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Pharmacokinetics of Vinylidene Chloride in the Rat

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The metabolism of inhaled vinylidene chloride in rats represents a balance of biotransformation pathways leading to the formation of a reactive alkylating species which is normally detoxified by conjugation with glutathione. Detoxification of the reactive intermediate formed from inhaled VDC is dependent upon the availability of hepatic glutathione (GSH); as VDC exposure concentrations are increased, the fraction of the dose detoxified by conjugation with GSH decreases markedly, commensurate with depletion of hepatic GSH. This reactive intermediate in the absence of GSH alkylates hepatic macromolecules and causes cell death. Similarly, hepatic GSH plays a vital role in the detoxification of the reactive metabolite formed from inhaled vinyl chloride (VC). However the dose–response relationships for the utilization of GSH and the accumulation of alkylating metabolites following inhalation exposure to either VDC or VC point to distinct differences which may explain the differing biological activities of the two materials. Finally, preliminary pharmacokinetic data for inhaled VDC in mice indicate an enhanced susceptibility to VDC by virtue of an increased ability for production of alkylating VDC metabolites over that observed in the rat. The importance of these findings in light of recent evidence for a carcinogenic effect of VDC in mice is discussed.

Introduction

Vinylidene chloride (1,1-dichloroethylene, VDC) is used extensively as a monomeric intermediate in the production of plastics. Studies by Jaeger et al. (*1*) have demonstrated that acute inhalation exposure to VDC results in a decrease in liver glutathione (GSH) concentrations of rats exposed to high VDC concentrations by inhalation. Further, VDC-induced hepatotoxicity is enhanced when hepatic GSH levels are lowered by fasting for 18 hr prior to VDC exposure.

Previous studies conducted in this laboratory (*2*) have described the pharmacokinetics and metabolism of VDC after oral and inhalation exposure of rats to ^{14}C-VDC. In these studies the fate of VDC was shown to be dependent upon the magnitude of the dose. An 18-hr fast prior to VDC administration decreased the excretion of VDC urinary metabolites associated with the GSH metabolic pathway in rats given a 50 mg/kg oral dose of VDC. The diminished ability of fasted rats to detoxify VDC correlates with the enhancement of VDC-induced toxicity in fasted rats observed by Jaeger, et al. (*1*).

The results of previous experiments have led to the hypothesis that the hepatotoxicity of VDC is mediated by a reactive electrophilic metabolite of VDC. At low levels of exposure this reactive intermediate is detoxified by conjugation with GSH. Exposure to toxic concentrations of VDC results in depletion of hepatic GSH and consequently the alkylation of tissue macromolecules rather than GSH. The toxicity of VDC may also be enhanced if GSH levels are depleted, by fasting or other means, prior to VDC exposure.

Vinyl chloride (VC) is also biotransformed to an intermediate which is detoxified by conjugation with GSH. The dose-dependent pharmacokinetics have been described in studies of vinyl chloride (VC) pharmacokinetics and metabolism (*3*, *4*). Similarities in the chemical structures of VC and VDC notwithstanding, marked differences in the biological actions of the two materials have been observed which may be accounted for by differences in their pharmacokinetics and metabolism in laboratory animals. Therefore the purpose of this

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report is to summarize our recent experiments on the fate of inhaled VDC in rats and provide a comparison with similar data for VC. In addition, preliminary data on the fate of VDC in mice are also presented.

Results and Discussion

Fate of ^{14}C-Vinylidene Chloride in the Rat Following Inhalation Exposure

Male Sprague-Dawley rats (normally fed or fasted for 18 hr) were exposed to 10 or 200 ppm ^{14}C-VDC for 6 hr. Immediately after exposure the animals were placed in individual glass metabolism cages and the elimination of ^{14}C-activity was followed for 72 hr. A detailed description of the experimental procedure has been published previously (*4*). Maintenance of a quantitative collection system for ^{14}C-activity and analysis of residual radioactivity at termination of the experiment (72 hr) enabled the calculation of a total ^{14}C-balance for each animal. The total recovery of ^{14}C-activity from each rat was assumed to approximate the total amount of ^{14}C-activity in the body (body burden) at the end of the inhalation exposure.

