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Fate of [^{14}C]Vinyl Chloride after Single Oral Administration in Rats[1]

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Fate of [^{14}C]Vinyl Chloride after Single Oral Administration in Rats. WATANABE, P. G., MCGOWAN, G. R., AND GEHRING, P. J. (1976). *Toxicol. Appl. Pharmacol.* 36, 339–352. Male rats were given single oral doses of 0.05, 1, and 100 mg/kg of [^{14}C]vinyl chloride (VC), and the routes and rates of elimination of ^{14}C activity followed for 72 hr. Following 0.05 and 1 mg/kg, excretion in the urine as nonvolatile metabolites and as $^{14}CO_2$ in expired air accounted for 59–68% and 9–13%, respectively of the administered dose. Only 1–2% of the dose was expired by the lungs as VC. Conversely, after 100 mg/kg, 67% of the dose was eliminated by the lungs as VC, while urinary nonvolatile metabolites and $^{14}CO_2$ comprised 11 and 3%, respectively. Pulmonary elimination after 100 mg/kg showed an apparent biphasic clearance with half-times ($t_{1/2}$) of 14.4 and 40.8 min for the respective fast and slow phases. Following 0.05 and 1 mg/kg the pulmonary clearance of VC was monophasic with $t_{1/2}$ of 53.3 and 57.8 min. The percentage of the dose remaining in the carcass after 72 hr was 10, 11, and 2% for the 0.05-, 1- and 100-mg/kg doses, respectively. The urinary radioactivity was separated by high pressure liquid chromatography into three major metabolites. Two of the three major urinary metabolites have been identified as *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine and thiodiglycolic acid by gas chromatography–mass spectrometry. The proportions of the urinary metabolites were not influenced by the dose. The fate of VC following an oral dose between 1 and 100 mg/kg was clearly dose-dependent. Consistent with our previous studies on the fate of VC following inhalation exposure in rats, the metabolism of VC appears to be a saturable process.

The hazard of industrial exposure to vinyl chloride (VC) during the production of polyvinyl chloride and other plastics has received considerable attention. Maltoni and Lefemine (1974) demonstrated the carcinogenic potential of VC in rats exposed daily by inhalation to concentrations ranging from 50–10,000 ppm. Subsequent epidemiologic data obtained from industrial workers with long-term exposure to high concentrations of VC demonstrated an association between exposure and various hepatic abnormalities including induction of angiosarcoma (Creech and Johnson, 1974).

The fate of inhaled VC has been studied previously in rats (Hefner *et al.*, 1975). Although some aspects of the fate of inhaled VC remain to be elucidated, the data indicated that in rats exposed to 50 ppm for 1 hr the inhaled VC was metabolized to polar products which were excreted predominantly in the urine. Using kinetic parameters and inhibitors of drug metabolism, additional evidence indicated that the fate

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of inhaled VC was concentration-dependent and that at least two metabolic pathways were involved in its metabolism. Consideration of these data led to the speculation that the carcinogenic activity of VC may be mediated through formation of alkylating metabolites such as chloroacetaldehyde and chloroethylene oxide. Even more important is the fact that the production of carcinogenic metabolites may increase disproportionately with the extent of exposure: or conversely, the capacity to detoxify alkylating metabolites may decrease disproportionately with the extent of exposure. Resolution of these possibilities is critical in assessing the hazard of exposure to low levels of VC.

The objective of this study was to determine the fate of orally administered VC in rats at various dose levels in order to provide data useful in evaluating the hazard of ingested VC.

METHODS

Compound. [1,2-^{14}C]Vinyl chloride ([^{14}C]VC) was synthesized directly from [1,2-^{14}C]-1,2-dichloroethane (lot no. 819-021, 3.4 mCi/mmol, New England Nuclear, Corp.) by the method of Wagner and Mueider (1975). The radiochemical purity of a representative sample of [^{14}C]VC, from this synthesis, has been reported to be 95–96% pure (Wagner *et al.*, 1975). The primary ^{14}C-containing contaminant (4–5%) in the [^{14}C]VC preparation was [^{14}C]acetylene. Nonlabeled VC (Matheson Gas Products) of 99.9% minimum purity was mixed with the ^{14}C material to obtain the desired specific activity.

Preparation of dose. The [^{14}C]VC was synthesized immediately prior to use. Typically 20 ml of the [^{14}C]VC, helium mixture (approx. 2.6 mCi/mmol) was bubbled directly into 15 g of USP corn oil in a sealed septum vial. An appropriate quantity of nonlabeled VC was then bubbled into the corn oil to obtain the desired concentration. One microliter of the corn oil-dosing solution was subjected to gas chromatography and the final concentration of VC in the corn oil was determined by comparison to standard gas samples.

