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Preliminary Studies on the Fate of Inhaled Vinyl Chloride Monomer (VCM) in Rats

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Rats were exposed to vinyl chloride monomer gas (VCM) in a closed recirculating system. The rate at which VCM was removed from the system via metabolism was determined for rats exposed to initial concentrations of VCM ranging from 50 to 1167 ppm. Upon exposure to initial concentrations of 50 to 105 ppm, the rate of metabolism was $8.04 \pm 3.40 \times 10^{-3}$ min^{-1}. Upon exposure to initial concentrations ranging from 220 to 1167 ppm, the rate constants were less; the mean value being $2.65 \pm 1.35 \times 10^{-3}$ min^{-1}. Regardless of concentration, the disappearance followed apparent first order kinetics.

Pretreatment of rats with pyrazole prior to exposure to initial concentrations of 65 and 1234 ppm VCM caused 71 and 87% reductions in the rate of metabolism. Ethanol caused 96% and 83% reductions in the rate of VCM metabolism by rats exposed to 56 and 97 ppm VCM, respectively. Ethanol was less effective in blocking the rate of metabolism by rats exposed to high concentrations of VCM; 46 and 36% in rats exposed to 1025 and 1034 ppm VCM. In rats exposed to an initial concentration of 65 ppm VCM, SKF-525-A administration caused no inhibition of the rate of VCM metabolism; however, a 19% inhibition was seen in rats exposed to 1038 ppm.

The nonprotein sulfhydryl content of the liver (glutathione and cysteine) of rats exposed to VCM concentrations ranging from 50 to 15,000 ppm VCM is reduced without a relationship to dose. With repeated daily exposure the degree of reduction is reduced. Preliminary results indicate that the primary metabolites of VCM react with the nonprotein sulfhydryl. Final metabolic products excreted in the urine appear to be *S*-(2-hydroxyethyl)cysteine and *S*-(2-carboxymethyl)cysteine and the respective *N*-acetyl derivatives. Monochloroacetic acid was identified as another potential metabolite.

Considering the results *in toto*, it is hypothesized that VCM is readily and extensively metabolized. Metabolism via the primary pathway, postulated to involve alcohol dehydrogenase, is swamped by exposures to concentrations exceeding 220 ppm. In rats exposed to concentrations at and exceeding this level, metabolism occurs via a secondary pathway(s), postulated to be epoxidation and/or peroxidation. These results are considered pertinent in assessing the potential hazard at low level exposures to VCM.

Introduction

Vinyl chloride monomer (VCM), extensively used for the production of poly(vinyl chloride) and other plastics, has been associated with the development of angiosarcoma and portal cirrhosis of the liver as well as other untoward effects in workers exposed to unknown but undoubtedly high concentrations of VCM. Angiosarcomas, zymbal gland carcinomas, and nephroblastomas developed in rats exposed to concentrations of VCM ranging from 50 to 10,000 ppm, 4 hr per day, 5 days per week for 12 months, and subsequently maintained and observed until death (*1*).

No information is available on the fate of VCM in the mammalian organism. Such information is essential for elucidating the toxicodynamics of VCM. This would provide a rationale to assess the potential hazard of exposure to low levels of VCM. Results of preliminary studies on the fate of VCM in rats exposed via inhalation are reported herein. Prior to undertaking these studies it was postu-

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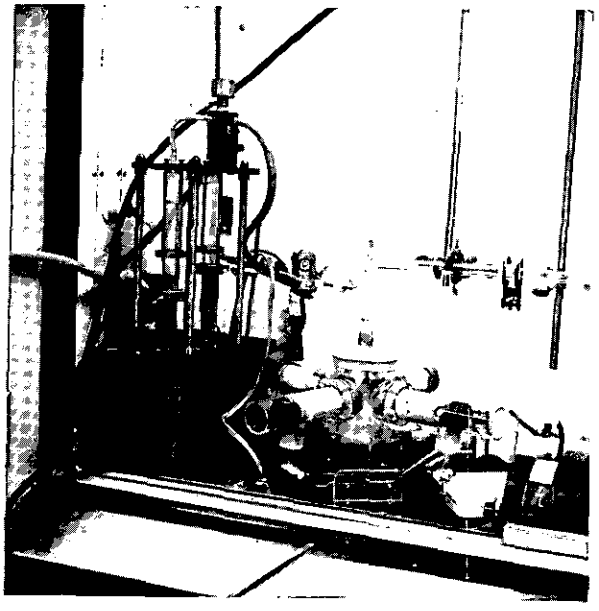


FIGURE 1. Closed recirculating inhalation apparatus used for exposing rats to VCM.

lated that VCM might be metabolized via the alcohol dehydrogenase pathway, and if so, this pathway would very likely be saturable and that conjugation of VCM or its metabolites with glutathione and cysteine might be expected. Therefore, much of the work conducted to date has been directed at evaluating these possibilities.

Methods

Kinetic Studies of the Uptake (Metabolism) of Inhaled Vinyl Chloride Monomer

Male Sprague-Dawley albino rats of Spartan strain weighing from 165 to 200 g were exposed to initial concentrations of VCM gas ranging from 51 to 1167 ppm (0.13 to 2.99 mg/l.). Exposures ranged from 52.5 to 356.3 min. Figure 1 depicts the closed, recirculating 4.7-l. inhalation apparatus in which a group of four rats were concurrently exposed. To minimize contamination of fur and skin, only the nares of the rats protruded through a rubber membrane into the chamber. Expired carbon dioxide was continuously removed from the chamber by absorption on an Ascarite column in the recirculating system. As carbon dioxide was removed from the system, the pressure drop was detected by a mercury manometer fitted with a photoelectric cell. This activated a solenoid valve and dual syringe pump to inject makeup oxygen into the system.

