TOXICITY OF VINYLIDENE CHLORIDE IN MICE AND RATS AND ITS ALTERATION BY VARIOUS TREATMENTS

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The toxicity of vinylidene chloride (VDC) was studied in [redacted] exposed to [redacted] addition, the ability of various compounds to alter parameters of toxicity was evaluated. Mice were more sensitive than rats to the lethal, hepatotoxic, and renal toxic effects of VDC. Disulfiram protected mice from these toxic effects of inhaled VDC and reduced the levels of covalently bound radioactivity in the liver and kidney after the ip administration of [^{14}C]VDC. Diethyldithiocarbamate and thiram also protected mice from the acute lethal effects of VDC.

INTRODUCTION

Vinylidene chloride (1,1-dichloroethylene; VDC) is used as a monomer in the production of plastics and an intermediate in the synthesis of other chemicals. The toxicity of VDC has been reviewed (Haley, 1975; Huffman and Desai-Greenaway, 1976).

A continuous 90 day inhalation of 189 mg/m^3 (48 ppm) of VDC produced deaths in monkeys and guinea pigs but not in dogs and rats (Prendergast et al., 1967). Morphological changes occurred in livers from monkeys, dogs, and rats and in kidneys from rats. The hepatic changes included focal necrosis, hemosiderin deposition, and fatty metamorphosis. The primary renal lesion was nuclear hypertrophy of the tubular epithelium.

Various parameters influenced the toxicity of VDC. Female rats were

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more sensitive than males to the toxic effects of VDC, which was administered by the oral route (Jaeger et al., 1973b). Nutritional status affected toxicity, since starved rats were more sensitive to VDC than fed rats (Jaeger et al., 1973c). This nutritional effect and a diurnal change in sensitivity to VDC (Jaeger et al., 1973a) were correlated with hepatic levels of reduced glutathione (GSH). Additional studies with diethylmaleate (Jaeger et al., 1974) and cysteine (Jaeger et al., 1975) pretreatment also indicated that hepatic levels of glutathione influenced the toxicity of VDC. Conjugation with GSH was a major pathway for the detoxification of VDC and hepatotoxicity was associated with the covalent binding of reactive metabolites (McKenna et al., 1977).

The purpose of this study was to evaluate the acute toxicity of continuously inhaled VDC in both mice and rats and to evaluate the effect of various treatments on toxicity. The treatments were selected to alter the metabolic activation or promote the detoxification of VDC. In addition, adrenergic blocking agents were used since VDC exposure sensitized rat hearts to catacholamines (Siletchnik and Carlson, 1974).

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 at all times during the study. [redacted] Control animals were similarly housed and exposed to room air.

VDC was obtained from [redacted], Milwaukee, Wisconsin, [redacted] monomethyl [redacted] No further analysis was performed on the VDC. [redacted] A stream of air carried the vapors from the flask to the chamber. The chamber atmosphere was sampled from a central point. Preliminary experiments, utilizing at least eight additional sampling points, indicated that there was a uniform distribution of VDC within 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. These determinations were made on the average every 2-3 hr during exposure.

Mice received one of several compounds in an effort to alter the toxicity of VDC. These compounds were disulfiram (0.10% in feed 2-3 days before and during exposure), diethyldithiocarbamate (DDC) (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 days before and during exposure), *N*-acetylcysteine (1,200 mg/kg po every day

during exposure), SKF 525-A (50 mg/kg ip every day during exposure), cobaltous chloride ($CoCl_2 \cdot 6H_2O$) (60 mg/kg ip daily for 2 days before 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 2 days before exposure and on first day of exposure). Control and treated mice were housed in the same chambers and, consequently, exposed to identical concentrations of VDC.

[redacted] Organ damage was assessed in terms of serum enzymes and histopathological changes in the liver and kidney. Serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) were determined in cardiac blood from mice and aortic blood from rats by the methods described by Amador and Wacker (1962) and Henry et al. (1960), respectively.

