

JUL 25 1977



THE DOW CHEMICAL COMPANY

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

July 21, 1977

Mr. Joseph Seawell
Manufacturing Chemist Assoc.
1825 Connecticut Avenue, NW
Washington, DC 20009

Dear Joe:

Because of comments received internally, Watanabe and Gehring have rewritten their May 28 report to better reflect what they feel is the relationship of animal to human data. I hope the Technical Panel finds this replacement draft useful. Will you:

- 1) Distribute the report to those indicated in my June 9, 1977 letter.
- 2) Indicate this is a replacement report and that the May 28 report should be destroyed.
- 3) Ballot for acceptance for the report by the panel.
- 4) Request authorization to pay \$12,000 to close this account.

Sincerely,

T. R. Torkelson, Sc.D.
Corporate Medical

MH

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14th Judicial District Court
Calcasieu Parish, Louisiana

P.S. Copies of attached report being sent under separate cover.



CHA 004679



THE DOW CHEMICAL COMPANY

June 9, 1977

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

Mr. Joseph Seawell
Manufacturing Chemist Assoc.
1825 Connecticut Avenue, NW
Washington, DC 20009

IMPORTANT: RESPONSE MAY
BE REQUIRED

Dear Joe:

As indicated in my letter of April 5, 1977, the final portion of the metabolic studies have been completed and 10 copies of the final report dated May 28 are enclosed. In addition 10 copies of an overall summary report dated May 31 with abstracts are also enclosed as requested by the Task Group.

Since this completes the contract, MCA will under separate cover, be billed for the balance of the cost which is \$12,000. Again, to expedite the distribution of the report, I am sending copies of the report and the final summary to each of the Task Group members.

Since it is the wish of Drs. Watanabe and Gehring to present this information at an international Symposium on Industrial Toxicology at the University of Surrey in Surrey England on July 25-30, I ask that you and each of the Task Group members immediately inform me if they have reasons as to why these reports should not be discussed at that meeting. If we do not have your comments by July 15, I will inform Drs. Watanabe and Gehring that they may proceed with the presentation.

I apologize for the short time provided for your review and ask your cooperation. You may wish to call me (517)636-5197, Dr. Watanabe (517)636-1313 or Dr. Gehring (517)636-1089.

Sincerely yours,

T. R. Torkelson, ScD
Chairman, MCA Technical Task Group
on Vinyl Chloride Research

ebg

cc: MCA Technical Task Group
on Vinyl Chloride Research
(Metabolism Studies, Dow Chemical)
P. J. Gehring
P. G. Watanabe

Enclosures

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CMA 004680

MCA Technical Task Group on Vinyl Chloride Research
(Metabolism Studies, Dow Chemical)

Air Products	W. M. Smith
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PPG	Z. G. Bell
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Stauffer	A. B. Lindquist
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Corporate Medical Department

A Memo From



T. R. Torkelson
(517) 636-5197

July 21, 1977

MCA Technical Penclon Vinyl
Chloride Research

Please destroy the May 28, 1977 report and replace with this draft. The tables of data are the same but the discussion has been expanded to include the relationship of animal to human response.

T. R. Torkelson

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RESOLUTION OF DOSE-RESPONSE TOXICITY DATA FOR CHEMICALS
REQUIRING METABOLIC ACTIVATION: EXAMPLE - VINYL CHLORIDE

BY: P. J. Gehring, P. G. Watanabe and C. N. Park

July 12, 1977

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14th Judicial District Court
Calcasieu Parish, Louisiana

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

This study was funded by the companies supporting the vinyl chloride projects being administered by the Manufacturing Chemists Association, Washington, D.C.

ABSTRACT

The toxicity of many chemicals results from biotransformation products formed from the chemical rather than to the chemical per se. In such cases, the incremental response may become diminishingly smaller with increasing dose or exposure because activation of the chemical to the toxic form follows apparent Michaelis-Menten rather than apparent first-order kinetics.