The chamber atmosphere was continuously analyzed for VCM (10.9 μ) by an in-line Miran-I infrared analyzer (Wilks). Using known concentrations of VCM prepared in 100-l. Saran bags, the absorbance versus concentration adhered to the Beer-Lambert Law. During the exposures, routine checks for carbon dioxide in the chamber atmosphere were made at 4.26 μ. Oxygen consumption for each experiment was determined directly by measuring the oxygen metered into the system using a dry test meter (American Meter Co.).

Prior to each exposure, the desired initial concentration of VCM (Matheson Gas Products, Joliet, Ill., 99.9% pure) was generated in the empty recirculating system and the decline in concentration was followed for a time equal to or exceeding that of the intended experiment. The decline in concentration was in accordance with first-order kinetics as described by

$$dC/dt = -K_1C \qquad (1)$$

where C (ppm) is the concentration of VCM at time t (min) and K_1 (min^{-1}) is the rate constant for the decline in VCM concentration from the empty recirculating system. Regression analysis of the logarithm of the concentration of VCM versus time yielded K_1. Initially values for K_1 were determined before and after inclusion of rats in the chamber. These values were reproducible, therefore K_1 was not redetermined after removal of the rats in every subsequent experiment. Between experiments, the system was disassembled, cleaned, and reassembled with inclusion of a new Ascarite column. The K_1 for each reassembled system was unique, within certain limits, which necessitated its redetermination for each experiment.

After determining the rate of decline of VCM concentration in the empty inhalation system, four rats were placed in the chamber. The chamber was then charged with the desired concentration of VCM, and the rate of decline of VCM concentration was determined as previously described. The rate of decline K_e followed apparent first-order kinetics. After an initial equilibration of the tissues of the rats,

the decline of VCM concentration from the closed system was assumed to occur via metabolism of VCM plus the background loss from the system. Since oxygen consumptions were within 10% for all experiments described herein respiratory parameters were not significantly changed. Therefore, respiratory parameters were not a factor influencing the rates of metabolism. The rate of metabolism K_c would therefore be given by eq. (2):

$$K_c = K_e - K_1 \quad (2)$$

To assess the effects of potential inhibitors of VCM metabolism, rats were pretreated by intraperitoneal injection with 320 mg/kg pyrazole (1,2-diazole) 1 hr prior to exposure, 5 ml/kg ethanol 1.5 hr prior to exposure, 75 mg/kg SK&F-525-A (β-diethylaminoethyldiphenylpropyl acetate) 0.5, 0.6, 1.0, or 2.25 hr prior to exposure, or 1000 mg/kg AT (3-amino-1,2,4-triazole) 3 hr prior to exposure. Without cleaning or reassembling the inhalation system, both untreated control rats and rats treated with the chosen potential inhibitor were exposed to the desired concentration of VCM. The inhibition was calculated from eq. (3):

$$\frac{K_c\ (\text{control}) - K_c\ (\text{treated})}{K_c\ (\text{control})} = \%\ \text{inhibition} \quad (3)$$

Effects of VCM on Liver Sulfhydryl Levels

Groups of male Sprague-Dawley albino rats of Spartan strain weighing from 193 to 250 g at initiation of the experiment were exposed to nominal concentrations of 15,000 ppm vinyl chloride for 5 days, 5000 or 500 ppm 5 days per week for 1, 3, or 7 weeks, and 50 ppm for 1 hr, 7 hr, or 5 days. The exposures were carried out in a glass-walled 160-l. chamber under dynamic conditions with the VCM being metered into the chamber airstream. For repeated daily exposures, 7-hr exposures were conducted on the first four of five consecutive days each week. On the fifth day, if the rats were to be sacrificed, the duration of exposure was reduced to 5 to 6 hr; however, if the rats were being continued on exposure, they received a full 7-hr exposure.

The body weights and food consumption of rats exposed to 500 and 5000 ppm VCM were determined before each daily exposure and the rats were observed periodically for signs of toxicity. Between 1 and 2 PM, immediately following the fifth exposure of the designated week, the rats were killed by cervical dislocation and the livers removed and assayed for sulfhydryl content. Gross pathological examinations were conducted.

The method used for the sulhydryl assay was a modification of that described by Sedlak and Lindsay (*2*). Exactly 500 mg of liver from each rat was homogenized for 1 min in a Dounce tissue homogenizer containing 8 ml of 0.02*M* disodium EDTA. For the total sulfhydryl assay, a 0.5-ml aliquot of each homogenate was mixed with 1.5 ml of 0.2*M* Tris HCl pH 9.2 buffer, 0.1 ml of 0.01*M* 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and 7.9 ml of methanol. A reagent blank without liver homogenate, and a sample blank, without DTNB, were also prepared. The color generated via the release of nitromercaptobenzoic acid anion was allowed to develop for 15 min, and the samples were centrifuged for 15 min at 4000*g*. Absorbance of each sample was read against the respective sample blank at 412 nm with a Beckman DB spectrophotometer. Subsequently, the molar concentration of total sulfhydryl in the sample was calculated using an extinction coefficient determined from standards of known concentrations of glutathione or cysteine. A plot of absorbance versus the concentration of cysteine or glutathione coincided with that reported by Sedlak and Lindsay (*2*).

