exposure. At a concentration of 1 mg/l, 9 of 24 mice succumbed during the first 24 hours. In most cases, autopsy disclosed no visible changes. The 24-hour LC_{50} for a 2-hour exposure was determined to be 15 mg/l. The author noted that earlier tests with the same sample of

vinylidene chloride showed a lower toxicity. It was suggested that the increase may have resulted from the formation of more toxic admixtures during standing (45).

In inhalation tests with guinea pigs, 2-hour exposure to 5 mg/l killed 1 of 3 animals after 36 hours, while 8 mg/l killed 3 of 3 within 24 hours. Death was caused by respiratory paralysis, and autopsy disclosed a congestive hyperemia of the laryngeal and pulmonary mucosae (45).

During a period of 3.1 to 4 months, one female and two male rabbits were exposed 5-7 times for 2-hours at intervals ranging from 8 to 37 days to increasing concentrations (2-30 mg/l) of vinylidene chloride vapors. The female died after 5 exposures (the last at 20 mg/l), while the males survived 7 exposures (the last at 30 mg/l). At autopsy the nasal mucosa of the female was covered with pus, the laryngeal mucosa exhibited a sharp hemorrhage and the larynx cavity contained a foamy liquid; the lungs were congested, hemorrhagic and edematous; the liver was congested and hypertrophied (45).

To determine the mimimum effective concentration of vinylidene chloride vapors, Rylova measured the changes in the rate of development of muscular stress and of reflex strength of 7 rabbits during inhalation of vinylidene chloride for 40 minutes. The average minimum effective concentration was found to be 1 mg/l (45).

Application of liquid vinylidene chloride for 5 minutes to the shaved abdominal skin of rabbits was observed to cause a slight, transient erythema. Following a 10-minute exposure, the erythema lasted about 1 hour. A rabbit ear concha placed in liquid vinylidene chloride for 1 minute developed an inflammatory edema (45).

Two-hour exposure of cats to vinylidene chloride vapors in a concentration of 1 mg/l resulted in only a slight irritation of the eye and nose mucosae. At 2 mg/l, the vapors produced a state of strong excitation

in 2 of 3 cats. One hour following exposure (2 mg/l) of the cats, a male, exhibited an unsteady gait, labored breathing and finally respiratory failure. Autopsy revealed the presence of an edematous fluid in the tracheal cavity, tracheal hyperemia, and pulmonary edema. A female cat which died a few hours after exposure to 2 mg/l showed only a slight hyperemia of the upper trachea. Autopsy of cats exposed to 6 mg/l for 2 hours disclosed a slight pulmonary edema, hemorrhages and foci of pneumonia (45).

In tests on human subjects, Rylova found the threshold of vinylidene chloride vapor for irritating action on the mucosae of the eye and upper respiratory tract to be 0.1 mg/l. The odor perception threshold was found to be 0.2 mg/l. The author suggested that the irritating effects may have been the result of decomposition products, such as formaldehyde and HCl (45).

Rylova noted that in the presence of air, vinylidene chloride forms explosive peroxide compounds which decompose slowly to give formaldehyde, phosgene and HCl (45).

In a test of the chronic toxicity of a copolymer of vinyl and vinylidene chloride, Seeler, et al., (cited by Wilson and McCormick, 1954) found that rats fed a diet containing 5% vinyl and vinylidene chloride copolymer for 2 years showed no toxic effects. Two dogs fed a diet containing 5% of the copolymer were also without evidence of toxic effects (51).

In 1955, Oppenheimer, et al., studied the carcinogenicity of a number of polymers by inserting, s.c., in Wistar rats, small squares of circles (1.5 cm wide) of film, one on each side of the abdominal wall just ventral to the fascia. One of the films used was Saran, a copolymer of vinyl chloride and vinylidene chloride. The first effect of the film was its encapsulation in a sac or pocket of connective tissue. This encapsulation was evident within 2-3 weeks after implantation, and was found in all animals except those in which a tumor was induced. In most cases, the tumors induced were fibrosarcomas and were located entirely in the subcutaneous layers. Saran induced tumors in 5 of 42 rats, with a latent period of 390 to 847 days (40).

A 1959 report by K. Miyasaki described an experiment in which rabbits were injected i.v. with copolymerization products of vinyl chloride and vinylidene chloride (EV) (no details given) according to the following schedule:

1% EV	1 cc/kg,	daily for 2 weeks:	9 rabbits
5% EV	1 cc/kg,	daily for 2 weeks:	5 rabbits
1% EV	1 cc/kg,	daily for 3 months:	9 rabbits

Throughout the experiment, all of the rabbits were healthy and maintained their normal body weight. Blood tests showed a reduction in red blood cell count, hemoglobin, hematocrit, erythrocyte resistance and whole blood specific gravity. The white blood cell count, color index, coagulation time, bleeding time and plasma specific gravity showed no significant changes. At autopsy the spleen and bone marrow appeared white, and the spleen swollen. Histological examination of the rabbits treated for 3 months revealed the cells belonging to the reticulo-endothelial system of the spleen, bone marrow, liver, lymphatic tissue, and lungs to be extremely hypertropic (39).

In a 1959 study, Meyer and Kerk tested the abdominal skin of mice for percutaneous permeability of 37 aliphatic compounds. The compounds were applied on a surface of 2.2 sq. cm. and their permeability measured as the time required to show the appearance of an eserine effect on the striated muscles. Dichloroethylene (isomer not specified) took 28 minutes. For comparison, time for some of the other compounds are shown below (38).

Methanol	-	n-Octanol (prim)	29
Ethanol	-	n-Octanol (sec)	49
n-Propanol	-	n-Nonylalcohol	64
i-Propanol	-	n-Decylalcohol	43
n-Butanol	73	Methyl glycol	-
i-Butanol	64	Ethyl glycol	-
n-Pentanol	43	1,2-Propylene glycol	-
i-Amylalcohol	47	1,3-Butylene glycol	-
n-Hexanol	21	Carbitol	-
n-Heptanol	26	Hexamethylene glycol	-

Glycerin	-	Tetrabromoethane	-
Hexane	-	Trichloroethylene	20
Trichloromethane	-	Tetrachloroethylene	23
Dibromoethane	-	Allyl bromide	12
Dichloroethane	-	n-Allyl chloride	28

D. Irish, 1962, discussed the toxicity of vinylidene chloride based on an unpublished report by the Biochemical Research Laboratory of the Dow Chemical Company. This 1962 report stated that vapor exposure (animal not stated; apparently human) of 4000 ppm vinylidene chloride results in central nervous system depression and the associated symptoms of drunkeness, with the development of unconsciousness during continued exposure. With an exposure of short duration complete recovery from the anesthetic effect is expected. The maximum single exposure in animals which could be tolerated without injuries was above 1000 ppm for up to 1 hour and 200 ppm for up to 8 hours (23).

Chronic vapor exposure, 5 days a week, 8 hours a day, for several months resulted in some kidney and liver injuries in animals (species not specified) at concentrations of 100 and 50 ppm. Minimal liver and kidney injuries resulted even at 25 ppm. Vapor exposure studies on carbon tetrachloride resulted in similiar findings. Studies on laboratory animals indicate that the quantitative vapor toxicity of vinylidene chloride is slightly greater than that of ethylene dichloride and slightly less than that of carbon tetrachloride (23, 35).

Eye contact studies showed that inhibited vinylidene chloride was moderately irritating to the eyes, causing pain, conjunctival irritation and some transient corneal injury, although permanent damage was rare. A high concentration of the phenolic inhibitor itself, however, caused serious and permanent eye injury (23).

The inhibitor content of liquid vinylidene chloride was held partially responsible for the skin irritation that develops after only a few minutes of direct contact to the skin (23).

Because vinylidene chloride has a high vapor pressure, the Dow study suggested a maximum possible concentration of 25 ppm in the work atmosphere. (8 hour per day, 5 days a week). Persons prone to kidney or liver disease, or who are excessive users of alcohol, should be forbidden to work even in areas mildly contaminated with vinylidene chloride (23, 35).

In 1963, A. Osbourn reported a case in which Saran wrap, a copolymer of vinylidene chloride and vinyl chloride, was implicated as the causitive agent of contact dermatitis of the body of a 30-year-old white male. The patient was given a steroid cream to treat an area of localized psoriasis, and was instructed to cover the area with Saran wrap for two to three hour periods every evening. The psoriasis improved, but the patient developed an inflammed, swollen, vesicular and exudative dermatitis on the area covered by Saran wrap (41).

Anderson and Saunders, 1963, of Lockheed Missiles and Space Company, identified contaminants in the atmospheres of the manned Mercury spacecraft. Vinylidene chloride, one of the contaminants, was believed to have originated as one of several impurities in a batch of contaminated breathing oxygen. The authors reported a value of 0-2 ppm as a probable minimum concentration which would have ensued had all the recovered contaminants been dispersed in the free volume of the cabin at one time (4).

Altman and Dittmer, 1966, reported that 2 ppm is the highest concentration of the vinylidene chloride normally found in the atmospheres of nuclear submarines (2).

Using rats, guinea pigs, dogs, rabbits and monkeys, Prendergast et al., 1967, studied the inhalation toxicity of vinylidene chloride. Animals were subjected to 2 types of studies: repeated, daily, 8-hour exposures, 5 days/week, for 6 weeks and continuous 90-day exposures. The animals were exposed to concentrations of 395, 189, 101, 61 and 20 mg/m^3 (42).

Repeated exposure to 395 mg/m^3 (100 ppm) did not result in a single animal death (out of a total 38) or visible signs of toxicity. However, the rabbits and monkeys were observed to have lost weight; autopsy revealed normal organs; however, one rat had a gelatinous material on the kidney and bloody urine in the bladder. Pneumonitis and congested lungs were found in an occasional rat or guinea pig. Guinea pig serum urea nitrogen concentration was 23 $\pm$ 3 mg/100 ml, which favorably compared with 24 $\pm$ 5 mg/100 ml obtained from control animals (42).

At continuous exposure to 189 mg/m^3, 7 out of 15 guinea pigs died in the beginning (day 4-day 9) of the experiment and 3 out of 9 monkeys died on days 26, 60 and 64. Surviving animals exhibited no visible signs of toxicity. Dogs and monkeys lost weight, while the rats gained less than the controls. Gross examination revealed mottled livers in many animals. Histopathologic examination of liver sections from dogs, monkeys and rats revealed morphologic changes which consisted of fatty metamorphosis, focal necrosis, hemosiderin deposition, lymphocytic infiltration, bile duct proliferation, fibrosis and pseudo-lobule formation. These changes were most severe in dogs. Kidney sections from all rats showed nuclear hypertrophy of tubular epithelium. Nonspecific inflammatory changes in the lungs of a majority of animals were also observed. One adrenal gland from a dog contained a cortical adenoma composed of cells of the zona glomerulosa type. The hepatic changes observed in dogs, monkeys and rats and the renal changes in rats were considered to be a direct result of the exposure. Liver alkaline phosphatase activity in surviving rats and guinea pigs was elevated, in comparision to control animals; serum glutamic-pyruvic transaminase activity was also elevated in rats and guinea pigs, but was more noticeable in guinea pigs. Although 2 rats had elevated liver lipid contents of 34.4 and 20.0%, the average value did agree well with the control animals (42).

Continuous exposure of 101 mg/m^3 did not result in visible toxic signs in the surviving animals, although 3 out of 15 guinea pigs died between day 3 and 6 of the experiment and 2 out of 3 monkeys died on days 39 and 47.

Loss in body weight was observed for rabbits, monkeys and dogs. Gross examination revealed white or bluish-grey spots and nodules on several guinea pig and rat lungs. Nonspecific inflammatory changes were noted in the lungs of all animals upon histopathologic study, but no changes were observed that could be attributed to the exposure. Guinea pig serum urea nitrogen concentration of 24 $\pm$ 3 mg/100 ml favorably compared with the control value of 24 $\pm$ 5 mg/100 ml (42).

Three out of 15 guinea pigs died on day 3 and 4 of the experiment when continually exposed to 61 mg/m^3, although visible signs of toxicity were not apparent in surviving animals. Weight loss was observed in monkeys, and the rats gained less than the controls. Gross examination revealed mottled livers and/or spleens in several animals of all species. Non-specific inflammatory changes, most marked in the lungs, and less so in the liver and kidneys, were observed in all species. These changes were not considered by the authors to have been induced by the exposure. Rat and guinea pig serum urea nitrogen levels of 20 $\pm$ 4 and 26 $\pm$ 3 mg/100 ml, respectively were not dissimiliar to the control values of 20 $\pm$ 4 and 24 $\pm$ 5 mg/100 ml, respectively (42).

