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GEHRING, P.J.	WATANABE, P.G.	YOUNG, J.D.	11
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1 2	61 62
	21
The Relevance of Dose-dependent Pharmacokinetics	22
in the Assessment of Carcinogenic Hazard of Chemicals --	23
Risk Assessment, Data,	24

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

1 2	61 62
Cold Spring Harbor Conferences on Cell Proliferation	31
Vol 4, 183-203, 1977	32

Brief Summary

1 2 10	61 62
SUMMARY:	61
	62
	63
	64

The Relevance of Dose-dependent Pharmacokinetics in the Assessment of Carcinogenic Hazard of Chemicals

0000123

P. J. Gehring, P. G. Watanabe and J. D. Young

Toxicology Research Laboratory, Health and Environmental Research
Dow Chemical, Midland, Michigan 48640

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Typically, the potential of chemicals to cause cancer is evaluated by administering daily a maximum tolerated dose of the agent to animals, usually rats or mice, for their lifetime. The maximum tolerated dose has been selected as the maximum dose not causing death or severe debilitation of the selected species in short-term studies lasting 6 to 12 weeks. Many times the chemical has been administered by routes other than that via which exposure occurs in practice. This methodological concept has resulted in the administration of massive doses of chemicals for which the real-life exposure is much smaller and often via a different route. In essence, this approach reveals whether the potential of the chemical to cause cancer is equivalent to or slightly greater than its potential to cause death or severe debilitation. Frequently the doses supersede those which produce clinical disease.

Although this approach may be acceptable for screening chemicals for further evaluation, translation of the data to predict the hazard of low-level exposure is presumptuous guesswork at best. The presumptuous guesswork has been sophisticated by the statistician who has applied his stochastic, statistical processes, and "stochastic" (which is defined as guesswork) is emphasized. Not considered in making such statistical extrapolations is the fact that the fate of many chemicals changes as the magnitude of the dose.

With some chemicals, metabolic changes are induced by large doses, rendering animals receiving such doses different from those receiving low doses. In either case, changes in fate of the chemical or the metabolic status of the animals preclude the use of routine statistical processes to predict the hazard of low doses. Indeed, this violates the a priori assumption for their use.

Toxicity, including carcinogenesis, is a dynamic process involving absorption of a chemical into the body, distribution to various tissues, reversible or irreversible reactions with cellular components, and ultimately clearance from the tissues and the body via metabolism and/or excretion. Therefore the predictability of animal toxicological data for assessing the hazard of a chemical to man is enhanced if the fate of the chemical per se and/or its

degradation products in animals is equated to the fate in man. By fate, we refer to the kinetics for absorption, distribution, reversible or irreversible reactions with cellular components, and elimination of the chemical per se and/or its degradation products. "Pharmacokinetics" is the term used to refer to such studies of the fate of a chemical. Over a selected range of doses, the pharmacokinetics of chemicals are commonly linear, i.e., the kinetics for absorption, distribution, reactions with cellular components, and elimination are proportional to the concentration or amount present.

In the simplest form, "linear pharmacokinetics" may be expressed by the linear differential equation

$$-\frac{\partial C}{\partial t} = k_e C,$$

where C is the concentration in a tissue at time t, and k_e is the rate constant for elimination. Solution of this differential equation gives

$$C = C_0 \exp(-k_e t)$$

or

$$\log C = \log C_0 - \frac{k_e t}{2.303},$$

where C_0 equals the concentration at time zero. A plot of C versus time on semilogarithmic paper will yield a straight line with slope $k_e/2.303$.

As long as the pharmacokinetics of a chemical remain linear, a tenfold increase in dose will increase the tissue concentration tenfold at any point in time. However, many metabolic and excretory processes are saturable, and as doses of the chemical begin to saturate or overwhelm these processes, it may be expected that there will be a disproportionate increase in the concentration in tissue and consequently in the toxicity. For these processes, nonlinear pharmacokinetics apply, which can be described by the Michaelis-Menten equation:

$$-\frac{\partial C}{\partial t} = \frac{V_m C}{K_m + C}.$$

In this equation, $\partial C/\partial t$ is the rate of change in the concentration of the chemical at time t; C is the concentration of chemical at time t; V_m is the maximum rate of the process; and K_m, the Michaelis constant, is equal to the concentration of the chemical at which the rate of the process is equal to $V_m/2$.