The nonprotein sulfhydryl content of liver was determined after precipitating out the protein by addition of 1 ml of a 50% solution of trichloroacetic acid in distilled water to a 5 ml sample of liver homogenate. Each sample was diluted with 4 ml of distilled water and after 15 min centrifuged at 4000*g* for 15 min. A 2-ml aliquot of the supernatant was mixed with 4 ml of 0.4*M* Tris HCl, pH 8.9 buffer. Immediately before reading the absorbance against a reagent blank, 0.1 ml of 0.01*M* DTNB was added. Subtraction of the nonprotein sulfhydryl content from the total sulfhydryl content yielded a value for protein-bound sulfhydryl.

Urine samples collected from rats exposed to 5000 ppm vinyl chloride for 4, 5, and 7 weeks were analyzed for the presence of *S*-(2-chloroethyl)cysteine, *S*-(2-hydroxyethyl)-cysteine, and *S*-(carboxymethyl)cysteine (5 and 7 week samples only). Urine was collected for analysis by applying pressure to the pos-

terior abdomen between the fifth and sixth hour of the fifth daily exposure on the designated week. Urine collected on the same day was pooled. For the three aforementioned compounds, 2 to 15 μl of urine were spotted directly on a 5 by 20 cm Baker-Flex silica gel plate. Also spotted were samples or urine collected from control rats and standard aqueous solutions as well as control urine to which approximately 1 μg/μl of each of the compounds had been added. The chromatograms were developed for 5 hr in a sealed glass tank containing *n*-butanol, acetic acid, and water (80:10:10 or 60:20:20). After being air dried, the plates were sprayed with Ninspray ninhydrin reagent and heated for 2 min at 80°C. The color of the spots and their R_f values were used to identify the compounds.

A urine sample collected from rats exposed to 5000 ppm vinyl chloride for 9 weeks was analyzed for the presence of chloroacetic acid. The 10 ml urine sample was acidified with 0.1 ml of 50% (*v/v*) H_2SO_4. Subsequently, the urine sample was extracted three times with 2 ml of diethyl ether. The diethyl ether extract was evaporated to 0.1 ml, and 2–15 μl of the concentrated extract and an aqueous standard containing 5% (w/v) chloroacetic acid were spotted on an Eastman fluorescent silica gel plate. The chromatograms were developed in a sealed glass tank for 5 hr using the aforementioned solvent systems. After air drying the plates, they were examined under ultraviolet light to determine the location of spots. Subsequently, the plates were treated with ninhydrin as described previously.

Urine collected from four rats for 12 hr after exposure to 7855 ppm ^{14}C-VCM (specific activity 0.2 mCi/mmole) for 62 min was pooled and analyzed for metabolites. A 3.0-ml portion of urine was applied to 22.2 by 1.5 cm Dowex 50 (4% crosslinked, sodium form, 200–400 mesh) column and eluted with a 0.01*M* Tris HCl, pH 7.0 to 3.0 gradient. A 10–12 μl portion of each fraction containing ^{14}C activity was spotted on a 5 by 20 cm silica plate. As standards, 2 μg of *S*-(2-hydroxyethyl)cysteine and *S*-(2-carboxymethyl)cysteine were spotted on the plates. The chromatograms were developed in a sealed glass tank containing *n*-propanol and ammonia solution (28%), 70:30. After air drying, the plates were analyzed for ^{14}C activity by using a Panax thin-layer chromatogram radioscanner and liquid scintillation counting techniques. A single minor fraction of ^{14}C activity was analyzed as previously described, however 2 μg of urea was spotted on the plates as a standard. Chromatograms were developed in sealed glass tanks containing *n*-propanol, 28% ammonia solution, 70:30; ethanol, 28% ammonia solution, 70:30; *n*-propanol, acetic acid, 90:10; or *n*-butanol, acetic acid, water, 60:20:20.

Results

Kinetic Studies on the Metabolism of Inhaled VCM

Typical declines in the concentration of VCM in the inhalation apparatus containing four rats and initial concentrations of approximately 50 or 1000 ppm are shown in Figure 2. Also shown are the corresponding declines in the

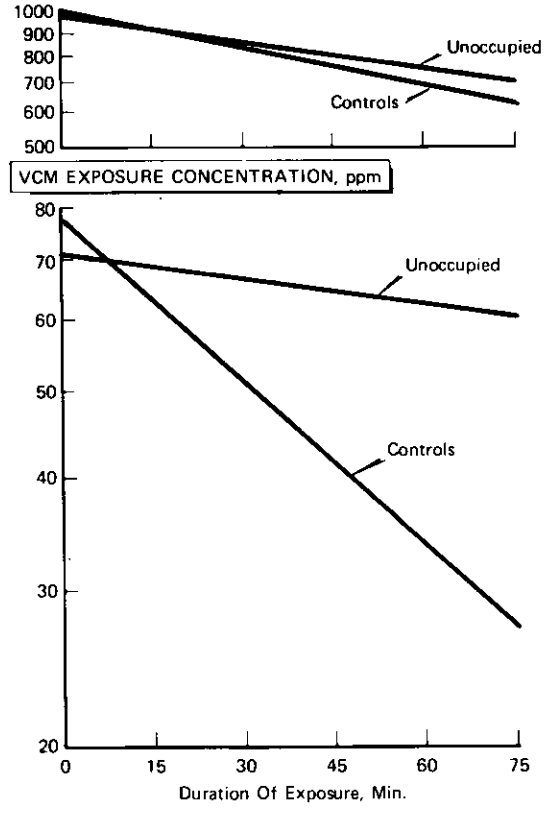


FIGURE 2. Typical declines in VCM concentration with time at approximately 50 and 1000 ppm VCM exposure concentrations. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

