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July 11, 1974

TO: Technical Task Group on Vinyl Chloride Research

SUBJECT: Manuscript of Dow Report on VCM Metabolism

Gentlemen:

Distributed herewith are copies of the manuscript of the subject paper presented by Dr. Gehring at the May 10-11 meeting of the New York Academy of Sciences.

Sincerely,

Kenneth D. Johnson, Ph.D.
Secretary
Technical Task Group on
Vinyl Chloride Research

KDJ/mb

Enclosure

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

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INTRODUCTION

Vinyl chloride monomer (VCM), extensively used for the production of polyvinyl 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 hours per day, 5 days per week for 12 months, and subsequently maintained and observed until death[9]. References to studies of the untoward effects of VCM in man and laboratory animals are located in this volume of The Annals of the New York Academy of Sciences.

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 conceived 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 grams were exposed to initial concentrations of VCM gas ranging from 50.5 to 1167.0 ppm (0.13 to 2.99 mg/ℓ). Exposures ranged from 52.5 to 356.3 minutes. Figure 1 depicts the closed, recirculating 4.7ℓ 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 (10.9μ) by an in line Miran-I infrared analyzer (Wilks).

Using known concentrations of VCM prepared in 100ℓ 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 4.26μ. 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 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

$$dC/dt = -K_1 C_o$$

In this equation, C(ppm) equals the concentration of VCM at time t(min), C_o(ppm) equals the initial concentration of VCM, 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 using 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 the equation

$$K_c = K_e - K_1$$

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 hour prior to exposure, 5 ml/kg ethanol 1.5 hours prior to exposure, or 75 mg/kg SK&F-525-A (β-diethylaminoethyldiphenylpropylacetate) 0.5 hour prior to exposure. Without clearing 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 percent inhibition was calculated from the equation

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

Effects of VCM on Liver Sulfhydryl Levels

Groups of male Sprague-Dawley albino rats of Spartan strain weighing from 193 to 250 grams 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 hour, 7 hours, or 5 days. The exposures were carried out in a glass-walled 160ℓ chamber under dynamic conditions with the VCM being metered into the chamber airstream. For repeated daily exposures, 7 hour 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 hours; however, if the rats were being continued on exposure, they received a full 7 hour 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 prepared for assay of sulfhydryl content. Gross pathological examinations were conducted.

The method used for the sulfhydryl assay was a modification of that described by Sedlak and Lindsay[12]. Exactly 500 mg of liver from each rat was homogenized for 1 minute in a Dounce tissue homogenizer containing 8 ml of 0.02M disodium EDTA. For the total sulfhydryl assay, a 0.5 ml aliquot of each homogenate was mixed with 1.5 ml of 0.2M tris HCl pH 9.2 buffer, 0.1 ml of 0.01M DTNB (5,5'-dithiobis(2-nitrobenzoic acid)), 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 minutes, and the samples were centrifuged for 15 minutes at 4,000 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[12].

The non-protein sulfhydryl content of liver was determined after precipitating out the protein by addition of 1 ml of 50% trichloroacetic acid to a 5 ml sample of liver homogenate. Each sample was diluted with 4 ml of distilled water and after 15 minutes centrifuged at 4,000 g. A 2 ml aliquot of the supernatant was mixed with 4 ml

of 0.4M tris HCl pH 8.9 buffer. Immediately before reading the absorbance against a reagent blank, 0.1 ml of 0.01M DTNB was added. Subtraction of the non-protein 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(2-carboxymethyl)cysteine (5 and 7 week samples only). Urine was collected for analysis by applying pressure to the posterior 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 of 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 hours in a sealed glass tank containing n-butanol, acetic acid, water 80:10:10 or 60:20:20. After being air dried, the plates were sprayed with Ninspray® ninhydrin reagent and heated for 2 minutes 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 3 times with 2 ml of diethyl ether. The diethyl ether extract was evaporated to 0.1 ml and 2 to 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 hours 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.