The VC was analyzed by gas chromatography (Hewlett-Packard Model 5750) on a Porapak Q column (80–100 mesh, 6 ft × ¼ in. stainless steel) with a carrier gas (He) flow rate of 15 ml/min. The flame ionization detector, injection and column temperatures were 280, 250, and 180°C, respectively.

The radioactivity of the dosing solution was determined by placing aliquots (0.050–0.100 g) into prefilled scintillation vials containing 20 ml scintillant, Conciluor (Mallinckrodt Chemical Works), 2-methoxyethanol:toluene, 6:11:83. The radioactivity was determined in a Nuclear Chicago Mark II liquid scintillation spectrometer (Searle, Inc.). External standard channel ratios were used to determine counting efficiency and the counts per minute (cpm) were converted to disintegrations per minute (dpm) with a standard quench curve. The specific activities for the 0.05-, 1- and 100-mg/kg dose solutions were 187.36, 20.50, and 0.18 μCi/mg VC, respectively. The VC–corn oil solution was administered by gavage with a glass syringe and stainless steel dosing needle in a volume not exceeding 5 ml/kg.

Animals and apparatus. Male Sprague–Dawley rats weighing from 180–224 g purchased from Spartan Research were used throughout the studies. All animals were fasted overnight, and the VC was administered between 8 and 10 AM the following morning.

The rats were housed in glass Roth-type metabolism chambers designed for the separate collection of urine, feces, and expired air. Room air was drawn by vacuum through the chambers at 400–500 ml/min. The air leaving the chamber was passed through a series of traps to collect the expired [^{14}C]VC and $^{14}CO_2$.

The air leaving the chamber was first passed 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, immersed in Dry Ice baths) and a single trap containing 120 ml of 5 M ethanolamine in 2-methoxyethanol (room temperature) enabled the collection of [^{14}C]VC and $^{14}CO_2$ respectively.

Experimental procedure. The animals were placed in the modified metabolism cages immediately after dosing. The VC traps were changed at 30-min intervals for the first 4 hr. Two subsequent changes at 4-hr intervals (8 and 12 hr) completed the collection of expired VC. The CO_2 trap, urine (immersed in a Dry Ice bath), and feces receptacles were changed at 12-hr intervals for 72 hr.

At termination of the study the animals were decapitated, exsanguinated and samples of tissue (liver, lung, perirenal fat, muscle, plasma) were collected for analysis of ^{14}C activity. The remaining carcass was skinned and homogenized (50%, w/v) in distilled water.

Sample preparation and assay. Aliquots of the VC traps (5 ml) were prepared for counting by addition of an equal volume of scintillating agent containing Concifluor (Mallinckrodt Chemical Works) 2-methoxyethanol, and toluene (6:11:33).

Five-milliliter samples of the solution from the CO_2 trap were added to 5 ml of 5 M ethanolamine in 2-methoxyethanol and 10 ml of the scintillating agent described above. The urine samples, 250 μl, were prepared by adding 1 ml of distilled water and 12 ml of Aquasol (New England Nuclear).

Aqueous homogenates (33 or 50%, w/v) of feces, tissues, and the remaining carcass were oxidized to CO_2 and H_2O in a Biological Material Oxidizer (Beckman Instruments). The $^{14}CO_2$ from the combustion was trapped in 8 ml of 5 M ethanolamine in 2-methoxyethanol and added to the Concifluor as described previously. Combustion of the samples of skin and fat was performed without homogenization. ^{14}C activity in all samples was determined by scintillation counting.

Isolation of urinary metabolites of VC by high pressure liquid chromatography (hplc). Urine (5 to 10 ml) containing 0.05 to 1.0 μCi of ^{14}C activity (ranging from 9–2000 μg VC equivalents) was lyophylized and the solid residue extracted once with 5 ml of methanol, once with 0.5 ml of water, and twice more with 5 ml of methanol. The solution was centrifuged after each extraction and the clear supernatants removed and combined. The extraction of ^{14}C activity into the combined supernatants was 100 ± 2 (SD)%. The combined supernatant fractions were evaporated to dryness under a stream of nitrogen at room temperature and the residue reconstituted in 1 ml of methanol.

A Corasil II (37–50 μm, Waters Associates, 2-mm i.d. × 50-cm glass) liquid chromatography column was used to separate the ^{14}C-containing urinary metabolites for both identification and routine quantitation. The pumping system used was a Waters Model 660 Solvent Programmer with two Waters Model 6000 Pumps. The flow rate was maintained at 2.0 ml/min while the solvent was programmed to form nonlinear gradient No. 7 from hexane:dioxane (7:1) to 2-propanol:methanol (3:2) over 20 min. The

column temperature was ambient temperature, about 23°C. Typically, from 10–75 μl of methanol solution containing from 1000–150,000 dpm of ^{14}C activity were injected onto the column followed by a hexane:dioxane (7:1) wash for about 10 sec.