To illustrate this concept, rats were exposed to concentrations ranging from 1.4 to 4600 ppm vinyl chloride for 6 hours and the total amount metabolized determined. The amount metabolized followed apparent Michaelis-Menten kinetics. For rats, the logarithmic probability incidence of angiosarcoma versus the amount of vinyl chloride metabolized rather than the exposure concentration of vinyl chloride is linear. Assuming no threshold inspite of evidence to the contrary, extrapolation of the data below the range of doses causing experimentally observable responses predicted an incidence of 0.01% hepatic angiosarcoma in rats exposed to 4.6 ppm vinyl chloride. Theoretical extention of the extrapolation to humans after adjusting for metabolic and body mass differences was undertaken. The theoretical extrapolation for man exposed daily for 8 hours to 1 ppm suggests an incidence of 1.5 per 100,000,000. This theoretical incidence, although a likely

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overestimate because of a less than predicted incidence in men exposed to 200 ppm and greater as well as evidence for a threshold in rats, is less than that expected to occur spontaneously. It is concluded that pharmacokinetic parameters must be elucidated before designing toxicological experiments or before interpreting the results therefrom.

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INTRODUCTION

There exists a great deal of uncertainty in predicting the potential response of exposure to chemicals at concentrations below those producing an experimentally discernible response. This is particularly true when the response to the chemical in question is oncogenesis. Statistical projections recommended for assessing the risk of exposure to doses of oncogenic chemicals less than those producing an observable response include those based on logarithm probability curves (probit curves), logistic curves or linear curves (one-hit curves), (FDA Advisory Committee on Protocols for Safety Evaluation, 1971). One of the most commonly used statistical projections for risk assessment has been that promoted by Mantel and Bryan (1961) in which a logarithm probability projection with a slope of one is utilized. A flaw innate to all of these methods is that the dose-response information used to make the projection is based on the dose of chemical administered to the animal rather than the quantity of the administered dose giving rise to the response; the latter may either increase or decrease disproportionately as the administered dose is increased.

The use of high doses to reveal the chronic toxicity incurred with exposure to a chemical is a common, scientifically

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defensible practice if judgment and scientific rationale is used in designing the experiments and in assessing the resulting data. Such doses overwhelm frequently the enzymatic processes for activation of the chemical to the toxic form or for deactivation of the toxic form to an innocuous form. In this paper, it is demonstrated how the dose-dependent activation of vinyl chloride to an oncogenic product must be considered in resolving the dose-response of rats exposed via inhalation to vinyl chloride.

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METHODS

Material.

Vinyl chloride (^{14}C -labeled) was synthesized from (1,2- ^{14}C) 1,2-dichloroethane (New England Nuclear, Lot #819-221 and 819-292, 5.0 and 4.8 mCi/mmol, respectively) directly prior to use (Wagner, et al., 1975). Non-labeled VC (Matheson Gas Products) of 99.9% purity was mixed with the ^{14}C -material to obtain the desired specific activity.

Animals.

Male, Sprague-Dawley rats (Spartan Research Laboratory) weighing 200-250 g were used throughout the study. Food and water were provided ad libitum except during the exposure. Exposures were conducted between 9:00 a.m. and 3:00 p.m. (EST). Groups of 3-6 rats were exposed to various concentrations of ^{14}C -VC for 6 hours.

Exposure and Procedure.

The rats were exposed by inhalation under dynamic conditions in a 30 l glass inhalation chamber. The mean analytical concentrations of VC measured by gas chromatography were 1.4 ± 0.3 (SD), 9.3 ± 0.2 , 24.7 ± 1.4 , 51 ± 2 , 109 ± 23 , 250 ± 2 , 511 ± 11 , 1020 ± 13 , and 4600 ± 311 ppm. Details of this exposure and the method of analytical determinations have been reported previously (Watanabe, et al., (1976a)). Immediately following

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the 6-hour exposure to various concentrations of ^{14}C -VC (1.4-4600 ppm) the rats were killed by a blow to the head, and the carcass was analyzed for total radioactivity (Watanabe, et al., 1976b). Since radioactivity found in the carcass was non-volatile, this radioactivity represented the total amount of VC metabolized.