There are two important limiting cases to the Michaelis-Menten equation: when the concentration is small compared to K_m,

$$-\frac{\partial C}{\partial t} = \frac{V_m C}{K_m}, \qquad C << K_m;$$

when the concentration is large compared to K_m,

$$-\frac{\partial C}{\partial t} = V_m, \qquad C >> K_m.$$

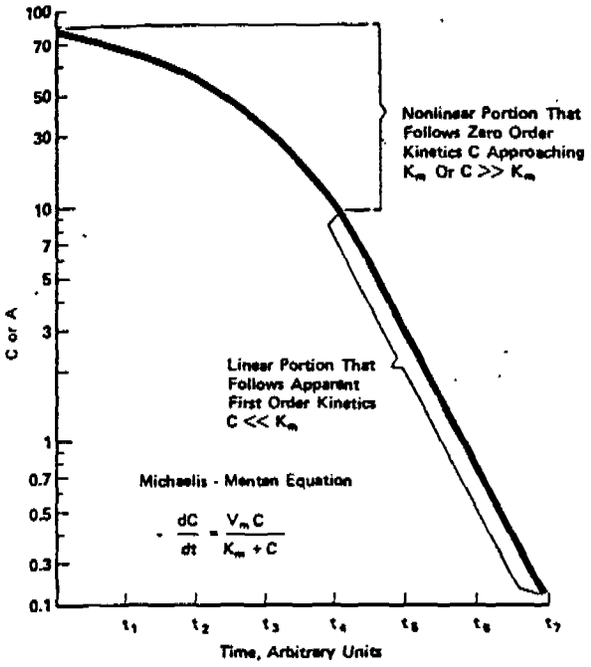


Figure 1
Simulated plasma concentration of a chemical (C) or the amount in the body (A) as a function of time for a chemical which displays dose-dependent or nonlinear pharmacokinetics described by the Michaelis-Menten equation. dC/dt is the change in concentration with time, V_m is the maximum rate of the process, and K_m is the Michaelis constant.

Figure 1 depicts a typical tissue concentration or amount in the body–time curve for a chemical whose elimination follows nonlinear or Michaelis-Menten kinetics. As long as the dose incurred via exposure to a chemical results in concentrations or amounts significantly less than K_m, the linear portion of the semilogarithmic plot is applicable. Doses that provide concentrations or amounts approaching or superseding K_m lead to the nonlinear portion of the curve.

Some criteria that serve as indications that nonlinear pharmacokinetics are applicable for describing the elimination of a chemical from the body were set forth by Levy (1968):

1. The decline of the levels of the chemical in the body is not exponential.
2. The rate constant for elimination decreases with increasing dose.
3. The area under the tissue concentration–time curve increases disproportionately with increasing dose.
4. The composition of the excretory products may be changed both quantitatively and qualitatively by dose.
5. Competitive inhibition by other chemicals metabolized or actively transported by the same enzyme system is likely.
6. Dose–response curves may show an unusually large increase in response

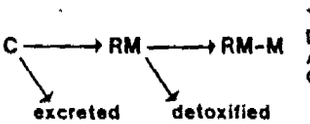


Figure 2
One-compartment model illustrating the chemical process for the fate of a chemical in the body. Each arrow represents a first-order process. (For a more elaborate treatise, see Gillette 1974b.)

with increasing dose, starting at the dose level where "saturation" effects become evident.

To this list of criteria should be added one more. The reaction of the chemical per se or reactive metabolites produced from it with macromolecules —DNA, RNA, or protein—increases disproportionately with dose. In carcinogenesis, this criterion is very important because it is generally believed that reactions of electrophiles with macromolecules lead to carcinogenesis.

Gillette (1974a,b) has given special consideration to the pharmacokinetics of reactive metabolites of foreign compounds that react with macromolecules. The concepts presented in these papers are very important to the toxicologist and oncologist because they elucidate plausible threshold mechanisms for toxicity, including carcinogenesis.

Figure 2 shows a simple, one-compartment model illustrating a typical chemical process for the fate of a chemical in the body. Each arrow represents a first-order rate process. The predominant process governing the fate of the chemical is different for different species, different chemicals, and frequently for different doses of the same chemical. For example, furosamide, a diuretic, is excreted predominantly via the kidney when low doses are administered. However, when high doses are given, the renal clearance of furosamide is overwhelmed, causing a disproportionate increase in the formation of toxic metabolites which react with macromolecules.

Bromobenzene is an example of a chemical whose fate in the body occurs almost entirely via biotransformation. Elimination of this compound is via formation of the chemically reactive arene oxide 3,4-bromobenzene epoxide. Detoxification of the epoxide occurs both enzymatically and nonenzymatically, with 70% of a nontoxic dose being converted to the glutathione conjugate by glutathione-*S*-epoxide transferase in the liver. Administration of toxic doses of bromobenzene depletes available glutathione, thus reducing conjugation of the reactive electrophilic metabolite with glutathione and, consequently, increasing alkylation of macromolecules and toxicity. Toxicity is not incurred until depletion of glutathione reaches a critical level. Here again is an example of nonlinear pharmacokinetics and a threshold for the toxic response.

For a summary of what we have presented thus far, refer to Figure 3. As increasing amounts of fluid flow into the barrel, elimination via the lower slit becomes overwhelmed, leading to disproportionate increases in the amount of fluid in the barrel and/or elimination via the upper slit. In the body, elimination via the upper slit corresponds to excretion via other routes, metabolism via other pathways, or, in some cases, reactions with macromolecules.