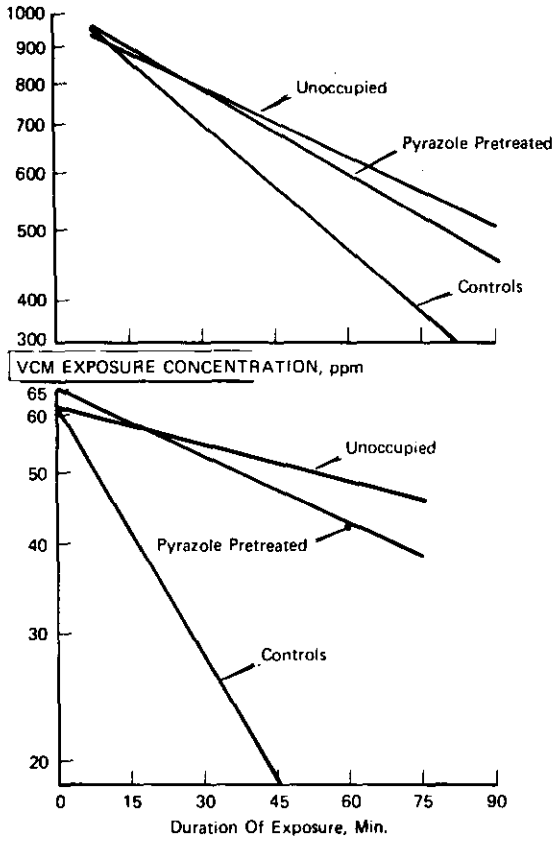


FIGURE 3. Declines in VCM concentration with time at 65 and 1234 ppm VCM exposure concentrations for both pyrazole pretreated and untreated control rats. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

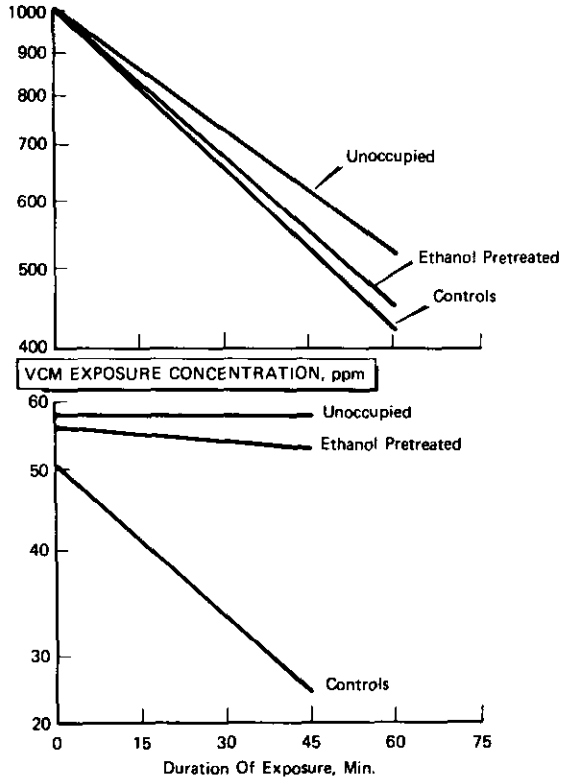


FIGURE 4. Declines in VCM concentration with time at 56 and 1034 ppm VCM exposure concentrations for both ethanol pretreated and untreated control rats. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

concentration of VCM from the unoccupied chamber. As indicated above, the rate of these declines, K_e and K_1, respectively, were determined by regression analysis. The rate of metabolism, K_c, was assumed equal to $K_e - K_1$. For seven separate exposures to concentrations of VCM ranging from 50 to 105 ppm, the mean and standard deviation for the apparent first-order rate constant K_c were $-8.04 \times 10^{-3} \pm 3.40 \times 10^{-3}$ min^{-1}. This corresponds to a half-life $t_{1/2}$ of 86 min.

For five separate exposures to concentrations of VCM ranging from 220 to 1167 ppm, K_c was $-2.65 \times 10^{-3} \pm 1.35 \times 10^{-3}$ min^{-1}. This corresponds to a $t_{1/2}$ of 261 min. As indicated by the standard deviations, there was variation of K_c within the indicated range of concentration, however, there was no consistent trend for the values of K_c within this range.

Administration of 320 mg/kg pyrazole, an inhibitor of alcohol dehydrogenase, xanthine oxidase, and other enzymes (*3*) 1 hr before exposure to 65 and 1234 ppm VCM resulted in 71.2 and 86.9% inhibition of metabolism of VCM, respectively (Fig. 3).