Covalently bound radioactivity was measured in male mice at 4 and 24 hr after the ip administration of 3 mg/kg (20 μCi/kg) [^{14}C]VDC (New England Nuclear, Boston, Massachusetts) with a specific activity of 0.652 mCi/mmol. The tissues were homogenized in cold water and an equal volume of 1 *N* perchloric acid (PCA) was added. The precipitate was washed with successive 5 ml portions of 0.2 *N* PCA, 0.2 *N* PCA, 95% ethanol saturated with sodium acetate, absolute ethanol, and ethanol and ether (3:1) and then heated for 1 hr at 37°C in 4 ml 0.5 *N* sodium hydroxide. Afterward, 3 ml of 30% trichloroacetic acid (TCA) was added and the precipitate was washed with 5 ml 5% TCA and heated in 5% TCA for 20 min on a boiling-water bath. The pellet was washed with 5 ml 5% TCA and dissolved in 10 ml 0.3 *N* sodium hydroxide. Radioactivity and protein (Lowry et al., 1951) were determined on this fraction and the results were expressed as dpm/mg protein.

Mortality data were evaluated in terms of the LC_{50} (Weil, 1952) and the LT_{50} (Litchfield, 1949) for VDC. Other data were analyzed by the two-sample rank test (Goldstein, 1969) or the Fisher exact probability test (Siegel, 1956). The level of significance selected was $p < 0.05$.

RESULTS

Rats and mice had reduced feed consumption and weight loss during exposure to VDC. After 2 days of exposure to 60 ppm VDC, 8 of 10 male mice and 0 of 10 male rats died. In addition, VDC produced more organ damage, as measured by elevated serum enzymes, in mice than in rats (Tables 1 and 2). A dose-related increase in both SGOT and SGPT occurred in male mice exposed to VDC for 1 day (Table 1). As the duration of exposure increased, these values tended to decline. The serum enzymes were elevated in male rats exposed to VDC; however, the increase was not as dramatic as that in mice and required a longer exposure period

TABLE 1. Effect of VDC Exposure on Serum Enzymes in Male Mice

Days exposed	Serum enzyme	VDC concentration (ppm) 0	15	30	60
1	SGOT	56 ± 8 (5)[a]	249 ± 28 (4)	466 ± 50 (5)	1,946 ± 271 (4)
	SGPT	32 ± 6 (5)	200 ± 19 (4)	474 ± 66 (5)	3,043 ± 209 (4)
2	SGOT	82 ± 30 (4)	91 ± 5 (5)	705 ± 120 (6)	751 ± 150 (2)
	SGPT	38 ± 4 (4)	189 ± 26 (5)	675 ± 81 (6)	1,112 ± 226 (2)
3	SGOT	64 ± 14 (5)	138 ± 16 (4)	448 ± 179 (4)	No survivors
	SGPT	29 ± 2 (5)	166 ± 33 (4)	514 ± 200 (4)	
5	SGOT	60 ± 5 (5)	118 ± 18 (6)	297 ± 50 (2)	No survivors
	SGPT	32 ± 2 (5)	47 ± 5 (6)	109 ± 8 (2)	

[a]Mean ± SE (number of mice).

(Table 2). Toxicity, as revealed by these biochemical indicators, was confirmed by histopathological observations. Hepatic and renal lesions were present in male mice exposed to 15, 30, and 60 ppm of VDC for various intervals (Table 3). In addition, hepatic but not renal lesions were observed in male rats similarly exposed to 60 ppm of VDC (Table 4). None of the lesions observed in mice and rats exposed to VDC occurred in control animals exposed to room air.

Groups of mice were given one of several compounds in order to investigate their effects on the toxicity of VDC. Among the various compounds tested, disulfiram, DDC, and thiram decreased the acute lethality of VDC (Tables 5 and 6). Disulfiram, in addition, protected male mice from the hepatotoxic effects of VDC as measured by serum enzymes (Table 7). However, this protective effect was evident after the first, but not the second or third, exposure day. Histopathological confirmation of this protective effect was complicated by the high mortality in nontreated animals exposed to VDC. However, the data indicated that disulfiram

TABLE 2. Effect of VDC Exposure on Serum Enzymes in Male Rats

Days exposed	Serum enzymes	VDC concentration (ppm) 0	60
1	SGOT	66 ± 10 (5)[a]	74 ± 6 (5)
	SGPT	28 ± 2 (5)	44 ± 7 (5)
2	SGOT	63 ± 4 (5)	264 ± 33 (5)
	SGPT	34 ± 4 (5)	198 ± 29 (5)
3	SGOT	81 ± 4 (5)	238 ± 47 (5)
	SGPT	34 ± 6 (5)	122 ± 29 (5)

[a]Mean ± SE (number of rats).