In the foregoing we have provided a conceptual basis for why metabolic

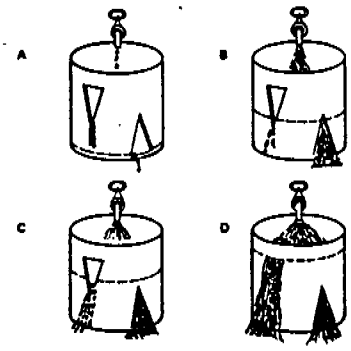


Figure 3
Diagrammatic representation of the elimination of a chemical via a primary saturable pathway and a secondary pathway whose significance increases upon saturation of the primary pathway.

thresholds may lead to a disproportionate increase in toxicity, inclusive of carcinogenesis. For a more complete treatise on the use of pharmacokinetics in assessing the hazard of chemicals, refer to Gehring et al. (1976). To elucidate the concept further, we will utilize the results of 1,4-dioxane and vinyl chloride pharmacokinetic studies conducted in the Toxicology Research Laboratory of The Dow Chemical Company.

1,4-Dioxane

1,4-Dioxane was reported to cause hepatomas and nasal carcinomas in rats maintained for over 13 months on drinking water containing 0.75% to 1.8% dioxane (Argus et al. 1965, 1973; Hoch-Ligeti et al. 1970). Kociba et al. (1974) confirmed these findings in rats maintained for 2 years on drinking water containing 1% dioxane. These rats also experienced an excessive number of deaths and exhibited severe renal and hepatic pathology. In rats maintained on water containing 0.1% dioxane, significant renal and hepatic damage was observed, but no increased incidence of tumors occurred. A further tenfold reduction in the concentration of dioxane in the water, to 0.01%, produced no discernible untoward effects. For the three levels of dioxane in the drinking water of rats, the approximate daily doses of dioxane were 1015 and 1599, 99 and 148, and 10 and 19 mg/kg/day for males and females, respectively.

The carcinogenic activity of dioxane, revealed only in rats receiving doses which superseded those necessary to cause death and severe pathology, raised the question of whether dioxane poses a carcinogenic hazard to individuals exposed to small amounts in their work environment. To provide additional information for making this judgment, a study was conducted to determine whether the fate of dioxane is influenced by dose (Young and Gehring 1976).

Figure 4 shows the plasma concentration–time curves for [^{14}C]dioxane in rats given single intravenous doses of [^{14}C]dioxane ranging from 3 to 1000 mg/kg. The clearance of dioxane from plasma is markedly dose-dependent and in accordance with Michaelis-Menten kinetics. The area under the curve increases disproportionately with dose, indicating that the elimination of dioxane is a saturable, dose-dependent or nonlinear pharmacokinetic process. The excretion of ^{14}C-activity by rats given various doses of [^{14}C]dioxane also demonstrated dose-dependent kinetics. As the dose was increased, more dioxane per se was eliminated via exhalation; at low doses, essentially all was excreted rapidly as β-hydroxyethoxyacetic acid in the urine. Thus the bio-