Average continuous exposure of 20 mg/m^3 resulted in the death of 2 out of 45 rats, 2 out of 45 guinea pigs, and 1 out of 21 monkeys, with no visible toxic signs in the survivors. Weight loss was observed in dogs, while the rats gained less than the controls animals. Mottled livers were observed in about one-third of all animals upon gross examination. Histopathologic examination revealed nonspecific inflammatory changes in the lungs of all species and in the livers and kidneys of monkeys. None of the pathologic changes noted were considered by the authors to have been caused by the exposure. Urea nitrogen concentrations in rat and guinea pig sera compared favorably with controls. In both rats and guinea pigs, liver lipid values fell within control limits (42).

According to Vallaud, et al., (cited by Lefaux, 1968), D. Matruchot regards vinylidene chloride to be slightly narcotic and highly toxic,

resulting in death, accompanied by convulsions and spasms, in minutes or hours (34).

Since 1959 health specialists in the U.S.S.R. have fixed a maximum concentration of 50 mg/m^3 for vinylidene chloride (34).

The inhalation toxicity of vinylidene chloride on rats was reported by Gage in 1970. Groups of 4 F and 4 M rats, weighing 200 g each, were exposed to an atmospheric concentration of 500 ppm or 200 ppm for periods of 6 hours, 5 days a week for up to 4 weeks. The rats exposed to 500 ppm of vinylidene chloride experienced nose irritation and retarded weight gain; histological examination at autopsy revealed liver cell degeneration. Rats exposed to 200 ppm of vinylidene chloride experienced slight nose irritation and histological examination at autopsy revealed normal organs (15).

In 1970, Broser, Henschler and Hopf reported the clinical findings in 2 patients with poisoning subsequent to handling an aqueous dispersion of vinylidene chloride copolymers. Within 8 to 30 hours of the exposure, both patients developed sensory disturbances in the trigeminal area of the face, mouth and tongue, spreading later on to the 2nd, or 2nd and 3rd cervical segments. One patient exhibited motor weakness of the jaw muscles, the lateral recti muscles of the eyes and of the tongue muscles. Electromyographic evaluation of the trigemino-facial reflexes revealed the functional disorder to involve mainly the interneuronal system (8).

In another report (Henschler, 1970) on the same case, the same authors concluded that the cause of the poisoning was monochloroacetylene and/or dichloroacetylene, both of which were present as highly toxic gas contaminants in the initial vinylidene chloride product or developed from contaminants (tetrachloroethane, trichloroethylene) during production or storage (21).

Sporn, et al., 1970, studied the effects of oral administration of oil and aqueous extracts of a vinylidene chloride copolymer on 60 mice and 32 white rats. The extracts were obtained by 3 months contact of the solvents with the polymer. Neither 1 ml of either extract, nor 4 repeated

doses of 10-fold concentrations influenced the viability of the animals or their body or organ weights. The aqueous extract, administered as 50% of the drinking water for 60 days, induced a slight decrease (13-17%) in aldolase, glutamic oxalacetic transaminase, succinoxidase, and acid and alkaline phosphatase activities, without influencing the blood picture, succindehydrogenase activity and the ascorbic acid content of the adrenals. The oil extract, administered as 8% of the food, had no apparent effect. The adverse effects induced by the aqueous extract were attributed to the presence of chlorhydrine in the polymer (49).

In a study published in 1971, Siegel, et al., determined the LC_{50} of vinylidene chloride using male NRMR : O (SD) Sprague-Dawley derived rats. The animals were exposed to vinylidene chloride vapors for 4 hours and then observed for 2 weeks. At 4900 ppm, 1 of 16 rats died, and at 6150 ppm, 7 of 16 died. From this data, the LC_{50} was estimated to be 6350 ppm (47).

In 1971, the American Conference of Government Industrial Hygienists set the threshold limit value for vinylidene chloride, for an 8-hour working exposure, at 10 ppm (3).

A 1972 report by Aleksandrowicz, et al., described the results of a study to test the possibility of using a vinyl chloride-vinylidene chloride copolymer in the surgical therapy of aneurisms of brain blood vessels. In 7 rabbits, weighing 3.5 to 5.5 kg, the copolymer was applied on the vascular mucosa of the brain and on the vascular nerve-bundle. This was covered with a layer of an epoxy resin to add strength. The animals were sacrificed 30, 60 and 90 days after treatment. Microscopic examination of the coated tissues disclosed no serious damage (1).

Using adult Holtzman rats weighing 200 to 470 g, Jenkins, et al., 1972, studied the biochemical effects of vinylidene chloride. The time-response and dose-response relationships of p.o. vinylidene chloride administration to enzyme activities are shown in Figure 1 and Table 1. Vinylidene chloride induced a decrease in liver glucose-6-phosphatase (G-6-Pase) activity and increased activities in liver alkaline phosphatase (AP) and tyrosine

transaminase (TT) and in plasma alkaline phosphatase and alanine transaminase (AT), all of which were dose-related. In a comparison of these responses between male and female rats, it was found that females exhibited a greater response in G-6-Pase and liver AP then males. Similar tests with CCl_4 and 1,2-dichloroethylene demonstrated that vinylidene chloride is more potent than either of those chemicals (32).

The effect of phenobarbital (PB) on vinylidene chloride toxicity was tested in rats which had received daily i.p. injections of 50 mg/kg of sodium phenobarbital for 5 days prior to administration of vinylidene chloride. When measured 20 hour after vinylidene chloride administration, PB pretreatment resulted in reduced effects on liver AP and TT and plasma AT activities. In contrast, PB pretreatment increased the effect of CCl_4 on these parameters (32).

To test the effect of adrenalectomy on vinylidene chloride, Jenkins, et al., determined the LD_{50} of vinylidene chloride, and for comparison CCl_4, in adrenalectomized and sham-operated rats (18-hour fasted), the results are presented in Table 2 (32).

Using male Sprague-Dawley derived rats, Carlson and Fuller (1972), investigated the effects of prior i.p. administration of phenobarbital (PB), 3-methylcholanthrene (3-MC), 2-diethylaminoethyl-2,2-diphenyl valerate hydrochloride (SKF 525A), and 2,4-dichloro-6-phenylphenoxyethyl-diethylamine hydrochloride (Lilly 18947) on the inhalation toxicity of vinylidene chloride. PB is an enzyme inducing agent which is known to potentiate CCl_4 toxicity; 3-MC is an enzyme inducer which protects aginst CCl_4 hepatotoxicity; SKF 525A and Lilly 18947 inhibit microsomal enzyme metabolism and protect against CCl_4 damage. PB was administered in doses of 50 mg/kg for four days, 3-MC in doses of 40 mg/kg for 2 days, SKF 525A in a single dose of 50 mg/kg and Lilly 18947 in a single dose of 30 mg/kg. Controls were injected with corn oil or saline. Exposures to vinylidene chloride were made 24 hours after the last dose of PB, 48 hours after the last dose of 3-MC and 30 minutes after dosage with SKF 525A and Lilly 18947.

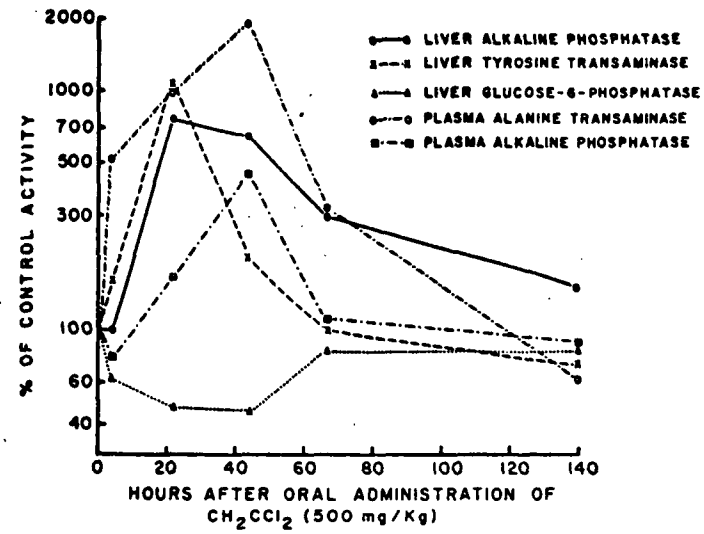


FIG. 1. Biochemical time-response to po administration of 1,1-dichloroethylene to male rats. Each point represents the mean percent of control obtained from a group of 3 rats based on the mean value for 3 control rats sacrificed at the same time. The ranges of control means for the time points studied were: liver AP, 0.34–0.41; liver TT, 10.1–19.1; liver G-6-Pase, 20.8–30.6; plasma AT, 0.08–0.16; plasma AP, 0.28–0.58 (μg product formed/hr/mg or ml).

TABLE 1

DOSE-RESPONSE RELATIONSHIPS OF BIOCHEMICAL PARAMETERS AT TIME OF MAXIMUM EFFECT AFTER ORAL ADMINISTRATION OF 1,1-DICHLOROETHYLENE TO MALE RATS

	Hours	Dose		
		100 mg/kg	300 mg/kg	500 mg/kg
Liver glucose-6-phosphatase	46	80 ± 5[a,b]	53 ± 2[b]	42 ± 3[b]
Liver alkaline phosphatase	22	194 ± 37[b]	468 ± 48[b]	774 ± 77[b]
Liver tyrosine transaminase	22	96 ± 16	380 ± 60[b]	1070 ± 119[b]
Plasma alkaline phosphatase	46	110 ± 15	150 ± 34	450 ± 46[b]
Plasma alanine transaminase	46	68 ± 20	150 ± 35[b]	1868 ± 202[b]

[a] All values are expressed as mean percent ± SE of controls given an equal volume of corn oil. Each dose group consisted of 3–6 rats. Control values were: liver G-6-Pase, 25.3 ± 1.0; liver AP, 0.34 ± 0.03; liver TT, 12.9 ± 1.2; plasma AP, 0.10 ± 0.01; plasma AT, 0.38 ± 0.05 (μg product formed/hr/mg or ml).

[b] Significantly different from next lower dose, $p < 0.05$.

TABLE 2

ORAL LD50 OF 1,1-DICHLOROETHYLENE AND CCl_4 IN ADRENALECTOMIZED AND SHAM-OPERATED MALE RATS

Operation	Time[b] (hr)	LD50 (mg/kg)	
		1,1-DCE	CCl_4
Adrenalectomy	24	84 (64–111)[a]	3260 (3070–3460)
	96	81 (70–94)	3200 (2870–3348)
Sham	24	1550 (1520–1581)	>7975
	96	1510 (1445–1578)	3250 (2928–3608)

[a] Values in parentheses are 95% confidence limits.

[b] Indicates time after administration of test compounds for LD50 calculation.

Hepatotoxicity was assessed by measuring serum glutamic oxalacetic (SGOT) and serum glutamic pyruvic (SGPT) transaminases and liver glucose-6-phosphatase (G-6-P). The lungs of the test animals were excised and weighed. Results of the tests are shown in Tables 3-7. All four of the compounds tested increased vinylidene chloride lethality. However, there was no increase in hepatotoxicity as evidenced by SGOT, SGPT or G-6-P, nor was any lung damage apparent through gross observation or weighing of the lungs (10).

C. Kramer and J. Mutchler, of the Dow Chemical Company, 1972, offered a technique of evaluating industrial exposure effects on a group of exposed workmen which would account for individual levels of exposure, rather than depending on a collective comparison with a control population. In the example given, a group of 98 workmen were exposed to vinyl chloride and vinylidene chloride; the vinyl chloride concentrations were higher than those of vinylidene chloride, due to the larger quantities of vinyl chloride used and its greater volatility. In more recent measurements of this study, infrared and gas chromatographic techniques have established that the vinyl chloride concentrations average 10 ppm, whereas almost all the vinylidene chloride concentrations amount to less than 5 ppm, and are most often detectable only in trace amounts (33).

Comparison of 95 parameters of the history, physical examination and laboratory work of the exposed workmen with those of a control group revealed no basic differences between the two populations with regard to general health nor the appearance of any significant disease as a result of work exposure (33).

Kramer and Mutchler concluded that repeated exposure to vinyl chloride at TWA (time-weighted average) levels of 300 ppm or above for a working lifetime, together with a very low level of vinylidene chloride may result in minor changes in certain physiologic and clinical laboratory parameters. Some impairment in liver function tests was implied, although no overt clinical disease was observed in any of the individuals studied (33).

