

BIO-MEDICAL RESEARCH
DOCUMENT DESCRIPTION FORM

63

68 69

76

77

78

Duplicate in all cards: →

1980

0000327

UC

year as-1961-

File number

[Right justify

[Numeric only]

Sub-Index

Code

Author(s), as Last Name FS (No Punctuation) and
codes for journal as JAMA preceded by one blank space

1	20 21	40 41	60	61 62
Westlake P.G.				11
Gehring P.J.				12
				13

Title of Report; end with space-hyphen-hyphen-space. Follow with Index Terms,
separated from each other with comma-space. Avoid other punctuation;
do not abbreviate.

1 2	61 62
	21
Issue-Dependent Fate of Urinal (Cisplatin) and	22
Possible Relationship to Oncogenicity in Rats --	23
Dow, Metabolism, Pharmacokinetics	24

Source (Journal, Vol., Number, Pages, Date)

1 2	61 62
Environmental Health Perspectives, 17 pp. 1975	31
	32

Brief Summary

1 2	10	61 62
SUMMARY:		61
		62
		63
		64

R&S 111598

Dose-Dependent Fate of Vinyl Chloride and Its Possible Relationship to Oncogenicity in Rats

by P. G. Watanabe* and P. J. Gehring*

Studies on the fate of ¹⁴C-labeled vinyl chloride (VC) following oral administration and inhalation exposure in rats demonstrated that the disposition of VC in the body is a function of the dose. More importantly, from the data available, it appears that a correlation exists between doses of VC which cause tumors and those that saturate metabolic or detoxifying pathways. Additional studies characterized the depression of liver non-protein sulfhydryl content (primarily GSH) with the duration and concentration of exposure to VC. The results of these investigations indicate that statistical projections utilizing data collected from rats exposed to high doses of VC are invalid for predicting the hazard of low level exposure, because such projections violate the *a priori* assumption that the dynamics governing the fate of VC in the body are unaltered.

Introduction

Once the carcinogenicity of a chemical has been established a primary consideration is the hazard of exposure to low levels of the given chemical. Studies on the pharmacokinetic and metabolic characteristics of such chemicals are essential in accurately assessing the hazard of low level exposures. Pharmacokinetics is the study of the dynamic processes involved in the absorption, distribution, metabolism and elimination of chemicals from the body. Pharmacokinetic studies alone are insufficient to assess toxicity. However, their principal value is in correlation of toxicity with the time-related disposition of chemicals in the body. An altered disposition of a chemical in the body with dose can explain in certain instances why toxicity, including carcinogenicity, is produced at high doses and not at low doses of the same chemical.

In the case of carcinogenicity, stochastic, statistical projections are made from the range of doses over which an increased incidence of cancer can be measured to predict what percentage of individuals may respond at lower

doses. Figure 1 shows a hypothetical cumulative dose-response curve for the percentage of individuals (triangles) in a population responding adversely in some manner to selected doses of a chemical. The sigmoid curve represents a response of a population described by a normal or Gaussian distribution. These adverse responses (cancer) are discernible only over a range of doses represented by the solid line, because the investigator is limited by the number of individuals he can include in such a study.

It should be emphasized that an *a priori* assumption for making such projections is that the chemical is handled in the same manner by the body as the dose decreases. If the dynamics for the fate of the chemical change, such extrapolation is not valid. Conceptually, it is not surprising that toxicity (including carcinogenicity) is expressed only after the capacity to detoxify the chemical in the body has been exceeded. In such cases, the response of the population may be more accurately described by the other two broken lines in the lower left-hand corner of Figure 1. The most important aspect is to determine if an altered disposition of a chemical with dose functions over a range from doses that cause toxicity to those that do not cause toxicity.

* Toxicology Research Laboratory, Health and Environmental Research, The Dow Chemical Company, Midland, Michigan 48640.