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RESULTS

Consistent with the results of previous studies (Watanabe, et al., 1976a and 1976b), the metabolism of VC by rats does not increase proportionately with increasing concentrations of VC being inhaled (Table 1). The nonlinearity of the amount of VC metabolized during 6 hours of exposure to various concentrations of VC appeared to be in accordance with Michaelis-Menten kinetics as described by the equation:

$$v = \frac{V_m S}{K_m + S} \quad (1)$$

In this equation, v and V_m , are the velocity and maximum velocity respectively for the biotransformation of VC expressed as μg equivalents VC metabolized per 6 hours. S and K_m are the concentration of VC being inhaled and the Michaelis constant expressed as $\mu\text{g VC/l air}$, respectively.

To ascertain whether Michaelis-Menten kinetics were applicable, the data in Table 1 were analyzed in accordance with the linear Woolf-Augustinson-Hofstee transformation of the Michaelis-Menten equation (Segel, 1976),

$$v = -K_m \frac{v}{S} + V_m \quad (2)$$

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It can be seen from the plot (Figure 1) that the data appear to lie along a straight line thus verifying, at least visually, the Michaelis-Menten model. V_m and K_m can be estimated by the ordinate intercept and the slope of the line or they can be estimated directly by fitting the nonlinear Michaelis-Menten model. Both procedures yield similar parameter estimates. The estimates derived by fitting the model directly are 8558 ± 1147 (SD) μg VC metabolized and 860 ± 159 (SD) μg VC/ ℓ air for V_m and K_m respectively.

Once a means is obtained to calculate the amount of VC metabolized by rats as a function of exposure, it is then possible to relate the untoward effects associated with VC exposure to the amount biotransformed rather than the exposure concentration of VC per se incurred by rats exposed to VC.

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Maltoni and Lefemine (1975) reported the incidence of hepatic angiosarcoma in rats exposed to different concentrations of VC, 4 hours/day, 5 days/week for 12 months and subsequently held for observation until death (Table 2). Before attempting to relate these data to the amount of VC biotransformed in accordance with the Michaelis-Menten equation using the previously determined values of V_m and K_m , the value for V_m must be adjusted for the shorter exposure duration used by

Maltoni and Lefemine, 4 hours versus 6 hours. This adjustment is accomplished by multiplying V_m by 4/6. Thus, the amount of VC biotransformed daily by rats exposed to the various concentrations used in the experiment of Maltoni and Lefemine can be calculated from the equation:

$$v = \frac{5706 \left(\frac{\mu g}{4 \text{ hr}} \right) \cdot S \left(\frac{\mu g}{l} \right)}{860 \left(\frac{\mu g}{l} \right) + S \left(\frac{\mu g}{l} \right)} \quad (3)$$

The resulting values for v are given in Table 2.

Figure 2 depicts a logarithm probability plot (probit plot) of the incidence of hepatic angiosarcoma observed in rats by Maltoni and Lefemine (1975) versus the amount of VC biotransformed for 4 hours of exposure, v , or the exposure concentration, S . The incidence of hepatic angiosarcoma in rats is linear with respect to $\log v$ but not $\log S$. The line drawn for $\log v$ versus tumor incidence (Figure 2) was determined by using a probit regression analysis program, and the equation relating the incidence of hepatic angiosarcoma to $\log v$ was:

$$\text{probit response} = -1.625 + 1.543 \log v \quad (4)$$

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Using the foregoing equation, a projection below the levels of exposure producing an experimentally discernible response has been made (dashed line). Assuming no threshold for the induction of angiosarcoma in rats exposed to VC, the exposure concentration producing one angiosarcoma in 10,000 rats can be calculated. The probit percent representing an incidence of 0.01% is 1.28. Substitution of this value into the equation (4) yields:

$$\text{Log } v = 1.8827$$

$$\text{Antilog } v = 76.33 \text{ } \mu\text{g VC metabolized/4 hours}$$

Using equation 3, the concentration of exposure to VC needed to give this value for v is 11.66 $\mu\text{g/l}$ or 4.6 ppm (95% confidence limits are from .08-22.2 ppm). Hence, exposure of rats to 4.6 ppm VC for 4 hours daily, 5 days/week for 1 year can be expected to produce one angiosarcoma per 10,000 rats if the dose-response curve remains valid at exposures less than those producing a discernible experimental response.

