REQUEST FOR APPROVAL OF PROPRIETARY TECHNICAL INFORMATION

DOW

B

1

Comparison of the Fate of Vinyl Chloride Following Single and Repeated Exposure in Rats

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

Toxicology & Applied Pharmacology

X

277 3894

R&S 103903

X

E. R. Smith
Inorg. Chem. R&D

X

V. K. Rowe
Toxicology Research

X

S. Walker
Legal

P. G. Watanabe | Toxicology Research | 656-1313
1803 | Midland

2

APPROVED
FOR USE IN DE

3

RECEIVED
JUN
CRL MIDLAND

COPIED IN MIDLAND CRL
JUL 16 1983

4

X

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

May 18, 1977

Toxicology Research Laboratory
Health and Environmental Research
Dow Chemical U.S.A.
Midland, Michigan 48640

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

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

ABSTRACT

Rats were exposed by inhalation to 5000 ppm nonlabeled vinyl chloride (VC) 6 hours/day, 5 days/week for 7 weeks. On the last day of repeated exposure ^{14}C-labeled VC was used. The fate of the ^{14}C-VC was compared in the group of rats exposed repeatedly to a group exposed simultaneously for a single 6 hour period 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. These results indicate 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., 1976). 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., 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 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., 1976).

Carbon 14 activity was determined by counting in a Mark II or Mark III liquid scintillation spectrometer. 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, 5 and 3 respectivley, were killed by a blow to the head immediately following exposure. A 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.

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 were repeatedly exposed to VC (4821 ppm ± 259, SD) for 8 weeks. On the last day of exposure ^{14}C-VC was used and an additional 4 rats (singly exposed group) were added. The mean analytical concentration of the ^{14}C-VC on the last day of exposure was 5065 ppm ± 30 (SD) and the specific activity was 34 dpm per microgram VC.

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} ± 0.02 (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 exposure to 5000 ppm 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 amount of VC biotransformed (B/A x 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 exposed to VC bind about 20-25% more reactive metabolite to hepatic macromolecules than rats exposed once. The magnitude of the binding was slightly greater in the second experiment. This was caused by a slightly higher concentration of ^{14}C-VC in the latter study (5065) ppm versus the former (4600 ppm). These results indicate that a larger fraction of the biotransformed VC reacts covalently with hepatic macromolecules in rats 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 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 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.

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 non-exposed

control rats. Thus, it did not appear than 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 amino-antipyrine and ethylmorphine were markedly depressed in rats following exposure to 50,000 ppm VC for 6 hours. The difference between our study and that of Reynolds, et al. (1975b) is that we used substrates which cause a "type II" binding spectra and they used substrates producing a "type I" binding spectra with hepatic microsomes. 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). Thus it appears that VC is metabolized by the hepatic microsomal enzymes and is capable of inhibiting metabolism of other substrates which interact with cytochrome P-450 producing similar type I binding spectra.

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-hour exposure to 5000 ppm ^{14}C-VC. Of particular significance was evidence that the binding of reactive metabolites of VC with hepatic macro-

molecules 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.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to M. M. Schlachter for technical assistance.

REFERENCES

Barbin, A., Bresil, H., Croisy, A., Jacquignon, P., Malaveille, C., Montesano, R. and Bartsch, H. (1975). Liver microsome mediated formation of alkylating agents from vinyl bromide and vinyl chloride. Biochem. Biophys. Res. Comm., 67, 596-603.

Bartsch, H., Malaveille, C., and Montesano, R. (1975). Human rat, and mouse liver mediated mutagenicity of vinyl chloride in Salmonella typhimurium strains. Int. J. Cancer, 15, 429-437.

Bolt, H. M., Kappus, H., Buchter, A., and Bolt, W. (1975). Metabolism of vinyl chloride. Lancet, 1425.

Hefner, R. E. Jr., Watanabe, P. G., and Gehring, P. J. (1975). Preliminary studies of the fate of inhaled vinyl choride monomer (VCM) in rats, Ann. N.Y. Acad. Sci., 246, 135-148.

Ivanetich, K. M., Aronson, I. and Katz, I. D. (1977). The interaction of vinyl chloride with rat hepatic microsomal cytochrome P-450 in vitro. Biochem. Biophys. Res. Comm., 74, 1411-1418.