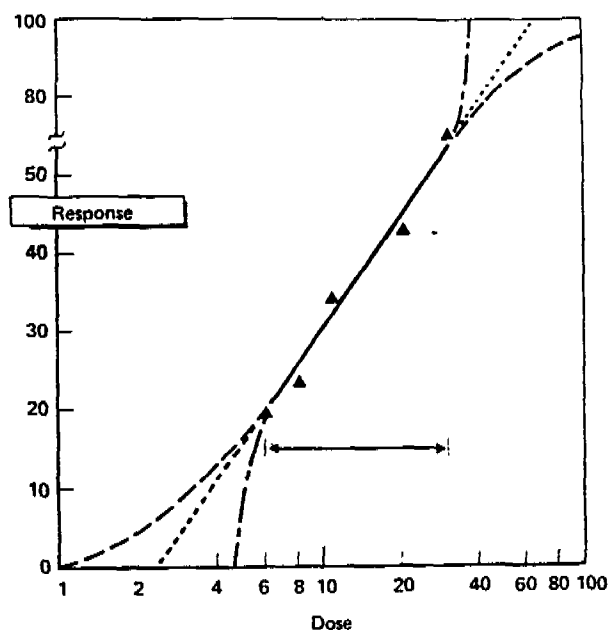


FIGURE 1. Plot of hypothetical log dose vs. per cent response curve. Measurable responses are represented by triangles. The sigmoid curve (---) represents a population described by a normal distribution; in theory the percentage responding never reaches zero on the low end or 100% on the high end. The other curves (---) and (-.-) represent simulated responses if there exists a threshold for the response.

In initial studies on the fate of vinyl chloride (VC), rats were exposed in a closed recirculating inhalation chamber to varying concentrations of VC (1). By monitoring the concentration of VC in the system by infrared spectrophotometry, the rate of uptake of VC by the rats was determined. Results on exposure to low and high concentrations of VC and on pretreatment of the animals with metabolic inhibitors indicated that VC was metabolized by at least two pathways. More importantly, it appeared that the primary pathway for the metabolism of VC became saturated as the exposure concentration increased.

These initial results suggested a dose-dependent fate of VC, and this motivated additional work to elucidate the fate of VC in rats following both oral administration and inhalation exposure. ^{14}C -labeled VC was utilized in subsequent investigations which greatly facilitated following the disposition of the administered VC.

Results and Discussion

Fate of VC Following Single Oral Administration

Table 1 shows the percentage of ^{14}C -activity eliminated via various routes following different single oral doses of VC in corn oil to rats (2). The ^{14}C -activity in urine, feces, and carcass and tissues represents nonvolatile metabolites of VC. If all the processes involved in the disposition of VC in the body could be described by first-order kinetics, implying that the rates of the processes were proportional to the amount of chemical available, then the proportions of ^{14}C activity eliminated by each route of excretion would be the same over the range of doses tested. If this were the case, then the fate of VC in the body would be independent of the dose administered. However, the results show that as the dose was increased from 0.05 and 1.0 mg/kg to 20 or 100 mg/kg, the percentage expired as VC increased markedly, while the other parameters, particularly urinary excretion of ^{14}C -activity, decreased. This demonstrates that the primary route for the elimination of VC from the body is dependent on the dose administered.

Since the urinary and pulmonary excretion of VC were altered dramatically as the dose increased from 1 to 100 mg/kg, the question was raised whether these processes of elimination may be a function of dose. Figure 2 shows a plot of the logarithm of the ^{14}C -activity eliminated via the urine as a function of time. Since the slopes or rates of elimination ($t_{1/2} = 4.5$ hr) are unchanged, it must be concluded that the rate of urinary excretion of nonvolatile metabolites of VC is unaltered by dose.

Figure 3 shows similarly the expiration of VC following various doses. Elimination following 0.05 or 1.0 mg/kg occurred in accordance with a first-order rate or monoexponential process with half-life of 53–58 min. When 100 mg/kg was given, the elimination was biexponential. The initial phase of elimination had a half-life of 14 min and was followed by a slower phase with a $t_{1/2}$ of 41 min. The rates or $t_{1/2}$ times for elimination at the 100 mg/kg dose correspond well with those reported by Withey (3) for blood. These results are indicative of a material which is bound reversibly to some site in the body having a finite capacity. As the dose increases, the availability of these binding