The body burden of ^{14}C-activity, inclusive of VDC *per se* and metabolites formed from VDC, for rats exposed to 10 or 200 ppm ^{14}C-VDC for 6 hr are given in Table 1. Little difference between fed and fasted rats was observed in the net retention and metabolism of ^{14}C-VDC during the 6-hr inhalation exposure to 10 ppm ^{14}C-VDC. However, fasted rats exposed to 200 ppm ^{14}C-VDC had significantly lower body burdens of ^{14}C-VDC following exposure than did fed rats of the same exposure group. Furthermore the increase in body burdens of both fed and fasted rats at 200 vs 10 ppm was less than the proportional increase in the VDC exposure concentration. Thus, if biotransformation of ^{14}C-VDC was a major factor in the accumulation of ^{14}C-activity during inhalation exposure, the data indicate that the capacity for VDC metabolism may have been exceeded at the higher VDC exposure level. This dose-dependent effect was enhanced by fasting prior to VDC inhalation exposure. Fasted rats exposed to 200 ppm ^{14}C-VDC showed definite signs of VDC-induced liver and kidney toxicity following exposure. These effects, characterized by hepatocellular degeneration and necrosis as well as proximal renal tubular epithelial degeneration, were evident only in fasted animals exposed to 200 ppm VDC. Microscopic examinations of liver and kidney tissues from all other animals were normal. Since the total amount of VDC biotransformed by fed rats exposed to 200 ppm was greater than that observed in fasted rats, the data clearly indicate that fasting augments the process whereby reactive intermediates formed from VDC induce tissue damage.

Table 1. Body burdens of ^{14}C-activity in rats exposed to 10 or 200 ppm ^{14}C-VDC for 6 hr.

	Exposure concentration, ppm	Body burden, mg-eq ^{14}C-VDC/kg[a]
Fed rats	10	2.89 ± 0.12
Fasted rats		2.30 ± 0.05
Fed rats	200	44.53 ± 3.05
Fasted rats		35.93 ± 0.30[b]

[a]$\bar{X}$ ± SE, n = 4. Defined as the total ^{14}C-activity (VDC plus metabolites) in the body at the end of the inhalation exposure.
[b]Significant fed-fasted difference, $p < 0.05$, t-test.

The disposition of ^{14}C-activity by rats exposed to ^{14}C-VDC for 6 hr is shown in Table 2. The elimination of ^{14}C-activity via the lungs was totally accounted for as unchanged ^{14}C-VDC and $^{14}CO_2$. No additional unchanged ^{14}C-VDC or volatile ^{14}C-activity was found in urine, feces, or tissues, indicating that the radioactivity in these samples represented nonvolatile metabolites of ^{14}C-VDC.

Table 2. Recovery of ^{14}C-activity from rats during 72 hr following ^{14}C-VDC exposure for 6 hr.

	Body burden, %[a]			
	10 ppm		200 ppm	
	Fed rats	Fasted rats	Fed rats	Fasted rats
Expired VDC	1.63	1.60	4.17[b]	8.36[c]
$^{14}CO_2$	8.74	8.27	8.22	7.24
Urine	74.72	78.19	74.66	70.41
Feces	9.73	6.75	6.39	2.72
Carcass	4.75	5.28	6.18[b]	10.52[c]
Cage wash	0.44	0.27	0.34	0.76

[a]$\bar{X}$ of 4 rats/group. Expressed as the percentage of the end-exposure body burden values given in Table 1.
[b]Significant difference from 10 ppm group, $p < 0.05$.
[c]Significant fed-fasted difference, $p < 0.05$.