RESULTS

Kinetic Studies on the Metabolism of Inhaled VCM

Typical declines in the concentration of VCM in the inhalation apparatus containing 4 rats and initial concentrations of approximately 50 or 1000 ppm are shown in Figure 2. Also shown are the corresponding declines in the concentration of VCM from the unoccupied chamber. As indicated in the methods, 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 one-half life ($t_{1/2}$) of 86 minutes.

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 minutes. As indicated by the standard deviations, there was little variation of K_c within the indicated range of concentration. Furthermore, 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 hour before exposure to 65 and 1234 ppm VCM resulted in 71.2 and 86.9% inhibition of metabolism of VCM, respectively (Figure 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 experiments conducted at extremes of initial VCM concentrations.

Pretreatment of rats with 75 mg/kg SK&F-525-A, an inhibitor of some types of microsomal oxidases[13] resulted in no inhibition

of metabolism of VCM in rats exposed to initial concentrations of 65 ppm and an 18.8% inhibition in those exposed to 1038 ppm (Figure 5).

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 non-protein 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 6, these data are summarized and expressed as the percent depression of the non-protein sulfhydryl content of the liver as a function of exposure concentration and duration of exposure. Significant reductions in the levels of non-protein sulfhydryl occurred in rats exposed to 50 ppm VCM for 7 hours, 500 and 5000 ppm for 1 and 3 weeks, and 15,000 ppm for 1

week. There is no difinitive association between exposure concentration and the degree of depression. The degree of depression decreases with continued exposure which suggests compensatory mechanisms 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[14].

Effect of Ethanol Administration of VCM Induced Depression of Non-Protein 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 minutes of exposure, the concentration of non-protein sulfhydryl in the liver was determined. For those pretreated with ethanol, the depression of non-protein sulfhydryl was 77.0 $\pm$ 12.8%, while for controls it was 95.0 $\pm$ 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 non-protein 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. 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 be present. 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 using the aforementioned solvent system). 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. In the gaseous state ^{14}C-VCM is relatively stable. Currently,

the methodology is being refined to allow synthesis of ^{14}C-VCM with reproducible specific activity.

Using ^{14}C-VCM, three 180 g. male rats have been exposed in the closed recirculating system for 65 minutes. 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 3 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 hours post 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 hours post 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 hours, 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 percent of the assumed dose recovered from the three rats was 97.9%.

Various chromatographic techniques are being evaluated for separation and identification of the ^{14}C labeled urinary metabolites. S(2-hydroxyethyl)cysteine and S(2-carboxymethyl)cysteine and their respective N-acetyl derivatives appear to be likely metabolites. Ultimate verification of metabolites will be determined by gas

chromatographic - mass spectroscopic analysis. In the experiment just described, monochloroacetic acid has not been detected.

In summary, metabolism studies have demonstrated tentatively the following:

a) 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 VCM are excreted in the expired air as carbon dioxide and in the feces. Very little is excreted in expired air as unchanged VCM.

b) A significant but small amount is retained in tissue, particularly liver, as long as 75 hours post exposure.

c) 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 non-protein free sulfhydryl levels in the livers of exposed rats. Preliminary in vitro experiments have shown that direct conjugation of vinyl chloride with cysteine or glutathione in aqueous solutions occurs to a small degree but very slowly.

d) 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 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:

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

b) When exposed to a concentration of VCM exceeding 200 ppm, its rate of metabolism was reduced, $t_{1/2}$ = 261 minutes. This indicates that the predominate pathway for metabolism of VCM by rats exposed to 100 ppm or less is saturable. Residual metabolism at concentrations exceeding 200 ppm may be via this pathway in conjunction with additional pathway(s).

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

d) Stronger evidence for the metabolism of VCM by alcohol dehydrogenase was its inhibition by the administration of etharol. 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.

e) SK&F-525-A does not affect the metabolism of VCM by rats exposed to 65 ppm VCM but there is an indication that it slightly depresses metabolism by rats exposed to 1038 ppm VCM. This suggests that in rats exposed to concentrations of VCM which exceed the capacity of the alcohol dehydrogenase pathways metabolism may occur via oxidases in the microsomes.