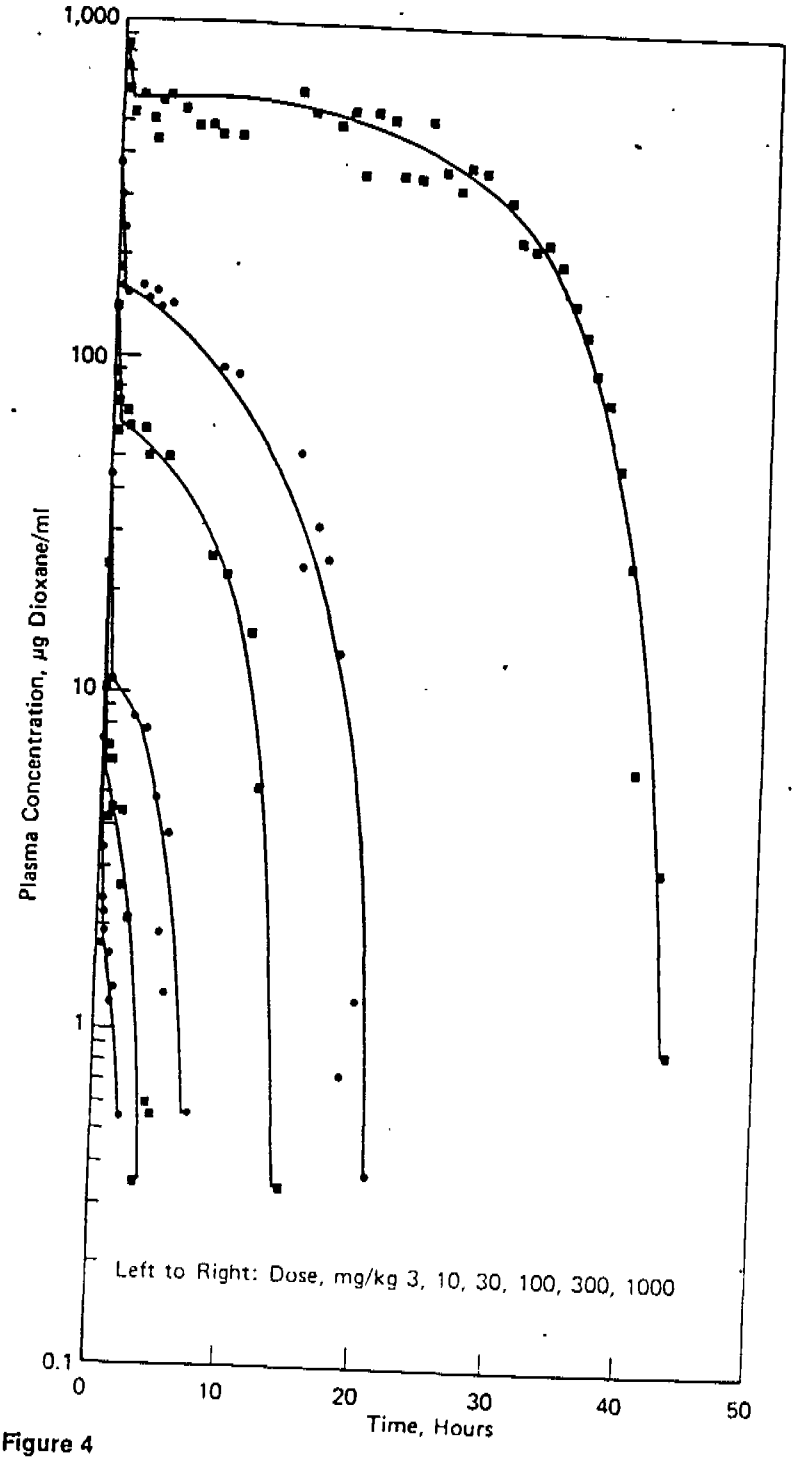


Figure 4

Concentration of dioxane per se in plasma of rats given various doses of dioxane intravenously.

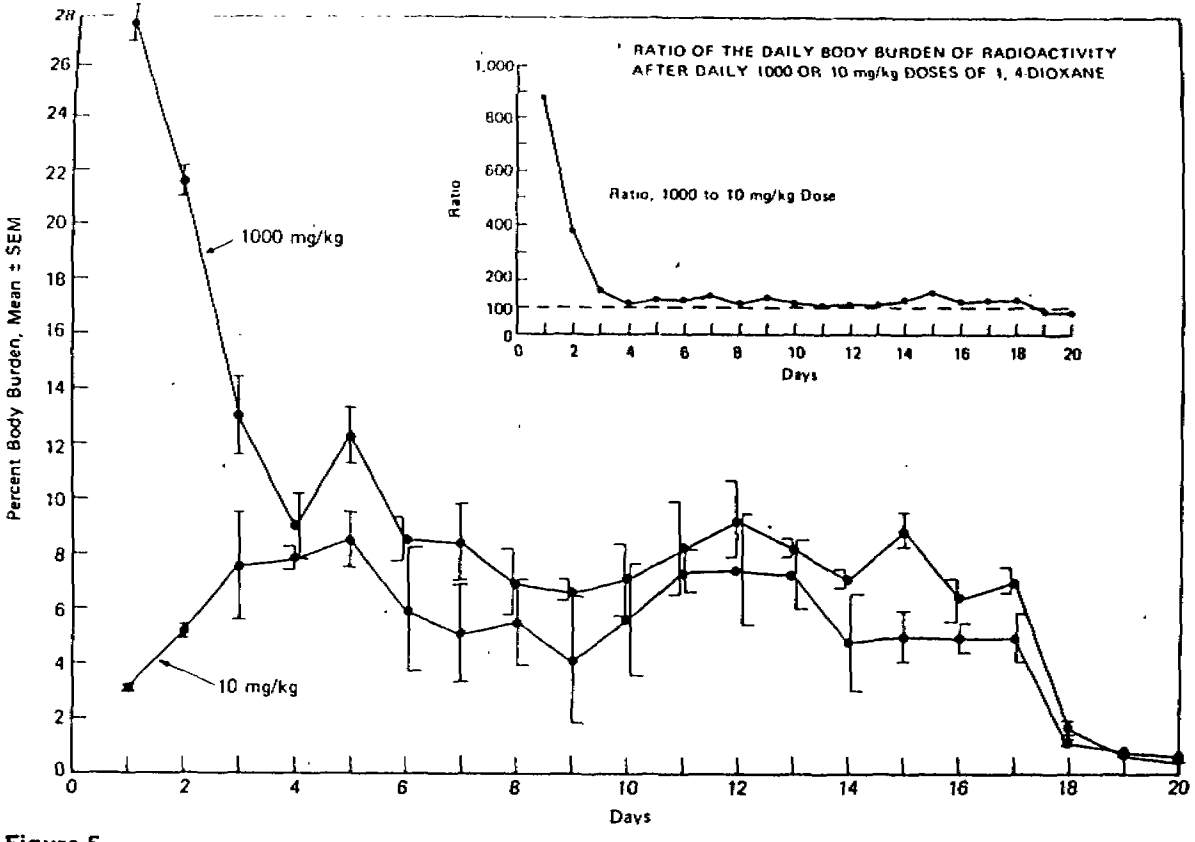


Figure 5

Daily body burden of ^{14}C-radioactivity of rats given oral doses of 10 or 1000 mg dioxane/kg/day. The body burden was calculated by subtracting the total cumulative excretion by all routes from the total cumulative dose and expressing the results as a percentage of the total cumulative dose. (*Inset*) Ratio of the daily body burden of ^{14}C-radioactivity after daily doses of 10 or 1000 mg/kg dioxane.