The data in Table 2 are presented as the percentage of the total ^{14}C-activity eliminated during 72 hr after exposure plus that remaining in the tissues at 72 hr following the inhalation exposure. If all of the processes involved in the disposition of ^{14}C-VDC in the rat could be described by first-order pharmacokinetics, i.e., if the rate constants for all processes were independent of the amount of VDC available, then the proportions of ^{14}C-activity eliminated or retained in the body would be the same for all animals regardless of the ^{14}C-VDC exposure concentration. The data in Table 2 indicate clearly that the fate of ^{14}C-VDC is dependent upon both the VDC exposure concentration and the physiological status of the rats.

Rats exposed to 200 ppm ^{14}C-VDC exhaled a greater percentage of their acquired body burden of ^{14}C-activity as unchanged ^{14}C-VDC than did the animals exposed to 10 ppm ^{14}C-VDC. More importantly, rats exposed to the higher concentration of VDC showed a greater percentage of the body burden remaining in the carcass at 72 hr after exposure. Retention of ^{14}C-activity in the carcass was greater in fasted rats exposed to 200 ppm ^{14}C-VDC, despite a smaller fraction of the body burden biotransformed by fasted than fed rats (Table 1). The increased elimination of unchanged VDC with increasing exposure concentrations indicates that the biotransformation of ^{14}C-VDC may be a saturable process. The effect is enhanced by fasting prior to exposure to ^{14}C-VDC, indicating that fasting prior to VDC exposure reduces the capacity for biotransformation and detoxification of VDC in the rat.

A comparison of the retention and metabolism of ^{14}C-VDC with that for ^{14}C-vinyl chloride (^{14}C-VC) is shown in Table 3. Inhalation exposures of normally fed rats to both radio-labeled chemicals were conducted under identical conditions. Values are given in micromole equivalents of ^{14}C-VDC or ^{14}C-VC per kilogram body weight to facilitate comparison of the data. Rats exposed to 10 ppm ^{14}C-VDC acquired approximately twice the body burden of those exposed to 10 ppm ^{14}C-VC. Although the percentage of the end-exposure body burden metabolized to nonvolatile ^{14}C-metabolites was comparable in both the VDC- and VC-exposed animals, the greater accumulation of ^{14}C-activity during exposure to ^{14}C-VDC suggests a faster rate of biotransformation for VDC in the rat than for VC. The reduction in the percentage of the body burden metabolized following the higher exposure concentrations of VDC and VC indicates the dose-dependent character of the pharmacokinetics of both materials.

Table 3. Body burdens and metabolism of ^{14}C-VC and ^{14}C-VDC in rats after inhalation exposure for 6 hr.[a]

	Exposure concentration, ppm	Body burden, μmole/kg	Metabolized, μmole/kg[b]	% Metabolized
VDC	10	30.10 ± 1.25	29.58 ± 1.35	98
	200	463.85 ± 63.02	445.10 ± 33.13	96
VC	10	15.97 ± 0.81	15.65 ± 0.81	98
	1000	433.23 ± 14.03	380.48 ± 16.29	88

[a] $\bar{X}$ ± SE, n = 4.
[b] Calculated as the body burden minus the ^{14}C-VDC or VC expired unchanged.