The eluant was monitored at 254 or 280 nm using a Chromatronix 220 uv monitor and collected in 2-ml fractions. Aliquots (25–250 μl) of the fractions from the HPLC were combined with 10 ml of Aquasol (New England Nuclear) and the ^{14}C activity determined by liquid scintillation counting. The fractions making up individual peaks of ^{14}C activity were combined, evaporated to dryness under N_2, and dissolved in a small volume of methanol.

A 1-m Porasil B (250) column (Waters Associates) was also used for preparative scale work-up of urine samples for structure identification. The Porasil column was eluted with a linear gradient from hexane:chloroform (1:1) to methanol. The results were similar to those obtained from the Corasil II column except for a higher column capacity and poorer peak resolution. With both columns the recovery of ^{14}C activity was quantitative and the columns could be reused numerous times before peak resolution decreased significantly.

For further purification of Metabolite A additional HPLC was done using alumina. The column was prepared by packing a 2-mm i.d. × 1-m glass column with acidic alumina AG-4 (40 μm, Bio-Rad Laboratories). The column was repacked for each run. The packed column was washed with methanol until a stable baseline was indicated by the uv monitor. About 50-μg VC equivalents of Metabolite A from the initial Porasil separation were injected on the column and eluted with 6% concentrated aqueous NH_4OH in methanol at a flow of 1 ml/min. The eluant was collected in fractions of 1–2.5 ml and an aliquot counted as before. The fractions making up the peak of ^{14}C activity were combined and evaporated to dryness under N_2.

Gas chromatography (gc). The collected eluant fractions from the initial hplc separation on Corasil II or Porasil B(250) were combined to give three fractions containing the three peaks of ^{14}C activity. The first two major peaks, designated Metabolites A and B, were evaporated to dryness under N_2, dissolved in methanol:diethylether (1:1), and methylated using diazomethane. They were then evaporated to dryness and dissolved in methanol to give a concentration of 0.5- to 1-μg VC equivalents and 500–1200 dpm of ^{14}C activity/ml.

The derivatized fractions were chromatographed on one of two columns: (A) 6-ft. × 2-mm i.d. glass packed with 10% UCW-98 on 80/100 Gas Chrom Q, or (B) 6-ft. × 2-mm i.d. glass packed with 3% OV-210 on 80/100 Chromsorb 750. Column A was programmed from 100 to 250°C at 10°C min and column B was run at 125°C isothermally.

The outlet of the column was routed into an effluent splitter using a 5:1 split ratio. One part was fed into the flame ionization detector while five parts exited through a 1 8-in. o.d. stainless steel heated exit line to a fraction-trapping apparatus. A Hewlett-Packard 5750B gas chromatograph was used with the injection port and flame ionization detector maintained at 250 and 275°C, respectively. The helium carrier gas flow rate was 35 ml/min.

When fractions were trapped from the gc for counting of ^{14}C-labeled metabolites, the glass capillary containing the condensed metabolite was washed into a scintillation vial using 2 ml of Aquasol. Eight additional milliliters of Aquasol were added to the vial for counting.

Mass spectroscopy (ms). Low resolution mass spectra were run on a Finnigan Model 3000D gc-ms operating at 70 keV using both direct insertion probe and gc inlets interfaced with the Model 6000 ms data system. When using the gc inlet the columns and gc conditioned were identical to those previously given. When using the direct insertion probe, samples (about 1 μg of metabolite) were placed in a quartz cup and the temperature of the probe was slowly raised from ambient to 250°C.

High resolution gc–ms were run on an AEI MS-30/DS-50 double beam mass spectrometer at Dow Corning Analytical Services. The resolution was 4300 and the mass measuring accuracy was generally within ±0.005 mass units over the range of interest. The gc conditions were as previously described.

Synthetic metabolite standards. Samples of *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine and thiodiglycolic acid were synthesized by N. Peet of Dow Lepetit, Pharmaceutical R & D. Samples of ^{14}C-labeled *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine and thiodiglycolic acid were synthesized by D. Gransden of Dow Environmental Sciences Research.

