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TITLE: COMPARISON OF THE FATE OF VINYL CHLORIDE FOLLOWING SINGLE AND REPEATED EXPOSURE IN RATS

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This report is: ☐ INTERIM ☑ FINAL and mainly: ☒ NEW ☐ REVIEW

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DESCRIPTIVE SUMMARY WITH CONCLUSIONS:

Rats were exposed to 5000 ppm nonlabeled vinyl chloride (VC) 6 hours/day, 5 days/week for 7 weeks. On the last day of exposure ^{14}C-labeled VC was used and the fate of the ^{14}C-VC was followed for 72 hours and compared with the fate in rats subjected to a single 6-hour exposure to 5000 ppm ^{14}C-VC. The routes and rates of excretion of ^{14}C-activity were the same for the two experimental groups. The activity of microsomal enzymes, as reflected by aniline hydroxylase and p-nitroanisole O-demethylase of 9000 x g liver supernatants was essentially the same in rats exposed once, repeatedly or in nonexposed control rats. Covalent binding to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. The hepatic nonprotein sulfhydryl level of the repeated and single exposed groups immediately following exposure was 79% and 39% of control, respectively. These results indicate that repeated exposure to VC does not induce its biotrasnformation. However, the increase in hepatic macromolecular binding indicates that repeated exposure augments the reaction of electrophilic metabolites with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

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COMPARISON OF THE FATE OF VINYL CHLORIDE FOLLOWING SINGLE AND REPEATED EXPOSURE IN RATS

by

P. G. Watanabe, J. A. Zempel and P. J. Gehring

September 28, 1976

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COMPARISON OF THE FATE OF VINYL CHLORIDE FOLLOWING SINGLE AND REPEATED EXPOSURE IN RATS

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ABSTRACT

Rats were exposed to 5000 ppm nonlabeled vinyl chloride (VC) 6 hours/day, 5 days/week for 7 weeks. On the last day of exposure ^{14}C-labeled VC was used and the fate of the ^{14}C-VC was followed for 72 hours and compared with the fate in rats subjected to a single 6-hour exposure to 5000 ppm ^{14}C-VC. The routes and rates of excretion of ^{14}C-activity were the same for the two experimental groups. The activity of microsomal enzymes, as reflected by aniline hydroxylase and p-nitroanisole O-demethylase of 9000 x g liver supernatants was essentially the same in rats exposed once, repeatedly or in nonexposed control rats. Covalent binding to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. The hepatic nonprotein sulfhydryl level of the repeated and single exposed groups immediately following exposure was 79% and 39% of control, respectively. These results indicat that repeated exposure to VC does not induce its biotransformation. However, the increase in hepatic macromolecular

binding indicates that repeated exposure augments the reaction of electrophilic metabolites with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

INTRODUCTION

While many pharmacokinetic and metabolic studies have been conducted on vinyl chloride (VC), most of these investigations have concentrated on the fate of VC during and following single exposure. Since cancer has been induced by long term repeated exposure of both experimental animals and man to VC, it is important to consider alterations in the disposition of VC in the body which may occur after repeated exposure.

In preliminary studies monochloroacetic acid was tentatively identified as a major urinary metabolite following repeated exposure (6 hours/day, 5 days/week for 9 weeks) to 5000 ppm nonlabeled VC (Hefner et al., 1975). However, this metabolite has not been found in rats given a single exposure to ^{14}C-VC (Watanabe et al., 1976a). This raised the question whether the biotransformation of VC may be altered upon repeated exposure.

A change in the biotransformation of VC after repeated exposure is particularly important in assessing the hazard of exposure to VC since evidence suggests that VC is metabolized to a reactive metabolite which is ultimately responsible for

its toxic manifestations. This concept is supported by studies showing an enhancement of the mutagenic activity of VC to bacteria when microsomal and soluble enzymes are added to the system to provide for metabolic transformation (Bartsch et al., 1975; Malavielle et al., 1975; Rannug et al. 1974). In addition, recent reports demonstrated that liver microsomal enzymes, in vitro, mediate the binding of VC metabolites to the microsomes (Kappus et al., 1975), protein sulfhydryl groups, RNA (Bolt et al., 1975), and adenosine (Barbin et al., 1975). That the biotransformation of VC is involved intimately in its toxic response was further substantiated when Reynolds et al. (1975a) showed that acute hepatotoxicity could be produced by VC if the animals were pretreated with phenobarbital or Arochlor 1254, inducers of microsomal enzyme activity. Reynolds et al. (1975b) also reported that a single 6 hour exposure to 5% VC deactivates cytochrome P-450 and other components of the mixed function oxidase system.