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DISCUSSION

For many chemicals, toxicity may not be a function of exposure to the chemical per se, but rather to a biotransformation product of the chemical. Frequently, production of a toxic metabolite is dependent upon enzymatically mediated reactions which are classically described by Michaelis-Menten kinetics. Since enzymatically mediated reactions are concentration-dependent and saturable, toxicity resulting from exposures to chemicals requiring activation to a toxic form cannot be related directly to the magnitude of exposure or dose. In such a case, it is necessary to determine the amount of the chemical undergoing biotransformation as a function of dose or exposure before a meaningful dose-response relationship can be established.

There is considerable evidence that vinyl chloride requires bioactivation to produce tumors. Metabolic activation is required to induce mutations in bacteria exposed to VC (Bartsch, et al., 1975; Malavielle, et al., 1975; Rannug, et al., 1974). Covalent binding of ^{14}C to hepatic macromolecules in rats (Watanabe, et al., 1977) exposed to ^{14}C -VC also requires bioactivation. Covalent binding of electrophiles to DNA has been associated with tumorigenesis.

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For vinyl chloride-induced hepatic angiosarcoma in rats, a logarithmic probability plot (probit plot) of the incidence versus the amount of vinyl chloride metabolized, v , over a range of exposures from 50 to 10,000 ppm VC gives a classical straight line, Figure 2. The dose-response relationship is not a straight line when plotted as a function of the exposure concentration, S . These results support further the conclusion that VC requires biotransformation to an active metabolite for tumorigenesis. Furthermore, a more reasonable evaluation of the dose-response data for vinyl chloride induced tumorigenesis requires knowledge of the amount of VC activated as a function of exposure.

After arbitrarily excluding data acquired from rats exposed to concentrations of VC exceeding 500 ppm in the experiment of Maltoni and Lefemine (1975), Schneiderman, et al (1975) extrapolated the remaining data to predict an incidence of 0.01% hepatic angiosarcoma in rats exposed to 1 ppm VC. This number is reasonably close to our prediction of 4.6 ppm VC for the same incidence. If Schneiderman, et al had used all of the data, a dose-response curve with an unrealistically shallow slope would have resulted and the predicted level causing 0.01% hepatic angiosarcoma would have been much smaller, on the order of 0.00001 ppm.

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The concepts developed herein allow use of all of the data presented by Maltoni and Lefemine (1975) to construct a dose-response curve on a scientifically defensible basis. Assuming that the resulting dose-response curve can be projected beyond the range of the experimentally discernible responses, the exposure concentration required to produce an incidence of 0.01% hepatic angiosarcoma in rats is 4.6 ppm.

Aside from interpreting toxicity data for chemicals requiring activation to a toxic form, there are some practical implications of the concepts presented herein for designing experiments to assess the toxicity, including carcinogenicity, of such chemicals. For these chemicals, increasing the concentration above the apparent K_m will produce diminishingly smaller increments in the response; no increase in the response is to be expected when the exposure concentration is 2 or 3 times K_m . Since total dose is a function of exposure time as well as concentration, it is important to determine the effect of exposure time on the response. As shown in Equation 3, the only parameter influence by exposure time is V_m , which is increased linearly with time. Therefore, after the concentration to which the animals are exposed becomes 2 to 3 times K_m , the amount metabolized, v , will increase linearly with increasing exposure time. For

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this reason, the gradation of incidence of angiosarcoma in rats exposed to high concentrations of VC will become a function of exposure time rather than concentration. This reasoning makes it imperative that the duration of exposure as well as exposure concentration be considered in evaluating the results of epidemiological studies of people exposed to high concentrations of VC in the work environment.