RESULTS

Disposition of [^{14}C]VC in Rats. Excretion of ^{14}C activity within 72 hr following a single oral dose of 0.05, 1, and 100 mg kg [^{14}C]VC is shown in Table 1. The percentage

TABLE 1

PERCENTAGE OF ADMINISTERED ^{14}C ACTIVITY RECOVERED FOLLOWING A SINGLE ORAL DOSE OF VINYL CHLORIDE (VC)[a]

	Dose (mg/kg)		
	0.05	1.0	100
Expired:			
As VC	1.43 ± 0.13[b]	2.13 ± 0.22	66.64 ± 0.67
As CO_2	8.96 ± 0.59	13.26 ± 0.47	2.52 ± 0.13
Urine	68.34 ± 0.54	59.30 ± 2.75	10.84 ± 0.95
Feces	2.39 ± 0.52	2.20 ± 0.39	0.47 ± 0.06
Carcass and tissues	10.13 ± 1.93	11.10 ± 0.47	1.83 ± 0.14
Cage wash[c]	0	0.84 ± 0.45	0
Total recovery	91.25 ± 2.47	88.83 ± 1.98	82.30 ± 0.43

[a] Percentage of dose excreted over 72 hr. Only the ^{14}C activity associated with the expired VC can be attributed to VC per se.

[b] Mean ± SE five rats per dose.

[c] Distilled water wash of metabolism cage at termination of the study.

of the dose expired as VC per se was 1, 2, and 67%, respectively. Due to the disproportionate pulmonary elimination of VC, a greater percentage of the dose was metabolized and eliminated in the urine, feces, and as expired $^{14}CO_2$ by rats given 0.05 and 1 mg/kg than by rats given 100 mg kg. No special precautions were taken to insure detection of volatile compounds when collecting and processing the urine, feces, and carcass. Thus, the ^{14}C activity in the excreta and carcass represent nonvolatile metabolites of [^{14}C]VC. The overall recovery of ^{14}C activity was 91.3, 88.8, and 82.3% at the 0.05-, 1-, and 100-mg kg dose levels, respectively. The primary radiochemical contaminant in the [^{14}C]VC preparation was ^{14}C-labeled acetylene (4–5%). Due to the

physicochemical properties of acetylene, the solvent cold traps used for the collection of expired VC would not trap the highly volatile [^{14}C]acetylene. Based on this assumption the total recovery of ^{14}C activity due solely to VC would be slightly higher than expressed in Table 1.

The characteristic pattern of pulmonary elimination of VC differed greatly between rats given 0.05 or 1 mg/kg than those given 100 mg/kg (Fig. 1). During the first 4 hr after administration of 100 mg/kg, the pulmonary elimination of VC was biphasic. The two linear portions of the curves were determined by regression analysis of the logarithmically transformed data. The data were feathered to obtain an approximation of the rate constant for the rapid phase of elimination. The apparent first-order rate

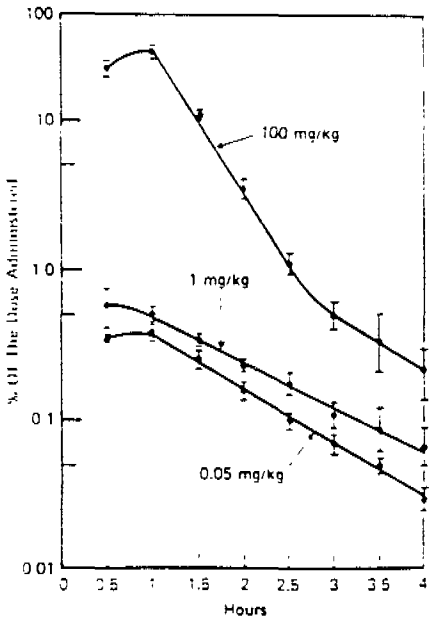


FIG. 1. Expired vinyl chloride expressed as percentage of the dose administered (0.05, 1, and 100 mg/kg) versus time (hr). Each point represents the mean ± SE of the mean of five rats. The linear phases of the curves were fit by linear regression analysis.

constants for the rapid and slow phases were 0.048 ± 0.005 and 0.017 ± 0.008 min^{-1} (±SD). These rate constants correspond to half-lives of 14.4 and 40.8 min. Following the two low doses of 0.05 and 1 mg/kg, pulmonary elimination of VC was monophasic with apparent first-order rate constants of 0.013 ± 0.001 and 0.012 ± 0.001 min^{-1} (±SD) corresponding to half-lives of 53.3 and 57.8 min, respectively. To assure that the ^{14}C activity collected in the cold traps was VC, the trapping solutions were analyzed by gas chromatography. Other than the components of the trapping solution the only compound detected had an identical retention time as a standard gas sample of VC.

The elimination of ^{14}C activity in the urine as a function of time after 1 and 100 mg/kg is shown in Fig. 2. The initial linear portions of the excretion curves from 12–36 hr were fit by regression analysis of the logarithmically transformed data. Similar biphasic elimination was evident at all dose levels. The curve for the 0.05-mg/kg dose was essentially identical to the 1-mg/kg dose and therefore was not graphically represented.

