Environmental Health Perspectives
Vol. 21, pp. 125–129, 1977

Effect of Various Treatments on Toxicity of Inhaled Vinylidene Chloride

by Robert D. Short*, Joseph M. Winston*, Jan L. Minor*, Joseph Seifter†, and Cheng-Chun Lee*

The toxicity of vinylidene chloride (VDC) was studied in mice and rats exposed to various concentrations of the vapors for 23 hr/day. In addition, the ability of various treatments to alter parameters of toxicity was evaluated. Mice were more sensitive than rats both to the acute lethal and hepatotoxic effects of VDC. Disulfiram treatment reduced the acute lethal and hepatotoxic effects of inhaled VDC and reduced the levels of covalent bound radioactivity in the liver and kidney after the intraperitoneal administration of ^{14}C-VDC. Treatment with diethyldithiocarbamate and thiram also protected mice from the acute lethal effects of VDC.

Introduction

Vinylidene chloride (1,1-dichloroethylene, VDC) is an intermediate used in the production of polymers and the synthesis of other chemicals. Since environmental contamination and human exposure are inevitable results of its widespread use, the risks of such exposures should be understood.

The toxicity of VDC, which was recently reviewed (*1, 2*), has been studied in several mammalian species (*3*). A continuous 90-day inhalation exposure to 189 mg/m^3 (48 ppm) of VDC produced deaths in monkeys and guinea pigs but not in dogs and rats. In addition, morphological changes occurred in livers from monkeys, dogs, and rats and kidneys from rats. The hepatic lesions included focal necrosis, hemosiderin deposition, and fatty metamorphosis. The primary renal lesion was nuclear hypertrophy of the tubular epithelium.

VDC toxicity was influenced by various parameters. Female rats were more sensitive than males to the oral toxicity of VDC (*4*). The nutritional status also influenced toxicity. For example, starved rats were more sensitive to VDC (*5*), and the diurnal change in toxicity was correlated with hepatic levels of glutathione (GSH) (*6*). Additional studies with diethyl maleate (*7*) and cysteine (*8*) pretreatment suggested that hepatic levels of GSH influenced VDC toxicity. Metabolic studies indicate that (a) a major pathway for detoxification of VDC was by conjugation with GSH and (b) hepatotoxicity was associated with covalent binding of VDC metabolites in the liver (*9*).

The purpose of this study was to determine the acute toxicity of continuously inhaled VDC and evaluate the effect of various treatments on this parameter. The treatments were selected to alter the metabolic activation of VDC or promote the detoxification of VDC. In addition, adrenergic blocking agents were used, since VDC exposure sensitized rat hearts to catacholamines (*10*).

Methods

CD-1 mice and CD rats (Charles River Breeding Laboratories, North Wilmington, Massachusetts) were used in these studies. Animals were given free access to feed (Wayne Lab-Blox, Allied Mills, Inc. Chicago, Illinois) and tap water. Mice received disulfiram (0.10% in feed 2 to 3 days before and during exposure), diethyldithiocarbamate (0.12% in feed 3 days before and during exposure), thiram (0.10% in feed 3 days before and during exposure), cysteine (0.10% or 0.50% in feed 3 days before and during exposure), methionine (0.10% or 0.50% in feed 3

*Pharmacology and Toxicology, Midwest Research Institute, Kansas City, Missouri 64110.
†Office of Toxic Substances, Environmental Protection Agency, Washington, D.C. 20460.

days before and during exposure), *N*-acetylcysteine (1,200 mg/kg orally every day during exposure), SKF 525-A (50 mg/kg IP every day during exposure), cobaltous chloride $6H_2O$ (60 mg/kg IP daily for 2 days before exposure), BAL (50 mg/kg SC daily during exposure), phenoxybenzamine (10 mg/kg IP daily for first 2 days of exposure), propranolol (10 mg/kg IP daily during exposure), Vitamin C (100 mg/kg IP daily during exposure), or DL-α-tocopherol acetate (Vitamin E, 750 mg/kg orally once 2 days before exposure and on first day of exposure).

