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.*, 1976). 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 ± 311 (SD) ppm. The specific activity was 50 dpm/μg of 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 adsorbed on activated charcoal. These traps were disposed as radioactive waste according to standard regulations.

Procedure. Eight rats were exposed repeatedly to VC as described previously. On the last day, five additional unexposed rats and the eight exposed repeatedly were exposed to 5000 ppm of [^{14}C]VC for 6 hr. Following this final exposure to [^{14}C]VC, three of the eight exposed repeatedly and two of the five 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.*, 1976).

Carbon-14 activity was determined by counting in a Mark II or Mark III liquid scintillation spectrometer (Searle). External standard-channels 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, five and three respectively, were killed by a blow to the head immediately following exposure. A piece of liver was used to prepare a 9000 *g* supernatant in 1.15% KCl in order to determine aniline hydroxylase (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). This method measures non-extractable radioactivity from a trichloroacetic acid precipitate which includes protein.

lipids, and nucleic acids. The carcass was analyzed for total radioactivity as described above.

A second experiment was conducted to confirm parameters obtained for binding of radioactivity to hepatic macromolecules. The methodology was the same as described above. A group of rats was repeatedly exposed to VC [4821 ± 259 (SD) ppm] for 8 weeks. On the last day of exposure [^{14}C]VC was used and an additional four rats (singly exposed group) were added. The mean analytical concentration of the [^{14}C]VC on the last day of exposure was 5065 ± 30 (SD) ppm and the specific activity was 34 dpm/μg of VC.

RESULTS

Excretion of ^{14}C activity within 72 hr after a single or repeated exposure to 5000 ppm of [^{14}C]VC is shown in Table 1. The percentage of ^{14}C activity excreted by each route as well as the total milligram equivalents of 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*.

TABLE 1

PERCENTAGE ^{14}C-ACTIVITY ELIMINATED BY RATS DURING 72 hr FOLLOWING INHALATION EXPOSURE TO 5000 ppm OF [^{14}C]VINYL CHLORIDE[a]

	Single exposure (2)[b]		Repeated exposure (3)[b]	
	(%)	(mg equiv of VC)	(%)	(mg equiv of VC)
Expired:				
As VC	54.5 ± 3.5	14.00	53.7 ± 2.1	12.94
As CO_2	8.0 ± 1.4	2.05	9.6 ± 1.6	2.27
Urine	27.1 ± 2.1	6.93	25.7 ± 1.4	6.21
Feces	3.2 ± 2.5	0.80	1.4 ± 0.4	0.32
Carcass and tissues	7.3 ± 2.5	1.89	9.7 ± 1.6	2.32
Total VC recovered		25.67		24.07

[a] Expressed as percentage of the total ^{14}C activity recovered, mean ± SD.
[b] Number of rats.

The time course for expiration of [^{14}C]VC *per se* (Fig. 1) and urinary excretion of ^{14}C activity (Fig. 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 ± 0.01 (SD) min^{-1} which corresponds to a half-life of 30 min. 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 to 36 hr was 0.155 ± 0.02 (SD) hr^{-1} which corresponds to a half-life of 4.47 hr. 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% 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 in *n*-butanol, acetic acid, H_2O (80:20:20) on silica gel. The profiles 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 hr after exposure revealed no statistically significant difference between rats exposed once or repeatedly to VC (Table