Role of Glutathione in VDC Metabolism

As mentioned previously, studies by Jaeger et al. (*1*) and those in this laboratory (*2*) were consistent with the hypothesis that hepatic glutathione plays a major role in the detoxification of VDC. Evidence for the conjugation of VDC or some reactive metabolite of VDC with glutathione was obtained in these studies by analysis of the urinary metabolites of ^{14}C-VDC in the rat. High pressure liquid chromatography of rat urine on a strong anion exchange column gave four major peaks of ^{14}C-activity, as shown in Figure 1. The chromatographic profiles of urinary ^{14}C-activity were qualitatively similar among all animals regardless of ^{14}C-VDC exposure concentration or pretreatment (fed vs. fasted rats). Metabolites B and C were identified by gas chromatography-mass spectrometry as *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycolic acid, respectively. The mass spectra of these metabolites were identical to those of synthesized reference materials. Their identification was further substantiated by quantitative coelution of the ^{14}C-labeled reference materials with the urinary metabolites using high pressure liquid chromatography. The presence of these mercapturic acid derivatives in rat urine following VDC exposure substantiates the proposed conjugation with glutathione as a major detoxification pathway for VDC. Combined, these two urinary metabolites account for 40–50% of the total urinary ^{14}C-activity following ^{14}C-VDC exposure. Both metabolites have also been identified as major metabolites in rat urine following inhalation exposure to ^{14}C-VC. Efforts to identify the two remaining urinary metabolites of VDC are still in progress.

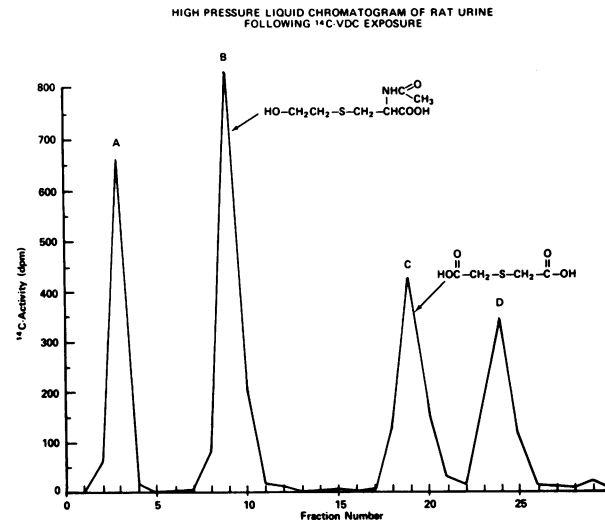


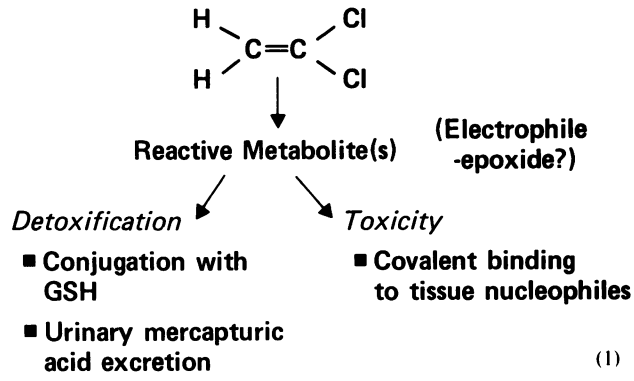
FIGURE 1. Typical separation of rat urinary ^{14}C-activity by high-pressure liquid chromatography, into four major fractions of urinary ^{14}C-activity: (*A*) unknown; (*B*) *N*-acetyl-*S*-(2 hydroxyethyl)cysteine; (*C*) thiodiglycolic acid; (*D*) unknown. Recovery of ^{14}C-activity from the column was 99.3 ± 1.65% ($\bar{X}$ ± SD, n = 30).