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

g) Exposure to VCM reduces the non-protein 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.

h) The administration of ethanol significantly reduces the depression of the concentration of non-protein sulfhydryl in the livers of rats caused by exposure to 1000 ppm VCM for 105 minutes.

i) In rats exposed to 49 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 hours post 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.

$$ClHC = CH_2 \longrightarrow ClH_2C\text{-}CH_2OH \xrightarrow{\text{alcohol dehydrogenase}}$$

$$ClH_2C\text{-}CHO \longrightarrow ClH_2C\text{-}COOH$$

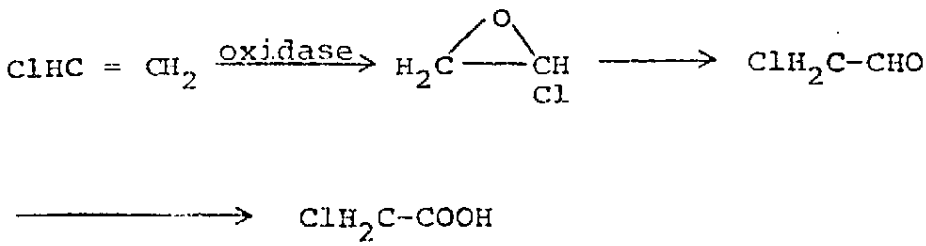
Only small amounts, if any, of monochloroacetic acid are formed at low doses because chloroacetaldehyde reacts rapidly with the sulfhydryl of glutathione and cysteine[8]. This conjugation accounts for the lack of dose related reduction in the non-protein sulfhydryl content of the liver of rats exposed 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 200 ppm, metabolism by the more rapid alcohol dehydrogenase pathway appears to be saturated, and metabolism via oxidation of the accumulating 2-chloroethanol may occur as follows:

$$ClH_2C{-}CH_2OH \xrightarrow[\text{catalase}]{H_2O_2} ClH_2C{-}CH_2OOH \rightarrow ClH_2C{-}CHO$$

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 expoxidation of VCM may occur as follows:

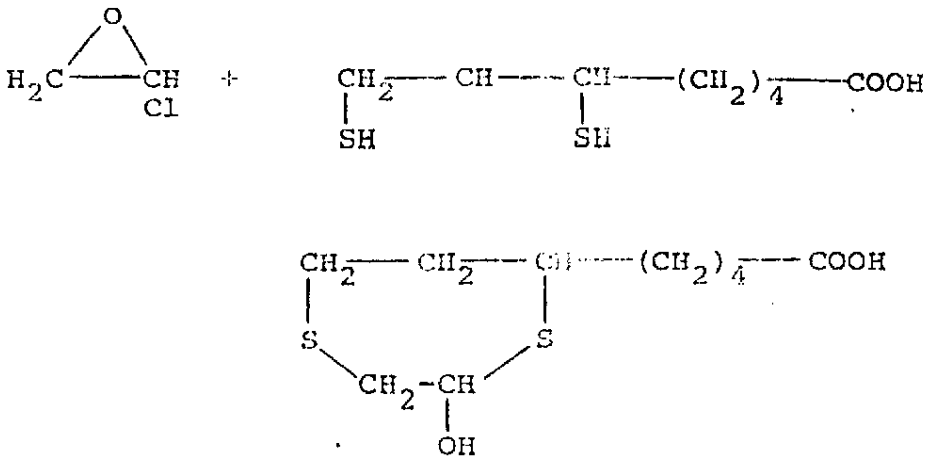


Zief, et. al.[15] have shown that chloroethylene oxide spontaneously rearranges to chloroacetaldehyde. Such mechanisms would explain why SK&F-525-A causes a slight inhibition of metabolism in rats exposed to 1038 but not 65 ppm VCM. Also it may explain why monochloroacetic acid may be excreted by rats 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 premature, 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 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.[1,2,5,10,14] 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 non-protein 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.[11] The mechanism of toxicity for arsenic has been shown to occur via its reaction with 6,8-dithiooctanoic acid (α-lipoic acid).[6] 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.