transformation of 1,4-dioxane to the detoxification product β-hydroxyethoxyacetic acid is a saturable process which is overwhelmed by increasing the magnitude of the dose. The marked retention of dioxane with increasing dose led us to conclude that the metabolism of dioxane must be markedly induced with repeated daily doses.

Figure 5 shows the body burden of radioactivity in rats given repeated daily oral doses of 10 or 1000 mg/kg for 17 days. The body burden was calculated by subtracting the total cumulative excretion by all routes from the total cumulative dose and expressing the results as a percentage of the total cumlative dose. The body burden in rats given 10 mg/kg/day dioxane averaged about 5% and ranged between 2% and 9%, with no apparent upward or downward trend. However, a striking decrease occurred in the body burden of rats given 1000 mg/kg/day dioxane during the first 4 days of administration. This indicates that a dose of 1000 mg/kg/day but not 10 mg/kg/day caused a marked induction of the elimination of dioxane. In essence, the rats receiving 1000 mg/kg/day dioxane had undergone marked biochemical alterations; their responses to dioxane, toxicological or carcinogenic, are no longer extrapolatable to rats receiving low doses.

Metabolic induction itself has been shown to increase tumorigenesis. Peraino et al. (1973a) demonstrated an enhancement of tumorigenesis in mice given 0.05% phenobarbital, a well-known inducer of metabolism, in their diet. Furthermore, induction of metabolism in rats by phenobarbital enhanced tumor production by 2-acetylaminofluorene (2AAF), a known hepatic carcinogen (Peraino et al. 1973b), suggesting that induction may enhance the expression of tumors by naturally occurring carcinogens.

The marked dose-dependent fate of dioxane and the strong metabolic induction by high doses, together with the toxicological results reported by Kociba et al. (1974), negate extrapolation of high-dose carcinogenesis to predict the hazard of low doses of dioxane. Such extrapolations violate common sense as well as the a priori assumptions for such statistical extrapolations. In rats, cancer induction occurs only when the doses are sufficient to cause marked pathology, metabolic alteration, and even death. These effects, including carcinogenesis, are correlative with the dose-dependent fate of dioxane. In light of this correlation, the hazard of low-level exposure to dioxane appears to be nil.

Vinyl Chloride

In our initial studies, four rats were exposed to varying starting concentrations of vinyl chloride (VC) in a closed, recirculating, 4.7-liter system (Hefner et al. 1975). The concentration of VC in the system was monitored by infrared analysis, and the rate at which the rats took up and chemically altered VC was determined as indicated in Figure 6. It is obvious that the rate of uptake, indicated by the slope, is markedly dependent on the initial exposure concentration. At an initial concentration of approximately 75 ppm VC, the rate of loss was much faster than the rate of removal from the chamber when the initial concentration was approximately 1000 ppm VC. The steeper the slope, the faster the rate of removal. In similar experiments, we demonstrated that the rates of removal between initial concentrations of 50 ppm and 105 ppm were the same, having a half-life ($t_{½}$) of 86 minutes. Between con-

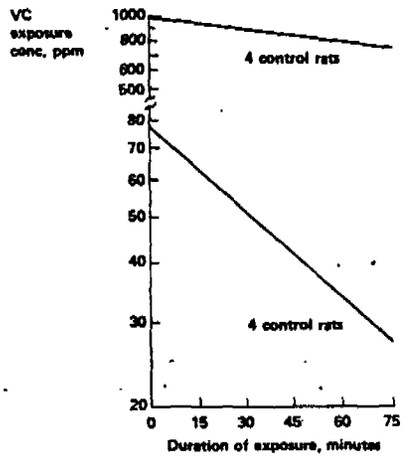


Figure 6
Semilogarithmic plot of the decline in the vinyl chloride concentration in a 4.7-liter, closed, recirculating chamber containing four rats (controls). Upper portion shows decline when initial concentration was approximately 1000 ppm; lower portion shows decline when initial concentration was approximately 75 ppm.

centrations of 220 ppm and 1167 ppm VC, the rates of removal were the same, with $t_{1/2} = 261$ minutes.

From the results of these studies, it was concluded that VC is metabolized or degraded readily at low concentrations, and that the primary pathway for degradation is swamped at concentrations exceeding 220 ppm.