TABLE 3

Effect of 3 MC or PB Pretreatment on 1, 1-Dichloroethylene Lethality

1, 1-Dichloroethylene (ppm for 1 hr)	Deaths in 24 Hours		
	Control	PB	3 MC
20,000	0/4	3/4	4/4
32,500	0/3	4/4	4/4

TABLE 4

Effect of PB and 3 MC on 1, 1-Dichloroethylene (1440 ppm for 1 hr) Hepatotoxicity 24 Hr Following Inhalation

Treatment	SGPT	SGOT
Saline - Air	6 ± 1.6[a]	17 ± 0.8
Saline - Dichloroethylene	18 ± 6.0	31 ± 7.5
PB - Dichloroethylene	41 ± 9.8	44 ± 16.3
3 MC - Dichloroethylene	19 ± 2.6	40 ± 3.6

[a] Reitman-Frankel Units.

TABLE 5

Effect of PB and 3 MC on 1, 1-Dichloroethylene Inhalation-Induced Changes in Liver Glucose-6-Phosphatase Activity

Pretreatment	1, 1-Dichloroethylene (ppm)	Glucose-6-Phosphatase[a]
Corn oil	0	16.8 ± 1.21
Corn oil	2270	12.9 ± 0.64[b]
3 MC	2270	14.4 ± 0.88
Saline	0	14.9 ± 0.99
Saline	2990	13.5 ± 0.31
PB	2990	13.5 ± 1.17

[a] umoles PO_4/g/min measured 48 hr after inhalation.

[b] $P < 0.05$.

TABLE 6

Effect of SKF 525A and Lilly 18947 on Survival Time During Inhalation of 1,1-Dichloroethylene

1,1-Dichloroethylene (ppm)	Survival Time (min)		
	Control	SKF 525A	Lilly 18947
42,000	90 ± 11.8	48 ± 10.3[a]	--
53,000	83 ± 14.5	--	32 ± 4.1[a]

[a] $P < 0.05$.

TABLE 7

Lungs Weights Following 1 Hr Exposure to 1,1-Dichloroethylene

Pre-treatment	1,1-Dichloroethylene (ppm)	$\frac{\text{Lung Wet Wt.}}{\text{Body Wt.}} \times 10^3$	$\frac{\text{Lung Dry Wt.}}{\text{Body Wt.}} \times 10^3$
Saline	0	4.76 ± 0.76 (4)[a]	1.25 ± 0.25 (2)
Saline	3590	4.54 ± 0.35 (4)	1.14 ± 0.08 (2)
PB	3590	5.05 ± 0.79 (4)	1.02 ± 0.90 (3)
Corn oil	0	8.25 ± 2.16 (4)	1.57 ± 0.36 (4)
Corn oil	3270	5.65 ± 0.25 (4)	1.14 ± 0.06 (4)
3 MC	3270	6.26 ± 0.57 (4)	1.36 ± 0.13 (4)

[a] Number of animals.

The 1972 threshold limit value (TLV) for vinylidene chloride, established by the Manufacturing Chemists Association, is 5 mg/m^3 (36).

In an effort to elucidate the mechanism of vinylidene chloride toxicity Jaeger, et al., 1973, compared the effects of vinylidene chloride with those of CCl_4, for which the mechanism of toxicity has been extensively studied. The parameters studied were glucose-6-phosphatase (G-6-Pase) and serum alanine-alpha-ketoglutarate transaminase (SAKT) activities, pentobarbital sleeping time (PST), liver triglyceride content, malonyldialdehyde formation in vitro, and conjugated diene levels in endoplasmic reticulum lipid. The tests were performed on male Holtzman rats, 250-350 g, to which vinylidene chloride and CCl_4 were administered by gavage. Results of the tests are presented in Figures 2-9. Vinylidene chloride administration resulted in decreased hepatic G-6-Pase, increased SAKT and liver triglycerides, and prolongation of PST. Little or no effect on hepatic lipoperoxidation was induced by vinylidene chloride, as evidenced by unchanged or reduced levels of malonyldialdehyde and conjugated dienes. The authors concluded that they found no evidence of a mechanistic similiarity between the hepatotoxicities of vinylidene chloride and CCl_4 (25).

The correlation between diurnal variation of hepatic glutathione (GSH) concentration and vinylidene chloride inhalation toxicity in rats was discussed in a 1973 paper by Jaeger, et al. In tests on male Holtzman rats weighing 250-350 g, the hepatic GSH concentration was found to vary as shown in Figure 10. To test the effects of this variation, two groups of rats were exposed to 2,000 ppm vinylidene chloride, one group between 10 a.m. and 2 p.m. and the other between 10 p.m. and 2 a.m. Serum alanine alpha-ketoglutarate transaminase (SAKT) activity was measured 23 hours after the end of exposure or at death. The 10 a.m. to 2 p.m. exposure caused only a slight increase in SAKT and no lethality, while the 10 p.m. to 2 a.m. exposure resulted in a marked increase in SAKT, and death in 2 of 5 rats. The animals which died exhibited bloody ascites, while those that survived showed no apparent signs of liver injury. The authors discussed the significance of these findings for industrial hygiene (25).

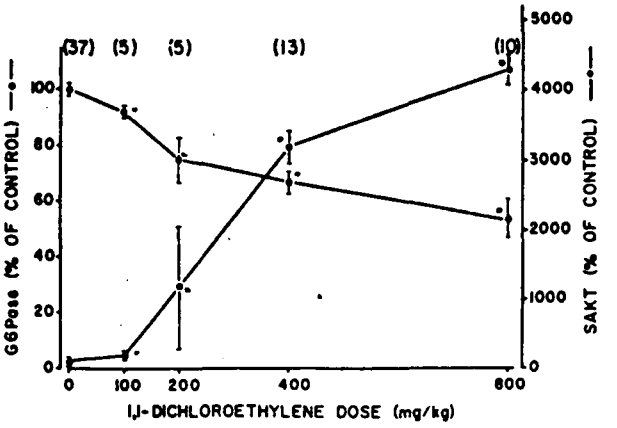


FIG. 2 Dose-response effect of 1,1-DCE on total hepatic G6Pase and SAKT. Rats, fasted and dosed as described in Methods, were sacrificed at 24 hr. In this and subsequent figures, an asterisk indicates significantly different from control at $p < 0.05$. The number in parentheses represents the number of animals used in each experiment. Control values for total liver G6Pase were 88.2 ± 1.5 mg P_i/hr/100 g body weight and for SAKT 0.28 ± 0.01 mg pyruvate/ml serum/hr.

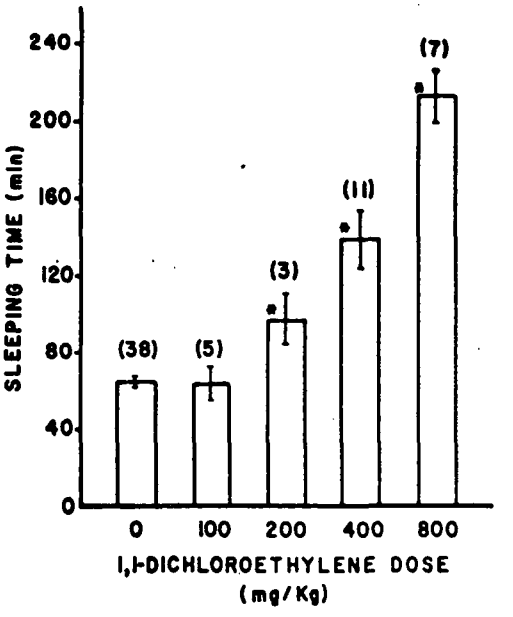


FIG. 3 Dose-response effect of 1,1-DCE on pentobarbital sleeping time (PST). The same rats used in Fig. 1 were used for these experiments. PST was determined between 20 and 24 hr.

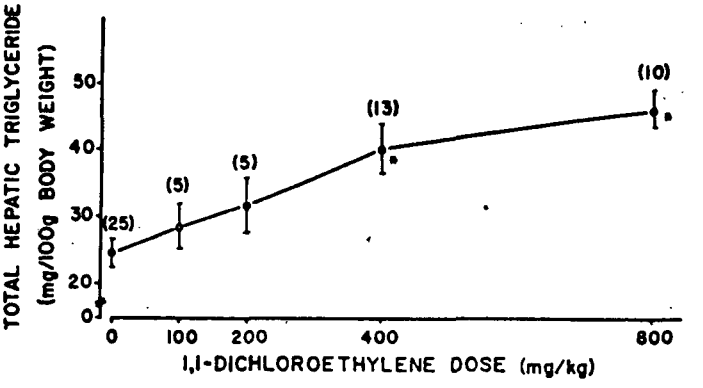


FIG 4 Dose-response effect of 1,1-DCE on total heptatic triglycerides, 24 hr after dosing. The values plotted are corrected for changes in liver to body weight ratio. The uncorrected data were also significantly elevated at 400 and 800 mg/kg of 1,1-DCE (8.2 ± 0.7 mg triglyceride/g wet tissue weight in controls versus 11.1 ± 1.0 and 13.0 ± 1.0 for the two higher doses of 1,1-DCE).

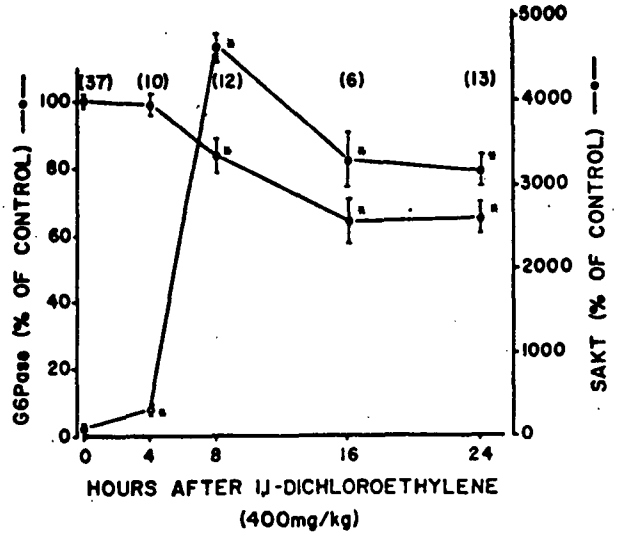


FIG. 5. Time-response effect of 1,1-DCE on total hepatic G6Pase and SAKT activity. All rats were fasted 28 hr before sacrifice. Values for control animals that had been given corn oil at various times before sacrifice were the same as in Fig. 1. There was no difference due to different times of corn oil treatment, and values for all control animals were pooled.

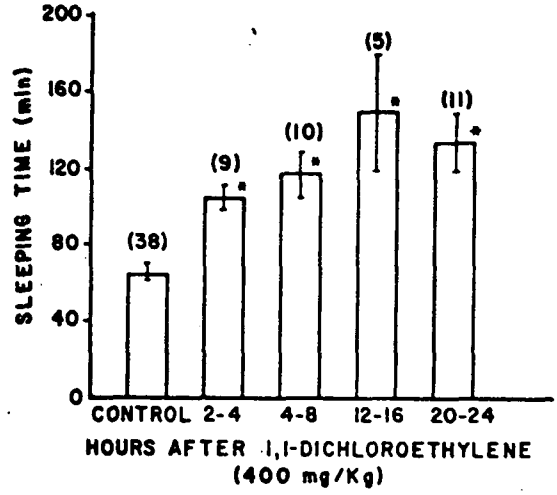


FIG. 6. Time-response effect of 1,1-DCE on pentobarbital sleeping time (PST). The rats described in Fig. 4 were given pentobarbital sodium, 30 mg/kg ip, 2–4 hr before sacrifice and the PST was determined.

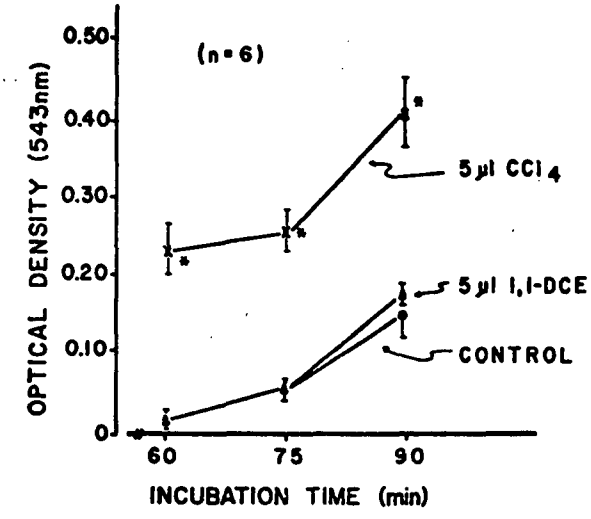


FIG. 7. Effect of CCl_4 and 1,1-DCE on in vitro lipid peroxidation. Whole liver homogenates from 6 male, nonfasted rats were treated with 5 µl of either 1,1-DCE or CCl_4. All values of OD_{543nm} are corrected for the blank tissue absorption.