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.

ACKNOWLEDGMENTS

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Captions for Figure and the Table

Figure 1 Closed Recirculating Inhalation Apparatus Used for Exposing Rats to VCM.

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.

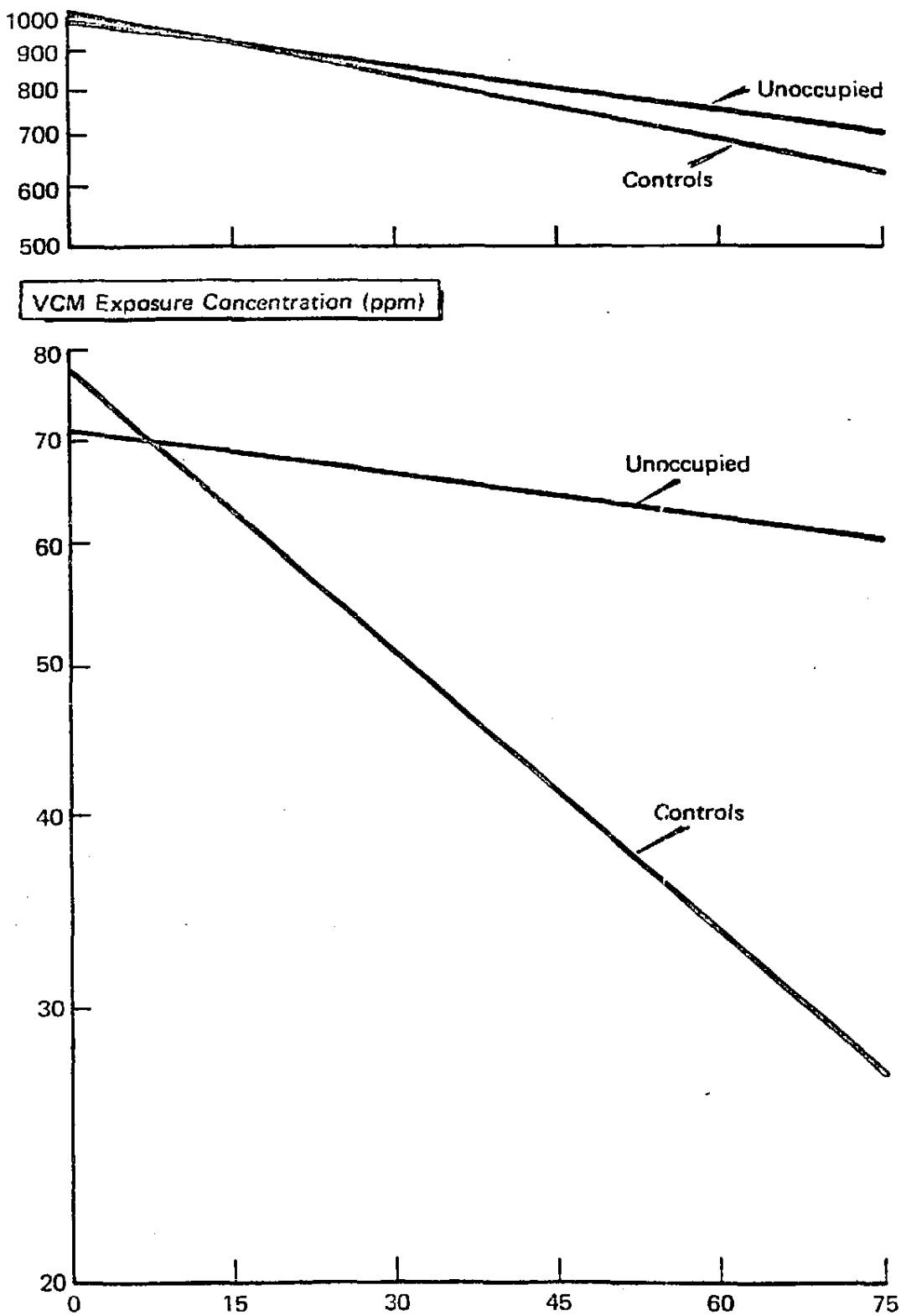
Figure 3 Declines in VCM concentration with time at 65 and 1234 ppm VCM exposure concentrations for both pyrazole pretreated and untreated control rats. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