Another objective means of demonstrating that the pathway for the metabolism or degradation of chemicals may be overwhelmed is to administer potential inhibitors of its metabolism. Figure 7 shows that the administration of 5 ml/kg of 95% ethanol inhibits profoundly the metabolism of VC by rats exposed to an initial concentration of 50 ppm ethanol but not that by rats exposed to approximately 1000 ppm. The percentages of inhibition were 96% and 40%, respectively. In other studies, we found that SKF-525A inhibited by approximately 20% the metabolism of VC by rats exposed to initial concentrations of approximately 1000 ppm but caused no inhibition in rats exposed to initial concentrations of less than 100 ppm. SKF-525A inhibits the mixed-function oxidase system of the cellular microsomes; this pathway is

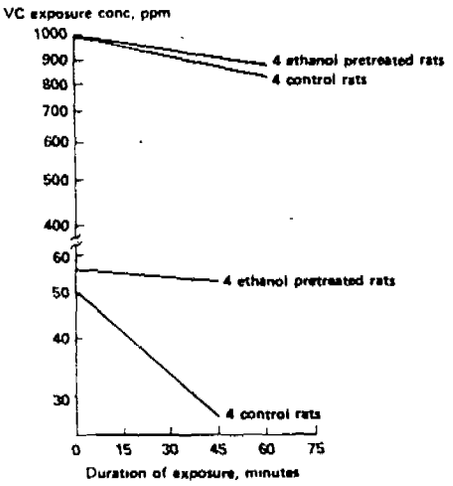


Figure 7
Semilogarithmic plot of the decline in the vinyl chloride concentration in a 4.7-liter, closed, recirculating chamber containing four control rats and four rats treated with 5 ml/kg ethanol.

Table 1
Percent of Administered ^{14}C-activity Eliminated following a Single Oral Dose of Vinyl Chloride

	Percent eliminated after doses (mg/kg) of			
Route	*0.05*	*1.0*	*20*	*100*
Expired as:				
VC	1.4	2.1	41.6	66.6
CO_2	9.0	13.3	4.8	2.5
Urine	68.3	59.3	22.6	10.8
Feces	2.4	2.2	1.0	0.5
Carcass and tissues	10.1	11.1	11.0	1.8
Total recovery	91.3	88.8	81.0	82.3

responsible for the metabolic degradation of many foreign compounds entering the body.

The results of these studies led to the conclusion that VC must be metabolized by at least two different pathways. The primary pathway for metabolism is overwhelmed as the exposure concentration is increased, until, at concentrations exceeding 220 ppm, the secondary pathway predominates. Using the barrel analogy, more escapes through the second slit as the flow into the barrel is increased.

In subsequent studies, we were fortunate to have radioactively tagged carbon-14 to follow the fate of VC. This greatly facilitated our ability to follow the disposition of the administered VC.

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 (Watanabe et al. 1976b). The ^{14}C-activity found in urine, feces, and carcass and tissues represents nonvolatile metabolites of VC. The key item to note in this table is that as the dose is increased from 0.05 and 1.0 mg/kg to 20 and 100 mg/kg, the percent expired as VC increases markedly, whereas the other parameters, particularly urinary excretion of ^{14}C-activity, decrease. Once again this demonstrates that the primary route for the elimination of VC from the body is dose-dependent, i.e., the primary route is overwhelmed and more begins to spill out via other routes (the upper slit in the barrel).

Figure 8 summarizes the dose-dependent excretion of VC via urinary excretion and pulmonary elimination. The area demarcated by the rectangle represents that range of doses over which distributive or metabolic saturation occurs. It is noteworthy that in ongoing carcinogenicity studies, Maltoni et al. (1975) have reported 19% and 14% incidences of tumors in rats given oral doses of 50 and 16.6 mg/kg/day, respectively. In rats given 3.33 mg/kg/day, no tumors have occurred, although extrapolation of the incidences of the two higher doses predicts an incidence of approximately 9%.

Although the data collected from studies in rats given oral doses of VC elucidate the dose-dependent fate of VC, the pharmacokinetics of inhaled VC are more pertinent to assessing the hazard of exposure in the industrial en-

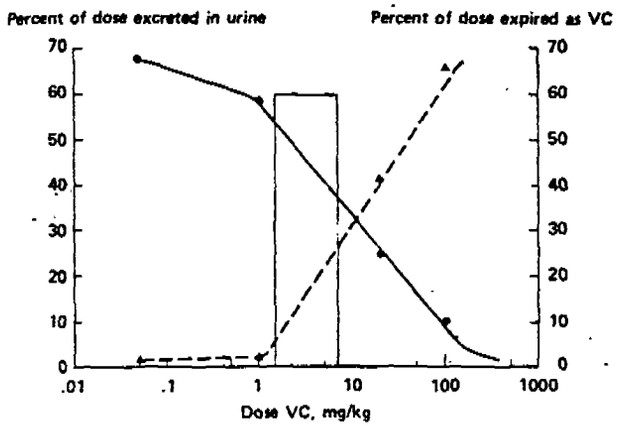


Figure 8
Summary of the dose-dependent excretion of VC via urinary excretion (——) and pulmonary elimination (– – –). Urinary excretion represents polar metabolites of VC, and pulmonary elimination is VC per se. The area demarcated by the rectangle represents the range of doses over which distributive and metabolic saturation occurs.