Metabolism of VDC Associated with Toxicity

In several instances, alkylation of tissue subcellular macromolecules either directly or by a reactive metabolite has been demonstrated to precede chemically induced tissue necrosis. Both the biotransformation of VDC to a reactive intermediate and its acute hepatotoxic action favored the possibility that evaluation of this phenomenon may prove a useful tool in assessing the biotransformation of VDC as associated with VDC induced hepatotoxicity. Initial experiments were conducted in the same animals employed in the ^{14}C-balance studies described earlier. Hepatic tissue obtained from rats 72 hr after ^{14}C-VDC exposure was repeatedly extracted as described by Jollow et al. (*5*). Values for nonextractable or so-called "covalently bound" ^{14}C-activity in hepatic tissue of rats exposed to 10 or 200 ppm ^{14}C-VDC are shown in Table 4. Values for total metabolism of ^{14}C-VDC are also presented to allow evaluation of the relationship between covalent binding and overall metabolism of ^{14}C-VDC. Metabolized ^{14}C-VDC was calculated from the total and end-exposure body burden minus the ^{14}C-activity expired as VDC. Fed rats showed a 15-fold increase in biotransformed ^{14}C-VDC with the 20-fold increase in exposure concentration (from 10 to 200 ppm). However, the concentration of covalently bound ^{14}C-activity in the liver increased approximately 26-fold, appreciably greater than the observed increase in VDC metabolism. Fasted rats exposed to 200 ppm ^{14}C-VDC metabolized less VDC than did fed rats. However, the concentration of covalently bound ^{14}C-activity in the liver was greater in fasted than in fed rats following the 200 ppm exposure. The increase in covalent binding in the livers of fasted rats was more apparent after normalization of the data to account for differences in metabolism of VDC (B/A ratio).

The data presented in Table 4 indicate that an increase in the covalent binding of VDC or some reactive metabolite to hepatic tissue is associated with VDC hepatotoxicity. The greater increase in macromolecular binding relative to that observed for metabolism of VDC with increasing exposure concentrations or after fasting may result from overwhelming the capacity to detoxify the reactive intermediate formed from VDC. Therefore as the capacity to detoxify VDC is exceeded (i.e., as tissue GSH stores are depleted), covalent binding to tissue constituents is enhanced.

The results of the experiments described above were formulated into a working hypothesis for the biotransformation of VDC which is represented schematically in Eq. (1). Apparently, VDC metabolism represents a balance between biotransformation pathways leading to detoxification via GSH or to covalent binding and subsequent tissue damage. Evidence for the initial biotransformation of VDC to one or more reactive intermediates has been discussed in several papers presented in this symposium. In addition experiments conducted on liver homogenates in our own laboratory have demonstrated that VDC conjugation with GSH requires the presence of a microsomal enzyme system. We have been unable to demonstrate conjugation of VDC with GSH either directly or using only the soluble fraction of a liver homogenate (GSH alkyl transferases) *in vitro*.



(1)

Table 4. Metabolism of ^{14}C-VDC and covalent binding of ^{14}C-activity to rat hepatic tissue after inhalation exposure to ^{14}C-VDC.[a]

Exposure concn, ppm	Pretreatment	(A) Metabolized ^{14}C-VDC mg-eq/kg	(B) ^{14}C-VDC bound, μg-eq/liver protein	(B)/(A)
10	Fed	2.84 ± 0.13	2.49 ± 0.17	0.88 ± 0.08[b]
	Fasted	2.26 ± 0.06	2.47 ± 0.29	1.10 ± 0.15
200	Fed	42.73 ± 3.18	64.18 ± 7.97	1.49 ± 0.10
	Fasted	32.92 ± 0.32[c]	79.46 ± 4.90[c]	2.42 ± 0.17[c]

[a]All values represent the $\bar{X}$ ± SE for four rats.
[b]Covalent binding data normalized to account for difference in metabolized VDC.
[c]Significant fed-fasted difference $p < 0.05$, *t*-test.

Subsequent experiments have been directed by defining the relationship between liver GSH depletion and covalent binding of ^{14}C-activity following inhalation exposure to ^{14}C-VDC. The reactive intermediate formed from vinyl chloride is also detoxified *in vivo* by conjugation with GSH and, in addition, some degree of covalent binding of ^{14}C-VC metabolites in the liver has been demonstrated (Watanabe et al., unpublished data). Therefore it was decided to conduct the experiments on ^{14}C-VDC in a manner which would allow direct comparison of the data for both materials.