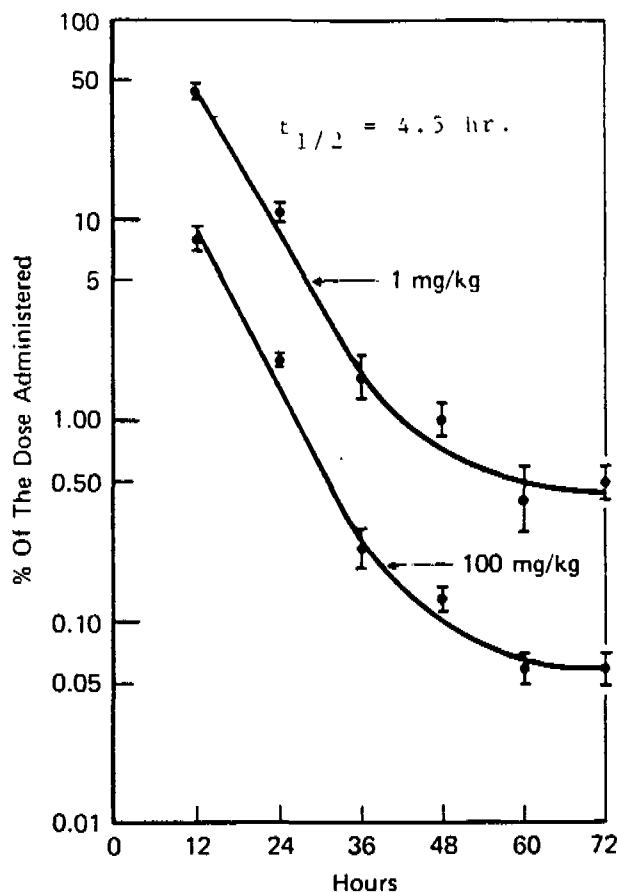


FIGURE 2. ^{14}C -activity excreted in the urine expressed as percent of the dose administered (1 and 100 mg/kg) vs. time. Each point represents the mean $\pm$ standard error of the mean for five rats. The initial linear segments of the curves (12-36 hr) were fit by linear regression analysis. $t_{1/2} = 4.5$ hr.

sites decreases and the chemical is free to find its way to other sites or to be eliminated. Thus, it may be concluded that the pulmonary excretion of VC is not a rate-limiting step. Even more importantly, the data indicate that the state in which VC exists in the body changes with dose.

Figure 4 summarizes the dose-dependent excretion of VC via urinary excretion (solid line) and pulmonary elimination (broken line). The area indicated by the rectangle represents the range of doses where evidence of distributive or metabolic saturation first occurs. Of particular significance is that in a current carcinogenesis study in rats by Maltoni (4) daily oral

doses of VC at 50 and 16.6 mg/kg-day have resulted in 19% and 14% induction of hepatic angiosarcoma, respectively, while at a dose of 3.33 mg/kg-day no tumors have been observed. These results are after 85 weeks. Figure 4, illustrating the occurrence of saturation as a function of a single oral dose of VC, shows that a correlation exists between doses of VC which cause tumors and those that saturate metabolic or detoxifying pathways. The dose-response curve generated from the two higher doses (50 and 16.6 mg/kg-day) predicts a 9% tumor incidence at the 3.33 mg/kg-day level. Since no tumors have been observed yet at this low level, this is an example of the use of pharmacokinetic data in interpretation of why high doses of a chemical may produce toxic