Animals were exposed to VDC for 22–23 hr/day for 7 days in stainless steel chambers. Control animals were similarly housed and exposed to room air. VDC vapors were generated by bubbling nitrogen into a flask that contained liquid VDC with a purity of 99% (Aldrich Chemical Co. Milwaukee, Wisconsin). A stream of air carried the vapors from the flask to the chamber. The concentration of VDC was measured with a Varian 2700 gas chromatograph equipped with a flame ionization detector and a stainless steel column packed with 0.4% Carbowax 1500 on Carbopak A.

Serum glutamic-oxaloacetic transaminase (SGOT) (*11*) and serum glutamic-pyruvic transaminase (SGPT) (*12*) were determined in cardiac blood from mice and aortic blood from rats. Hepatic nonprotein sulfhydryl concentration (*13*) was determined in the livers of male mice that received various treatments for a total of 10 days (i.e., 3 days before exposure and 7 days during exposure to room air).

Covalent bound radioactivity was measured in male mice after the intraperitoneal administration of 3 mg/kg ^{14}C-VDC (20μCi/kg) which was obtained from New England Nuclear (Boston, Massachusetts) with a specific activity of 0.652 mCi/mmole. The ^{14}C-VDC was slowly bubbled into the peanut oil vehicle with nitrogen. The tissues were homogenized in cold water and macromolecules were precipitated with an equal volume of 1*N* perchloric acid (PCA). The precipitate was washed with 5 ml of 0.2*N* PCA, 0.2*N* PCA, 95% ethanol saturated with sodium acetate, absolute ethanol, ethanol:ether (3:1), heated 1 hr at 37°C in 4 ml of 0.5*N* sodium hydroxide, and afterwards 3 ml of 30% thichloroacetic acid (TCA) was added. The precipitate was washed with 5 ml of 5% TCA and heated in 5% TCA for 20 min on a boiling water bath. The pellet was washed with 5 ml of 5% TCA and dissolved in 10 ml of 0.3*N* sodium hydroxide. Radioactivity and protein (*14*) were determined on this fraction, and the results were expressed as DPM/mg protein.

Mortality data were evaluated in terms of the concentration of VDC required to kill 50% of the animals (LC_{50}) (*15*) and the time required to kill 50% of the animals at 20 ppm of VDC (LT_{50}) (*16*). Other data were analyzed by the two-sample rank test (*17*) with a level of significance selected as $p<0.05$. Data are reported as the means ± SE.

Results

VDC was more toxic in male mice than male rats both in terms of hepatotoxicity, as measured by SGOT and SGPT, and lethality (Table 1). In addition, male mice were more sensitive to the lethal effects of VDC than females, since at the end of 3 and 7 days exposure to 40 ppm VDC 5/10 and 7/10 males were dead, respectively, while no females were dead. Exposed mice had a reduced feed consumption (Table 2), weight loss, rough coats, and were lethargic. In addition, the body temperature of debilitated mice was reduced by 5 to 7°C.

Table 1. Toxicity of 60 ppm VDC in male mice and rats.

Species	Days exposed	SGOT, IU/l.[a]	SGPT, IU/l.[a]	Ratio dead/ exposed
Mouse	1	1,946 ± 270	3,045 ± 209	2/10
	2	751 ± 150	1,112 ± 226	8/10
Rat	1	74 ± 6	44 ± 7	0/10
	2	263 ± 33	198 ± 29	0/10

[a]Mean ± SE for 2 to 5 determinations.

Table 2. Feed consumption in male mice exposed to VDC.

Treatment	Feed Consumption, g/mouse/day[a]		
	VDC=0	VDC=41 ppm	VDC=80 ppm
None	4.4 ± 0.1	1.5 ± 0.3	1.7 ± 0.1
Cysteine (0.1%)	4.6 ± 0.2	1.8 ± 0.6	1.8 ± 0.3
Methionine (0.1%)	4.8 ± 0.1	1.5 ± 0.1	1.8 ± 0.1
Disulfiram (0.1%)	4.2 ± 0.1	3.3 ± 0.3	2.8 ± 0.1

[a]Determined on a cage basis for a total of 10 mice, housed 5 mice/cage.