Unless the dose-dependent, Michaelis-Menten type pharmacokinetic parameters are resolved prior to designing the experiment, the results may be useless for characterizing the dose-response function for the untoward effects observed. Thus, the current approach using the maximum tolerated dose as defined presently and fractions thereof, may be scientifically unsound if the objective is to assess the potential toxicity of exposure to much lower doses or exposures.

For some chemicals detoxification of the chemical per se or reactive metabolites formed from the chemical may also be dose-dependent and saturable leading to a build-up of toxic materials. In such cases, the incremental responses to increasing doses or exposures will become disproportionately larger rather than smaller (see Gehring and Blau, 1977).

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In the foregoing analysis of the dose-response data for the induction of angiosarcoma in the rat, no threshold for the response was assumed. As indicated extrapolation below the range of doses causing an observable response may overestimate the response in rats because there is evidence that detoxification of reactive metabolites of VC may occur more efficiently in rats exposed to concentrations of VC below 50 ppm (Watanabe, et al, 1976c). Indeed, further analysis of the data reported by Maltoni and Lefemine (1975) also provides an indication of a practical threshold. In a subsequent presentation of these data, it was revealed that the latency for the development of hepatic angiosarcoma was respectively 64, 70, 78, 81, 79 and 135 weeks for rats exposed to 10,000, 6,000, 2,500, 500, 250 and 50 ppm VC (Maltoni, 1975). These results indicate that at the low level of exposure the time required for induction exceeds considerably the mean life expectancy for rats of approximately 104 weeks. This is consistent with the work of others suggesting that multiples of a lifetime may be required for expression of cancer in response to low doses of a carcinogen (Druckney, 1967 and Albert and Altshuler, 1973). Thus, extrapolation of the data obtained for rats below the range of exposures causing a discernible response may be expected to overestimate the projected incidence.

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The ultimate objective of a toxicological study is to develop data which can be used to assess the potential risk in man. Although such extrapolation is fraught with uncertainties, it is worthwhile to utilize the concepts presented herein to achieve this objective. The basic assumptions made are:

- 1) Induction of angiosarcoma is related to the amount of reactive metabolite of VC per unit of mass.
- 2) Exposure of rats for 12 months approximates exposure of workers for their working life.
- 3) There is no threshold for the induction of angiosarcoma in either rats or man which probably overestimates the assumption of risk as discussed above.
- 4) The efficiency of the metabolic processes involved in the conversion of VC to the reactive form is proportional to the body surface area. Since data for the biotransformation of VC by man are not available, the most logical basis for translation of the animal data to man would seem to be on the basis of body surface area.

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The last assumption deserves comment. There are considerable data in the literature showing that metabolism in general and other physiological parameters as well are relatable directly to the surface area of the body (see Schmidt-Nielsen, 1970 and Pinkel, 1958). For this reason, administration of biologically active chemicals to various species frequently gives an equivalent response when the dose is administered in proportion to the surface area of the body, that is dose per square meter of body surface (Pinkel, 1958). This relationship is gaining recognition in estimating the risk incurred by man from exposure to chemicals in the environment (Committee on Safe Drinking Water, National Research Council, 1977). In utilizing this relationship it must, however, be recognized that the original relationship was developed for biologically active agents. Since metabolism and other physiological processes involved in detoxification are more active in smaller animals, the dose of a biologically active chemical per unit of mass required to produce a given effect increases as the body mass decreases, while the dose per unit surface area remains relatively constant. However, for a chemical requiring activation to the biologically active toxic form, the total amount transformed will be roughly proportional to the body surface area. Since toxicity is a function of the concentration of the active form in tissue, this total amount transformed must then be normalized for mass to estimate an equivalent response.