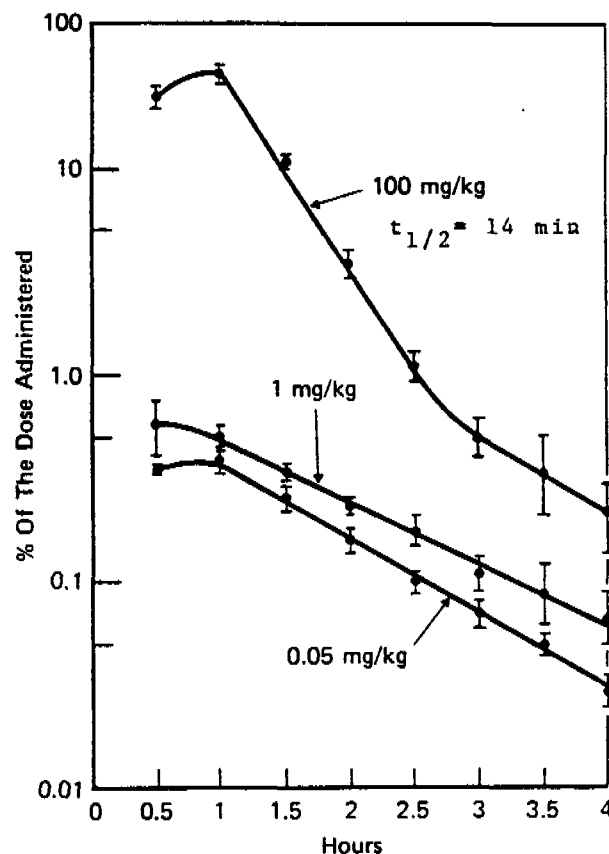


FIGURE 3. Expired vinyl chloride expressed as percent of the dose administered (0.05, 1, and 100 mg/kg) vs. time. Each point represents the mean $\pm$ standard error of the mean of five rats. The linear phases of the curves were fit by linear regression analysis.

Table 1. Percentage of administered ^{14}C activity eliminated during 72 hr following a single oral dose of vinyl chloride.

	% of ^{14}C activity ^a			
	0.05 mg/kg	1.0 mg/kg	20 mg/kg	100 mg/kg
Expired as				
VC	1.4 ± 0.1 ^a	2.1 ± 0.2	41.6 ± 5.7	66.6 ± 0.7
CO ₂	9.0 ± 0.6	13.3 ± 0.5	4.8 ± 0.7	2.5 ± 0.1
Urine	68.3 ± 0.5	59.3 ± 2.8	22.6 ± 1.2	10.8 ± 1.0
Feces	2.4 ± 0.5	2.2 ± 0.4	1.0 ± 0.1	0.5 ± 0.1
Carcass and tissues	10.1 ± 1.9	11.1 ± 0.5	11.0 ± 2.7	1.8 ± 0.1
Total recovery	91.3 ± 2.5	88.8 ± 2.0	81.0 ± 2.9	82.3 ± 0.4

^a Mean ± standard error, five rats/dose except three rats at 20 mg/kg level.

Table 2. Percentage of ^{14}C activity eliminated during 72 hr following inhalation exposure to ^{14}C -vinyl chloride for 6 hr.^a

	% of ^{14}C activity ^a	
	10 ppm	1000 ppm
Expired as		
VC	1.61 ± 0.16 (4)	12.26 ± 0.96 (814)
CO ₂	12.09 ± 0.43 (30)	12.30 ± 0.63 (817)
Urine	67.97 ± 1.71 (169)	56.29 ± 1.96 (3739)
Feces	4.45 ± 0.22 (11)	4.21 ± 1.05 (280)
Carcass and tissues	13.84 ± 1.16 (34)	14.48 ± 0.52 (977)
Cage wash ^b	0.15 ± 0.08 (<1)	0.23 ± 0.09 (15)
Total VC recovered, μg	(248)	(6642)

^a Expressed as percentage of the total ^{14}C -activity recovered. All values are means ± standard error from four rats. Values in parenthesis are microgram equivalents of vinyl chloride.

^b Water and acetone wash of the metabolism cage at termination of the experiment.

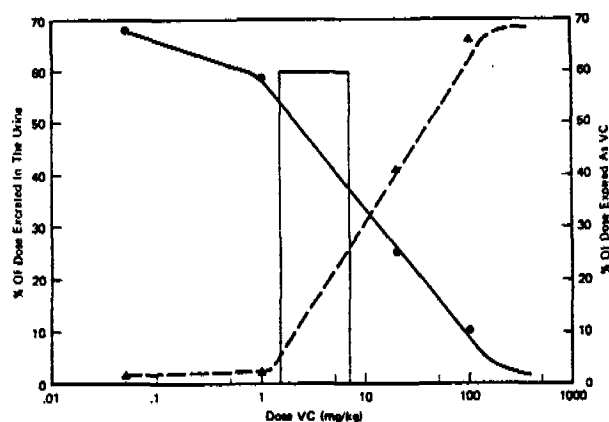


FIGURE 4. Summary of the dose-dependent excretion of VC: (—) urinary excretion; (---) pulmonary elimination. Urinary excretion represents polar metabolites of VC while pulmonary elimination is VC per se. Area demarcated by the rectangle represents range of doses over which distributive and metabolic saturation occurs.

city and extrapolation of the same toxic effect at lower levels is invalid because the fate of the chemical has changed.