The effects of various treatments on the LC_{50} value of VDC determined at the end of 1 and 2 days exposure are presented in Tables 3 and 4, respectively. The only compounds that dramatically altered the toxicity of VDC were disulfiram, diethyldithiocarbamate (DDC), and thiram. Although the exposure lasted for 7 days, the pattern of deaths did not permit a calculation of the LC_{50} value for each treatment on the same day. If the treatments were evaluated in terms of the LT_{50} value (Table 5), then the 0.50% methionine and cysteine diets also would provide a degree of protection. The hepatic nonprotein sulfhydryl concentration was not increased in control mice that received various treat-

Table 3. One-day LC_{50} of VDC in mice.

Treatment	LC_{50} ppm[a] Male	Female
Control	98 (82-118)	105 (92-121)
Disulfiram (0.10%)	>320	>320
Cysteine (0.10%)	98 (76-127)	92 (74-113)
Methionine (0.10%)	113 (93-138)	113 (93-138)
$CoCl_2$	113 (81-157)	123 (85-179)

[a]LC_{50} in ppm (95% confidence limits) or approximation of LC_{50}.

Table 4. Two-day LC_{50} of VDC in male mice.

Treatment	LC_{50}, ppm[a]
Control	35 (25–47)
Disulfiram (0.10%)	> 160
DDC (0.12%)	> 160
Thiram (0.10%)	> 160
N-Acetylcysteine	20 (7–25)
Methionine (0.50%)	38 (28–51)
Cysteine (0.50%)	43 (31–58)
SKF 525-A	26 (17–35)
Phenoxybenzamine	35 (25–47)
Propranolol	28 (19–37)
Vitamin C	35 (25–46)
Vitamin E	35 (25–47)

[a]LC_{50} in ppm (95% confidence limits) or approximation of LC_{50}.

ments for a total of 10 days (Table 6).

Disulfiram protected male mice from the hepatotoxic effects of 60 ppm VDC as measured in terms of SGOT and SGPT values (Table 7). Protection was observed after 1 day exposure. However, after 2 days exposure there was no evidence of protection, as measured in terms of these enzymes. At the end of the second day of exposure to 60 ppm VDC there were 8/10 dead in the group receiving the control diet and 2/10 dead in the group receiving the disulfiram diet.

Table 5. LT_{50} at 20 ppm of VDC for male mice.

Treatment	LT_{50}, days[a]
Control	4.0 (3.6–4.4)
Disulfiram (0.10%)	>7
DDC (0.12%)	>7
Thiram (0.10%)	>7
Cysteine (0.50%)	>7
Methionine (0.50%)	~7
N-Acetylcysteine	1-2
SKF 525-A	2.4 (1.7–3.4)
Phenoxybenzamine	3.4
Propranolol	2.7 (2.0–3.7)
Vitamin C	2.6 (1.9–3.5)
Vitamin E	3.4

[a]LT_{50} in days (95% confidence limits) or approximation of LT_{50}.

The interaction of disulfiram and VDC was also evaluated in terms of covalent bound radioactivity after the intraperitoneal administration of ^{14}C-VDC to male mice (Table 8). Covalent bound radioactivity was detected in the liver and kidney in control mice at 4 and 24 hr after ^{14}C-VDC. Disulfiram treatment reduced these values in both tissues at both times.

Table 6. Hepatic nonprotein sulfhydryl concentration in male mice.

Treatment	Concentration, % of control[a]
None	100 ± 3
N-Acetylcysteine	91 ± 2
Methionine (0.50%)	87 ± 1
Cysteine (0.50%)	84 ± 2
Disulfiram (0.10%)	102 ± 2
DDC (0.12%)	100 ± 3
Thiram (0.10%)	95 ± 2

[a]Mean ± SE for 4 determinations.

Table 7. Toxicity of 60 ppm VDC in control and disulfiram-treated male mice.

Diet	Days Exposed to VDC	SGOT, IU/l.[a]	SGPT, IU/l.[a]
Control	1	1,946 ± 270	3,045 ± 209
	2	751 ± 150	1,112 ± 226
Disulfiram	1	140 ± 38	66 ± 11
	2	784 ± 332	1,236 ± 668

[a]Mean ± SE for 2 to 5 determinations.