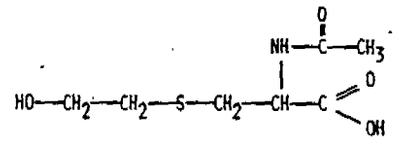
vironment (Watanabe et al. 1976c). Table 2 depicts the fate of [^{14}C]VC in rats exposed for 6 hours to 10 ppm or 1000 ppm VC. Immediately following the exposure, the rats were placed in cages equipped for collection of ^{14}C-activity in the expired air, feces, and urine during the subsequent 72 hours. 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 note in this table that the percent ^{14}C-activity found in the tissues and

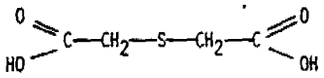
Table 2
Percent ^{14}C-activity Eliminated during 72 Hours following Inhalation Exposure to ^{14}C-labeled Vinyl Chloride for 6 Hours

Route	*Percent eliminated after exposure to*	
	10 ppm	*1000 ppm*
Expired as:		
VC	1.61 ± 0.16[a] (4)[b]	12.26 ± 0.96[a] (814)[b]
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[c]	0.15 ± 0.08 (<1)	0.23 ± 0.09 (15)
Total μg equivalents VC recovered	(248)	(6642)

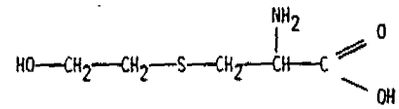
Values are percent of the total ^{14}C-activity recovered.
[a] Mean ± standard error from four rats.
[b] Microgram equivalents of vinyl chloride.
[c] Water, acetone wash of the metabolism cage at termination of the experiment.



(A) N-ACETYL(S-2-HYDROXYETHYL)CYSTEINE



(B) THIODIGLYCOLIC ACID



S-(2-HYDROXYETHYL)CYSTEINE

Figure 9
Metabolites of vinyl chloride found in the urine of rats. Metabolites A and B comprise approximately 30% and 25%, respectively, of the metabolites of vinyl chloride found in urine. A third metabolite, comprising 35% of the metabolites present in the urine, has been isolated but remains unidentified. These metabolites were isolated using high-pressure liquid chromatography. Authentic standards of each have been synthesized and cochromatographed. The unidentified metabolite was thought to be *S*-(2-hydroxyethyl)cysteine but it did not cochromatograph with an authentic standard.

carcass, though not statistically significant, increases slightly as the exposure is increased from 10 ppm to 1000 ppm. This is remarkable since 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 these tissues. This aspect is currently being investigated as such reactivity may explain carcinogenesis.

The results of these studies support the conclusions of previous studies that (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.

Since the metabolism of VC appears to occur via at least two pathways, a considerable effort has been made to identify the metabolites of VC. It had been demonstrated previously that a measurable amount of VC was metabolized to the end product of mammalian metabolism CO_2. By means of 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 shown in Figure 9. Metabolite A is *N*-acetyl(*S*-2-hydroxyethyl)cysteine. Metabolite B is thiodiglycolic acid. Together these metabolites comprise 50% to 60% of the radioactivity found in urine. Both of these metabolites are likely formed from the compound shown at the bottom, S-(2-hydroxyethyl)cysteine. At one point it appeared that the third major metabolite in urine, comprising about 30% of the radioactivity, was this compound. Even though some analytical comparisons between the isolated metabolite and S-(2-hydroxyethyl)cysteine favored this conclusion, others failed to establish identity.

The finding of these metabolites of VC in urine indicates that VC is trans-

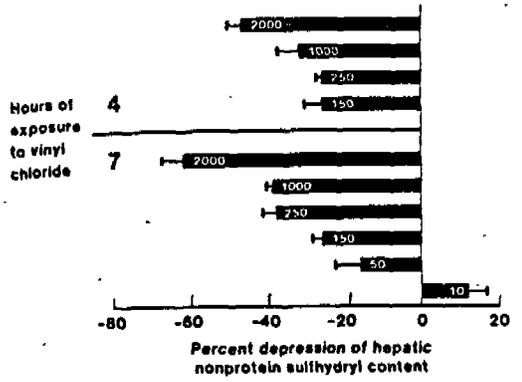


Figure 10
Percent depression of hepatic nonprotein sulfhydryl content vs. duration of exposure to 2000, 1000, 250, 150, 50, and 10 ppm vinyl chloride. Bars represent the mean ± standard error for five animals, except the point for 50 ppm which represents 25 animals. Depressions were significantly different for exposure concentrations of 150 ppm and greater but not for 50 ppm.

formed in the body to a reactive intermediate metabolite. This reactive metabolite is detoxified by reaction with glutathione. Subsequently the glutamic acid and glycine moieties of glutathione are cleaved, and the cysteine conjugate of the reactive metabolite of VC is either acetylated or further oxidized and excreted as the aforementioned metabolites.