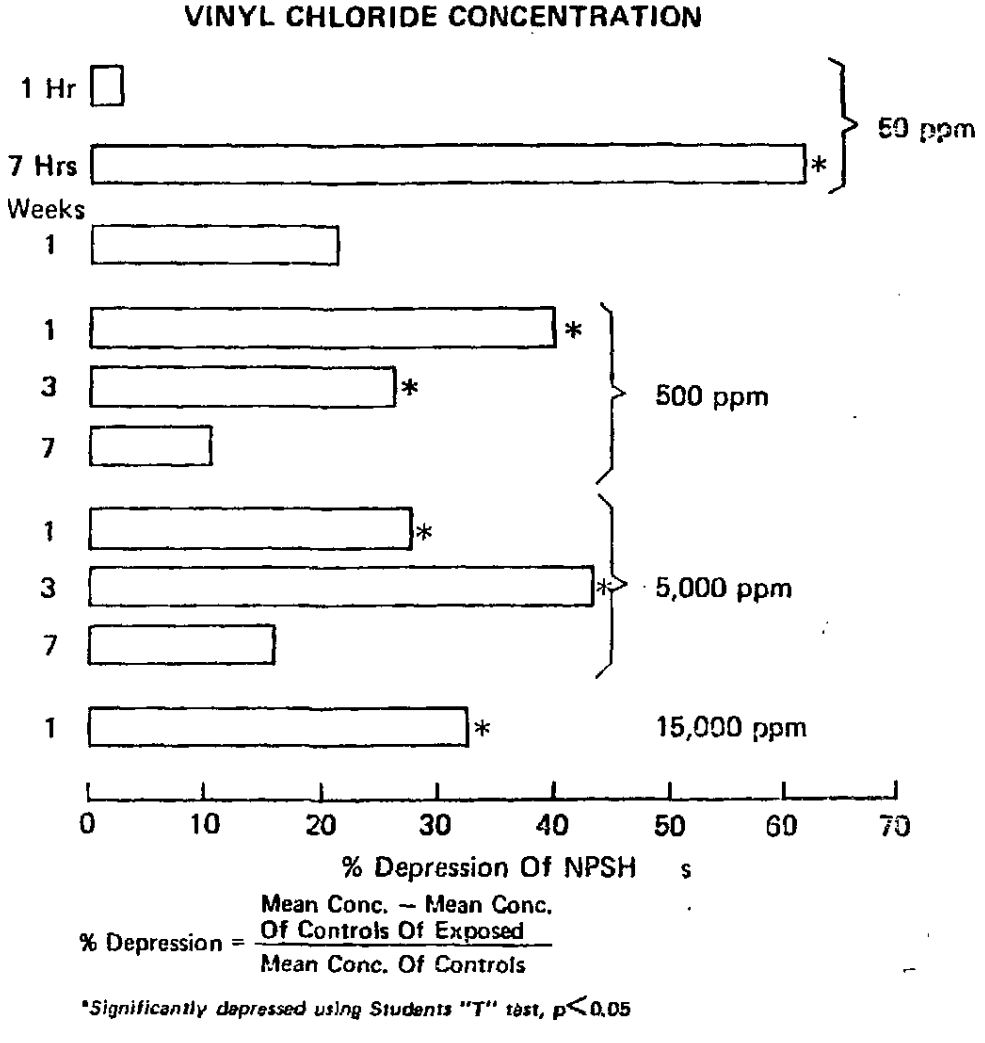
Figure 4 Declines in VCM concentration with time at 56 and 1034 ppm VCM exposure concentrations for both ethanol pretreated and untreated control rats. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