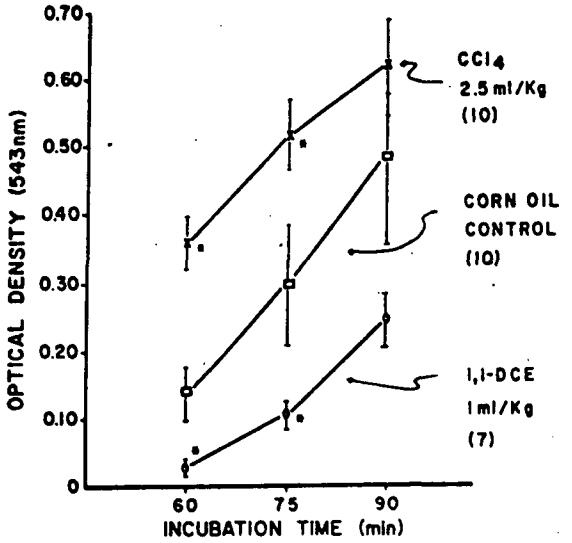


FIG. 8 Effect of in vivo treatment with 1,1-DCE or CCl_4 on in vitro lipid peroxidation. Rats were fasted overnight and dosed the following morning with corn oil (2.5 ml/kg), 1,1-DCE (1 ml/kg) or CCl_4 (2.5 ml/kg). One hour after dosing, the animals were sacrificed, and the liver homogenates were prepared. The experimental procedure was identical to the experiment in Fig. 6 except that exogenous addition of the chlorinated hydrocarbons was omitted.

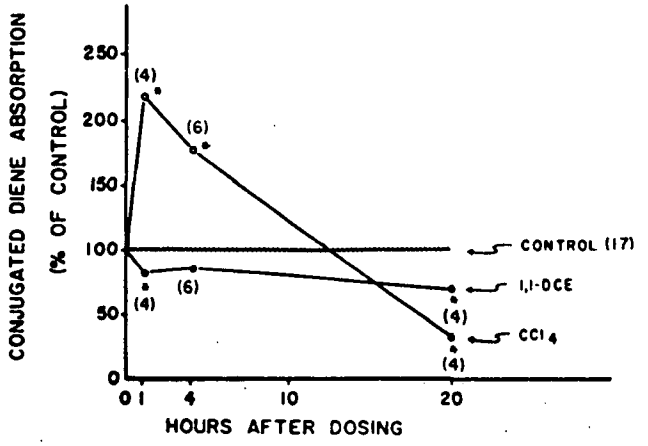


FIG. 9 Effect of CCl_4 and 1,1-DCE on microsomal conjugated dienes. Rats, fasted 20–28 hr before sacrifice, were dosed with corn oil, 1,1-DCE or CCl_4 as described for Fig. 7. At the indicated times after dosing, the animals were sacrificed (1–2 PM), their livers were removed, and conjugated dienes were measured as described in methods. The results shown in this figure represent the weight-corrected amounts expressed as a percentage of pooled control values (0.21 ± 0.01 OD_{243nm}/100 g body weight).

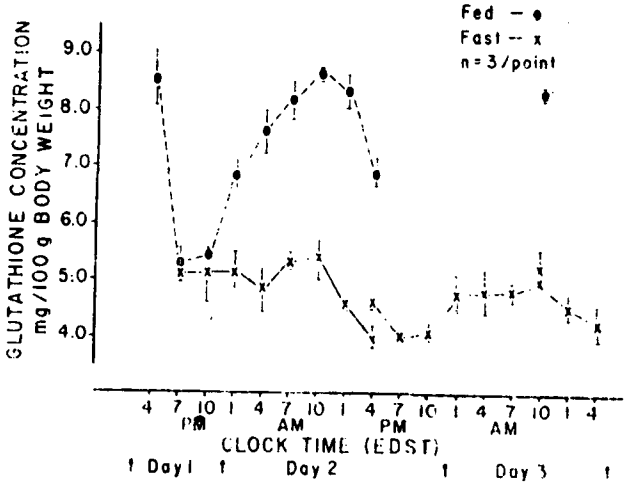


Figure 10

DIURNAL VARIATION OF HEPATIC GLUTATHIONE CONCENTRATION IN RATS

In tests using male Holtzman rats, 225-325 g, Jaeger and Murphy, 1973 found that vinylidene chloride (400 mg/kg, p.o.) prolonged pentobarbital sleeping time between 2 and 4 hours after administration. Liver injury, as measured by glucose-6-phosphatase depression was not detected at this time, and hexobarbital sleeping time was not affected. At 17 to 22 hours after dosing, both pentobarbital and hexobarbital sleeping times were prolonged, and hepatic injury was apparent. The authors suggested that orally administered vinylidene chloride affects the absorption or distribution of pentobarbital. The metabolism of pentobarbital did not appear to be affected, since the rate of its disappearance was determined to be similar in vinylidene chloride-treated and control rats (26).

In a brief communication published in 1973, Jaegar, et al., reported that following a single oral dose (400 mg/kg) of vinylidene chloride, both adrenalectomized (ADX) and sham rats had similar 24-hour mortalities. The ADX rats, however, exhibited an apparent decrease in hepatic injury as measured by serum AKT elevation and the degree of bloody ascites and hemorrhagic congestion. Corticosterone treatment, which significantly elevated serum and AKT liver activity in ADX rats, resulted in a slight decrease of lethal effects of vinylidene chloride in both ADX and sham rats. Prolonged (45 hour) fasting potentiated hepatic injury as measured by the 4-hour elevation of serum AKT. Glucose (25% ad lib) provided some protection against lethality in ADX rats. When vinylidene chloride was administered by inhalation for 4 hours, the LC_{50} for fed rats was estimated to be between 10,000 and 15,000 ppm, while the LC_{50} for fasted rats was between 500 and 2500 ppm (30).

In 1973, Bando and Rosenbaum reported that when HeLa cell cultures, in tubes, were covered with Saran Wrap, a copolymer of vinyl and vinylidene chlorides, no cytotoxicity was induced. However, when the plastic wrap was cut to fit inside plastic petri dishes and the HeLa cell suspension cultured thereupon, extensive degeneration was observed at 72 hours, but not before, in the cells overlaid on the Saran (5).

In a 1974 report, Jaeger, et al., related findings on the effects of fasting and glutathione depletion on vinylidene chloride toxicity. Male Holtzman rats weighing between 250 and 400 g were used in the experiments. By exposing fed rats to vinylidene chloride vapors for 4 hours and then observing them for 24 hours, the LC_{50} was determined to be 15,000 ppm. Similar treatment of rats that had been fasted for 18 hours prior to exposured yielded an LC_{50} of 600 ppm. The minimum lethal concentrations for the fed and fasted rats were 10,000 and 200 ppm, respectively. Determination of the 24-hour serum alanine-alpha-ketoglutarate transaminase (AKT) activity after a 4-hour vinylidene chloride inhalation exposure disclosed a dose-related increase which was much greater in fasted than in fed rats. Reversal of the feeding schedule (refeeding fasted rats or fasting fed rats) 2 hours prior to vinylidene chloride exposure did not alter the toxic effects. Analysis of blood and liver samples from vinylidene chloride treated rats demonstrated that altered distribution of the compound cannot account for the difference in sensitivity between fed and fasted animals (27).

Jaeger, et al., also performed in vitro experiments on perfused rat livers to determine if vinylidene chloride-induced hepatotoxicity results from a direct effect on the liver. The perfused livers were exposed to a concentration of 20,000 ppm vinylidene chloride in the gas phase. After 3 hours the livers of fed rats showed no change in weight or perfusate AKT. The livers of fasted rats, however, began to appear grossly damaged within 2 hours, becoming pale and swollen, with decreased flow. At the end of 3 hours both their weight and the perfusate AKT had increased markedly. In experiments to determine a biochemical difference between fed and fasted rats which could account for the difference in sensitivity to vinylidene chloride, alanine, sodium oleate and glucose were added to the perfusate without effect. Exposure of fed animals to cold stress and epinephrine prior to vinylidene chloride exposure also failed to effect the sensitivity (27).

Measurement of hepatic glutathione (GSH) concentration revealed a significant reduction in fasted rats, compared to fed rats. Vinylidene chloride exposure caused a reduction of hepatic GSH in both fed and

fasted rats. The degree of reduction, however, did not correlate with hepatic injury. To test the possibility that GSH depletion causes enhanced sensitivity to vinylidene chloride, Jaeger, et al., pretreated fed rats with diethylmaleate (DEM) (0.25 ml/kg, i.p.), a compound which depletes hepatic GSH but is not known to cause liver injury. Upon exposure to vinylidene chloride at 1000 ppm for 4 hours, the DEM-treated rats showed a 42-fold elevation of serum AKT, while vehicle-treated rats showed only a 3.8-fold increase. The DEM-treated rats exhibited bloody ascites and hemorrhagic liver enlargement. One of these rats died at 5 hours, although the vinylidene chloride concentration was well below the minimum lethal concentration (10,000 ppm) for fed rats (27).

To further test the hypothesis that depletion of hepatic GSH is responsible for the alteration of sensitivity to vinylidene chloride, the in vitro effect of DEM was tested in the isolated rat liver system. The addition of DEM (25 mcl) to the perfusate of vinylidene chloride-treated livers from fed rats resulted in an increase of perfusate AKT comparable to that seen in the perfused livers from fasted rats (27).

In a 1974 article, Siletchnik and Carlson reported the results of experiments on the cardiac sensitizing effects of vinylidene chloride, and its enhancement by phenobarbital (PB) pretreatment. The test animals were male Charles River albino rats weighing between 250 and 400 g. The animals were lightly sedated with 25 mg/kg sodium pentobarbital i.p. and restrained in a supine position. The animals were placed in an air chamber and injected with epinephrine at a dose of 4 mcg/kg. The rats were then exposed to 25,600 ppm vinylidene chloride for 10 minutes or more and the dose of epinephrine titrated to determine the minimum concentration necessary to produce arrhythmias or demonstrate a difference between pairs of animals. The effect of PB pretreatment was studied in pairs of rats, one of which was pretreated with PB 50 mg/kg i.p. for 4 days (controls received saline) and exposed to vinylidene chloride 24 hours after the last dose (48).

Exposure to vinylidene chloride alone caused progressive sinus bradycardia accompanied by arrythmias which took the form of AV-block,

multiple continuous premature ventricular contractions and ventricular fibrillation. The dose of epinephrine necessary to elicit cardiac arrhythmias in vinylidene chloride-exposed rats was as low as 0.5 mcg/kg, while doses of 4 mcg/kg failed to elicit arrhythmias in the air-exposed rats. The dose of epinephrine necessary to elicit arrhythmias in vinylidene chloride-exposed rats was time-dependent, as shown in Figure 11. The data in Table 8 indicates that PB pretreatment enhances the ability of vinylidene chloride, thereby hastening the amount of cardiotoxic metabolite formed (48).

The death of 4 workers in a B.F. Goodrich PVC plant prompted labor unions, in 1974, to demand emergency controls on the occupational exposure to vinyl chloride or other chemicals involved in its production or use. The 4 workers had average exposures of some 19 years to vinyl chloride and 10 years to vinylidene chloride, with variable exposures to such chemicals as vinyl acetate, methyl acrylate, ethyl acrylate, methanol, and chlorinated solvents. Death was attributed to angiosarcoma of the liver, a disease so rare that NIOSH believes that it causes only 20 to 30 deaths a year throughout the U.S. (52).