TABLE 3. Microscopic Lesions in Organs of Male Mice Exposed to VDC

VDC:	15 ppm				30 ppm				60 ppm	
Exposure days:	1	2	3	5	1	2	3	5	1	2
Number of mice examined	5	5	5	7	5	5	4	2	4	2
Liver lesions										
Midzonal necrosis										
Mild	0[a]	0	0	0	20	40	50	0	0	50
Moderate	0	0	0	0	0	0	0	0	50	50
Marked	0	0	0	0	0	0	0	0	50	0
Mitotic figures of hepatocytes										
Slight increase	0	60	40	0	0	0	0	0	0	0
Moderate increase	0	0	0	0	0	0	25	0	0	0
Hepatocellular degeneration	20	40	20	86[b]	20	40	0	50	25	0
Kidney lesions										
Tubular nephrosis										
Moderate	0	0	0	57	0	0	0	0	0	0
Marked	0	0	0	43	0	0	0	0	0	0
Severe	100[b]	100[b]	100[b]	0	100[b]	100[b]	100[b]	100[b]	50	100[b]
Tubular regeneration	0	0	0	86[b]	0	0	0	0	0	0

[a] Percentage of mice examined with the indicated lesion. None of these lesions were observed in the control group (five mice a day) exposed to room air.
[b] Significantly different from control group (Fisher exact probability t

protected mice from the hepatic and renal toxicity of VDC (Table 8). Additional evidence supporting a protective effect of disulfiram was obtained in covalent binding studies (Table 9). Radioactivity, which was covalently bound to proteins, was measured in the liver and kidney at 4 and 24 hr after the ip administration of [^{14}C]VDC. These levels of radioactivity were reduced at both times in mice treated with disulfiram.

TABLE 4. Microscopic Lesions in Organs of Male Rats Exposed to 60 ppm VDC

	Exposure days		
	1	2	3
Number of rats examined	5		
Liver lesions			
Centrilobular degeneration and/or necrosis			
Mild	40[a]	80[b]	40
Moderate	0	0	60
Mild hyperplasia of bile duct	60	40	0

[a] Percentage of rats examined with the indicated lesion. None of these lesions were observed in the control group (five mice a day) exposed to room air.
[b] Significantly different from control group (Fisher exact probability test).

TABLE 5. One Day LC_{50} (ppm) of VDC in Mice

Treatment	Male	Female
Control	98 (82–118)[a]	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 6. Two Day LC_{50} (ppm) of VDC in Mice

Treatment	Male
Control	35 (25–47)[a]
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}.

TABLE 7. Toxicity of 60 ppm VDC in Control and Disulfiram-Treated Male Mice

Diet	Days exposed to VDC	SGOT (IU/liter)	SGPT (IU/liter)
Control	1	1,946 ± 271 (4)[a]	3,043 ± 209 (4)
	2	751 ± 150 (2)	1,112 ± 226 (2)
	3	No survivors	No survivors
Disulfiram	1	140 ± 38 (5)[b]	66 ± 11 (5)[b]
	2	784 ± 332 (4)	1,236 ± 668 (4)
	3	2,292 ± 965 (3)	3,182 ± 1,488 (3)

[a] Mean ± SE (number of observations).
[b] Significantly different from control (two-sample rank test).

TABLE 8. Microscopic Lesions in Organs of Male Mice Exposed to 60 ppm VDC and Treated with Disulfiram

Treatment:	None		Disulfiram[a]		
Exposure days:	1	2	1	2	3
Number of mice examined	4	2	5	5	3
Liver lesions					
Midzonal necrosis					
Mild	0[b]	50	0	0	0
Moderate	50	50	0	0	0
Marked	50	0	0	60	0
Severe	0	0	0	0	67
Hepatocellular degeneration	25	0	0	20	33
Kidney lesions					
Tubular nephrosis					
Mild	0	0	20	40	0
Moderate	0	0	20	20	0
Marked	50	0	0	40	100
Severe	50	100	0	0	0

[a]Disulfiram (0.1%) in feed 3 days before and during exposure to VDC.
[b]Percentage of mice examined with the indicated lesion. None of these lesions were observed in groups (five mice a day) fed either control or disulfiram diets and exposed to room air.