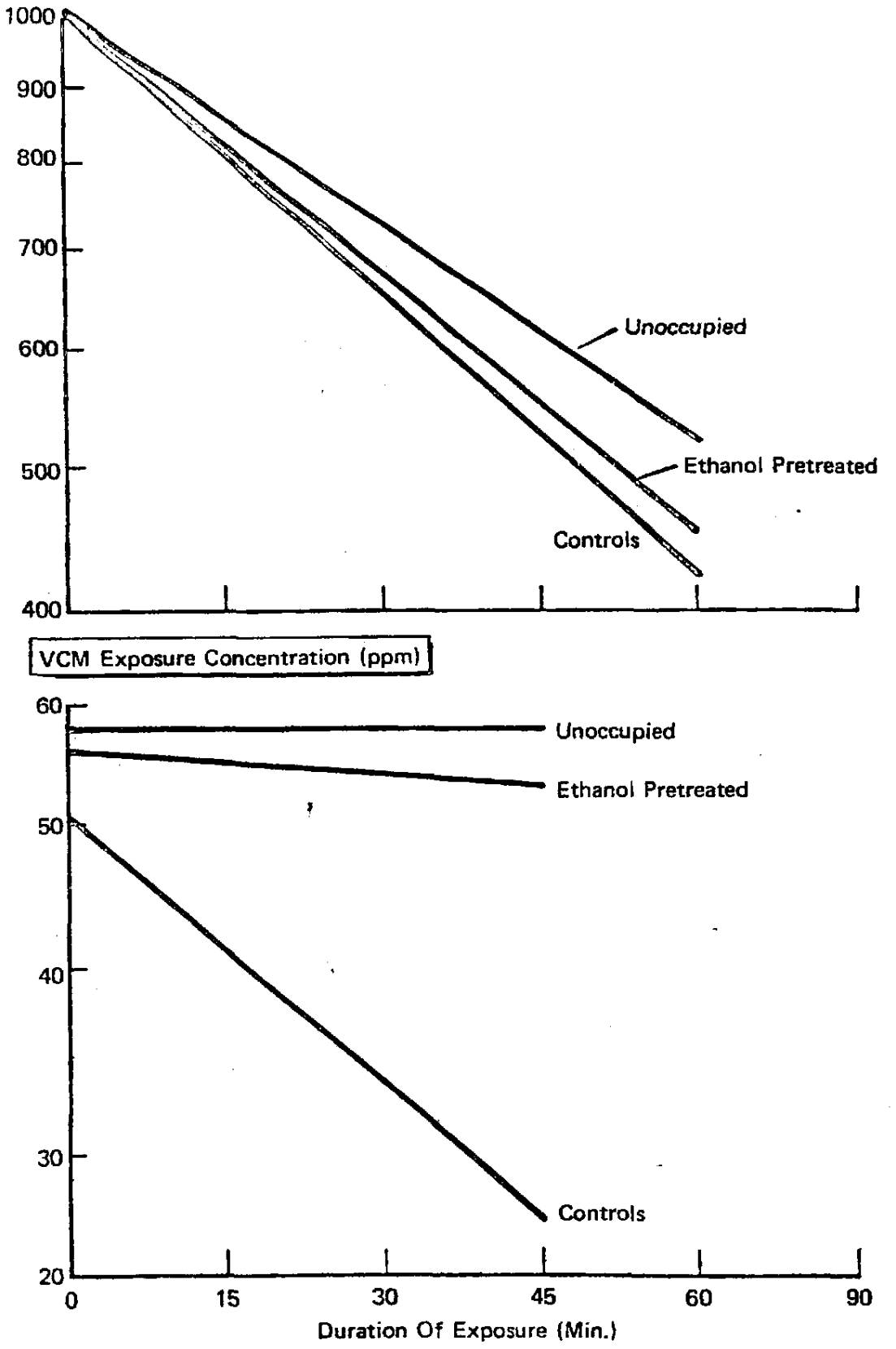
Figure 5 Declines in VCM concentration with time at 65 and 1038 ppm VCM exposure concentrations for both SK&F-525-A pretreated and untreated control rats. Also shown are the respective declines in VCM concentration for the unoccupied inhalation apparatus.

Figure 6 Percent depression of non-protein sulfhydryl in the liver of rats as a function of VCM exposure concentration and duration of exposure.