Male rats were exposed to constant concentrations of ^{14}C-VDC ranging from 5 to 200 ppm. Exposure duration was 6 hr. Immediately after exposure the animals were sacrificed and hepatic nonprotein sulfhydryl levels and covalent binding of ^{14}C-activity to hepatic tissue were assayed by the methods of Sedlak and Linsay (*6*) and Jollow et al. (*5*), respectively. The results of these experiments are shown in Figure 2.

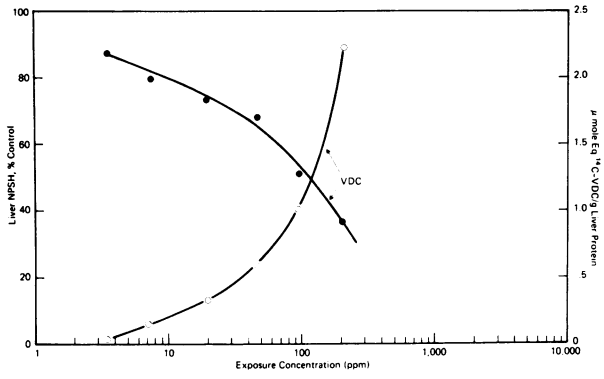


FIGURE 2. Dose-response relationship for (•) hepatic NPSH depletion and (o) covalent binding of ^{14}C-VDC metabolites to liver protein following inhalation exposure of rats to ^{14}C-VDC. Each point represents the mean for three rats.

Hepatic NPSH concentrations declined with increasing VDC exposure concentrations. The dose-dependent or saturable character of VDC detoxification is apparent, indicating an initial deviation from first-order or linear kinetics at about 50 ppm. However, appreciable covalent binding of ^{14}C-activity to liver protein was found only after VDC exposure which depleted hepatic glutathione by 30% or greater. Thus the metabolic events associated with VDC-induced hepatotoxicity continue to increase markedly when the VDC exposure concentration is sufficiently high to produce enough reactive metabolite to exceed the availability of glutathione for detoxification. The data are consistent with the hypothesis that covalent binding of VDC metabolites to tissue macromolecules represents a biochemical event which precedes the development of VDC hepatotoxicity.

A comparison of dose-response relationships for hepatic glutathione depletion and covalent binding to liver protein following ^{14}C-VDC or ^{14}C-VC inhalation exposure is shown in Figure 3. Inhalation exposure of rats to ^{14}C-VC is less effective in producing hepatic NPSH depletion than observed with VDC. Furthermore covalent binding of ^{14}C-VC metabolites to liver protein approaches saturation at VC exposure concentrations greater than about 500 ppm. Thus at VC exposure concentrations as high as 5000 ppm the accumulation of reactive VC metabolites is not sufficiently great enough to result in hepatotoxicity.

The dose-response relationships shown in Figure 3 indicate that VDC is more rapidly metabolized than VC to reactive metabolites which can deplete hepatic glutathione or covalently bind to liver macromolecules. The apparent saturation of covalent binding of VC metabolites in the liver likely results from saturation of the biotransformation of VC to a reactive intermediate.

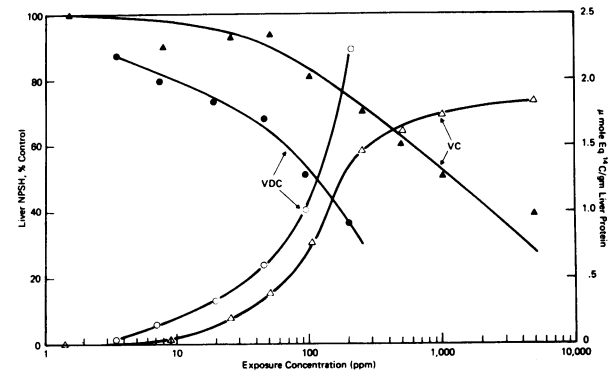


FIGURE 3. Comparison of dose–response relationships for (•) hepatic NPSH depletion and (o) covalently bound ^{14}C-activity in the liver following ^{14}C-VDC or ^{14}C-VC exposures. Each point represents the mean for three or five rats.