Fate of VC Following Inhalation Exposure

Table 2 shows the fate of ^{14}C -VC in rats exposed for 6 hr to 10 or 1000 ppm VC (5). Immediately following the exposure, the rats were placed in cages providing for collection of ^{14}C -activity in the expired air, feces, and urine over the subsequent 72 hr. As in the experiments in which oral doses were given, the percentage of ^{14}C -activity expired as VC increased as the exposure increased.

Also to be noted in Table 2 is that the percentage ^{14}C -activity in the tissues and carcass increases slightly as the exposure is increased from 10 to 1000 ppm. Although not statistically significant, this is remarkable because a much larger fraction was expired as VC. In particular, the normalized amount of ^{14}C -activity in

the liver and skin increased. This may mean that a larger fraction is being bound to the macromolecules of the tissues. This aspect is currently being investigated, since such reactivity may explain the carcinogenic effect of VC.

Figures 5 and 6 show respectively the elimination of ^{14}C -nonvolatile metabolites in the urine and VC per se in the expired air. Neither elimination process is rate-limiting or overwhelmed by increasing the exposure concentration. However, it is noteworthy that expired VC increased with increasing dose, whereas excretion of urinary metabolites decreased, suggesting a saturation of the metabolism of VC. The results of these studies as those of previous studies support the conclusions that

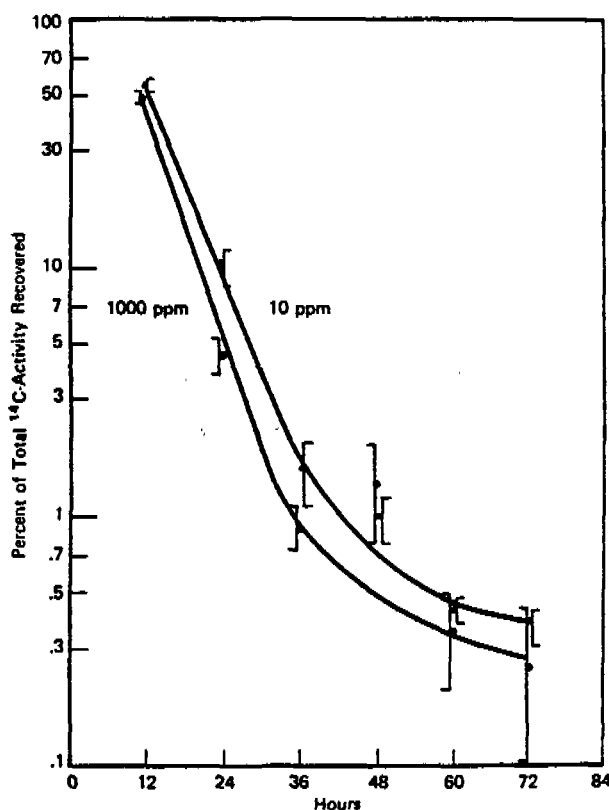


FIGURE 5. ^{14}C -activity excreted in the urine expressed as percentage of the recovered radioactivity vs. time following a 6-hr exposure to 10 and 1000 ppm VC. Each point represents the mean $\pm$ standard error of the mean for four rats. The initial log linear phase of the curves (12–36 hr) were fit by linear regression analysis.

(1) the fate of VC changes with dose and (2) this occurs because the primary pathway for the metabolism of VC is saturated at high doses or exposures.