Table 1 Non-protein sulfhydryl content (1×10^{-8} moles SH/mg of liver) of rats exposed to 50, 500, 5000 or 15,000 ppm VCM and concurrent controls.



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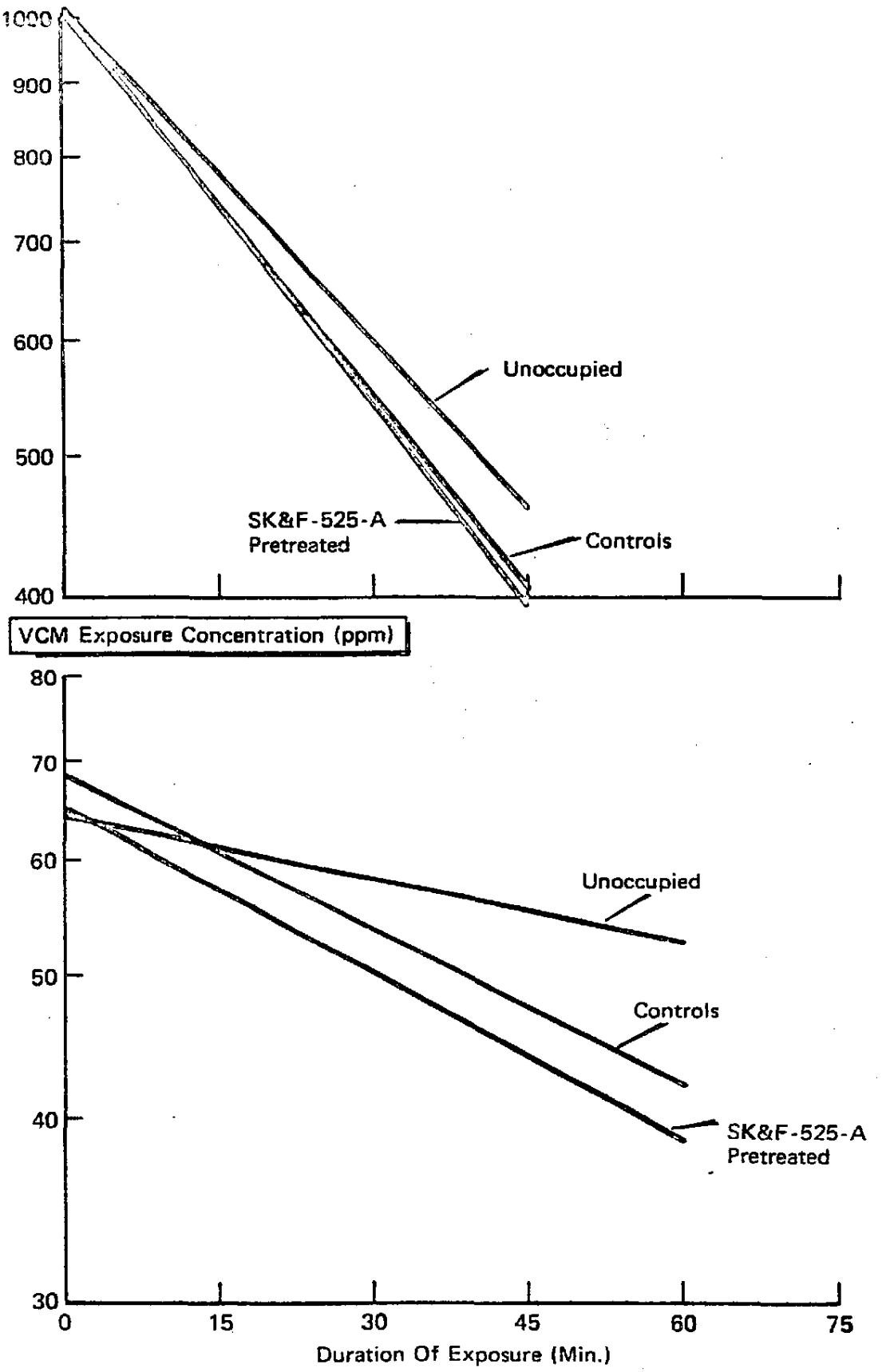
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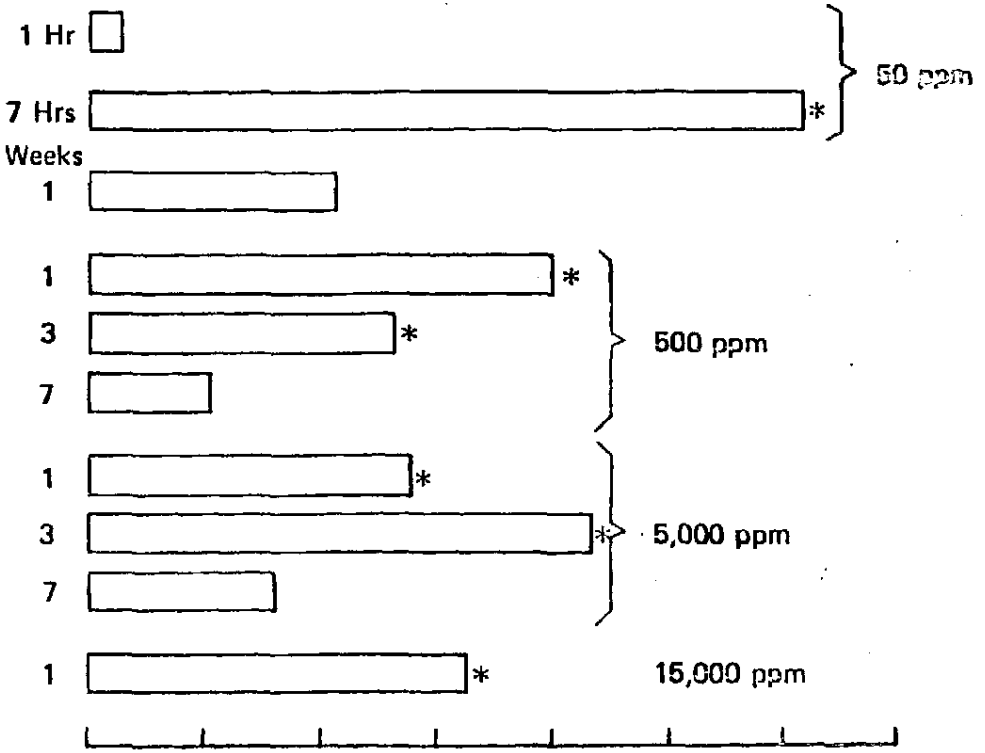
Duration Of Exposure (Min.)