Fate of ^{14}C-VDC in the Mouse

The results of preliminary pharmacokinetic experiments on the fate of inhaled ^{14}C-VDC in mice are summarized in Table 5. In these experiments, male Ha(ICR) mice were exposed to 10 ppm ^{14}C-VDC for 6 hr. Immediately after exposure, these animals were placed in glass metabolism cages and excreta collected for 48 hr according to the ^{14}C-balance study protocol outlined for rats (*4*).

Table 5 shows the disposition of ^{14}C-VDC in rats and mice following exposure to 10 ppm ^{14}C-VDC. The single 6-hr exposure to ^{14}C-VDC resulted in a

body burden of 5.3 mg-eq. ^{14}C-VDC/kg in the mouse, nearly twice that obtained in the rat in an identical experiment. Total metabolism of ^{14}C-VDC was more efficient in the mouse than the rat, the former eliminated less than 1% of the body burden as unchanged VDC in expired air.

The higher body burden and more rapid metabolism of VDC by mice suggested that production of toxic metabolites of VDC by mice may be greater than that observed in rats and thus render them more susceptible to the effects of VDC exposure. A comparison of values obtained for covalently bound ^{14}C-activity in liver and kidney of mice and rats immediately following ^{14}C-VDC exposure is shown in Table 6. This data indicate clearly the enhanced production of reactive metabolites of VDC in mice as evidenced by the marked increase in covalently bound ^{14}C-activity in both liver and kidney when compared to the rat. In the context of previous findings which indicated the relationship between covalent binding and VDC-induced tissue damage in rats, the data clearly indicate that the mouse is much more susceptible than the rat to the adverse effects of VDC.

Table 5. Disposition of ^{14}C-activity in rats and mice following inhalation exposure to 10 ppm ^{14}C-VDC.

	Mice	Rats
Expired VDC, %[a]	0.65 ± 0.07	1.63 ± 0.14
Expired $^{14}CO_2$, %[a]	4.64 ± 0.17	8.74 ± 3.72
Body burden, %[a]		
Urine	80.83 ± 1.68	74.72 ± 2.30
Feces	6.58 ± 0.81	9.73 ± 0.10
Carcass	5.46 ± 0.41	4.75 ± 0.78
Cage Wash	1.83 ± 0.84	0.44 ± 0.28
Body burden, mg-eq ^{14}C-VDC/kg	5.30 ± 0.75	2.89 ± 0.24
Total metabolized VDC, mg-eq ^{14}C-VDC/kg	5.27 ± 0.74	2.84 ± 0.26

[a] $\bar{X}$ ± SE, n = 4.

Table 6. Covalently bound ^{14}C-activity in rat and mouse tissue following exposure to 10 ppm ^{14}C-VDC.

	^{14}C-VDC, μg-eq./g protein ($\bar{X}$ ± SE, n = 4)	
	Liver	Kidney
Mice	22.29 ± 3.77	79.55 ± 19.11
Rats	5.28 ± 0.14	13.14 ± 1.15

Two studies reported by Maltoni and Lee at this conference have found VDC to be carcinogenic in mice. Maltoni (*7*) has reported kidney tumors in Swiss mice exposed chronically to 25 ppm but not 10 ppm VDC. In Lee's study (*8*), mice (CD-1) exposed to 55 ppm VDC for one year showed hepatomas, angiosarcoma of the liver and pulmonary adenomas. Despite the marked difference in the response of the two mouse strains employed in these studies, both investigators have reported moderate to severe chronic tissue damage indicative of VDC toxicity in the tumor-bearing organs. Only in Maltoni's study was a "no-effect" level for VDC-induced renal damage realized (10 ppm), and at this exposure concentration no kidney tumors were observed.