A 1975 report by Reynolds, et al., examined the role of the mixed function oxidase system (MFOS) in the acute toxic responses of animals to vinylidene chloride. Differential induction of MFOS components was achieved by treating 200 g male Sprague-Dawley rats with 400 mcM/kg doses of phenobarbital (PBT), 3-methylcholanthrene (3-MC), hexachlorobenzene (HCB), spironolactone (SNL), or prenenolone-16-alpha-carbonitrile (PCN) or 150-300 mcM/kg doses of Arochlor 1254 (1254) by gavage once daily for 7 days. On the 8th day the animals were exposed to 200 ppm vinylidene chloride in the atmosphere for 4 hours, and sacrificed 2 hours thereafter (44).

Vinylidene chloride exposure resulted in extensive liver injury, which occurred earlier and was more extensive in fasted than in fed rats. In fasted rats, parenchymal cell injury, characterized by retraction of cell borders and the formation of pericellular lacunae became apparent within 2 hours of the onset of exposure. Such cells showed loss of perinuclear

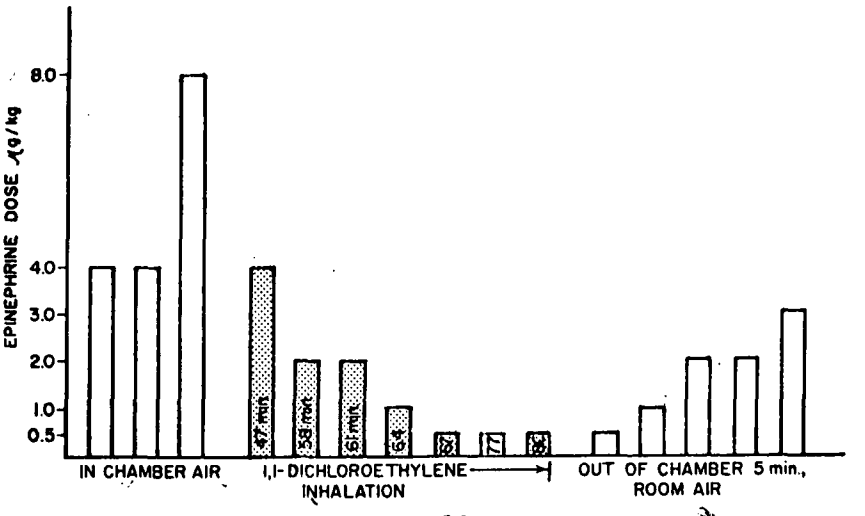


FIG. 11

Increased cardiac sensitization as a function of time exposed to DCE vapors. The bars illustrate the dose of epinephrine injected. The number within the bar represents minutes of exposure time to the DCE. Open bars represent no gross arrhythmias although reflex bradycardia may have been present. Darkened bars represent frank and life threatening arrhythmias.

TABLE 8

Effect of Phenobarbital on Epinephrine Induced Arrhythmias in Rats Exposed to 1,1-Dichloroethylene (1) *Vapor*

Pretreatment	Exposure Time (min)	Dose of Epinephrine Producing Arrhythmia (2) (µg/kg)
PB (3)	21	3
Saline	21	None @ 3
	25	None @ 4
	39	None @ 4
PB	48	3
	65	2
Saline	48	None @ 3
	65	None @ 2
PB	50	3
	67	2
Saline	50	None @ 3
	67	None @ 4
PB	53	3
Saline	53	None @ 3
PB	49	0 (4)
Saline	49	None

(1) 25,600 ± 700 ppm. — (2) Continuous premature ventricular contractions. — (3) PB injected 50 mg/kg i.p. for 4 days. — (4) Spontaneous, no epinephrine injected.

chromatin and clumping and coalescence of perinuclear chromatin into temporary cresentic deposits of electron-opaque material against the nuclear envelope. Mitochondria appeared swollen, with ruptured outer membranes. Following vinylidene chloride exposure, the zone of injured midzonal parenchymal cells became confluent, forming a prominent midzonal stripe of necrosis; hemorrhagic centrolobular necrosis rapidly ensued, becoming readily apparent within 6 hours. Necrosis was minimized by PBT and abolished by 1254 pretreatment; HCB, 3-MC, SNL and PCN were without effect. In contrast, PBT, 1254 and HCB potentiated the hepatotoxic action of vinyl chloride monomer (VCM; 5,000 ppm, 6 hour). Liver glutathione content was decreased and serum alanine alpha-ketoglutarate transaminase activity, an indicator of hepatic injury, greatly increased by vinylidene chloride exposure in all rats except those pretreated with 1254. The authors found a strong correlation between the degree of injury induced by vinylidene chloride, or VCM, and mean total microsomal cytochrome P-450 content (44).

The histological changes induced in the livers of adult male Holtzman rats, 250-350 g, by exposure to 200 ppm atmospheric vinylidene chloride for 4 hours were described in a 1975 report by Jaeger, et al. Within 2 hours after exposure, massive midzonal hepatic necrosis with hepatic thrombosis and chromatolysis were apparent in the livers of fasted, but not fed, rats. Subsequently, the central portion of the lobule collapsed, accompanied by congestion, ascites and an increased hematocrit. Early changes were observed in the nucleus, mitochondria and plasma membrane, indicating that these organelles are primarily affected by vinylidene chloride (31).

The results of an investigation of the interaction of acetone and vinylidene chloride are shown in Table 9. A 2-hour exposure to 10,000 ppm acetone either prior to or concurrent with vinylidene chloride exposure (2,000 ppm, 4 hours) resulted in a significant increase in serum AKT activity at 6 hours in fed rats (31).

Table 9 Effect of acetone exposure on the serum AKT response to inhaled 1,1-DCE in fed rats.[a]

	N	Serum AKT, mg pyruvate/ml-hr[b]	
		6 hr	24 hr
Air+1,1-DCE	10	0.37±0.05	0.43±0.03
Acetone before 1,1-DCE	5	1.10±0.20[c]	1.39±0.34[c]
Acetone with 1,1-DCE	5	1.02±0.41[c]	0.54±0.15
Fasted rats	3	13.45±1.92[d]	—

[a] Acetone was ca. 10,000 ppm for 2 hr before or simultaneous with 1,1-DCE; 1,1-DCE concentration was 2000 ppm for 4 hr.
[b] Control AKT: 0.20-0.40 mg pyruvate/ml-hr.
[c] $p < 0.05$ when compared to air+1,1-DCE.
[d] $p < 0.01$ when compared to air+1,1-DCE.

Jaeger, et al., also examined the effect of pretreatment with SKF-525A or cysteine on vinylidene chloride toxicity to rats. The results shown in Table 10 indicate that SKF-525A (50 mg/kg, i.p.) had no effect and that cysteine (500 mg/kg, i.p.) protected fasted rats from the hepatotoxic and lethal effects of vinylidene chloride (1000 ppm, 4 hour) (31).

Table 10 Effect of SKF-525A or cysteine on serum AKT in rats exposed to 1,1-DCE (1000 ppm, 4 hr).

Expt.		Serum AKT, mg pyruvate/ml-hr	
		Fed	Fasted
I	Saline	0.54±0.07 (5)[a]	9.88± 4.96[b] (4)[a] (1.22–23.58)[c]
	SKF-525A[d]	0.60±0.08 (5)[a]	18.34 (2)[a] (9.10+27.58)[c]
II	Saline	0.32±0.04 (5)	17.72±1.47[b] (3)[a] (2 died)
	Cysteine[e]	0.75±0.09: (5)[a]	2.43±0.75[d, f, g] (5)[a]

[a] Numbers in parentheses indicate number of animals in groups *N*.
[b] $p < 0.05$ when compared to fed rats.
[c] Values in parentheses are ranges.
[d] 50 mg/kg, IP.
[e] 500 mg/kg, IP.
[f] $p < 0.05$ when compared to saline control rats.
[g] $p < 0.05$ when compared to fed saline pretreated rats.

To further test the interaction between vinylidene chloride and VCM, rats were exposed to 10,600 ppm VCM for 5 hours prior to a 4 hour exposure to 2000 ppm vinylidene chloride. As shown in Table 11, VCM, which depleted

liver GSH, significantly enhanced vinylidene chloride hepatotoxicity, as evidenced by increased serum AKT and SDH activities (31).

Table 11 Effect of prior VCM exposure on serum AKT+ SDH after 1,1-DCE exposure in fed rats.

Exposure conditions	*N*	Serum AKT, mg pyruvate/ml-hr[a]	Serum SDH, units/ml serum-min[a]
Unexposed controls	50	0.20-0.40	5-10
Air+1,1-DCE	5	0.38±0.01 (0.35-0.43)	6.2±1.0 (4.4-10.0)
VCM (5 hr)+1,1-CDE	5	3.77±1.39[b] (0.94-8.22)	548±190[b] (42-1178)

[a] Values in parentheses are ranges.
[b] $p<0.05$ compared to animals also given 1,1-DCE but previously exposed to air. See text for details.

The results of a further study by Jaeger, et al., 1975, on the interaction of vinylidene chloride (DCE) and vinyl chloride monomer (VCM) are presented in Table 12. Adult male Holtzman rats were used in this study. The combined exposure to VCM and vinylidene chloride in molar ratios of 5:1 and 3:1 resulted in complete protection; at an equimolar ratio, protection was not complete. Measurement of serum sorbitol dehydrogenase, another index of hepatotoxicity, confirmed the protective action of VCM (28).

Table 12 Effect of a 4-hr VCM and/or DCE Exposure on Serum AKT Activity In Fed or Fasted Rats (Killed) at or Before 6 hr

Atmosphere Concentration, ppm		Serum AKT Activity, mg pyruvate/ml/hr		
VCM	DCE	Fed	(N = 5)	Fasted
...	...		0.2-0.40*	
1,122 ± 46	...	...		0.24 ± .01
...	205 ± 7	0.80 ± 35		16.00 ± 8.95†
1,056 ± 68	195 ± 15	0.21 ± .03		0.16 ± .02
671 ± 116	210 ± 9	0.21 ± .01		0.21 ± .02
201 ± 12	190 ± 7	0.24 ± .05		2.00 ± 1.40‡
...	1,980 ± 76	0.22 ± .01		9.73 ± 2.45†§
12,093 ± 929	1,971 ± 50	0.21 ± .01		0.18 ± .03

* Control range, see footnote Table 2.
† $P<.05$ when compared to air control.
‡ Two rats in this group had severe liver injury while the other three were normal.
§ These rats were killed in extremis before 6 hr.

In a recent (1975) communication, R.J. Jaeger briefly reported on a series of experiments to determine the interaction between vinyl chloride monomer (VCM) and vinylidene chloride. Exposure of fasted rats to 0.02% (v/v) atmospheric vinylidene chloride for 4 hours produced a 50-fold increase in serum alanine alpha-ketoglurate transaminase (SAKT) activity at 2 hours after the end of exposure. Exposure to 0.1% VCM was without effect on SAKT. When 0.02% vinylidene chloride and 0.1% VCM were administered to fasted rats simultaneously for 4 hours, there was a complete lack of injury in the rats killed 2 hours later. Thus, VCM prevented vinylidene chloride injury. This protection was concentration dependent, and was still apparent at a 1:1 molar ratio (24).

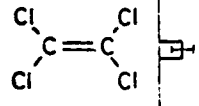
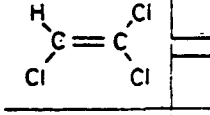
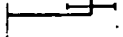
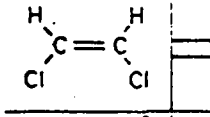
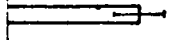
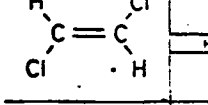
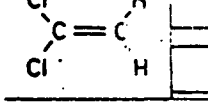
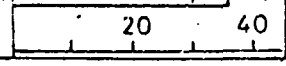
The findings of a study by Bonse, et al., on the uptake and metabolism of chlorinated ethylenes in relation to their acute liver toxicity were reported in an article published in 1975. Livers of female Wistar rats, 170-230 g, were exposed to the test chemicals by adding the chemicals as vapors to the carbogen, a 5% CO_2 and 95% O_2 mixture, used for oxygenating blood in the perfusion. Results of the tests are shown in Table 13. Vinylidene chloride was readily taken up by the system and resulted in increased hepatic enzyme activities. No metabolites of vinylidene chloride were determined (7).

In a 1975 review of the literature on vinylidene chloride, T.J. Haley postulated the biotransformation pathway for vinylidene chloride presented in Figure 12 (18).

Van Esch and Van Logten, 1975, suggested that the presence of the double bond in vinylidene chloride could lead to free radical formation during metabolism, with the resultant product acting as an alkylating agent (50).