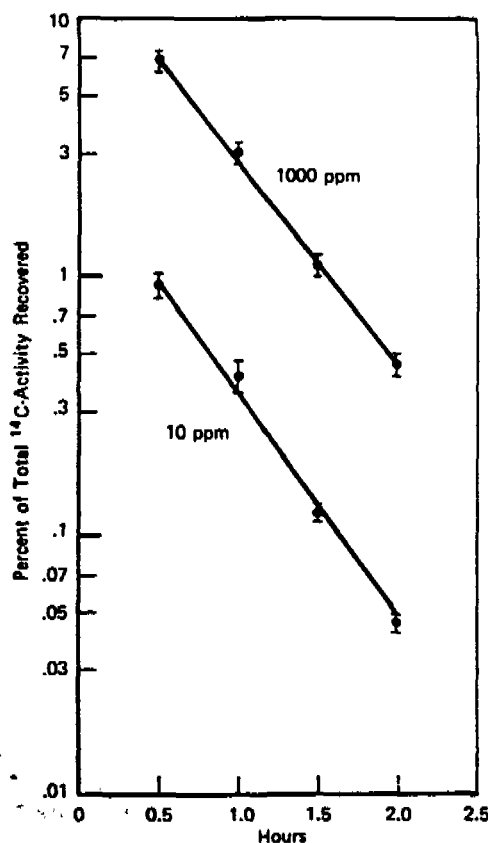


FIGURE 6. Expired vinyl chloride expressed as percentage of the recovered radioactivity vs. time following a 6-hr. exposure to 10 and 1000 ppm VC. Each point represents the mean $\pm$ standard error of the mean of four rats. The curves were fit by linear regression analysis.

Since the metabolism of VC appears to occur via at least two pathways, an effort was made to identify the urinary metabolites of VC. Already it had been demonstrated that a measurable amount of VC was metabolized to CO_2 . Using high-pressure liquid chromatography, three major metabolites have been isolated from urine. Two of the three have been identified by gas chromatography-mass spectroscopy. These are metabolite A, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, and metabolite B, is thiodiglycolic acid. Together these metabolites

comprise 50 to 60% of the radioactivity found in urine. A third metabolite, comprising 35% of the metabolites present in the urine has been isolated but remains unidentified. Both metabolites A and B are likely formed from *S*-(2-hydroxyethyl)cysteine. At one time, it appeared that the third major urinary metabolite, comprising about 30% of the radioactivity, was *S*-(2-hydroxyethyl)cysteine. Although some analytical comparisons between the isolated metabolite and 2-hydroxyethylcysteine favored this conclusion, other failed to confirm the identity of this metabolite.

Identification of these metabolites of VC in urine indicates that VC is transformed in the body to a reactive intermediate metabolite, which is then detoxified by reaction with glutathione, (γ -glutamylcysteinylglycine). Subsequently, the glutamic acid and glycine moieties of the tripeptide are cleaved, and the cysteine conjugate of the reactive metabolite of VC is either acetylated or further oxidized and excreted as the aforementioned metabolites.

The urinary metabolites of VC were not changed either qualitatively or quantitatively as the dose or exposure level was increased. Since evidence has been presented for several metabolic pathways being involved in the biotransformation of VC, the lack of alteration in the urinary metabolites with dose may seem inconsistent. However, it should be emphasized that toxicity is a result of the balance between the relative rates of intoxicating to detoxicating metabolic pathways, and while this param-

eter may change with dose it need not be reflected by the urinary metabolites which constitute ultimate end products of metabolism.

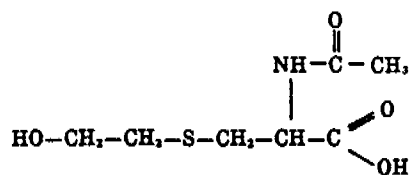
Our initial work and subsequently that of others (6-8) indicates that one pathway involves oxidation of VC by microsomal enzymes to chloroethylene oxide. Other pathways which involve either nonenzymatic or enzymatic conjugation with GSH, mediated by soluble enzymes, and dechlorination reactions, mediated by both soluble and microsomal enzymes, are all possibly involved in the overall metabolism of VC. The relative contribution of these enzyme systems in the metabolism of VC are currently under investigation.

Depression of Hepatic Nonprotein Sulfhydryl Content by VC

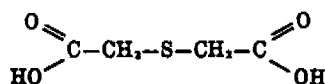
A very important aspect of the metabolism of VC is the detoxification reaction with hepatic nonprotein sulfhydryl groups (composed of primarily GSH). When high doses of some chemicals, for example bromobenzene and acetaminophen, are given, the glutathione is used up at a faster rate than it can be produced by conjugation with the reactive intermediate. As the level of glutathione in the liver is progressively depleted, the reactive metabolites react with macromolecules such as protein, DNA and RNA leading to toxicity (9, 10). Generally, it is accepted that one mechanism for chemical carcinogenesis may involve such reactions.