Jollow, D, J., Thorgeirsson, S. S., Potter, W. Z., Hashimoto, M. and Mitchell, J. R. (1974). Acetaminophen-induced hepatic necrosis VI., Pharmacology, 12, 251-271.

Kappus, H., Bolt, H. M., Buchter, A. and Bolt, W. (1975). Rat liver microsomes catalyze covalent binding of ^{14}C-vinyl chloride to macromolecules, Nature, 257, 134-135.

Kinoshita, F. K., Frawley, J. P. and DuBois, K. P. (1966). Quantitative measurement of induction of hepatic microsomal enzymes by various dietary levels of DDT and toxaphene in rats. Toxicol. Appl. Pharmacol., 9, 505-513.

LaDu, B. N., Mandel, H. G. and Way, E. L. (ed.) (1971). Fundamentals of Drug Metabolism and Drug Disposition, pp. 566-569, The Williams and Wilkins Co., Baltimore, Maryland.

Malaveille, C., Bartsch, H., Barbin, A., Camus, A. M. and Montesano, R. (1975). Mutagenicity of vinyl chloride, chloroethyleneoxide, chloroacetaldehyde and chloroethanol. Biochem. Biophys. Res. Comm., 63, 363-370.

Rannug, U., Johansson, A., Ramel, C. and Washtmeister, C. A. (1974). The mutagenicity of vinyl chloride after metabolic activition, Ambio, 3, 194-197.

Reynolds, E. S., Moslen, M. T., Szabo, S., Jaeger, R. J., and Murphy, S. D. (1975a). Hepatotoxicity of vinyl chloride and 1,1-dichloroethylene. Am. J. Path., 81 (1), 219-231.

Reynolds, E. S., Maslen, M. T., Szabo, S. and Jaeger, R. J. (1975b). Vinyl chloride induced deactivation of cytochrome P-450 and other components of the liver mixed function oxidase system: on in vivo study. Res. Comm. Chem. Path. Pharmacol., 12(4), 685-693.

Salmon, A. G. (1976). Cytochrome P-450 and the metabolism of vinyl chloride. Cancer Letters, 2, 109-117.

Wagner, E. R. and Muelder, W. W. (1975). A procedure for preparing ^{14}C-labeled vinyl chloride. Ann. N.Y. Acad. Sci., 246, 152-153.

Wagner, E. R., Muelder, W. W., Watanabe, P. G., Hefner, R. E., Jr., Braun, W. H., and Gehring, P. J. (1975). Gas chromatographic method for the preparation of ^{14}C-labeled vinyl chloride, J. Labeled Compounds, 11, 535-542.

Watanabe, P. G., McGowan, G. R., Madrid, E. O. and Gehring, P. J. (1976). Fate of ^{14}C-vinyl chloride following inhalation exposure in rats. Toxicol. Appl. Pharmacol., 37, 49-59.

TABLE 1

Percentage ^{14}C-Activity Eliminated During 72 Hours Following Inhalation Exposure to 5000 ppm ^{14}C-Vinyl Chloride[a]

	Single Exposure(2)[b]		Repeated Exposure(3)[b]	
	%	(mg equiv. VC)	%	(mg equiv. VC)
Expired:				
as VC	54.5±3.5	(14.0)	53.7±2.1	(12.94)
as CO_2	8.0±1.4	(2.05)	9.5±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 mg equivalents VC recovered		(25.67)		(24.07)

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

[b]Number of rats.

Table 2

Percentage ^{14}C-Activity per Gram Tissue 72 Hour Following Inhalation Exposure to 5000 ppm Vinyl Chloride[a]

Tissue	Percentage ^{14}C-Activity/g Tissue	
	Single Exposure(3)[b]	Repeated Exposure(2)[b]
Liver	0.119±0.022	0.157±0.028[d]
Kidney	0.062±0.026	0.070±0.006
Fat	N.D.[c]	N.D.[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/group.

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

Table 3

Effect of Vinyl Chloride on Drug Metabolism By A 9,000 x g Supernatent Fraction of Liver[a]

	ug product/g liver/hour	
	Aniline Hydroxylase	p-Nitroanisole O-Demethylase
Control (4)[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 assayed. Values are means ± SD.

[b]Number of rats/group.