DISCUSSION

Mice are more sensitive than rats to the lethal, hepatotoxic, and renal toxic effects of VDC. Disulfiram reduces the severity of these effects in mice. In addition, DDC and thiram protect mice from the lethal effects of VDC. The compounds that protected against the toxicity of VDC are dithiocarbamates. Disulfiram is used clinically in alcohol therapy programs and thiram is used in the agricultural and rubber industries. Disulfiram is metabolized to DDC (Stromme, 1965) and both compounds alter the metabolism of xenobiotics (Zemaitis and Green, 1976). These compounds,

TABLE 9. Covalently Bound Radioactivity after [^{14}C]VDC in Control and Disulfiram-Treated Male Mice

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

[a]Mean ± SE for four determinations.
[b]Significantly different from control (two-sample rank test).

in addition, protect against several types of drug-induced toxicities (Lutz et al., 1973; Wattenberg, 1974). Dithiocarbamates also have radioprotective properties (Barnes et al., 1975).

Speculations concerning the toxicity of vinyl chloride (Watanabe and Gehring, 1976) form a basis for similar speculations concerning the toxicity of VDC. VDC is metabolically activated to a reactive metabolite that is detoxified by combining with GSH (McKenna et al., 1977). As the levels of GSH are depleted, the reactive metabolites are able to combine with macromolecules and produce toxicity or carcinogenicity. This proposal is supported, in the present study, by the presence of covalently bound ratioactivity in the liver and kidney after [^{14}C]VDC.

There are a variety of mechanisms by which the dithiocarbamate compounds tested could reduce the toxicity of VDC. These mechanisms include a decrease in metabolic activation and an increase in detoxification. If protection occurred as a result of reduced activation by the hepatic microsomal mixed-function oxidase system, then SKF 525-A (Conney et al., 1966) and cobaltous chloride (Tephly and Hibbeln, 1971) should have produced similar effects. If protection resulted from an increase in sulfhydryl groups for the detoxification of VDC metabolites, then *N*-acetylcysteine (Piperno and Berssenbruegge, 1976) and other sulfhydryl-containing compounds should also have protected. Since these interaction studies are complicated by dosage and pharmacokinetic considerations, it is possible that we failed to detect an interaction because of these factors. In addition, since death and organ damage may not be related, it is possible that compounds that failed to protect against death provided protection against organ damage.

The results suggested that the mechanism of protection provided by the dithiocarbamates involves more than an inhibition of activation or an increase in detoxification of VDC. Possibly both mechanisms are operating at the same time. For example, these compounds may have reduced the activation of VDC and increased the detoxification of reactive metabolites. In this regard, dithiocarbamates may serve as more effective molecules for detoxifying VDC metabolites than some of the sulfhydryl-containing compounds that were tested.

REFERENCES

Amador, E. and Wacker, W. D. C. 1962. Serum glutamic-oxaloacetic transaminase activity: A new modification and an analytical assessment of current assay techniques. *Clin. Chem.* 8:343–348.

Barnes, J. H., Fatome, M., Esslemont, G. F., Andriev, L. and Bargy, E. 1975. Syntheses et effets radioprotecteurs d'alkanebisdithiocarbamates disodiques, d'acides ω-aminoalkyldithiocarbamiques et de leurs derives N,N'-dimethyles. *Eur. J. Med. Chem. Chim. Ther.* 10:619–622.

Conney, A. H., Sansur, M., Soroko, F., Koster, R. and Burns, J. J. 1966. Enzyme induction and inhibition in studies on the pharmacological actions of acetophenetidin. *J. Pharmacol. Exp. Ther.* 141:133–138.

Goldstein, A. 1969. *Biostatistics, an introductory text.* New York: Macmillan.

Haley, T. J. 1975. Vinylidene chloride: A review of the literature. *Clin. Toxicol.* 8:633–643.