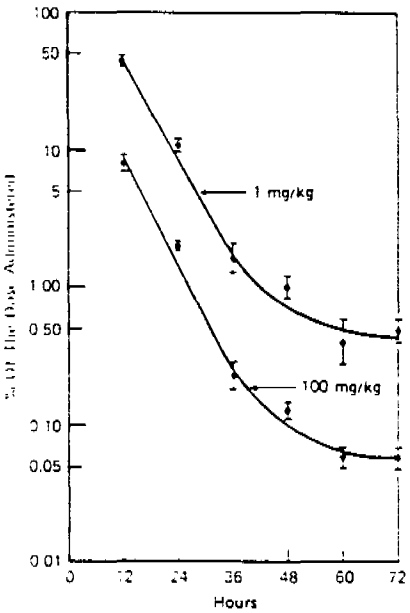


FIG. 2. ^{14}C activity excreted in the urine expressed as percentage of the dose administered (1 and 100 mg/kg) versus time (hr). Each point represents the mean ± SE of the mean for five rats. The initial linear segments of the curves (12–36 hr) were fit by linear regression analysis.

For rats given 0.05, 1, and 100 mg/kg, respectively, estimates of the apparent first-order rate constants for the initial phase of elimination were 0.155 ± 0.006, 0.150 ± 0.020, and 0.152 ± 0.011 (±SD) hr^{-1}. These correspond to half-lives of 4.5, 4.6, and 4.6 hr. The data for the secondary phase of the urinary excretion curves were extremely variable and since this phase accounted for less than 3% of the total urinary radioactivity, no attempt was made to estimate the rate constants.

The liver contained the highest concentration of ^{14}C activity after 72 hr at all dose levels (Table 2). The concentration in the liver expressed on a percentage dose per gram

TABLE 2

PERCENTAGE OF THE ADMINISTERED ^{14}C ACTIVITY PER GRAM OF TISSUE AFTER ADMINISTRATION OF [^{14}C]VINYL CHLORIDE[a]

Tissue	Dose (mg/kg)		
	0.05	1.0	100
Liver	0.172 ± 0.025[b]	0.182 ± 0.005	0.029 ± 0.002
Skin	0.070 ± 0.023	0.076 ± 0.010	0.010 ± 0.002
Carcass	0.027 ± 0.007	0.046 ± 0.002	0.007 ± 0.001
Plasma	0.041 ± 0.004	0.053 ± 0.007	ND[c]
Muscle	0.028 ± 0 003	0.031 ± 0.003	0.006 ± 0.001
Lung	0.050 ± 0.003	0.061 ± 0.003	0.011 ± 0.001
Fat	0.030 ± 0.004	0.045 ± 0.008	0.006 ± 0.001

[a] Remaining in the body after 72 hr.
[b] Mean ± SE, five rats per dose.
[c] Not detectable above background.

tissue basis was three- to fivefold greater than muscle, lung, or fat. Consistent with the proportionally greater metabolism at the 0.05- and 1-mg/kg level, the proportion of the dose remaining in the tissues after 72 hr was considerably higher in rats given the low doses than those given 100 mg/kg.

Isolation and identification of urinary metabolites of VC. Using hplc on a Corasil II column, methanol extracts of urine from rats given 0.05 to 100 mg/kg [^{14}C]VC orally

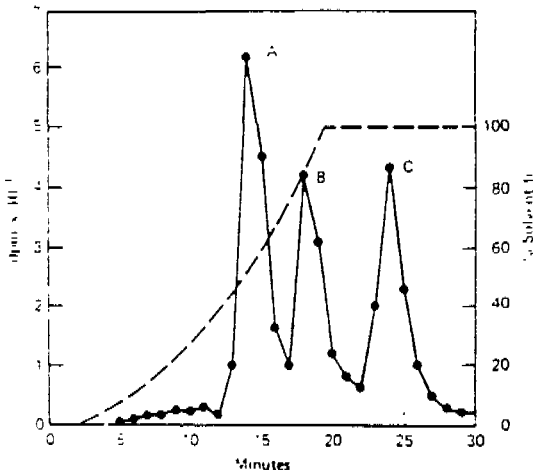


FIG. 3. Separation of urinary metabolites by high pressure liquid chromatography (hplc) on a Corasil II column. The profile formed by the solid line shows the separation of radioactivity representing metabolites A, B, and C, by hplc versus time (min, flow rate = 2 ml/min). The dashed line depicts the nonlinear gradient profile used to effect the separation expressed as percentage solvent b. Solvent a, hexane:dioxane (7:1); solvent b, 2-propanol:methanol (3:2).

were separated into three major peaks containing about 95% of the applied ^{14}C activity and several minor peaks. Figure 3 shows a chromatogram of a typical sample. The metabolites were designated by their elution order from the column as A, B, and C. The proportions of radioactivity determined by the three metabolites were not influenced by the dose (Table 3).