Table 4

Total Metabolism and Hepatic Macromolecular Binding Following Single or Repeated Exposure to 5000 ppm Vinyl Chloride[a]

	A µg VC Equivalents Metabolized	B µg VC Equivalents Bound per g Protein	Binding Corrected For Metabolism, B/A x 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 means ± SD (3-5 rats/group).

[b]The ratio of B/A x 100 was calculated from the individual animal data.

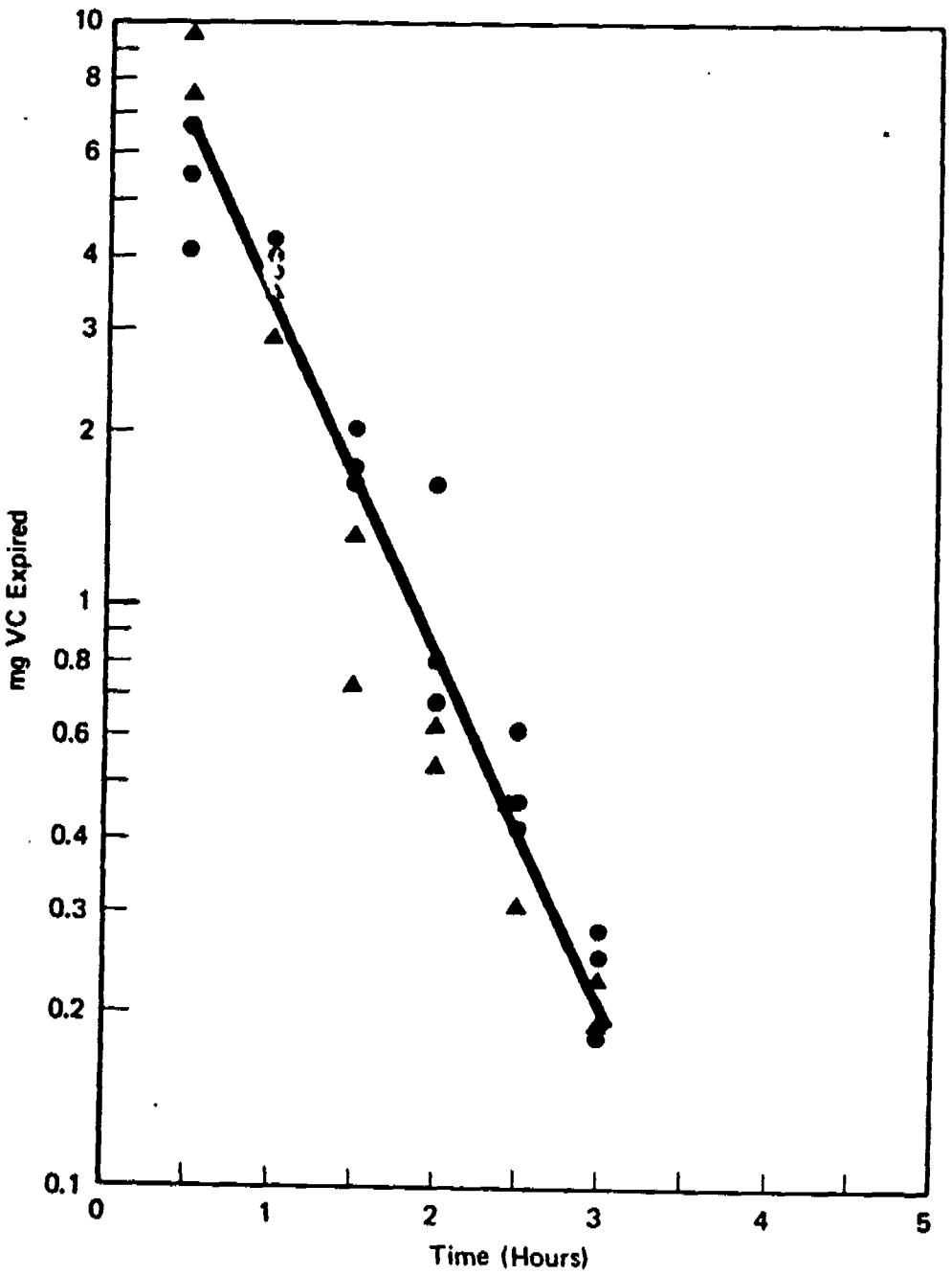
[c]Statistically significant from the single exposure, Student t-test ($p < 0.05$).