Table 8. Covalently bound radioactivity after ^{14}C-VDC in control and disulfiram-treated male mice.

Tissue	Time after ^{14}C-VDC, hr	DPM/mg protein[a] Control	Disulfiram
Liver	4	88 ± 14	31 ± 7
	24	58 ± 2	23 ± 5
Kidney	4	134 ± 9	32 ± 7
	24	88 ± 2	27 ± 4

[a]Mean ± SE for 4 determinations.

Discussion

The results of this study demonstrate that (1) mice are more sensitive than rats to the lethal and hepatotoxic effects of VDC, (2) disulfiram reduces the acute lethal and hepatotoxic effects of inhaled VDC and reduces the levels of covalent bound radioactivity in the liver and kidney after ^{14}C-VDC (3) diethyldithiocarbamate, thiram and, to a lesser extent, methionine and cysteine also protect mice from the lethal effects of VDC.

Disulfiram, diethyldithiocarbamate, and thiram are structurally related dithiocarbamates. Disulfiram is used clinically in alcohol therapy programs

and thiram is used both in the agricultural and rubber industry. Disulfiram is metabolized to diethyldithiocarbamate (*18*), and both compounds alter the metabolism of xenobiotics (*19*) and protect against several types of drug-induced toxicities (*20, 21*). In addition, members of the dithiocarbamate class have radioprotective properties (*22*).

Although the mechanisms by which the dithiocarbamates tested protected against VDC toxicity is uncertain, speculations may be offered concerning such mechanisms. For these speculations, it was assumed that VDC was metabolized by the hepatic mixed-function oxidase system to a compound that produced hepatotoxicity which resulted in death. If disulfiram reduced the metabolic activation of VDC, then SKF 525-A (*23*) and cobaltous chloride (*24*) should have provided protection. If treatments protected by providing additional sulfhydryl groups for the detoxification of VDC epoxides then doses of *N*-acetylcysteine that protected mice from acetaminophen toxicity (*25*) should also have protected against VDC toxicity. Although cysteine and methionine provided a degree of protection the effect was not as dramatic as with the dithiocarbamates. The failure of compounds to alter the hepatic nonprotein sulfhydryl concentration may be due to (a) pharmacokinetic properties of the compounds and/or (b) adaptation of the liver to an increased supply of sulfhydryl containing compounds.

The results suggest that disulfiram protects against toxicity by a mechanism that involves more than an inhibition of VDC activation or an increase in VDC detoxification. Possibly, disulfiram is superior to the other nondithiocarbamate compounds because both mechanisms are operating simultaneously. In other words, disulfiram and its metabolites may not only reduce the activation of VDC but also increase the extent of detoxification. In this regard, dithiocarbamates may serve as more effective molecules for detoxifying VDC metabolites than some of the sulfhydryl containing compounds that were tested.

We would like to acknowledge the helpful discussions with Dr. Frederick Sperling, Department of Pharmacology, Howard University Medical School, Washington, D.C. and the competent technical assistance of Brett Ferguson and Timothy Unger. These studies were supported by EPA contract No. 68-01-3242.