Because of the lack of specificity of pyrazole as an enzyme inhibitor, 5 ml/kg 95% ethanol was administered to rats in an attempt to specifically inhibit alcohol dehydrogenase activity. In rats exposed to initial concentrations of 56 and 97 ppm VCM, ethanol pretreatment caused 96.0 and 82.9% inhibition of VCM metabolism, respectively. When rats were pretreated with ethanol and exposed to initial concentrations of 1025 and 1034 ppm VCM, inhibition of metabolism was 46.5 and 35.7%, respectively. Figure 4 illustrates the inhibition for separate

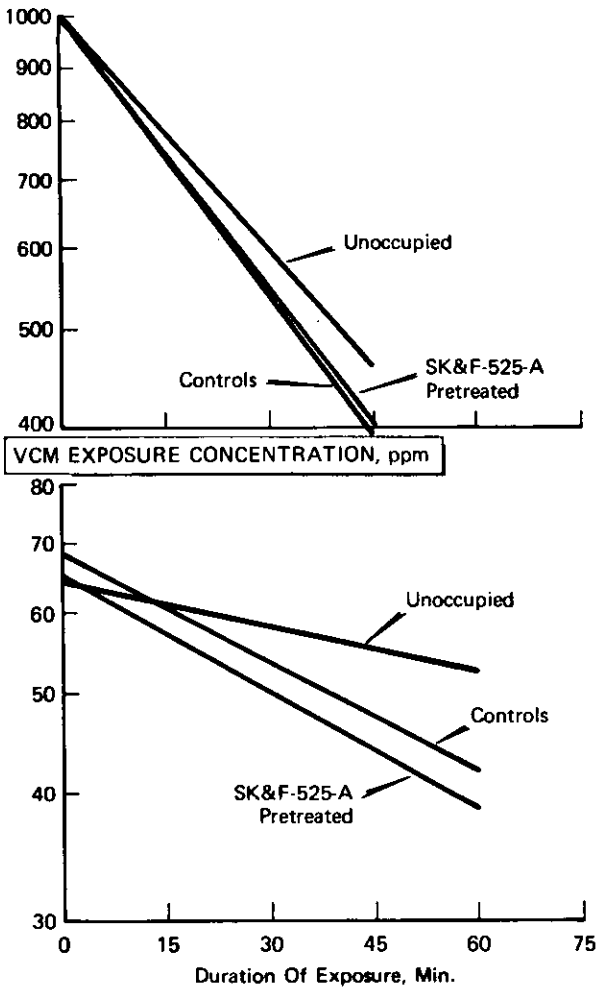


FIGURE 5. Declines in VCM concentration with time at 65 and 1038 ppm VCM exposure concentrations for both SK&F-525-A pretreated and untreated control rats. Also shown are the respective deadlines in VCM concentration for the unoccupied inhalation apparatus.

experiments conducted at extremes of initial VCM concentrations.

Pretreatment of rats 0.5 hr previous to exposure to VCM with 75 mg/kg SK&F-525-A, an inhibitor of some types of microsomal oxidases (*4*), resulted in no inhibition of metabolism of VCM in rats exposed to initial concentrations of 61 or 65 ppm and an 18.8% inhibition in those exposed to 1038 ppm (Fig. 5). Additional exposures of rats pretreated 0.6, 1, or 2.25 hr with 75 mg/kg SK&F-525-A to approximately 1000 ppm VCM resulted in 8.9, 6.5, and 4.5% inhibition of VCM metabolism, respectively.

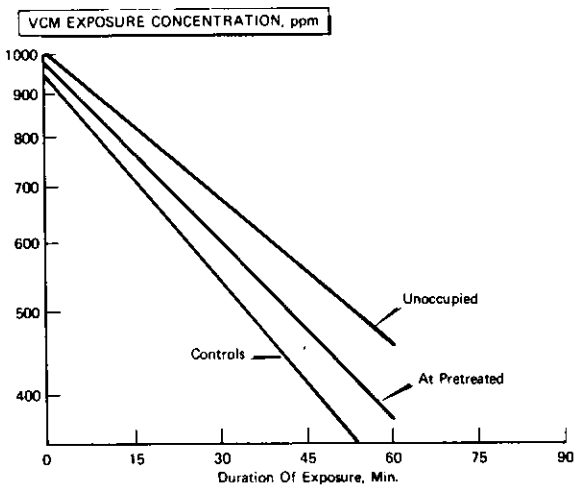


FIGURE 6. Declines in VCM concentration with time at 940.6 ppm VCM exposure concentrations for both AT pretreated and untreated control rats. Also shown is the respective decline in VCM concentration for the unoccupied inhalation apparatus.

Pretreatment of rats with 1000 mg/kg AT, an inhibitor of liver catalase activity (*5*), resulted in a 16.4% inhibition of VCM metabolism in rats exposed to an initial concentration of 941 ppm VCM (Fig. 6).

Effect of Inhaled VCM on Liver Sulfhydryl Levels

No antemortem or postmortem signs of toxicity were noted in rats exposed to any concentration of VCM used in these experiments. Analysis of food consumption data for rats exposed to nominal concentrations of 500 or 5000 ppm VCM, and unexposed controls revealed no statistically significant differences. In addition, no significant difference was found in the water consumption of rats exposed to 5000 ppm and controls. Throughout the durations of observation, the mean body weights of rats exposed to the various concentrations of VCM were essentially the same as those of the respective controls. No gross pathological lesions related to exposure of VCM, were found in any of the rats.