LEGENDS

Figure 1 — Expired vinyl chloride (mg) versus time following a 6 hour exposure to 5000 ppm ^{14}C-VC. Repeatedly exposed animals (x) and singly exposed animals (·).

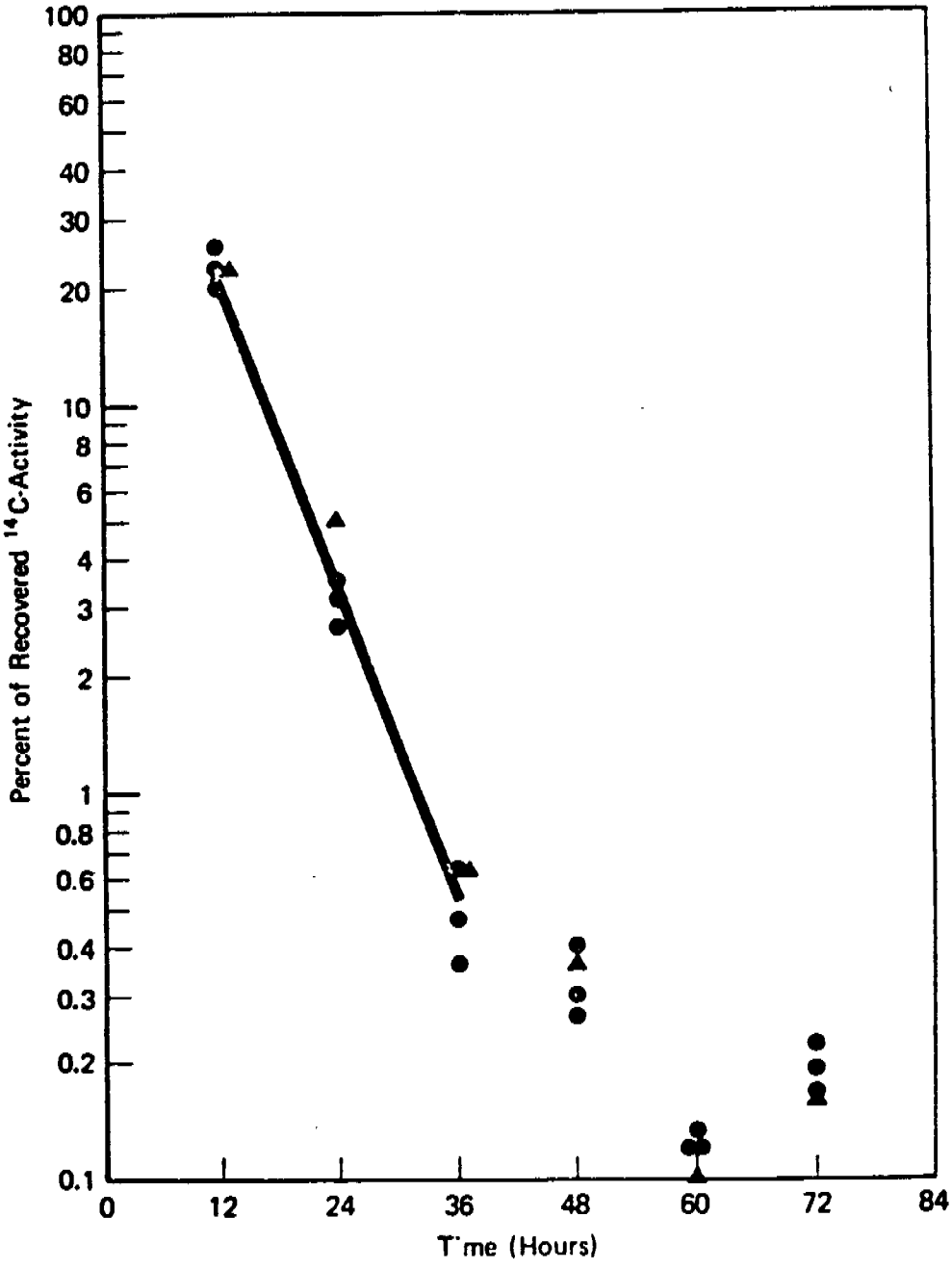
Figure 2 — ^{14}C-activity excreted in the urine expressed as percentage of the recovered radioactivity versus time following a 6 hour exposure to 5000 ppm ^{14}C-VC. Repeatedly exposed animals (x) and singly exposed animals (·).