Henry, R. J., Chiamori, N., Golub, O. J. and Berkman, S. 1960. Revised spectrophotometric methods for the determination of glutamic oxaloacetic transaminase, glutamic-pyruvic transaminase, and lactic dehydrogenase. *Am. J. Clin. Pathol.* 34:381–387.

Huffman, R. D. and Desai-Greenaway, P. 1976. *Health and environmental impacts. Task 1, vinylidene chloride.* Springfield, Va.: National Technical Information Service, PB-258 855

Jaeger, R. J., Conolly, R. B. and Murphy, S. D. 1973a. Diurnal variation of hepatic glutathione concentrations and its correlation with 1,1-dichloroethylene inhalation toxicity in rats. *Res. Commun. Chem. Pathol. Pharmacol.* 6:465–471.

Jaeger, R. J., Trabulus, M. J. and Murphy, S. D. 1973b. Biochemical effects of 1,1-dichloroethylene in rats: Dissociation of its hepatotoxicity from a lipoperoxidative mechanism. *Toxicol. Appl. Pharmacol.* 24:457–467.

Jaeger, R. J., Trabulus, M. J. and Murphy, S. D. 1973c. The interaction of adrenalectomy, partial adrenal replacement therapy, and starvation with hepatotoxicity, and lethality after 1,1-dichloroethylene intoxication. *Toxicol. Appl. Pharmacol.* 24:abstr. 133.

Jaeger, R. J., Conolly, R. B. and Murphy, S. D. 1974. Effect of 18 hr fast and glutathione depletion on 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. *Exp. Mol. Pathol.* 20:187–190.

Jaeger, R. J., Conolly, R. B., Reynolds, E. S. and Murphy, S. D. 1975. Biochemical toxicology of unsaturated halogen monomers. *Environ. Health Perspect.* 11:121–128.

Litchfield, J. T. 1949. A method for rapid graphic solutions of time-percent effect curves. *J. Pharmacol. Exp. Ther.* 97:399–408.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurements with Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Lutz, L. M., Glende, E. A. and Recknagel, R. O. 1973. Protection by diethyldithiocarbamate against carbon tetrachloride lethality in rats and against carbon tetrachloride-induced lipid peroxidation in vitro. *Biochem. Pharmacol.* 22:1729–1734.

McKenna, M. J., Zempel, J. A., Madrid, E. O. and Gehring, R. J. 1977. The fate of [^{14}C]vinylidene chloride following inhalation exposure and oral administration in rats. Presented at the 16th Annual Meeting of the Society of Toxicology, Toronto, Canada.

Piperno, E. and Berssenbruegge, D. A. 1976. Reversal of experimental paracetamol toxicosis with N-acetylcysteine. *Lancet* 2:738–739.

Prendergast, J. A., Jones, R. A., Jenkins, L. J. and Siegel, J. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethylene. *Toxicol. Appl. Pharmacol.* 10:270–289.

Siegel, S. 1956. *Nonparametric statistics.* New York: McGraw-Hill.

Siletchnik, L. M. and Carlson, G. P. 1974. Cardiac sensitizing effects of 1,1-dichloroethylene: Enhancement by phenobarbital treatment. *Arch. Int. Pharmacodyn. Ther.* 210:359–364.

Stromme, J. H. 1965. 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.* 14:393–410.

Tephly, T. R. and Hibbeln, P. 1971. The effect of cobalt chloride administration on the synthesis of hepatic microsomal cytochrome P-450. *Biochem. Biophys. Res. Commun.* 42:489–595.

Watanabe, P. G. and Gehring, P. J. 1976. Dose-dependent fate of vinyl chloride and its possible relationship to oncogenicity in rats. *Environ. Health Perspect.* 17:145–152.

Wattenberg, L. W. 1974. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by several sulfur-containing compounds. *J. Natl. Cancer Inst.* 52:1583–1587.

Weil, C. S. 1952. Tables for convenient calculations of medium-effective dose (LD_{50} or ED_{50}) and instructions in their use. *Biometrics* 8:249–263.

Zemaitis, M. A. and Green, F. F. 1976. Impairment of hepatic microsomal drug metabolism in the rat during daily disulfiram administration. *Biochem. Pharmacol.* 25:1355–1360.

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