Table 1 shows the nonprotein sulfhydryl content of the liver of rats exposed to 50, 500, 5000, or 15,000 ppm VCM for the indicated durations. Values for concurrent controls are also given. In Figure 7, these data are sum-

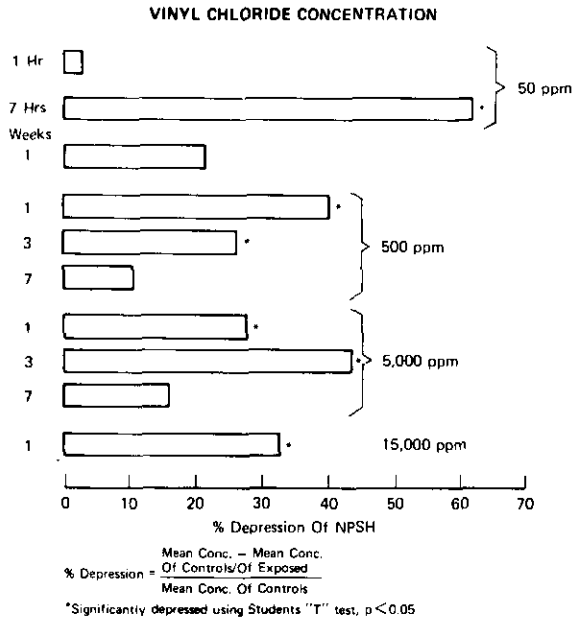


FIGURE 7. Depression of nonprotein sulfhydryl in the liver of rats as a function of VCM exposure concentration and duration of exposure.

marized and expressed as the per cent depression of the nonprotein sulfhydryl content of the liver as a function of exposure concentration and duration of exposure. Significant reductions in the levels of nonprotein sulfhydryl occurred in rats exposed to 50 ppm VCM for 7 hr, 500 and 5000 ppm for 1 and 3 weeks,

Table 1. Nonprotein sulfhydryl content in livers of rats exposed to VCM and in concurrent controls.

VCM concn, ppm	Sulfhydryl content×10^8, mole/mg liver after various exposure times		
	7 hr/day, for 5 days	7 hr/day, 5 days/wk, for 3 weeks	7 hr/day, 5 days/wk, for 7 weeks
15,000	0.29±0.08[a]	—	—
Control	0.42±0.07	—	—
5,000	0.33±0.02[a]	0.41±0.03[a]	0.59±0.08
Control	0.46±0.03	0.72±0.03	0.70±0.02
500	0.35±0.09[a]	0.45±0.06[a]	0.58±0.06
Control	0.58±0.06	0.61±0.08	0.64±0.08
50[b]	0.34±0.01	—	—
Control	0.43±0.09	—	—

[a] Significantly different by using Student's t test, $p<0.05$.
[b] A single 1-hr exposure to 50 ppm VCM resulted in 0.64±0.05 and 0.65±0.06 for the concurrent controls. A single 7-hr exposure to 50 ppm VCM resulted in 0.17±0.07 and 0.44±0.08 for the concurrent controls.

and 15,000 ppm for 1 week. There is no definitive association between exposure concentration and the degree of depression. The degree of depression decreases with continued exposure which suggests compensatory mechanisms(s) are responding to relieve this biochemical effect induced by VCM.

The protein-bound sulfhydryl content of the liver of rats exposed to 50, 500, 5000, or 15,000 ppm VCM for the indicated durations were not significantly different from those of the concurrent controls. This was not unexpected because the sulfhydryl groups of protein have been shown not to be readily akylated unless denaturation of the protein renders them available for alkylation (*6*).

Effect of Ethanol Administration on VCM-Induced Depression of Nonprotein Sulfhydryl in Liver

In this experiment, four rats pretreated with 5 ml/kg ethanol and four untreated control rats were exposed to 1070 ppm VCM. Following 105 min of exposure, the concentration of nonprotein sulfhydryl in the liver was determined. For those pretreated with ethanol, the depression of nonprotein sulfhydryl was 77.0 ± 12.8%, while for controls it was 95.0 ± 3.4%. These levels of depression were significantly different as determined by Student's t test, $p<0.05$. Johnson (*7*) has reported that ethanol alone does not affect the nonprotein sulfhydryl content of the liver.

Metabolites of VCM

Preliminary results which must be viewed with considerable reservation have been obtained from studies in which rats were exposed to 5000 ppm unlabeled VCM for 4, 5, or 7 weeks. Thin-layer chromatograms of urine collected from rats after each of these exposure durations were comparable. *S*-(2-Hydroxyethyl) cysteine (R_f = 0.26–0.28 in 80:10:10 *n*-butanol, acetic acid, water) appeared to present. *S*-(2-Hydroxyethyl)cysteine was not present in a thin-layer chromatogram of urine collected from control rats. *S*-(2-Chloroethyl)cysteine and *S*-(2-carboxymethyl)cysteine were not detected; however, it is conceivable that the latter compound was not adequately resolved from the urine background.

In another experiment, chromatograms of urine from rats exposed to 5000 ppm VCM for 9 weeks revealed the presence of monochloroacetic acid (R_f = 0.74 in *n*-butanol, acetic acid, water, 80:10:10). The spots for monochloroacetic acid were visualized under ultraviolet light on the fluorescent plates and did not develop with ninhydrin reagent.

Studies of the metabolism of VCM were terminated until methods were developed to synthesize ^{14}C-VCM. ^{14}C-VCM in a liquid state polymerized even when an inhibitor was present and a temperature of —70°C was maintained. Recently, a method has been developed to synthesize ^{14}C-VCM in a gaseous state from 1,2-dichloroethane (*8*). In the gaseous state, ^{14}C-VCM is stable.