To assess the effect of VC exposures on hepatic glutathione levels, rats were exposed to concentrations of 10, 50, 150, 250, 1000, or 2000 ppm for 1-7 hr (11). The results are shown in Figure 7. Exposure to 150, 250, 1000, or 2000 ppm VC caused a progressive depression of the hepatic nonprotein sulfhydryl content. Exposure to 50 ppm for 7 hr produced a small and inconsistent depression. No depression was observed in rats exposed to 10 ppm VC. These results indicate that there is a measurable biological threshold for the depression of hepatic glutathione levels induced by exposures to vinyl chloride. Unequivocal depressions are produced by concentrations exceeding 50 ppm, while exposure to 50 ppm seems to be a transition zone and exposure to 10 ppm causes no depression.

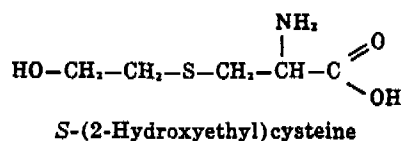
How do these results relate to the carcinogenicity of VC? In the studies of Maltoni



Metabolite A: *N*-Acetyl(*S*-2-hydroxyethyl)cysteine



Metabolite B: Thiodiglycolic acid



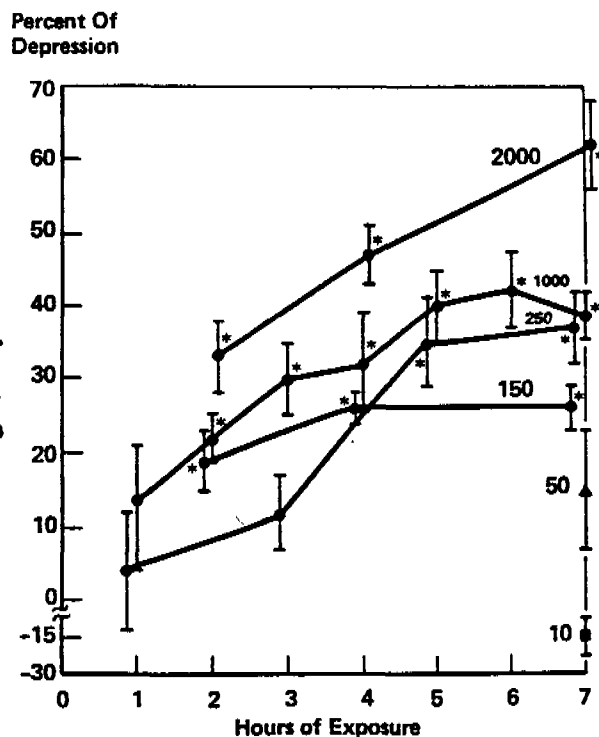


FIGURE 7. Depression of hepatic nonprotein sulfhydryl content vs. duration of exposure to 2000, 1000, 250, 150, 50, and 10 ppm vinyl chloride. Each point represents the mean $\pm$ standard error of five animals except the point for 50 ppm which represents 25 animals. The asterisks (*) represent values statistically different from controls ($p < 0.05$).

Lefemine (12), the reported incidence of angiosarcoma of the liver in rats exposed 4 hr/day, 5 days/week to 2500 or 6000 ppm was 22%. The incidence in rats exposed to 500 and 250 ppm were, respectively, 12 and 7%. Reference to Figure 7 indicates that the depression of the hepatic nonprotein sulfhydryl content observed after 4 hr of exposure coincides with the increased incidence of angiosarcoma.

In the same study (12) an incidence of only 2% angiosarcoma of the liver occurred in rats exposed to 50 ppm VC. As indicated previously, exposure to 50 ppm for 7 hr caused a small and inconsistent depression of the hepatic nonprotein sulfhydryl content. This exposure appeared to be in the transition zone of the threshold for this biological effect. In a recent publication by Maltoni (13), not only the incidences of angio-

sarcomas of the liver were given but also the latency periods for their development were provided. The latency periods were 64, 70, 78, 81, and 79 weeks in rats exposed to 10,000, 6000, 2500, 500, and 250 ppm, respectively. In rats exposed to 50 ppm, the latency period was 135 weeks. Indeed tumors were discovered in these aged rats when they were killed at the end of the study. Since the tumors in the former groups of rats were discovered as they died spontaneously, the discrepancy is even greater than the values indicate. The latency period for the development of other types of tumors showed the same discrepancy. Consideration of these results leads to the conclusion that, in rats, exposure to 50 ppm VC 4 hr/day is in the threshold transition zone for not only hepatic nonprotein free sulfhydryl depression but for tumor induction as well.