Since there is evidence suggesting that repeated exposure to VC may alter its biotransformation and since the metabolism of VC is associated with its toxicity, the objective of the current study was to determine whether the fate of VC is

indeed altered with repeated exposure. This was accomplished by exposing rats to nonlabeled VC for 7 weeks and on the last exposure to ^{14}C-labeled VC. The fate of the ^{14}C-VC was then followed for 72 hours and compared to animals receiving a single exposure.

METHOD

Material. Vinyl chloride gas (Matheson Gas Products) of 99.9% minimum purity was used throughout the study. ^{14}C-labeled VC was synthesized from (1,2 ^{14}C) 1,2-dichloroethane (New England Nuclear, Lot #819-292, 4.8 mCi/mmole) immediately prior to use (Wagner and Muelder, 1975). The synthesized ^{14}C-VC has been reported to be 95-96% radiochemically pure (Wagner et al., 1975). Nonlabeled VC (Matheson Gas Products) was mixed with the ^{14}C-material to obtain the desired specific activity. Typically, 40 ml of the ^{14}C-VC, helium gas mixture was injected into a 10 liter Saran bag (Anspec, Inc.) containing the desired quantity of nonlabeled VC.

Animals. Male Sprague-Dawley rats (Spartan Research Laboratory) with an initial weight of 160-180 g were used in the study. All animals were housed in rooms in which a constant humidity, temperature and 12 hour light-dark cycle (8 AM-8 PM) were maintained. Food and water was provided ad libitum

except during the exposure. Exposures were conducted between 9:00 AM and 4:00 PM (EST).

Exposure. All rats were exposed under dynamic conditions to a nominal concentration of 5000 ppm VC (treated) or room air (control) in 30 ℓ glass inhalation chambers. VC was metered into the chamber air flow (~6 l/min) with a dual syringe pump. The nominal concentration of VC was determined from the ratio of the rate at which the VC gas was dispensed and the total chamber air flow. The analytical concentration of VC was monitored continuously by recirculating a fraction of the chamber atmosphere through an infrared spectrophotometer (Wilks) set at 10.6 μ. The rats repeatedly exposed to VC were exposed 6 hours/day, 5 days/week for 7 weeks (32 exposures in 44 days). The mean analytical concentration of VC over the 7 week exposure period was 4775±908 (SD) ppm.

On the final day of exposure the animals were subjected to a 6 hour exposure to ^{14}C-labeled VC generated in the same manner as described above. On this final day of exposure, the chamber atmosphere was also analyzed at hourly intervals by gas chromatography, and at the same times the ^{14}C-activity was determined by bubbling 1 ml aliquots of the chamber atmosphere into a scintillation solution containing Concifluor (Mallinckrodt Chemical), 2-methoxyethanol, toluene

(6:11:83) (Watanabe et al., 1976a). The radioactivity was determined by counting in a liquid scintillation spectrometer. The mean analytical concentration determined on the final exposure day when the animals were exposed to ^{14}C-labeled VC was 4600 ppm ± 311 (SD). The specific activity was 50 dpm per microgram VC.

The inhalation chamber was operated in a laboratory fume hood to prevent contamination of the working environment. After transit through the inhalation chamber the ^{14}C-VC was absorbed on activated charcoal. These traps were disposed of as radioactive waste according to standard regulations.

Procedure. Eight rats were exposed repeatedly to VC as described previously. On the last day, 5 additional unexposed rats and the 8 rats exposed repeatedly were exposed to 5000 ppm ^{14}C-VC for 6 hours. Following this final exposure to ^{14}C-VC, 3 of the 8 exposed repeatedly and 2 of the 5 exposed once were placed in glass Roth-type metabolism cages for the collection of urine, feces and expired air.