Three 180-g male rats were exposed to ^{14}C-VCM in the closed recirculating system for 65 min. The initial concentration of ^{14}C-VCM with a specific activity of 1.155 mCi/mmole was 49 ppm. Assuming equivalent uptake of VCM by the three rats, each rat received 0.49 mg/kg VCM. Immediately following exposure, the rats were removed from the chamber and placed in Roth type metabolism cages which allow separate collection of urine, feces, expired carbon dioxide, and any expired VCM.

Within 15 hr after exposure, a mean of 58.0% of the ^{14}C activity had been excreted in the urine, 2.7% in the feces, and 9.8% as expired carbon dioxide. By 75 hr after exposure, 67.1% had been excreted in the urine, 3.8% in the feces, and 14.0% as expired carbon dioxide. Only a trace, 0.02% of the dose, was expired as VCM and trapped on activated carbon. After 75 hr, 1.6% of the dose remained in the liver, 3.6% in the skin, 0.2% in the kidneys and 7.6% in the remaining carcass. The mean recovery of the assumed dose from the three rats was 97.9%.

Chromatograms of the major fraction (97.9%) of ^{14}C activity obtained from ion-exchange separation of urine of rats exposed to 7855 ppm ^{14}C-VCM for 62 min revealed the presence of three major VCM urinary metabolites. R_f values for these urinary metabolites do not exactly coincide with those for the *S*-(2-hydroxyethyl)cysteine or *S*-(2-carboxymethyl)cysteine standards; thus, if these compounds are present, they are likely present as mercapturic acids (*N*-acetylated) or other derivatives (sulfoxide, sulfone). Chromatograms of a single minor fraction (1.2%) of ^{14}C activity indicated the presence of ^{14}C urea as a minor VCM urinary metabolite.

In summary, metabolism studies have demonstrated tentatively the following.

VCM is quite readily metabolized to polar metabolites which are excreted predominantly in the urine of rats exposed via inhalation to an initial concentration of 50 ppm. Smaller amounts of ^{14}C activity are excreted in the expired air as carbon dioxide and in the feces. Very little is excreted in expired air as unchanged VCM.

A significant but small amount of ^{14}C activity is retained in tissue, particularly liver, as long as 75 hr after exposure.

Metabolites excreted in the urine appear to be conjugated with glutathione and/or cysteine through covalent linkage to the sulfhydryl group. This is consistent with the reduction of the nonprotein free sulfhydryl levels in the livers of exposed rats. Preliminary *in vitro* experiments (unpublished data) have shown that direct conjugation of vinyl chloride with cysteine or glutathione in aqueous solutions occurs to a small degree but very slowly.

Monochloroacetic acid also appears to be a metabolite of VCM, when rats were exposed to 5000 ppm for an extended time.

Discussion

Because of the preliminary nature of some of the studies reported herein, the results and subsequent discussion must not be considered conclusive. Justification for premature publication of some of the data contained herein is the magnitude of the impact of recently revealed toxicological effects of VCM and the urgent need for communication of even preliminary results.

The studies reported herein have demonstrated reliably in some instances and tentatively in others the following.

Rats exposed to concentrations of VCM below 100 ppm metabolize the compound quite readily and in accordance with first-order rate kinetics, $t_{1/2}$ = 86 min.

When exposed to a concentration of VCM exceeding 220 ppm, its rate of metabolism was reduced, $t_{1/2}$ = 261 min. This indicates that the predominant pathway for metabolism of VCM by rats exposed to 100 ppm or less is saturable.

Residual metabolism at concentrations exceeding 220 ppm may be via this pathway in conjunction with additional pathway(s).

Pyrazole inhibits the metabolism of VCM suggesting that metabolism of VCM is via alcohol dehydrogenase.

Stronger evidence for the metabolism of VCM by alcohol dehydrogenase was its inhibition by the administration of ethanol. This inhibition was less pronounced in rats exposed to 1000 ppm than in rats exposed to 100 ppm or less, suggesting that metabolism via pathways other than that of alcohol dehydrogenase occurs in rats exposed to 1000 ppm VCM.

β - Diethylaminoethyldiphenylpropyl acetate (SK&F–525–A) does not affect the metabolism of VCM by rats exposed to approximately 65 ppm VCM but there is an indication that it slightly depresses metabolism by rats exposed to approximately 1000 ppm VCM. This suggests that in rats exposed to concentrations of VCM which exceed the capacity of the alcohol dehydrogenase pathway metabolism may occur via oxidases in the microsomes.

3-Amino-1,2,4-triazole (AT) slightly depresses the metabolism of VCM by rats exposed to 1021 ppm of VCM. This suggests that in rats exposed to concentrations of VCM which exceed the capacity of the alcohol dehydrogenase pathway, metabolism may occur via catalase.

Monochloracetic acid was found in urine of rats exposed to 5000 ppm VCM daily for 9 weeks.

Exposure to VCM reduces the nonprotein sulfhydryl concentration of liver. This reduction is not definitively associated with the exposure concentration of VCM in a range of 50 to 15,000 ppm. There is a tendency for the reduction to become less pronounced with repeated daily exposures. These results are consistent with a saturable mechanism for the metabolism of VCM followed by conjugation of the metabolites of VCM with glutathione and/or cysteine.

The administration of ethanol significantly reduces the depression of the concentration of nonprotein sulfhydryl in the livers of rats caused by exposure to 1000 ppm VCM for 105 min.