Conclusion

In summarizing the studies on the pharmacokinetics and metabolism of VC, the data indicate that the fate of VC in rats is dose-dependent following either single oral administration or inhalation exposure. More importantly, it appears from the data available that a correlation exists between doses of VC that cause tumors and those that saturate metabolic or detoxifying pathways.

The primary detoxification pathway for VC involves conjugation of its reactive metabolites with nonprotein sulfhydryl groups. Therefore, it seems reasonable to postulate that as the nonprotein sulfhydryl groups are depleted, reactive metabolites will be free to react with other macromolecules (DNA, RNA, protein, lipids), resulting in toxicity and carcinogenicity. Recent reports have demonstrated that in the presence of fortified microsomal enzyme preparations reactive metabolites of VC are produced which covalently bind to rat liver microsomes (6), protein sulfhydryl groups, RNA (7), and adenosine of DNA (8). Inclusion of glutathione in the system will decrease or preclude these reactions depending on the concentration. It is highly significant that exposure to 10 ppm VC for 7 hr caused no depression of hepatic nonprotein sulfhydryl content. This indicates that there is a threshold of exposure in rats at which the ability to replace sulfhydryl groups is not overwhelmed and physiologic defense mechanisms remain fully operative. Further-

more, this suggests that thresholds exist for toxic effects which are expressed with greater intensity as this protective mechanism is depressed. Studies currently in progress are designed to characterize the *in vivo* macromolecular binding of VC to protein and nucleic acids following exposure to various concentrations of ^{14}C -VC.

Finally, it must be emphasized strongly that stochastic, statistical projections utilizing data collected from rats exposed to high doses of VC are invalid for predicting the hazard of low level exposures. Such projections violate the *a priori* assumption that the dynamics governing the fate of the compound are unaltered.

REFERENCES

- Hefner, R. E., Watanabe, P. G., and Gehring, P. J. Preliminary studies of the fate of inhaled vinyl chloride in rats. *Ann. N. Y. Acad. Sci.* 246: 135 (1975).
- Watanabe, P. G., McGowan, G. R., and Gehring, P. J. Fate of ^{14}C -vinyl chloride after single-oral administration in rats. *Toxicol. Appl. Pharmacol.* 36: 339 (1976).
- Withey, R. J. Uptake and pharmacodynamic of vinyl chloride administered to rats by different routes. *Toxicol. Environ. Health* 1: 381 (1976).
- Maltoni, C., Gli effetti onogeni del cloruro di vinile somministrato per via orale nel ratto. *Gli Ospedali della Vita*, 2: 102 (1975).
- Watanabe, P. G., et al. Fate of ^{14}C -vinyl chloride following inhalation exposure in rats. *Toxicol. Appl. Pharmacol.* 37: 49 (1976).
- Kappus, H., et al. Rat liver microsomes catalyze covalent binding of ^{14}C -vinyl chloride to macromolecules. *Nature* 257: 134 (1975).
- Bolt, H. M., et al. Metabolism of vinyl chloride. *Lancet*: 1425 (1975).
- Barbin, A., et al. Liver-microsome-mediated formation of alkylating agents from vinyl bromide and vinyl chloride. *Biochem. Biophys. Res. Commun.* 67: 596 (1975).
- Gillette, J. R. A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity—I. Correlation of changes in covalent binding of reactive metabolites with changes in the incidence and severity of toxicity. *Biochem. Pharmacol.* 23: 2785 (1974).
- Gillette, J. R. A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity—II. Alterations in the kinetics of covalent binding. *Biochem. Pharmacol.*, 23: 2927 (1974).
- Watanabe, P. G., Hefner, R. E., Jr., and Gehring, P. J. Vinyl chloride induced depression of hepatic nonprotein sulfhydryl content and effects on bromosulphalein (BSP) clearance in rat. *Toxicology* 6:1 (1976).
- Maltoni, C., and Lefemine, G. Carcinogenicity bioassays of vinyl chloride: Current results. *Ann. N.Y. Acad. Sci.* 246: 195 (1975).
- Maltoni, C. The value of predictive experiments in environmental carcinogenesis. An example: vinyl chloride. *Ambio*, 4: 18 (1975).