VINYL CHLORIDE CONCENTRATION



$$\% \text{ Depression} = \frac{\text{Mean Conc. Of Controls} - \text{Mean Conc. Of Exposed}}{\text{Mean Conc. Of Controls}}$$

*Significantly depressed using Students "T" test, $p < 0.05$

	VCM CONCENTRATION							
Duration of Exposure	15,000 ppm	Concurrent Controls	5,000 ppm	Concurrent Controls	500 ppm	Concurrent Controls	+50 ppm	Concurrent Controls
7 hours per day for 5 days	*0.29 ±0.08	0.42 ±0.07	*0.33 ±0.02	0.46 ±0.03	*0.35 ±0.09	0.58 ±0.06	0.34 ±0.01	0.43 ±0.09
7 hours per day 5 days per week for 3 weeks			*0.41 ±0.03	0.72 ±0.03	*0.45 ±0.06	0.61 ±0.08		
7 hours per day 5 days per week for 7 weeks			0.59 ±0.08	0.70 ±0.02	0.58 ±0.06	0.64 ±0.08		

+A single 1 hour exposure to 50 ppm VCM resulted in 0.64±0.05 and 0.65±0.06 for the concurrent controls. A single 7 hour exposure to 50 ppm VCM resulted in 0.17±0.07 and 0.44±0.08 for the concurrent controls.

*Significantly different using Students "t" test, $p<0.05$.