TABLE 3

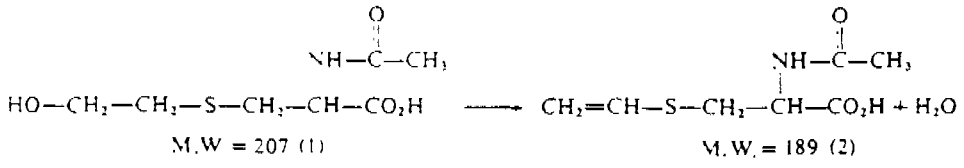
SEPARATION OF ^{14}C-CONTAINING URINARY METABOLITES FROM RATS GIVEN VINYL CHLORIDE[a]

Compound	Dose (mg/kg)		
	0.05(4)[b]	1.0(5)	100(5)
(A) N-acetyl-S-(2-hydroxyethyl)-cysteine	30.4 ± 2.0[c]	36.2 ± 3.9	29.1 ± 2.0
(B) Thiodiglycolic acid	25.6 ± 1.9	23.7 ± 1.1	25.4 ± 0.9
(C) Unidentified	38.6 ± 2.9	34.5 ± 4.6	36.6 ± 2.0
Total	94.6	94.4	91.1

[a] Metabolites were separated and quantitated by high pressure liquid chromatography. Values are expressed as percentage of total urinary radioactivity.
[b] ( ) = Number of animals per dose.
[c] Mean ± SE.

Additional purification of the metabolite A fraction from the initial Corasil II or Porasil separation was carried out on an acidic alumina hplc column with a methanol: aqueous NH_4OH eluant. The ^{14}C activity eluted as a single peak containing over 90% of the applied radioactivity. A mass spectrum of the combined fraction was determined using a direct sample introduction probe. The sample was shown to have an apparent weak ion of $m/e = 189$ and a prominent peak at $m/e = 130$. Earlier work with *S*-(2-hydroxyethyl)-cysteine had shown that the highest mass peak found corresponded to the dehydrated molecular ion (M-18).

If a similar dehydration had occurred in the metabolite, the mass spectrum obtained corresponded to that expected for *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine (1).



The mass spectrum of a synthesized sample of *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine was found to be virtually identical to that of the material found in the Metabolite A fraction (Fig. 4).

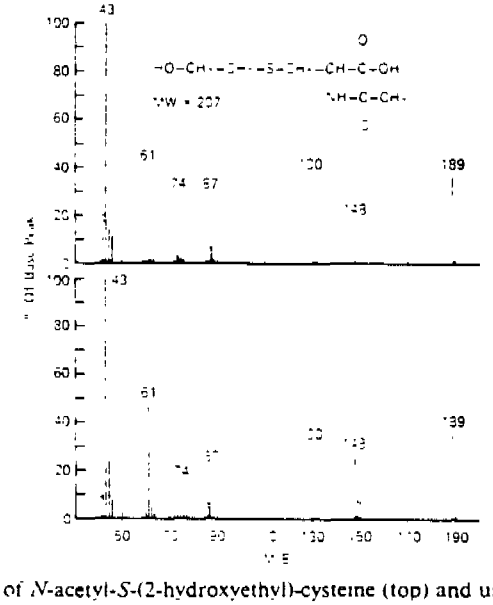


FIG. 4. Mass spectra of *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine (top) and urinary Metabolite A (bottom). All peaks *m/e* greater than 140 were expanded by a factor of 10.

Using a 10% UCW-98 column programmed from 120 to 250°C, the methylated Metabolite A fraction was shown to contain a peak which had the identical retention time and mass spectrum as that of the methyl ester of the previously synthesized *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine standard.

Additional confirmation of the identity of Metabolite A was obtained by coinjection of a synthetic ^{14}C-labeled *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine with the urinary

metabolites using hplc. It was found that the added ^{14}C activity coeluted quantitatively with Metabolite A on both the Corasil II and acidic alumina columns described previously.

When the methylated Metabolite B fraction from the initial Porasil separation was run on the gas chromatograph it was possible to associate a single peak in the chromatogram with the ^{14}C activity in the sample by trapping the material at the column outlet. The best separation was obtained using an OV-210 column at 125°C (isothermal). Initial gc–ms analysis of methylated Metabolite B indicated that it was a sulfur containing carboxylic acid. By high resolution gc–ms Metabolite B was shown to be a carboxylic acid (methyl ester) with a nominal molecular weight 178 and molecular formula $C_6H_{10}O_4S$. A listing of the important peaks is shown in Table 4.