Before leaving the subject of metabolism, it is important to state that the metabolites of VC were not changed either qualitatively or quantitatively as the dose or exposure level was increased. Since evidence exists that there are at least two pathways for the metabolism of VC, this indicates that both pathways produce reactive metabolites leading to the same end products. Our initial work and subsequently that of others (Kappus et al. 1975; Bolt et al. 1975; Barbin et al. 1975) indicate that one pathway involves oxidation of VC by microsomal enzymes to chloroethylene oxide.

As indicated previously, the reactive metabolites of VC are detoxified by reaction with glutathione. This is very important because it has been demonstrated that when high doses of some chemicals (e.g., bromobenzene and acetaminophen) are given, the glutathione is used up at a rate faster than it can be produced by conjugation with the reactive intermediates. As the level of glutathione in the liver is progressively depleted, the reactive metabolites react with macromolecules such as protein, DNA, and RNA, which leads to toxicity (Gillette 1974a,b). It is generally accepted that at least one mechanism for chemical carcinogenesis occurs through such reactions.

To assess the effect that exposures to VC may have on hepatic glutathione levels, rats were exposed to concentrations of 10, 50, 150, 250, 1000, or 2000 ppm for durations of from 1 to 7 hours (Watanabe et al. 1976a). The results are shown in Figure 10. Exposure to 150, 250, 1000, or 2000 ppm VC caused a progressive depression of the hepatic nonprotein sulfhydryl content; the hepatic nonprotein sulfhydryl content is primarily glutathione. Exposure to 50 ppm for 7 hours produced a small and inconsistent depression. No de-

Table 3
Correlation of the Incidence of Angiosarcoma and the Depression of Hepatic Nonprotein Sulfhydryl in Rats Exposed to Vinyl Chloride

Exposure concentration (ppm)	*Percent angio-sarcoma*[a]	*Mean latency*[b] *(weeks)*	*Nonprotein sulfhydryl depression*	
			4 hr	*7 hr*
10,000	19	64	—	—
6000	22	70	—	—
2500	22	78	—	—
2000	—	—	45	65
1000	—	—	30	45
500	12	81	—	—
250	7	79	25	35
150	—	—	20	20
50	2	135	0	15 (?)
10	—	—	0	0

[a] Maltoni and Lefemine (1975).
[b] Maltoni (1975).

pression 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, whereas 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? Maltoni and Lefemine (1975) reported the incidence of liver angiosarcoma in rats exposed to various concentrations of VC 4 hours/day, 5 days/week for 1 year and subsequently maintained until death. A summary of these data together with our data on the depression of the nonprotein sulfhydryl content of the liver is given in Table 3. At exposure concentrations of 250 ppm VC and greater, the incidence of angiosarcoma correlates well with the depression of hepatic nonprotein sulfhydryl content.

In the study of Maltoni and Lefemine (1975), a liver angiosarcoma incidence of 2% occurred in rats exposed to 50 ppm VC. As indicated previously, exposure to 50 ppm for 7 hours 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 (1975), not only are the incidences of liver angiosarcomas given but also the latency periods for their development. 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 latencies 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 hours/day is in the threshold transition zone not only for hepatic nonprotein free sulfhydryl depression but for tumor induction as well.

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, which appears saturable at high dose levels, 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 (Kappus et al. 1975), protein sulfhydryl groups, RNA (Bolt et al. 1975), and adenosine of DNA (Barbin et al. 1975). 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 hours caused no depression of hepatic nonprotein sulfhydryl content. This indicates that there is a *threshold* of exposure in rats where the ability to replace sulfhydryl groups is not overwhelmed and physiologic defense mechanisms remain fully operative. Furthermore, 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 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 concentrations exceeding 100 ppm VC are invalid for predicting the hazard of lower level exposures. Such projections violate the a priori assumption that the dynamics governing the fate of the compound are unaltered.

As an overall conclusion, the dose-dependent alterations in the fate of chemicals must be considered when using toxicological data, including carcinogenesis, obtained at high doses to assess the hazard of low doses. For many chemicals, large doses exceed metabolic and physiological thresholds, leading to prolonged retention in the body, formation of different metabolites, and in some cases disproportionate increases in reactions between reactive electrophilic metabolites and macromolecules, and, consequently, there is a disproportionate increase in toxicity, including carcinogenesis. Other compounds for which there is evidence indicating that their fate may be dose-dependent include styrene, ethylene glycol, aniline, carbon disulfide, 2-naphthylamine, benzopyrene, bis-hydroxycoumarin, salicylamide, amphetamine, and sulfobromophthalein (Gehring et al. 1976). It is likely that as more compounds are evaluated, dose-dependent fate will be found to be the rule rather than the exception.

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