Room air was drawn through the cages at 400-500 ml/min. The exiting air was passed through a series of traps to collect the expired ^{14}C-VC and $^{14}CO_2$. The air leaving the chamber

was passed first through a glass tube containing about 40 g of Drierite (W. A. Hammond Drierite Co.) to remove moisture. Subsequent transit through a series of two cold finger traps containing 50 ml of toluene, 2-methoxyethanol (80:20), and a single trap containing 120 ml of 5 M ethanolamine in 2-methoxyethanol enabled the collection of ^{14}C-VC and $^{14}CO_2$, respectively. The cold finger traps were immersed in 2-methoxyethanol, dry ice baths throughout the collection periods. The trap for CO_2 was maintained at room temperature.

Samples of excreta were collected for 72 hr after termination of exposure and analyzed for ^{14}C activity. Expired VC was collected at 0.5 hr intervals for 3 hr; the CO_2 trap and urine receptacle (immersed in dry ice bath) were changed at 12 hr intervals for 72 hr; and feces were collected every 24 hr. At the termination of the study (72 hr) the animals were killed by a blow to the head, and samples of tissues (fat, kidney, liver) were collected for analysis of ^{14}C activity. The remaining carcass was skinned and homogenized (50% w/v) in distilled water and analyzed for ^{14}C activity. The samples of excreta and tissue were prepared for scintillation counting as described previously (Watanabe et al., 1976a).

Carbon 14 activity was determined by counting in a Mark II or Mark III liquid scintillation spectrometer. External standard channel ratios were used to determine the counting efficiency. The counts per minute were converted to disintegrations per minute using a standard quench curve.

The remaining rats in the groups exposed repeatedly and singly to VC, 5 and 3 respectively along with a group of 4 controls exposed to room air for 6 hours were killed by a blow to the head immediately following exposure. A piece of liver was sampled and used for determining hepatic non-protein sulfhydryl content by a modification of the method of Sedlak and Lindsay (1968). Another piece of liver was used to prepare a 9000 x g supernatent in 1.15% KCl in order to determine aniline hydrolyxlase (LaDu et al., 1971) and p-nitroanisole O-demethylase (Kinoshita et al., 1966) activity. Macromolecular binding of radioactivity to hepatic tissue was determined by the method of Jollow et al. (1974). The carcass was analyzed for total radioactivity as described above.

RESULTS

Excretion of ^{14}C-activity within 72 hours after a single or repeated exposure to 5000 ppm ^{14}C-VC is shown in Table 1.

The percentage of ^{14}C-activity excreted by each route as well as the total mg equivalents VC recovered were essentially identical for the singly and repeatedly exposed groups. The majority of ^{14}C-activity eliminated was expired as VC per se.

The time course for expiration of ^{14}C-VC per se (Figure 1) and urinary excretion of ^{14}C-activity (Figure 2) were also essentially identical for the singly and repeatedly exposed rats. The curves were fit by linear regression analysis of the logarithmically transformed data. The estimate of the apparent first order rate constant for expiration of VC was 0.023 min^{-1} ± 0.01 (SD) which corresponds to a half-life of 30 minutes. The elimination of urinary ^{14}C-activity was biphasic. An estimate of the apparent first order rate constant for the initial portion of the urinary excretion curve from 12-36 hours was 0.155 hr^{-1} ± 0.002 (SD) which corresponds to a half-life of 4.47 hours. The data for the slow phase of urinary excretion were extremely variable; therefore no attempt was made to estimate the excretion rate. Less than 1 percent of the radioactivity excreted in the urine occurred during the slow phase.

Urinary ^{14}C-activity was separated by thin layer chromatography in n-butanol, acetone, H_2O (50:20:30) on cellulose

and n-butanol, acetic acid, H_2O (80:20:20) on silica gel. The profile of radioactivity for rats exposed repeatedly or singly were qualitatively similar and no significant radioactivity was associated with the R_f value of a standard of monochloroacetic acid.

The concentration of radioactivity detected in tissue 72 hours after exposure revealed no statistically significant difference between rats exposed once or repeatedly to VC (Table 2). It does appear that in those exposed repeatedly more radioactivity may have been retained in the liver and skin; however, the number of animals used does not provide for an adequate statistical evaluation.

The effect of VC on xenobiotic drug metabolism by liver 9000 x g supernatents as reflected by aniline hydroxylase and p-nitroanisole-O-demethylase activity is presented in Table 3. Neither single or repeated exposures to 5000 ppm VC altered discernibly the enzyme activity in either system when compared to air exposed controls.