TABLE 4

HIGH RESOLUTION gc–ms OF METABOLITE B

m/e	Molecular formula	Structure[a]
178.0302	$C_6H_{10}O_4S$	$[CH_3O{-}C{-}CH_2{-}S{-}CH_2C{-}OCH_3]^+$
146.0064	$C_5H_6O_3S$	$[M^a$ minus $-CH_3OH]^+$
119.0165	$C_4H\text{-}O_2S$	$[M^a$ minus $-C{-}OCH_3]^+$
118.0127	$C_4H_6O_2S$	
91.0247	$C_3H\text{-}OS$	$[-CH_2{-}S{-}CH_2{-}O{-}CH_3]^+$
74.0393	$C_3H_6O_2$	$[CH_3C{-}OCH_3]^+$
61.0142	C_2H_5S	
59.0118	$C_2H_3O_2$	$[{-}C{-}OCH_3]^+$
45.9905	CH_2S	
45.0379	C_2H_5O	$[CH_2CH_2OH]^+$

[a] Molecular ion.

When a sample of thiodiglycolic acid (dimethyl ester) (3) was run on the Finnigan gc–ms, the mass spectrum was found to be identical to that of Metabolite B (Fig. 5).

$$CH_3O{-}C{-}CH_2{-}S{-}CH_2{-}C{-}OCH_3$$

(3)

DISCUSSION

The results of the current study establish that the fate of VC following ingestion by rats is dose-dependent. Following a dose of 0.05 or 1 mg kg $[^{14}C]$VC, most of the ^{14}C activity was excreted in the urine as nonvolatile metabolites and as $^{14}CO_2$ in expired air. After 100 mg kg, the predominant mode of excretion was by expiration of VC. There-

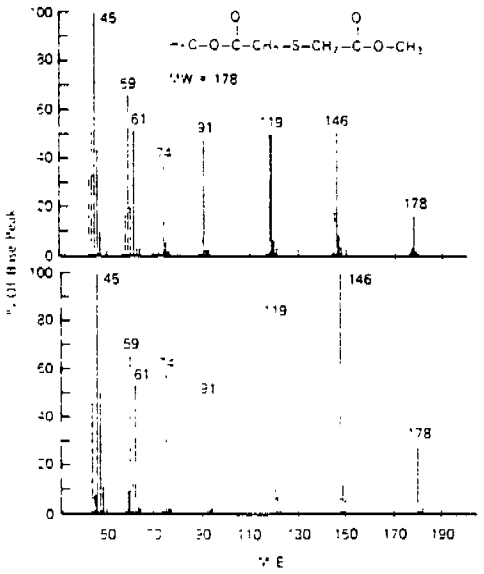


FIG. 5. Mass spectra of the methyl ester of thiodiglycolic acid (top) and the methyl ester of metabolite B.

fore, it appears that the metabolism of VC is a dose-dependent, saturable process. Similar results on the excretion of [^{14}C]VC following oral ingestion in rats have been found recently by T. Green and D. E. Hathway (personal communication).

The fate of VC following oral administration is consistent with its fate after inhalation (Hefner *et al.*, 1975). In those studies, the rate of VC metabolism was more rapid in rats exposed to 100 ppm or less than in rats exposed to 220 ppm or greater. Because of the experimental procedure used by Hefner *et al.* (1975), evidence was obtained indicating that at least two pathways may be involved in the metabolism of VC and that the degree of their involvement was concentration-dependent.

Recently, R. J. Withey (personal communication) has found a biphasic clearance of VC from the plasma of rats after cessation of inhalation exposure to concentrations of 500 to 7000 ppm or after iv injection of 50 to 75 mg/kg VC. The half-lives of the biphasic process were 4 to 9 min and approx. 40 min. These results deviate to some degree from those reported here. In this study the biphasic pulmonary excretion of VC following a dose of 100 mg/kg had half-lives of 14.4 and 40.8 min for the two phases. The half-lives for the slow component are similar. With regard to the differences for the half-lives of the first phase, 4 to 9 min vs 14.4 min, our value may be somewhat slower because of delayed absorption from the gastrointestinal tract. In any case, the results of this study confirm and complement those of R. J. Withey (personal communication).

Of paramount importance to assessing the hazard of exposure to VC is the fate of that portion metabolized. In the previous study (Hefner *et al.*, 1975) it was speculated that potential alkylating metabolites such as chloroacetaldehyde and chloroethylene oxide may be formed *in vivo* from VC. Two of the three major urinary metabolites of VC have been identified as *N*-acetyl-*S*-(2-hydroxyethyl)-cysteine and thiodiglycolic acid. The identification of these metabolites is consistent with the proposed pathways for metabolism of VC. Chloroacetaldehyde and chloroethylene oxide will conjugate with gluta-

thione and cysteine leading ultimately to the types of metabolites identified in the urine. Göthe (1974) reported recently the trapping of acetaldehyde formed from VC by an *in vitro* microsomal preparation. This provides additional support for the formation of chloroacetaldehyde.