REFERENCES

1. Haley, T. J. Vinylidene chloride: a review of the literature. Clin. Toxicol. 8: 633 (1975).
2. Huffman, R. D., and Desai-Greenaway, P. Health and environmental impacts: task 1, vinylidene chloride. U.S. Department of Commerce, National Technical Information Service, Washington, D.C., 1976.
3. Prendergast, J. A., et al. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethylene. Toxicol. Appl. Pharmacol. 10: 270 (1967).
4. Jaeger, R. J., Trabulus, M. J., and Murphy, S. D. Biochemical effects of 1,1-dichloroethylene in rats: dissociation of its hepatotoxicity from a lipoperoxidative mechanism. Toxicol. Appl. Pharmacol. 24: 457 (1973).
5. Jaeger, R. J., Trabulus, M. J., and Murphy, S. D. The interaction of adrenalectomy, partial adrenal replacement therapy, and starvation with hepatotoxicity, and lethality after 1,1-dichloroethylene intoxication. Toxicol. Appl. Pharmacol. 24: abstr. no. 133 (1973).
6. Jaeger, R. J., Conolly, R. B., and Murphy, S. D. Diurnal variations of hepatic glutathione concentrations and its correlation with 1,1-dichloroethylene inhalation toxicity in rats. Res. Commun. Chem. Pathol. Pharmacol. 6: 465 (1973).
7. Jaeger, R. J., Conolly, R. B., and Murphy, S. D. Effect of 18 hr fast and glutathione depletion of 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. Exp. Mol. Pathol. 20: 187 (1974).
8. Jaeger, R. J., et al. Biochemical toxicology of unsaturated halogen monomers. Environ. Health Perspect. 11: 121 (1975).
9. McKenna, M. J., et al. The fate of [^{14}C] vinylidene chloride following inhalation exposure and oral administration in rats. Paper presented at 16th Annual Meeting, Society of Toxicology, Toronto, Canada, 1977; Toxicol. Appl. Pharmacol. 41: abst. 206 (1977).
10. Siletchnik, L. M., and Carlson, G. P. Cardiac sensitizing effects of 1,1-dichloroethylene: enhancement by phenobarbital treatment. Arch. Int. Pharmacodyn. 210: 359 (1974).
11. Amador, E., and Wacker, W. E. C. Serum glutamic-oxaloacetic transaminase activity: a new modification and an analytical assessment of current assay techniques. Clin. Chem. 8: 343 (1962).
12. Henry, R. J., et al. Revised spectrophotometric methods for the determination of glutamic oxaloacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase. Amer. J. Clin. Path. 34: 381 (1960).
13. Sedlak, J., and Lindsay, R. H. Estimation of total, protein bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25: 192 (1968).
14. Lowry, O. H., et al. Protein measurements with folin phenol reagent. J. Biol. Chem. 193: 265 (1951).
15. Weil, C. S. Tables for convenient calculations of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. Biometrics 8: 249 (1952).
16. Litchfield, J. T. A method for rapid graphic solutions of time-percent effect curves. J. Pharmacol. Exptl. Therap. 97: 399 (1949).
17. Goldstein, A. Biostatistics: An Introductory Text. MacMillan, New York, 1969.
18. Stromme, J. H. Metabolism of disulfiram and diethyldithiocarbamate in rats with demonstration of an *in vivo* ethanol-induced inhibition of the glucuronic acid conjugation of the thiol. Biochem. Pharmacol. 14: 393 (1965).
19. Zemaitis, M. A., and Green, F. E. Impairment of hepatic microsomal drug metabolism in the rat during daily disulfiram administration. Biochem. Pharmacol. 25: 1355 (1976).
20. Lutz, L. M., Glende, E. A., and Recknagel, R. O. Protection by diethyldithiocarbamate against carbon tetrachloride lethality on rats and against carbon tetrachloride-induced lipid peroxidation *in vitro*. Biochem. Pharmacol. 22: 1729 (1973).
21. Wattenberg, L. W. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulfur-

containing compounds. J. Nat. Cancer Inst. 52: 1583 (1974).
22. Barnes, J. H., et al. Synthèse et effects radioprotecteurs d'alkanebisdithiocarbamates disodiques, d'acides ω-aminoalkyldithiocarbamiques et de leurs derivés *N,N'*-diméthylés. Eur. J. Med. Chem-Chim. Ther. 10: 619 (1975).
23. Conney, A. H., et al. Enzyme induction and inhibition in studies on the pharmacological actions of acetophenetidin. J. Pharmacol. Exptl. Therap. 151: 133 (1966).
24. Tephly, T. R., and Hibbeln, P. The effect of cobalt chloride administration on the synthesis of hepatic microsomal cytochrome P-450. Biochem. Biophys. Res. Commun. 42: 589 (1971).
25. Piperno, E., and Berssenbruegge, D. A. Reversal of experimental paracetamol toxicosis with *N*-acetylcysteine. Lancet 2: 738 (1976).