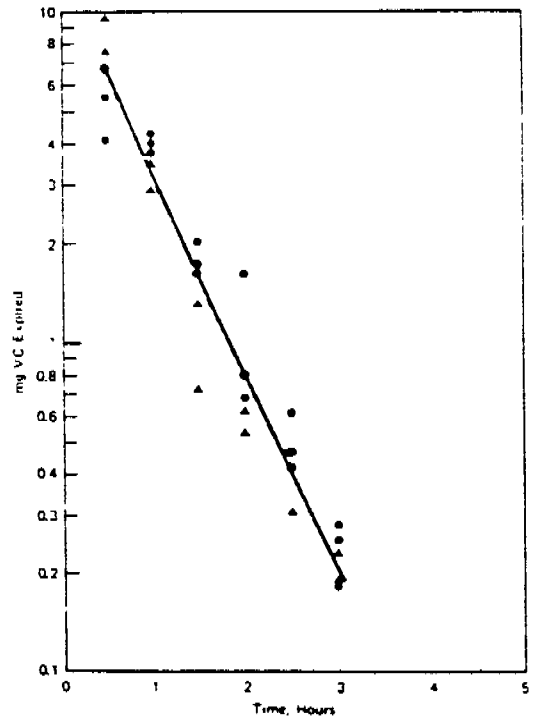

FIG. 1. Expired vinyl chloride (milligrams) versus time following a 6 hr exposure to 5000 ppm of $[^{14}C]VC$. Repeatedly exposed rats (▲) and singly exposed rats (●).

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 *g* supernatants, as reflected by aniline hydroxylase and *p*-nitroanisole *O*-demethylase activity, is presented in Table 3. Neither single nor repeated exposure to 5000 ppm of VC altered discernibly the enzyme activity in either system when compared to air-exposed controls.

The total amount of VC biotransformed and the hepatic macromolecular binding of ^{14}C activity 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 in the first experiment appeared to be increased in the rats exposed repeatedly. When the protein binding was corrected for the

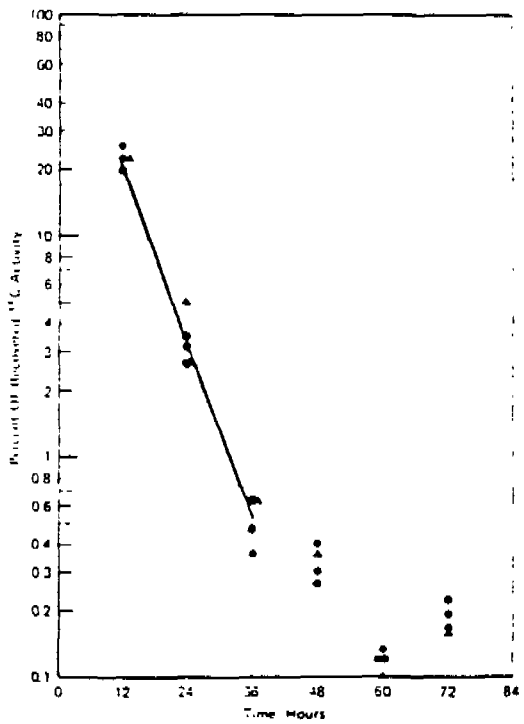

FIG. 2. ^{14}C-Activity excreted in the urine expressed as percentage of the recovered radioactivity versus time following a 6 hr exposure to 5000 ppm of [^{14}C]VC. Repeatedly exposed rats (▲) and singly exposed rats (●).

amount of VC biotransformed (B/A × 100) a statistically significant increase was found. Because the increased binding in repeatedly exposed rats was not definitive in the first experiment, the experiment was repeated and the results are shown in the lower portion of Table 4. The second study confirmed the observation that rats repeatedly

TABLE 2

PERCENTAGE ^{14}C-ACTIVITY 72 hr FOLLOWING INHALATION EXPOSURE OF RATS TO 5000 ppm OF VINYL CHLORIDE[a]

Tissue	^{14}C Activity g of tissue (%)	
	Single exposure (3)[b]	Repeated exposure (2)[b]
Liver	0.119 ± 0.022	0.157 ± 0.028
Kidney	0.062 ± 0.026	0.070 ± 0.006
Fat	ND[c]	ND[c]
Skin	0.046 ± 0.015	0.080 ± 0.019
Carcass	0.030 ± 0.014	0.039 ± 0.011

[a] Expressed as percentage of the total ^{14}C activity metabolized, mean ± SD.