Recently, T. Green and D. E. Hathway (personal communication) have shown that after multiple dosing of [^{14}C]VC in rats (50 mg/kg orally, three times at 3-hr intervals), thiodiglycolic acid was the major metabolite of vinyl chloride (about 47% of the total urinary ^{14}C activity). The present work confirms that thiodiglycolic acid is one of the major metabolites of vinyl chloride.

Two other major urinary metabolites identified by T. Green and D. E. Hathway (personal communication) were *S*-(2-chloroethyl)cysteine and its acetylated analog, *N*-acetyl-*S*-(2-chloroethyl)cysteine. Since we have identified a major metabolite as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine the question is raised whether the vinyl chloride metabolites exist in the urine as hydroxyethyl or chloroethyl conjugates.

S-(2-chloroethyl)-Cysteine is a monofunctional sulfur mustard and has been shown to be mutagenic (Fahmy and Fahmy, 1970). It seems doubtful, however, that it could be detected intact in the urine even if formed because of its susceptibility to hydrolysis to *S*-(2-hydroxyethyl)-cysteine. Jones (1973) reviewing the metabolism of 1,2-dibromoethane reported that the initially formed *S*-(2-bromoethyl)-glutathione is unstable and spontaneously hydrolyzes to *S*-(2-hydroxyethyl)-glutathione producing *S*-(2-hydroxyethyl)-cysteine as the primary metabolite. A similar hydrolysis would be expected for the chloroethyl conjugate. More direct evidence for the instability of *S*-(2-chloroethyl)-cysteine is its reported half-life of 7 min in aqueous solution at 37°C and pH 7 (Ross, 1962).

T. Green and D. E. Hathway (personal communication) identified the vinyl chloride metabolites by preparing the *N*-trifluoroacetyl *n*-butyl esters by the method of Gehrke and Stalling (1967). This involves the formation of the methyl ester using 1.25 M HCl (gas) in methanol at room temperature followed by the transesterification to the *n*-butyl ester using 1.25 M HCl (gas) in *n*-butanol at 100°C. It has been reported by Connors and Ross (1958) and Carson and Wong (1964) that *S*-(2-chloroethyl)-cysteine can be prepared by heating *S*-(2-hydroxyethyl)-cysteine with concentrated HCl. It seems likely, therefore, that if the urinary vinyl chloride metabolites were present as *S*-(2-hydroxyethyl)-cysteine and its acetylated analog they may be converted into the corresponding chloroethyl compounds by the derivatization procedure used by T. Green and D. E. Hathway (personal communication).

Therefore, it appears that the vinyl chloride metabolites identified by T. Green and D. E. Hathway (personal communication) correspond to those identified in this study but that the chloroethyl conjugates were in fact artifacts of the derivitization procedure. This does not, however, rule out the initial formation of the chloroethyl conjugate in the animal followed by hydrolysis to the corresponding hydroxyethyl compounds before excretion.

As a final point for discussion, it is important to consider how the data gathered by this laboratory and others may relate to assessing the hazard of exposure to VC. Such a discussion has been made previously (Hefner *et al.*, 1975). Data gathered since that report continue to support the hypothesis that the carcinogenicity of VC is related to the metabolic formation of alkylating metabolites. Numerous studies have reported

the enhancement of the positive mutagenic response in *Salmonella typhimurium* exposed to VC if microsomal enzymes or fortified liver homogenates are present (Rannug *et al.*, 1974; Bartsch *et al.*, 1975; Malavielle *et al.*, 1975).

The metabolites of VC identified in the urine indicate that the primary deactivating mechanism is by conjugation with the nonprotein free sulfhydryl compounds, glutathione and cysteine. Studies in this laboratory have shown that the nonprotein free sulfhydryl groups of the liver are depleted in rats exposed to VC both as a function of concentration and exposure duration (Watanabe *et al.*, 1976). As the nonprotein free sulfhydryl concentrations are depleted, the alkylating metabolites are more likely to react with protein, DNA, and RNA, eliciting proportionally greater toxicity. This phenomena has been demonstrated to markedly influence the toxicity of compounds such as bromobenzene, furosemide, and acetaminophen (Gillette, 1974a,b; Mitchell *et al.*, 1973; Jollow *et al.*, 1974). The threshold for the toxicity of these materials coincides with their reactions with tissue macromolecules (protein, DNA, and RNA). Reactions with these cellular components and discernible toxicity occurs only after the glutathione content is sufficiently depleted to preclude deactivation of the reactive metabolites of these agents.

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