The total amount of VC biotransformed, the hepatic macromolecular binding of ^{14}C-activity and the hepatic nonprotein sulfhydryl content following single and repeated exposure

are shown in Table 4. The total amount of VC biotransformed was not significantly different between the two groups. However the hepatic macromolecular binding appears to be increased in the rats exposed repeatedly. When the protein binding was corrected for the amount of VC biotransformed (B/A x 100) a statistically significant increase was found. This parameter indicates that a larger fraction of the biotransformed VC reacts covalently with hepatic macromolecules in rats exposed repeatedly. This result strengthens the significance of the previous observation that 72 hours after the last exposure more radioactivity was found in the liver of rats exposed repeatedly than those exposed once.

In contrast to the results of macromolecular binding, hepatic nonprotein sulfhdyryl content was depressed to a greater extent in rats receiving a single exposure than in those exposed repeatedly.

DISCUSSION

Repeated exposure of rats to 5000 ppm ^{14}C-VC did not alter discernibly the routes or rates of excretion of radioactivity or qualitatively the excretory products formed from VC or VC per se. These results negate the previous preliminary observation that monochloroacetic may be a major biotransformation producer of VC (Hefner et al., 1975).

No differences were found in the activity of the enzymes, aniline hydrolylase and p-nitroanisole O-demethylase, in the liver of rats exposed repeatedly to 5000 ppm VC, in rats exposed once and in nonexposed rats. Thus, exposure to VC at this concentration does not appear to influence microsomal metabolism. In contrast to this conclusion, are the findings of Reynolds et al. (1975b) that the cytochrome P-450 content and the oxidative N-demethylation of aminoantipyrine and ethylmorphine were markedly depressed in rats following exposure to 50,000 ppm VC for 6 hrs. However, the extremely high exposure concentration renders suspect the relevance of any conclusion based on the Reynolds study.

A most significant finding in the study reported herein was a significantly increased amount of radioactivity bound covalently to macromolecules of rats exposed repeatedly to VC versus those exposed once. An associated observation was the retention of an apparently greater level of radioactivity in the liver of repeatedly exposed rats 72 hours after exposure than those exposed once. These results indicate that toxic manifestations, including carcinogenicity,

associated with the reaction of reactive metabolites of VC with macromolecules may be enhanced by repeated exposure to VC.

In rats exposed repeatedly, the enhanced covalent binding of VC with macromolecules occurred in spite of a less significant depression of the nonprotein sulfhydryl content of the liver when compared to those rats exposed once. Previous studies have demonstrated that detoxification of the reactive metabolites of VC occurs via their enzymatic conjugation with glutathione (Watanabe et al., 1976b; Watanabe et al., 1976c) and subsequent excretion. Glutathione is reportedly the major constituent of the nonprotein sulfhydryl content of the liver.

The foregoing results are somewhat perplexing because an increased covalent binding of radioactivity is normally thought to be associated with either an enhanced biotransformation to reactive metabolites or a decreased detoxification of the reactive metabolites. The former possibility is negated by the absence of a qualitative or

quantitative change in the biotransformation of VC in rats exposed repeatedly as well as the lack of evidence that microsomal enzymes are induced by repeated exposure to VC. The finding that the hepatic nonprotein sulfhydryl content was reduced less in rats exposed repeatedly to VC suggests that detoxification of reactive metabolites formed from VC by conjugation with GSH should not be depressed. Therefore, both logical reasons for the increased covalent binding of reactive metabolites of VC to macromolecules appear non-operative. However, not to be overlooked is the fact that the nonprotein sulfhydryl content of the liver rather than GSH content was measured. It is conceivable that the GSH level of the liver was actually decreased. If this were true, the binding of reactive metabolites to macromolecules may be enhanced inspite of an apparently normal hepatic nonprotein sulfhydryl content. It may be expected that nonprotein sulfhydryl compounds other than GSH will be less effective in detoxifying reactive metabolites of VC.

In conclusion, the results of these studies show that repeated exposure to high levels of VC cause a persistent but partially compensated depression of the nonprotein sulfhdyryl

content of the liver. More significantly, the binding of reactive metabolites of VC with hepatic macromolecules appears to be enhanced by repeated exposure to high levels of VC. Associated with this may be expected an enhanced toxicity, including carcinogenicity. The reason for the enhanced covalent binding with repeated exposure is under investigation.

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