Vinylidene chloride was tested for mutagenicity by McCann et al., 1975, using the Salmonella/microsome test. Petri plates containing several specially constructed mutants of Salmonella typhimuriun (to which homogenates of rat or human liver had been added to provide metabolic conversion of

Table 13 Comparative metabolism of chlorinated ethylenes in the isolated perfused rat liver under equimolar substrate concentrations (55.0±2.5 nmol/ml). Each value represents the mean of 3 to 5 experiments.

Compound (conc. gas-phase ppm)	Uptake[a] (nmol/ml)	Solubility[b] in liver tissue (%)	Metabolites[b] (%)	Enzyme activities (perfusate) lactate/pyruvate 0'	60'	180'	GPT (mU/ml·g liver^{-1}) 0'	60'	120'	180'	GOT (mU/ml·g liver^{-1}) 0'	60'	120'	180'
		41 ± 5	CCl_3COOH 10-15 (perfusate) CCl_3COOH 3-5 (bound in tissue)	7.3 ± 2.2	7.4 ± 2.2	7.9 ± 2.4	1.9 ± 0.6	2.2 ± 0.8	2.2 ± 0.8	2.4 ± 0.8	1.8 ± 0.6	1.9 ± 0.8	2.2 ± 0.8	2.2 ± 0.8
		4 ± 1	CCl_3CHO 2-4 CCl_3COOH 15-20 CCl_3CH_2OH[c] 65-75	7.9 ± 2.5	7.9 ± 2.5	8.0 ± 2.5	2.0 ± 0.8	2.1 ± 0.8	2.1 ± 0.8	2.1 ± 0.8	1.9 ± 0.8	1.9 ± 0.8	2.2 ± 0.8	2.3 ± 0.9
		3 ± 1	$CHCl_2COOH$ 1-3 $CHCl_2CH_2OH$ 8-10	8.0 ± 2.5	7.1 ± 2.2	17.4 ± 4.0	1.8 ± 0.8	2.0 ± 0.8	7.7 ± 3.0	23.0 ± 4.5	1.8 ± 0.8	2.0 ± 0.8	5.2 ± 2.0	14.0 ± 3.5
		6 ± 2	$CHCl_2COOH$ $CHCl_2CH_2OH$ 0.5-1	7.4 ± 2.2	6.7 ± 2.1	12.3 ± 3.0	1.4 ± 0.4	1.5 ± 0.6	1.5 ± 0.6	3.6 ± 1.2	1.9 ± 0.8	2.4 ± 0.8	3.1 ± 1.0	5.4 ± 1.4
		1 ± 0.5	d)	7.4 ± 2.3	6.6 ± 2.2	11.7 ± 2.9	1.2 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	4.0 ± 0.8	1.2 ± 0.3	1.4 ± 0.3	2.3 ± 0.8	4.6 ± 1.0

a) uptake (at 60 min), conditions: perfusate flow 1.0 ± 0.05 ml/min·g liver^{-1}.

b) all values as percent of total uptake.

c) total = free (5%) and conjugated (95%) forms.

d) not determined.

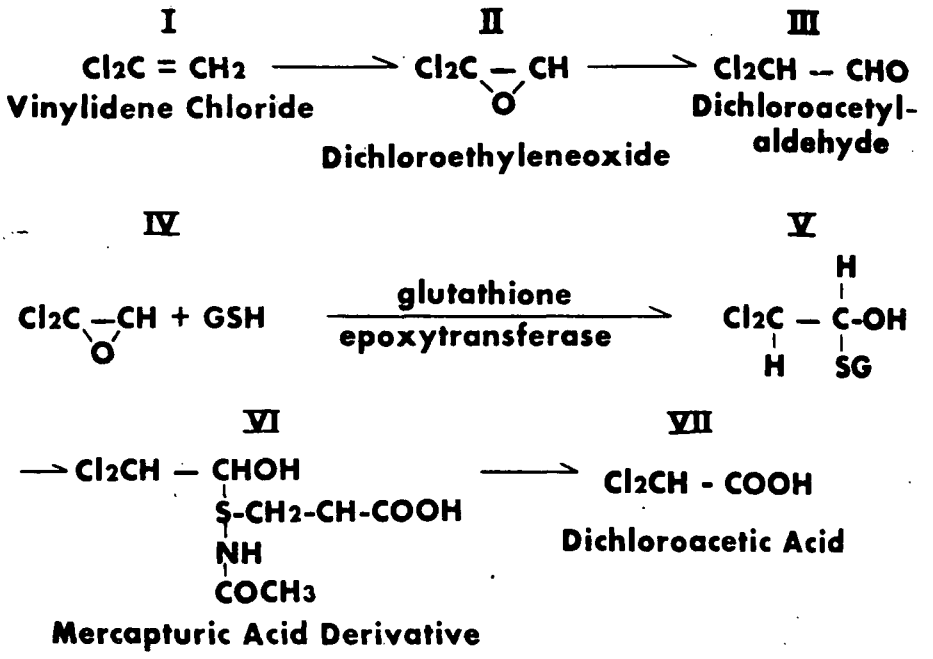


FIG. 12 Postulated biotransformation of vinylidene chloride by hepatic mixed function oxidases.

the chemical to an active form) were exposed to an atmosphere of vinylidene chloride (concentration and duration not specified). By counting the number of histidine revertants per plate, it was found that vinylidene chloride is weakly mutagenic. Upon testing 174 carcinogens, the authors reported that this test method shows a high (90%) correlation between mutagenicity and carcinogenicity (37).

To study the mutagenicity of vinylidene chloride, Greim, et al., 1975, employed a metabolizing in vitro system with E coli K 12. Cultures of this bioauxotrophic strain were suspended for 2 hours in an incubate containing mouse liver microsomal enzymes, an NADPH-generating system and 2.5 mM vinylidene chloride. A mutation rate of slightly more than twice the spontaneous rate was detected in the arginine genes. Other genes tested showed little or no mutation. When the tests were performed without the addition of microsomal enzymes, no mutagenic activity was observed. This indicates that it is not vinylidene chloride itself but a metabolite that is the mutagenic agent (17).

Using the histidine-auxotroph strains of Salmonella typhimurium TA1530 and TA100, Bartsch, et al., 1975, examined the tissue-mediated mutagenicity of vinylidene chloride. Incubates containing the baceria, an NADPH-generating system and mouse or rat liver, kidney or lung fractions were exposed to vinylidene chloride vapor in a desiccator at atmospheric concentrations of 0.2, 2 or 20% for up to 4 hours. The concentrations of vinylidene chloride in the aqueous phase after 2 hours of exposure to 0.2, 2 and 20% in the air were 0.33, 3.3 and 33 mM, respectively. Results of the tests are shown in Figure 13 and Table 14. The mutagenic response of both strains increased after exposure to up to 2% vinylidene chloride. The authors suggested that the lower mutagenic response observed with 20% vinylidene chloride may have resulted from an inhibitory action of the compound or its metabolites on the microsomal enzymes responsible for its metabolic activation. A linear increase, with time, in mutagenic response was observed when TA100 strain was exposed to 2% vinylidene chloride for periods of up to 4 hours. Ommission of the NADPH-generating system from the incubate resulted in a complete absence of mutagenic response (6).

In further studies, Bartsch, et al., found that pretreatment of female BD-VI rats with phenobarbitone (0.1% in the drinking water for 7 days) increased the mutagenic effect of exposure to 2% vinylidene chloride in air on S. typhimurium; pretreatment with pregnenolone-16alpha-carbonitrile (50 mg/kg, 5 times orally at 12 hour intervals) or aminoacetonitrile (500 mg/kg, single s.c. dose, 24 hours before the assay) or the addition of N-acetyl-cysteine and N-acetyl methionine (12 mcM of each per ml soft agar layer) resulted in a reduction of mutagenic response (6).

Prof. P.L. Viola of Regina Elena Institute for Cancer Research, in Rome, has reported (1975) that vinylidene chloride may be carcinogenic to rats in high concentrations via inhalation. Viola allegedly has found tumors in animals exposed to the chemical (53).

The Dow Chemical Corporation has stated (1975) that the average exposure of a production facility employee to vinylidene chloride monomer rarely if ever reaches the TLV of 10 ppm (7).

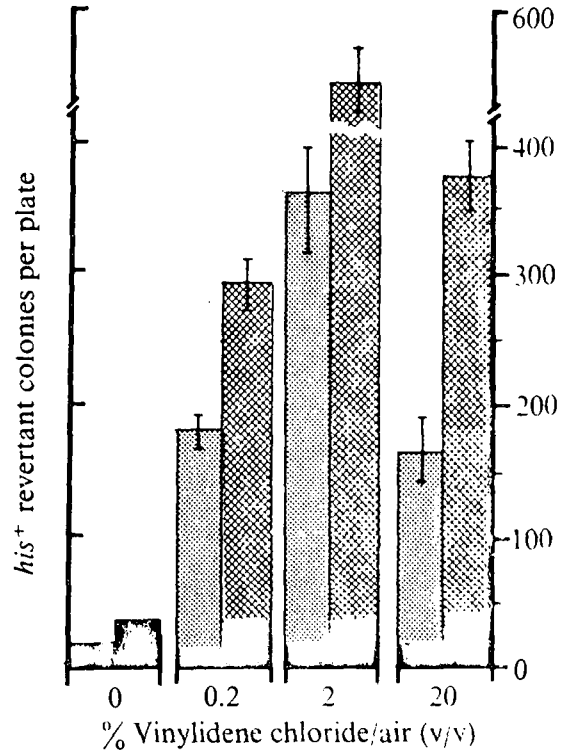


Fig. 13 VDC concentration-dependent induction of reverse mutations in *S. typhimurium* strains TA1530 and TA100. Petri dishes containing 9,000*g* liver supernatant from phenobarbitone-pretreated male OF-1 mice, $NADP^+$ (2 µmol per plate), glucose-6-phosphate (2.5 µmol per plate) and the bacteria in a histidine-deficient medium were exposed to 0, 0.2, 2 or 20% VDC in air (by volume) for 4 h in a desiccator at 37 °C in the dark. VDC was removed under vacuum and replaced by air, and the incubation was continued up to 48 h at 37 °C. Bacterial survival was estimated by seeding TA1530 (10^{-5} dilution) on a histidine-enriched medium. Control assays were carried out by omitting the cofactors ($NADP^+$, glucose-6-phosphate). Mean values ± s.e. from 1-4 series of experiments, each on a pool of five mouse livers, are plotted. Bacteria were plated in triplicate. VDC, containing 0.3% 4-methoxyphenol as antioxidant, was obtained from Merck-Schuchardt (Darmstadt, Federal Republic of Germany). Black: control; stippled, TA 1530; cross-hatched, TA100.

Table 14 Mouse and rat tissue mediated mutagenicity of VDC in *S. typhimurium* TA100*

Experiment no.	Species	Phenobarbitone pretreatment	Tissue (9,000*g* supernatant†)	Cofactors‡	2% VDC in air *his*+ revertants per plate§	2% VDC in air Relative activity¶	20% VDC in air *his*+ revertants per plate§	20% VDC in air Relative activity
1		Yes		+	500 ± 23	150	330 ± 29	75
2		Yes		−	23 ± 10	5	7 ± 5	2
3		No	Liver	+	330 ± 49	100	435 ± 46	100
4		No		−	16 ± 4	5	1 ± 3	0
5		Yes		+	147 ± 15	45	173 ± 5	40
6		Yes		−	31 ± 7	9	17 ± 2	4
7	OF-1 mouse♂	No	Kidney	+	67 ± 2	20	125 ± 5	29
8		No		−	20 ± 3	6	16 ± 1	4
9		Yes		+	34 ± 4	10	48 ± 5	11
10		Yes		−	5 ± 4	1	10 ± 1	2
11		No	Lung	+	21 ± 5	6	37 ± 3	8
12		No		−	6 ± 9	2	14 ± 8	3
13		No		+	95 ± 7	30	77 ± 5	18
14		No	Liver	−	0	0	2 ± 2	0
15		No		+	18 ± 4	5	16 ± 2	4
16	BDVI rat ♀	No	Kidney	−	18 ± 2	5	21 ± 4	5
17		No		+	9 ± 2	3	11 ± 2	3
18		No	Lung	−	9 ± 7	3	12 ± 6	3

*Assays carried out as described in Fig. 1.
†Equivalent to 38 mg wet tissue per plate.
‡$NADP^+$ (2.0 µmol per plate) and glucose-6-phosphate (2.5 µmol per plate).
§Mean values ± s.e. from 1-4 experiments, each using pooled tissues from 4 mice or 3 rats. The number of spontaneous mutations per plate (49 ± 2) has been subtracted from each value.
¶Relative mutagenic activity was expressed by taking the value obtained in experiment 3 as 100.