In rats exposed to 49 or 847 ppm, VCM is metabolized to polar products which are excreted predominantly in the urine. These products appear to be derived following initial metabolism of VCM and subsequent conjugation of the products with glutathione and/or cysteine through covalent binding with the sulfhydryl. A small but significant fraction of VCM is metabolized to CO_2 and expired. An even smaller but significant amount of ^{14}C activity appears to be retained in the liver primarily, but also in other tissues as long as 75 hr after exposure.

Considering the results *in toto*, we hypothesize that in rats exposed to concentrations of VCM below 100 ppm, VCM is predominantly metabolized via sequential oxidation to 2-chloroethanol, chloroacetaldehyde, and monochloroacetic acid by the alcohol dehydrogenase pathway [eq. (4)].

$$\mathrm{ClHC{=}CH_2} \rightarrow \mathrm{ClCH_2{-}CH_2OH} \xrightarrow[\text{dehydrogenase}]{\text{alcohol}} \mathrm{ClH_2C{-}CHO} \rightarrow \mathrm{ClCH_2{-}COOH} \qquad (4)$$

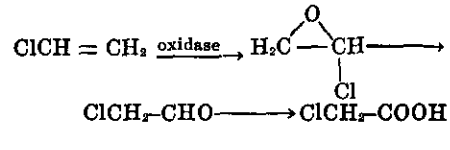
Only small amounts, if any, of monochloroacetic acid are formed at low doses because chloroacetaldehyde reacts rapidly with the sulfhydryl of glutathione and cysteine (*9*). This conjugation accounts for the lack of dose related reduction in the nonprotein sulfhydryl content of the liver of rats exposed to 50 through 15,000 ppm VCM.

Alternate pathways of VCM metabolism which may be involved at high doses are at this time purely speculative. However, at 220 ppm, metobolism by the more rapid alcohol dehydrogenase pathway appears to be saturated, and metabolism via oxidation of the accumulating 2-chloroethanol may occur as in eq. (5).

$$\mathrm{ClCH_2{-}CH_2OH} \xrightarrow[\text{catalase}]{\mathrm{H_2O_2}} \mathrm{ClHC_2{-}CH_2OOH} \rightarrow \mathrm{ClCH_2{-}CHO} \qquad (5)$$

Carter et al. (*3*) have demonstrated that microsomal oxidation of ethanol *in vitro* proceeds via the formation of hydrogen peroxide and catalase which subsequently forms a peroxide of ethanol. Acetaldehyde is the end product of this oxidation. It is conceivable that a similar oxidation of 2-chloroethanol occurs.

In addition to this series of reactions, a direct epoxidation of VCM may occur [eq. (6)].



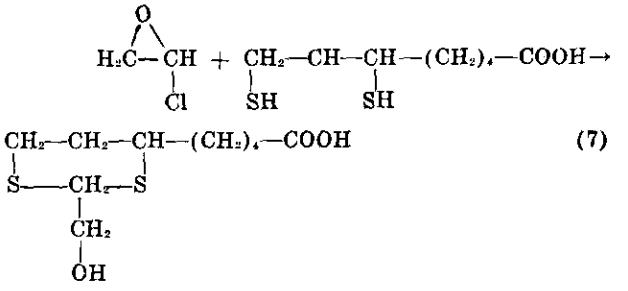
(6)

Zief et al. (*10*) have shown that chloroethylene oxide spontaneously rearranges to chloroacetaldehyde. Such mechanisms would explain why SK&F–525–A causes a slight inhibition of metabolism in rats exposed to approximately 1000 but not 65 ppm VCM. Also it may explain why monochloroacetic acid may be excreted by rats exposed to 5000 ppm but not 50 ppm VCM. In the former case, chloroacetaldehyde is produced by each of the hypothesized pathways which may result in a greater amount being oxidized to monochloroacetic acid than being conjugated with glutathione and/or cysteine.

Inferences from the results of these studies about the toxicodynamics of VCM are preliminary, but worth mentioning. First, the saturation of a primary metabolic pathway for VCM degradation and redirection through other pathways provides some hope that a threshold concentration for the untoward effects of VCM may exist. Metabolites of VCM formed only via the alternate pathways may constitute the ultimate toxin and carcinogen.

It has been reported (*6,11–14*) that the administration of cysteine or glutathione provides protection against the untoward effects of various aliphatic and aromatic mustards, triethylenemelamine, x-rays, and ionizing radiation. Conceivably, cysteine and glutathione may provide a natural defense against tumor producing free radicals generated within the body, as well as synthetic or naturally occurring alkylating agents which are absorbed into the body. Therefore, reduction of the nonprotein sulfhydryl content of the liver in animals exposed to VCM may constitute predisposition to toxicity and carcinogenicity mediated via other materials.

Finally, the speculated formation of chloroethylene oxide seems particularly pertinent insofar as postulation of the mechanism of carcinogenesis. This compound is undoubtedly a very active difunctional alkylating agent. It is most interesting that inorganic arsenicals have been reported to cause untoward hepatic effects, portal cirrhosis and angiosarcoma, like those reported for VCM (H. Popper, National Institutes of Health, Bethesda, Md., personal communication). The mechanism of toxicity for arsenic has been shown to occur via its reaction with 6,8-dithiooctanoic acid (α-lipoic acid) (*15*). In this reaction arsenic forms a stable bridge between the two sulfhydryl groups. If chloroethylene oxide were formed, it would readily react with α-lipoic acid, bridging the sulfhydryl groups like arsenic [eq. (7)].



(7)

Although this postulated mechanism is highly speculative, the rarity of materials known to produce untoward hepatic effects like those of VCM must be given some weight.

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