[b] Number of rats per group.

[c] Not detectable, detection limit of 3 μg of VC equiv/g of fat or 0.03% ^{14}C activity metabolized g of tissue.

exposed to VC bind about 20–25% more reactive metabolite to hepatic macromolecules than do rats exposed once. It is not clear why the magnitude of the binding was slightly greater in the second experiment. Nonetheless, these results indicate that a larger fraction of the biotransformed VC reacts covalently with hepatic macromolecules in rats exposed repeatedly.

TABLE 3

EFFECT OF VINYL CHLORIDE ON DRUG METABOLISM BY A 9000 *g* SUPERNATANT FRACTION OF LIVER OF RATS[a]

	Amount (μg of product/g of liver/hr)	
	Aniline hydroxylase	*p*-Nitroanisole *O*-demethylase
Control (3)[b]	65 ± 16	226 ± 22
Single VC exposure (3)[b]	71 ± 7	254 ± 45
Repeated VC exposure (5)[b]	83 ± 10	217 ± 31

[a] Animals were killed immediately following the last exposure and enzyme activity was assayed. Values are mean ± SD.
[b] Number of rats per group.

TABLE 4

TOTAL METABOLISM AND HEPATIC MACROMOLECULAR BINDING FOLLOWING SINGLE OR REPEATED EXPOSURE OF RATS TO 5000 ppm OF VINYL CHLORIDE[a]

	A VC equivalents metabolized (μg)	B VC equivalents bound (μg/g of protein)	Binding corrected for metabolism (B/A × 100)[b]
Single exposure	9265 ± 1467	114 ± 10	1.12 ± 0.13
Repeated exposure	8718 ± 895	124 ± 10	1.43 ± 0.16[c]
Experiment was repeated			
Single exposure	8746 ± 882	148 ± 25	1.69 ± 0.28
Repeated exposure	9421 ± 482	195 ± 24[c]	2.07 ± 0.25[c]

[a] Values are the mean ± SD (three to five rats per group).
[b] The ratio of B/A × 100 was calculated from individual animal data.
[c] Statistically significantly different from the single exposure, Student *t* test ($p < 0.05$).

DISCUSSION

Repeated exposure of rats to 5000 ppm of [^{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 do not substantiate the previous preliminary observation that monochloroacetate may be a major biotransformation product of VC (Hefner *et al.*, 1975).

A most significant finding in the study 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 hr 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.

While the binding of reactive metabolites of VC to hepatic macromolecules was enhanced following repeated exposure, no differences were observed in the activity of hepatic microsomal enzymes to the substrates aniline or *p*-nitroanisole in any of the treatment groups when compared to nonexposed control rats. Thus, it did not appear that exposure to VC at this concentration influenced microsomal metabolism. However, in contrast to these findings was the observation by 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 of VC for 6 hr. The difference between our study and that of Reynolds *et al.* (1975b) is that we used substrates which cause a "type II" binding spectra compared to their use of substrates producing a "type I" binding spectra with hepatic microsomes. In addition Reynolds *et al.* (1975b) used a 10-fold greater concentration of VC. The apparent discrepancy can be explained by the recent observation that VC causes a "type I" binding spectra when incubated with hepatic microsomes (Salmon, 1976; Ivanetich *et al.*, 1977), and furthermore it has been demonstrated (Ivanetich *et al.*, 1977) that high concentrations of VC can degrade cytochrome *P*-450. Thus it appears that VC is metabolized by the hepatic microsomal enzymes and is capable of inhibiting metabolism of other substrates by competitive inhibition or by degradation of cytochrome *P*-450.

In conclusion, the results of these studies showed that repeated exposure to high levels of VC did not alter discernibly the routes or rates of excretion of radioactivity when compared to rats subjected to a single 6 hr exposure to 5000 ppm of [^{14}C]VC. Of particular significance was evidence that the binding of reactive metabolites of VC with hepatic macromolecules was 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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