Therefore, given the enhanced susceptibility of the mouse to VDC, it appears uncertain as to whether the tumorigenic responses observed are truly the direct result of VDC exposure or whether they arose subsequent to chronic insult which resulted in appreciable tissue damage. Based upon the preliminary pharmacokinetic data obtained thus far, it is conceivable that VDC-induced toxicity may well occur long before a tumorigenic response to VDC is realized.

Future studies on the fate of VDC in mice will be oriented towards hazard assessment and the relationship between the development of the aforementioned biochemical and morphological events in VDC-induced hepatic and renal toxicity. It is expected that these studies will provide data which will aid in interpreting the results obtained in chronic toxicity and carcinogenicity studies of VDC in mice.

Summary and Conclusions

The data presented in this report indicate that the pharmacokinetics of inhaled VDC in the rat is dose dependent. Detoxification of VDC in the rat occurs primarily via conjugation of one or several VDC metabolites with glutathione and subsequent urinary excretion of the mercapturic acid derivatives *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and thiodiglycolic acid. The diminished ability to detoxify VDC is enhanced by fasting, and is associated with a reduction in available hepatic GSH prior to VDC inhalation.

VDC-induced centrolobular hepatic necrosis observed in fasted but not normally fed rats exposed to 200 ppm ^{14}C-VDC was associated with an increase in covalently bound ^{14}C-VDC metabolites in livers of the affected animals. Subsequent experiments have shown that significant accumulation of covalently bound VDC metabolites occurs when hepatic glutathione concentrations are depleted by greater than 30% following VDC exposure. The data are consistent with the hypothesis that VDC is rapidly metabolized to one or more alkylating metabolites which are detoxified via conjugation with GSH. When the availability of GSH is exceeded the reactive species may accumulate in the tissue by alkylation of subcellular macromolecules and thereby result in toxicity.

Comparison of the pharmacokinetics of inhaled VDC and VC in rats points to several marked differences in the fate of the two chemicals which may account for their differing biological actions. Inhaled VC is less readily metabolized, and hence exposure to equivalent concentrations results in less hepatic GSH depletion and alkylation of tissue macromolecules than with VDC. Although the reactive metabolic intermediates formed from both materials are detoxified via conjugation of their respective reactive metabolites with GSH, the data indicate that the biotransformation of VDC during inhalation exposure is quantitatively greater than that seen with similar exposure concentrations of VC.

Furthermore covalent binding of VC metabolites in rat liver approaches saturation at exposure concentrations of greater than 500 ppm VC. This observation for VC is of particular significance since it explains the lack of a hepatotoxic effect of VC at exposures as high as 5000 ppm. In addition this non-linear or saturable character of VC pharmacokinetics correlates well with previously reported data indicating a relatively constant incidence of hepatic angiosarcoma in rats exposed to VC concentrations ranging from 2500 to 10,000 ppm (*9*). Thus, although the hepatocyte is not the primary target site for VC tumorigenesis, the relationship between VC metabolism and tumor incidence appears to be well supported by these experiments

The preliminary data on the pharmacokinetics of inhaled VDC in the mouse deserve particular comment. In the context of our findings in experiments on the relationship between the metabolism and toxicity of VDC in rats, the data for mice suggest an enhanced capacity for metabolism of VDC. Furthermore, a potentially greater susceptibility of mice to VDC is evidenced by an increased production of reactive VDC metabolites capable of alkylating renal and hepatic tissue constitutents over that observed in rats exposed to the same concentration (10 ppm) of VDC. The data suggest that accumulation of VDC metabilites covalently bound to target organ macromolecules may be sufficient to produce tissue damage and necrosis appreciably sooner than the onset of neoplasia. To date this hypothesis is supported by the findings reported by both Maltoni and Lee. In neither study were tumors in mice observed in the absence of nontumor pathology attributed to VDC exposure. Further studies of the metabolism and pharmacokinetics of VDC in mice are indicated to fully explain the relationships between metabolism, toxicity and potential carcinogenicity of this material. Such studies are currently underway in our laboratory.

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