FIGURE 1



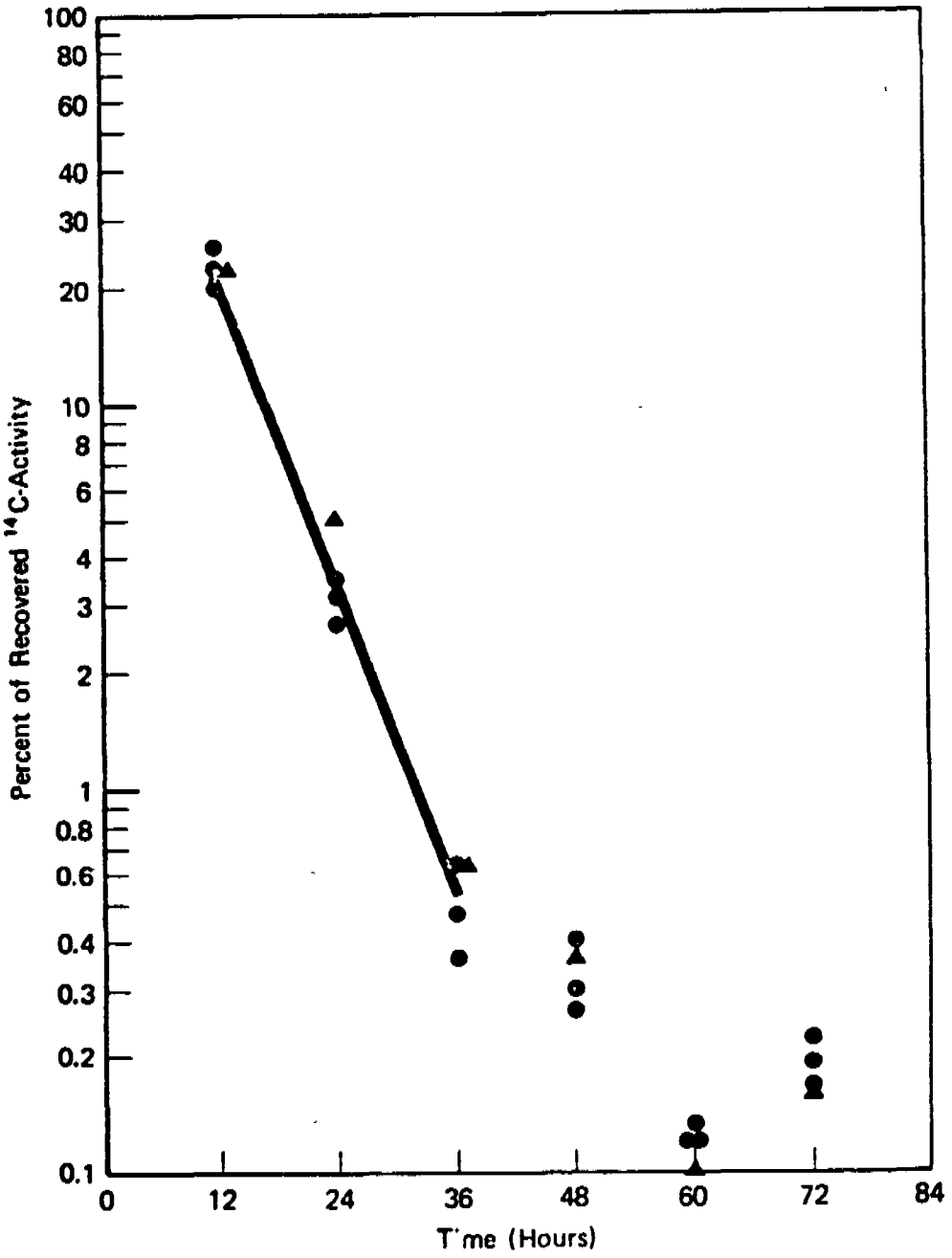
R&S 103926

FIGURE 2



R&S 103927

FIGURE 2



R&S 103927