To estimate the persistance of low-molecular-weight chlorinated hydrocarbons in natural water bodies, Dilling, et al., 1975, carried out laboratory studies on evaporation and reaction rates at the 1-ppm level in water under ambient conditions. Vinylidene chloride evaporated to the extent of 50% in 22 minutes and 90% in 89 minutes when stirred in water at 25°C. The hydrolytic-oxidative reaction half-life of vinylidene chloride was not determined, but that for related chlorinated hydrocarbons was 6-18 months. The authors concluded that 1 ppm concentrations of low-molecular-weight chlorinated hydrocarbons would not persist in agitated natural water bodies due to evaporation (14).

To derive information on the atmospheric degradation of halogenated compounds, Gay, et al., 1976, studied the photooxidation of chlorinated ethylenes in air in the presence of nitrogen dioxide with ultraviolet light. At a concentration of 4.85 ppm, in the presence of 2.26 ppm NO_2, vinylidene chloride was found to decompose rapidly (83% in 140 min). The reaction products identified were formic acid, hydrochloric acid, carbon monoxide, formaldehyde, ozone, phosgene, chloroacetyl chloride, formyl chloride and nitric acid. Possible reaction mechanisms were discussed (16).

On the basis of laboratory studies on photolysis rates under simulated atmospheric conditions (100 ppm vinylidene chloride with 5 ppm NO_2; 27°C), Dilling, et al., 1976, calculated the half-life of atmospheric vinylidene chloride to be 2.1 hours (13).

The gas-phase room-temperature oxidation of haloethylenes was reviewed by Heicklen, et al., in 1975. Tests have been performed on five types of oxidation: (1) chlorine atom initiation, (2) Hg $6(^3P)$ sensitization, (3) reaction with $O(^3P)$, (4) reaction with $O(^3P)$ in the presence of O_2, and (5) reaction with O_3 (20).

The Cl-atom initiated and Hg $6(^3P)$ sensitized oxidations of vinylidene chloride proceed by a long-chain free radical process. Reaction with $O(^3P)$ results in double-bond cleavage. Ozonolysis proceeds by a chain oxidation, carried by a di-radical mechanism, but is inhibited in the presence of O_2 (20).

The authors concluded that chloroolefins in the atmosphere will generate chlorine atoms and oxidize in a chain process. For vinylidene chloride, the relative imporatnce of C=C cleavage, excited molecule and rearrangement processes were calculated to be 31:55:14, respectively (20).

Sweden has set its exposure level for vinylidene chloride at a recommended 1 ppm, with a maximum of 5 ppm weighted average for 15 minute periods (22).

Local hemolysis tests on rat spleen samples in gel, performed by L.Shmuter in 1976, demonstrated that dichloroethylene (isomer not specified) at a concentration of 25 mg/l stimulates, while 5 mg/l inhibits, the formation of cells producing antibodies to typhoid antigen, as well as the plasmocytic reaction of the spleen following immunization of the O-antigen from Sal. typhi (46).

Harms, et al., 1976, reported on the action of chlorinated hydrocarbons on bile duct-pancreatic fluid in laboratory rats. Male Sprague-Dawley rats were treated with 0.5 ml/kg vinylidene chloride i.p., in 4 volumes of corn oil one day before testing; this dose approximated the LD_{25}. The first test consisted of a retrograde intrabiliary injection of ^{3}H-inulin followed by a 6-minute period of occlusion, holding the ^{3}H-inulin solution in the biliary tree, then collecting and analyzing each drop of bile. Dilution of the ^{3}H-inulin content in the drops from the distant portion of the biliary tree was accomplished by increased excretion of bile duct-pancreatic fluid into the duct system during the 6-minute occlusion period. Recovery of this retrogradely inject inulin was not significantly different from controls, so a double cannulation method was utilized (19).

The second method consisted of cannulating the common bile duct at the proximal bifurcation to collect hepatic bile and distally at the duodenum to collect bile duct-pancreatic fluid. Pretreatment with vinylidene chloride brought about a marginal depression in hepatic bile flow, but significantly increased bile duct-pancreatic fluid flow at the same time. Histopathologic examinations indicated that one out of 5 rats had liver necrosis, the remaining ones showed slight congestion. Only mild nonspecific interstitial inflammation and congestion were noted in the pancreas and common bile duct (19).

Recovery in hepatic bile of ^{3}H-ouabain administered intravenously 1 hour earlier was marginally depressed in vinylidene chloride treated rats (19).

In a current investigation sponsored by the Manufacturing Chemists Association in cooperation with Dow Chemical Corporation, the following projects are being conducted:

1. A toxicological study on the effects of vinylidene chloride included in the drinking water of rats for 90 days and two years.
2. A toxicological study of vinylidene chloride in peanut oil fed to dogs for 90 days.
3. A 90 day and two year vapor inhalation study of vinylidene chloride on rats.
4. A study of the effects of maternally inhaled or ingested vinylidene chloride on rat and rabbit embryonal and fetal development.
5. A study of absorption, distribution, metabolism and excretion of ingested and inhaled vinylidene chloride in rats.

To date, only the first two 90 day studies have been completed, and those showed no evidence of carcinogenicity of vinylidene chloride (22).

The teratogenicity of vinylidene chloride is currently being studied by Midwest Research Institute under contract to the EPA office of Toxic Substances. A report is expected by January 1977 (9).

Vinylidene chloride is produced by only 3 plants in the U.S. - PPG, Lake Charles, La.; Dow Chemical, Freeport, Texas and Plaquemine, La. Production losses from these plants are estimated to release 3.355 million pounds of vinylidene chloride into the environment annually (See Table 15). An additional 709,400 pounds are estimated to be released during polymer synthesis and processing (See Table 16) (22).

TABLE 15

EMISSIONS OF VINYLIDENE CHLORIDE

Producer	Location	Total annual emmisions (lbs/yr)	χmg /m^3	Emmission in lbs/100lbs VDC monomer
PPG	Lake Charles, La.	2,920,000*	3.06	1.72 - 1.67
		175,000**	.1 83	.10
Dow Chemical	Freeport Texas	289,000	.3 03	.51 - .48
	Plaquemine, La.	146,000	.1 53	
	TOTAL	3,355,000*		
		610,000**		

*Emissions using an existing control technology.

**Emissions reflecting new control technology at PPG plant by late 1975.

TABLE 16

ESTIMATED ANNUAL EMISSIONS OF VINYLIDENE CHLORIDE IN THE U.S.A.

		lbs/yr
A.	Monomer Synthesis	3,355,000* 611,000**
B.	Polymer Synthesis	
	Total	679,000
1.	Latex for Burner Coatings	120,000
2.	Latex for Miscellaneous Coatings	150,000
3.	Synthetic Fibres	160,000
4.	Coating Resin for Cellophane	182,000
5.	Extrusion Resin (Emulsion)	27,000
6.	Extrusion Resin (Suspension)	40,000
C.	Fabrication Polymer Processing	
	Total	30,400
1.	Coating Cellophane	1,600
2.	Coating Plastics, Paper and Glassine	16,400
3.	Extrusion	400
4.	Miscellaneous Coating	12,000
	TOTAL	4,064,000 lbs/yr.

*Emissions using an existing control technology.

**Emissions reflecting new control technology at PPG plant by late 1975.

Research Request No. 1
Contract No. 68-01-4116

Information to be derived in the literature search shall include:

I. Health Impact

A. Toxicological data.

1. Absorption, excretion, transport, and distribution.

2. Metabolic effects.

3. Pharmacology.

4. Biochemical descriptions of effects on organs, cells, enzyme systems, and nucleic acids and proteins.

5. Acute, subacute, and chronic toxicity.

6. Sensitization from repeated doses.

7. Teratogenicity and mutagenicity.

8. Carcinogenicity.

9. Dose-response relationships.

10. Behavioral effects.

11. Synergisms and related interactive effects with other chemicals.

B. Epidemiological data.

1. Occupational exposure studies.

2. Environmental incidents, poisonings, and case histories.

3. Clinical and subclinical manifestations.

4. Statistical or risk-comparison studies.

5. Other controlled studies.

II. Environmental Impact.

A. Environmental fate.

1. Chemical and biochemical reactions, including degradation and chemical interconversion, in the environment.

2. Transport in soils, aquatic systems, and biota.

B. Environmental/ecosystem effects

1. Fish and other aquatic organisms.

2. Birds.

3. Mammals of economic importance.

4. Other terrestrial organisms.

[Note: To the extent, these impacts should be related to the categories under I, A, above.]

5. Atmosphere and/or climate.

6. Manmade structures.

III. Monitoring Data and Exposure Levels.

A. Human exposure profile, defined in geographic terms, if necessary.

B. Exposure of other organisms, and assessment of degree of risk.

C. Monitoring data.

N.B. It is recognized that all of these subjects may not be presented in the literature. This listing reflects information needed, but a determination of nonavailability is also important. The contractor should notify the Project Officer of gaps in the literature, as soon as the determination has been made.

Appendix B. Sources Used in Vinylidene Chloride Search

Secondary Journals (1950-present)

- Air Pollution Abstracts
- Biological Abstracts
- Bioresearch Index
- Chemical Abstracts (1907-present)
- Chemische Zentralblatt
- Current Contents (1976 only)
- Excerpta Medica
  - Pharm and Toxicol
  - Public Hlth., Soc. Med. and Hygiene
  - Occup. Hlth. and Ind. Med.
  - Devel. Biol. and Teratol.
- FDA Clinical Experience Abstracts
- Index Medicus
- Industrial Hygiene Digest
- Pollution Abstracts
- Referativnyi Zhurnal

On-line Data Bases

- CAIN
- CANCERLINE
- ENVIRONLINE
- MEDLINE
- NTIS
- SCISEARCH
- TOXLINE

Bibliography

1. Aleksandrowicz, R., W. Mazurowski, J. Wislawski, J. Lozinski and K. Gralinska. 1972. Influence of polymers used for reinforcing of blood vessels on tissues of test animals. Polimery Med., 2(1):77-81.

2. Altman, P.L., D.S. Dittmer. 1966. Environmental Biology. Federation of American Societies for Experimental Biology, Bethesda, Md., pp. 326, 328.

3. American Conference of Governmental Industrial Hygienists. Documentation of the Threshold Limit Values for Substances in Workroom Air. Cincinnati, Ohio, pp. 351-352, 1971.

4. Anderson, W.L. and R.A. Saunders. 1963. Evolution of materials in the closed system. Symposium on Toxicity in the Closed Ecological System (M. Honma and H.J. Crosby, eds.), Lockheed Missiles and Space Co., Palo Alto, California, pp. 9-18.

5. Bando, B.M. and M.J. Rosenbaum. 1973. Toxicity to HeLa cells mediated by plastic wraps. Lab. Pract., 22:26.

6. Bartsch, H., C. Malaveille, R. Montesano, L. Tomatis. 1975. Nature (London), 255(5510):641-3.

7. Bonse, G., T. Urban, D. Reichert, D. Henschler. 1975. Chemical reactivity, metabolic oxirane formation, and biological reactivity of chlorinated ethylenes in the isolated perfused rat liver preparation. Biochem. Pharmacol., 24(19):1829-34.

8. Broser, F. 1970. Irreversible lesions of the trigeminal nerve caused by chlorinated acetylenes in two patients. Deut. Z. Nervenheilk, 197(2):163-170.

9. Brunner, P.: Personal Communication

10. Carlson, G.P. and G.C. Fuller. 1972. Interaction of Modifiers of hepatic microsomal drug metabolism and the inhalation toxicity of 1,1-dichloroethylene. Res. Commun. Chem. Pathol. Pharmacol., 4(3):553-560.

11. Carpenter, C.P., H.F. Smyth, Jr. and U.C. Pozzani. 1949. The assay of acute vapor toxicity, and the grading and interpretation of results on ninety-six chemical compounds. J. Ind. Hyg. Toxicol., 31:343-6.

12. Cranch, A.G. 1945. Toxicity of some of the newer plastics. Industrial Medicine, 15(2):168-170.

13. Dilling, W.L., C.J. Bredeweg and N.B. Tefertiller. 1976. Simulated atmospheric photodecomposition rates of methylene chloride, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene and other compounds. Environ. Sci. Technol. 10(4):351-356.

14. Dilling, W.D., N.B. Tefertiller and G.J. Kallos. 1975. Evaporation rates and reactivities of methylene chloride, chloroform, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene and other chlorinated compounds in dilute aqueous solutions. Environ. Sci. Technol. 9(9):833-838.

15. Gage, J.C. 1970. Subacute inhalation toxicity of 109 industrial chemicals. Brit. J. Ind. Med., 27(1):1-18.

16. Gay, B.W., Jr., P.L. Hanst, J.J. Bufalini and R.C. Noonan. 1976. Atmospheric oxidation of chlorinated ethylenes. Environ. Sci. Technol. 10(1):58-67.

17. Greim, H.,G. Bonse, Z. Radwan, D. Reichert and D. Henschler. 1975. Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation. Biochem. Pharmacol., 24:2013-2017.

18. Haley, T.J. 1975. Vinylidene Chloride: A Review of the Literature. Clinical Toxicology, 8(6):633.

19. Harms, M.S., R.E. Peterson, J.M. Fujimoto and C.P. Erwin. 1976. Increased "bile duct-pancreatic fluid" flow in chlorinated hydrocarbon-treated rats. Toxicol. Appl. Pharmacol., 35(1):41-9.

20. Heicklen, J.P., E. Sanhueza, E.C. Hisatsune, R.K.M. Jayanty, R. Simonaitis, L.A. Hull, C.W. Blume and E. Mathias. 1975. Oxidation of Halocarbons. EPA-650/3-75-008.

21. Henschler, D., F. Broser and H.C. Hopf. 1970. "Polyneuritis cranialis: following poisoning with chlorinated acetylenes while handling vinylidene copolymers. Arch Toxikol, 26(1):62-75.

22. Hushon, J. and M. Kornreich. 1976. Air Pollution Assessment of Vinylidene Chloride. U.S. Environmental Protection Agency Report.

23. D. Irish. 1972. Industrial Hygiene and Toxicology, Vol. II. F.A. Patty, ed., Wiley (Interscience), New York, pp. 1305-1307.

24. Jaeger, R.J. 1975. Vinyl chloride monomer. Hepatotoxicity and interaction with 1,1-dichloroethylene. Ann. N.Y. Acad. Sci., 246: 150-1.

25. Jaeger, R.J., R.B. Conolly and S.D. Murphy. 1973. Diurnal variation of hepatic glutathione concentration and its correlation with 1,1-dichloroethylene inhalation toxicity in rats. Res. Commun. Chem. Pathol. Pharmacol., 6(2):465-71.

26. Jaeger, R.J. and S.D. Murphy. 1973. Alterations of barbiturate action following 1,1-dichloroethylene, corticosterone, or acrolein. Arch. Int. Pharmacodyn. Ther., 205(2):281-92.

27. Jaeger, R.J., R.B. Conolly and S.D. Murphy. 1974. Effect of 18 hr fast and glutathione depletion of 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. Exp. Mol. Pathol., 20(2):187-98.

28. Jaeger, R.J., R.B. Conolly and S.D. Murphy. 1975. Short-term inhalation toxicity of halogenated hydrocarbons. Effects on fasting rats. Arch. Environ. Health, 30(1):26-31.

29. Jaeger, R.J., M.J. Trabulus and S.D. Murphy. 1973. Biochemical effects of 1,1-dichloroethylene in rats. Dissociation of its hepatotoxicity from a lipoperoxidative mechanism. Toxicol. Appl. Pharmacol. 24(3):457-67.

30. Jaeger, R.J., M.J. Trabulus and S.D. Murphy. 1973. The interaction of adrenalectomy,partial adrenal replacement therapy and starvation with hepatotoxicity and lethality after 1,1-dichloroethylene intoxication. Toxicol. Appl. Pharmacol., 25(3):491.

31. Jaeger, R.J., R.B. Conolly, E.S. Reynolds and S.D. Murphy. 1975. Biochemical toxicology of unsaturated halogenated monomers. Environ. Health Perspect, 11:121-8.

32. Jenkins, L.J., Jr., M.J. Trabulus and S.D. Murphy. 1972. Biochemical effects of 1,1-dichloroethylene in rats. Comparison with carbon tetrachloride and 1,2-dichloroethylene. Toxicol. Appl. Pharmacol., 23(3):501-10.

33. Kramer, C.G. and J.E. Mutchler. 1972. The correlation of clinical and environmental measurements for workers exposed to vinyl chloride. Am. Ind. Hyg. Assoc. J., 33(1):19-30.

34. Lefaux, R. Industrial toxicology of monomers and various metals. Practical Toxicology of Plastics. Chemical Rubber Company, Cleveland, Ohio, pp. 66-153.

35. Leonard, E.C. 1971. High Polymers. XXIV. Vinyl and Diene Monomers. Part 3. Wiley-Interscience, New York, pp. 1255-1289.

36. Manufacturing Chemists Association. Guide for Safety in the Chemical Laboratory, 2nd ed., Appendix 4, Van Nostrand-Reinhold, New York, p. 441.

37. McCann, J., E. Choi, E. Yamasaki and B.N. Ames. 1975. Detection of carcinogens in the Salmonella/Microsome Test: Assay of 300 chemicals. Proc. Natl. Acad. Sci. U.S.A., 72:5135-5139.

38. Meyer, Fr. and L. Kerk. 1959. Permeability of the skin to some aliphatic carrier substances. Arch. exptl. Pathol. Pharmakol., 235:267-79.

39. Miyasaki, K. Experimental studies on the reticulo-endothelial system by intravenous injection of high molecular synthetic vinyl compounds in rabbits. Acta Pathologica Japonica, 9(1):109-131.

40. Oppenheimer, B.S., E.T. Oppenheimer, I. Danishefsley, A.P. Stout and F.R. Eirich. 1955. Further studies of polymers as carcinogenic agents in animals. Cancer Res. 15:333-40.

41. Osbourn, R.A. Contact dermatitis caused by saran wrap. Journal of the American Medical Association, 188(13):141.

42. Prendergast, J.A., R.A. Jones, L.J. Jenkins, Jr. and J. Siegel. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol. Appl. Pharmacol., 10(2):270-89.

43. Reinhardt, R.C. 1947. Handling vinylidene chloride. Chem. Eng. News, 25:2136.

44. Reynolds, E.S., M.T. Moslen, S. Szabo, R.J. Jaeger and S.D. Murphy. 1975. Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene. Role of mixed function oxidase system. Am. J. Pathol., 81(1): 219-36.

45. Rylova, M.L. 1953. Toxicity of 1,1-ethene dichloride. Farmakol. i Toksikol., 16(1):47-50.

46. Shmuter, L.M. 1976. Influence of acute and chronic poisoning with dichloroethylene on thekinetics of antibody-forming cells and the plasmocytic reaction in the spleen of rats in immunization with the O-antigen of Sal. typhi. Farmakol. Toksikol., 39(1):104-8.

47. Siegel, J., R.A. Jones, R.A. Coon and J.P. Lyon. 1971. Effects on experimental animals of acute, repeated, and continuous inhalation exposures to dichloroacetylene mixtures. Toxicol. Appl. Pharmacol. 18(1):168-174.

48. Siletchnik, L.M. and G.P. Carlson. 1974. Cardiac sensitizing effects of 1,1-dichloroethylene. Enhancement by phenobarbital pretreatment. Arch. Int. Pharmacodyn. Ther. 210(2):359-64.

49. Sporn, A., A. Cirstea, D. Ilean, L.D. Ghizelea and L. Stoenescu. 1970. Contributions to investigations on the toxicity of vinylidene chloride polymer extracts. Igiena (Bucharest), 19:587.

50. Van Esch, G.J. and M.J. Van Logten. 1975. Vinyl chloride: A report of a European assessment. Toxicology, 4:1.

51. Wilson, R.H. and W.E. McCormick. 1954. Toxicology of plastics. Plastomers and monomers. Ind. Med. and Surg., 23:479-86.

52. Anon. 1974. Battle lines drawn on vinyl chloride issue. Chem. Eng. News, 52(8):16.

53. Anon. 1975. Health hazards of monomers probed. C&E News, 53(3):8.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA 560/6-76-023		

4. TITLE AND SUBTITLE	5. REPORT DATE
Health and Environmental Impacts: Task 1, Vinylidene Chloride	October 1976
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Randall D. Huffman Purna Desai-Greenaway	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Tracor Jitco, Inc. 1776 East Jefferson Street Rockville, Maryland 20852	11. CONTRACT/GRANT NO. 68-01-4116, Task 1

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Toxic Substances U.S. Environmental Protection Agency Washington, D.C. 20460	Final
	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

16. ABSTRACT

A comprehensive survey of the world literature was conducted to prepare this report on the health and environmental impacts of vinylidene chloride. The available information indicates that vinylidene chloride may have significant health effects, but the information shows inconsistencies and is insufficient for the formulation of conclusions. Very little information is available on the environmental impacts of vinylidene chloride.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Vinylidene Chloride 1,1-Dichloroethylene Health Effects Environmental Impacts		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release Unlimited	Unclassified	55
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (9-73)

INSTRUCTIONS

1. **REPORT NUMBER**
   Insert the EPA report number as it appears on the cover of the publication.

2. **LEAVE BLANK**

3. **RECIPIENTS ACCESSION NUMBER**
   Reserved for use by each report recipient.

4. **TITLE AND SUBTITLE**
   Title should indicate clearly and briefly the subject coverage of the report, and be displayed prominently. Set subtitle, if used, in smaller type or otherwise subordinate it to main title. When a report is prepared in more than one volume, repeat the primary title, add volume number and include subtitle for the specific title.

5. **REPORT DATE**
   Each report shall carry a date indicating at least month and year. Indicate the basis on which it was selected *(e.g., date of issue, date of approval, date of preparation, etc.)*.

6. **PERFORMING ORGANIZATION CODE**
   Leave blank.

7. **AUTHOR(S)**
   Give name(s) in conventional order *(John R. Doe, J. Robert Doe, etc.)*. List author's affiliation if it differs from the performing organization.

8. **PERFORMING ORGANIZATION REPORT NUMBER**
   Insert if performing organization wishes to assign this number.

9. **PERFORMING ORGANIZATION NAME AND ADDRESS**
   Give name, street, city, state, and ZIP code. List no more than two levels of an organizational hirearchy.

10. **PROGRAM ELEMENT NUMBER**
    Use the program element number under which the report was prepared. Subordinate numbers may be included in parentheses.

11. **CONTRACT/GRANT NUMBER**
    Insert contract or grant number under which report was prepared.

12. **SPONSORING AGENCY NAME AND ADDRESS**
    Include ZIP code.

13. **TYPE OF REPORT AND PERIOD COVERED**
    Indicate interim final, etc., and if applicable, dates covered.

14. **SPONSORING AGENCY CODE**
    Leave blank.

15. **SUPPLEMENTARY NOTES**
    Enter information not included elsewhere but useful, such as: Prepared in cooperation with, Translation of, Presented at conference of, To be published in, Supersedes, Supplements, etc.

16. **ABSTRACT**
    Include a brief *(200 words or less)* factual summary of the most significant information contained in the report. If the report contains a significant bibliography or literature survey, mention it here.

17. **KEY WORDS AND DOCUMENT ANALYSIS**
    (a) DESCRIPTORS - Select from the Thesaurus of Engineering and Scientific Terms the proper authorized terms that identify the major concept of the research and are sufficiently specific and precise to be used as index entries for cataloging.

    (b) IDENTIFIERS AND OPEN-ENDED TERMS - Use identifiers for project names, code names, equipment designators, etc. Use open-ended terms written in descriptor form for those subjects for which no descriptor exists.

    (c) COSATI FIELD GROUP - Field and group assignments are to be taken from the 1965 COSATI Subject Category List. Since the majority of documents are multidisciplinary in nature, the Primary Field/Group assignment(s) will be specific discipline, area of human endeavor, or type of physical object. The application(s) will be cross-referenced with secondary Field/Group assignments that will follow the primary posting(s).

18. **DISTRIBUTION STATEMENT**
    Denote releasability to the public or limitation for reasons other than security for example "Release Unlimited." Cite any availability to the public, with address and price.

19. & 20. **SECURITY CLASSIFICATION**
    DO NOT submit classified reports to the National Technical Information service.

21. **NUMBER OF PAGES**
    Insert the total number of pages, including this one and unnumbered pages, but exclude distribution list, if any.

22. **PRICE**
    Insert the price set by the National Technical Information Service or the Government Printing Office, if known.