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Using aforementioned rationale, the maximum velocity, V_m , for a 70 kg man can be estimated by calculation using the V_m value obtained for a 0.250 kg rat. The V_m of man for VC will be :

$$V_m \text{ (man)} = V_m \text{ (rat)} \left(\frac{1.85 \text{ sq m}}{0.045 \text{ sq m}} \right)$$

or

$$V_m \text{ (man)} = (8558 \text{ } \mu\text{g/6 hr}) \left(\frac{1.85 \text{ sq m}}{0.045 \text{ sq m}} \right) = 351829 \text{ } \mu\text{g/6 hr}$$

where the values 1.85 and 0.045 sq m are the body surface areas of a 70 kg man and a 0.250 kg rat, respectively (Pinkel, 1958). For an 8 hour exposure, the value is 469105 $\mu\text{g/8 hr}$. In order to use this number to theoretically estimate the response in man using data collected in rats, the V_m for man must be adjusted to a mass equivalent to that of rats since toxicity is a function of concentration in tissue. To do this the number is divided by 70 kg/0.25 kg or 280. The resulting V_m for man on a mass equivalent basis to that of rats is 1675 $\mu\text{g/8 hr}$. Using this value, the amount of VC transformed to a reactive form by man on a mass equivalent basis to rat is given by the equation:

$$V \text{ } \mu\text{g/8 hr} = \frac{1675 \text{ } \mu\text{g/8 hr} \cdot S \text{ } \mu\text{g/l}}{860 \text{ } \mu\text{g/l} + S \text{ } \mu\text{g/l}} \quad (5)$$

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Using this equation, the amount of VC transformed by man on a mass equivalent basis to rats was calculated as a function of exposure concentration and the expected incidence of angiosarcoma estimated from equation 4 (Table 3).

For men exposed to greater than 200 ppm VC, the incidence of angiosarcoma has been reported to be 0.02% (Fox and Collier, 1977). Hence, the theoretical calculated incidence using data from rats exceeds that currently detected by approximately 50 fold. This may indicate that people are less sensitive than rats to the induction of angiosarcoma or it may indicate that a practical threshold for the induction of angiosarcoma had been attained. Consistent with this latter possibility is that V on a mass equivalent basis to rats for men exposed to 200 ppm is 625. This number lies below that for rats exposed to 50 ppm. As indicated previously, angiosarcoma observed in rats exposed to 50 ppm occurred only in a rat that lived 135 weeks. Further, this rat did not die as a result of the angiosarcoma but was killed. Thus, as indicated previously, projection of the data collected in rats below the range of exposures producing an observable response may overestimate the incidence.

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Inspite of the likelihood of overestimating the incidence of angiosarcoma in rats or man, the incidence predicted for people exposed to 1 ppm, the current OSHA (Occupational Safety and Health Act) standard, is very small (1.5 per 100 million). This value which is likely an overestimate is below the expected incidence of spontaneous angiosarcoma reported to be 20 to 25 cases in the U.S. annually (Makk, et al, 1976).

In summary, it has been demonstrated that the incidence of VC induced angiosarcoma in rats is relatable not to the concentration of exposure but rather to the amount of VC biotransformed. Biotransformation of VC by rats is a dose-dependent process characterized by Michaelis-Menten type kinetics. The concepts evolved from this analysis reveals why pharmacokinetics must be considered in designing toxicology experiments as well as in interpretation of the resulting data. Having characterized the dose-response for induction angiosarcoma in rats as a function of the amount of VC biotransformed, a theoretical estimate of the incidence expected to occur in exposed men was undertaken using the data collected in rats. For daily 8 hr exposures to 1 ppm, the predicted incidence is 1.5 in 100,000,000 which is less than that expected to occur spontaneously. This predicted

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incidence is likely an over-estimate of that which will occur as a result of exposure to 1 ppm because there is some evidence for at least a practical threshold in both rats and man. There are no illusions that this estimate by extrapolation of data outside the range of doses causing experimentally observable responses and subsequently to man is without flaws. However, the rationale used represents a new approach which utilizes more logic than methods employed currently for such extrapolation.

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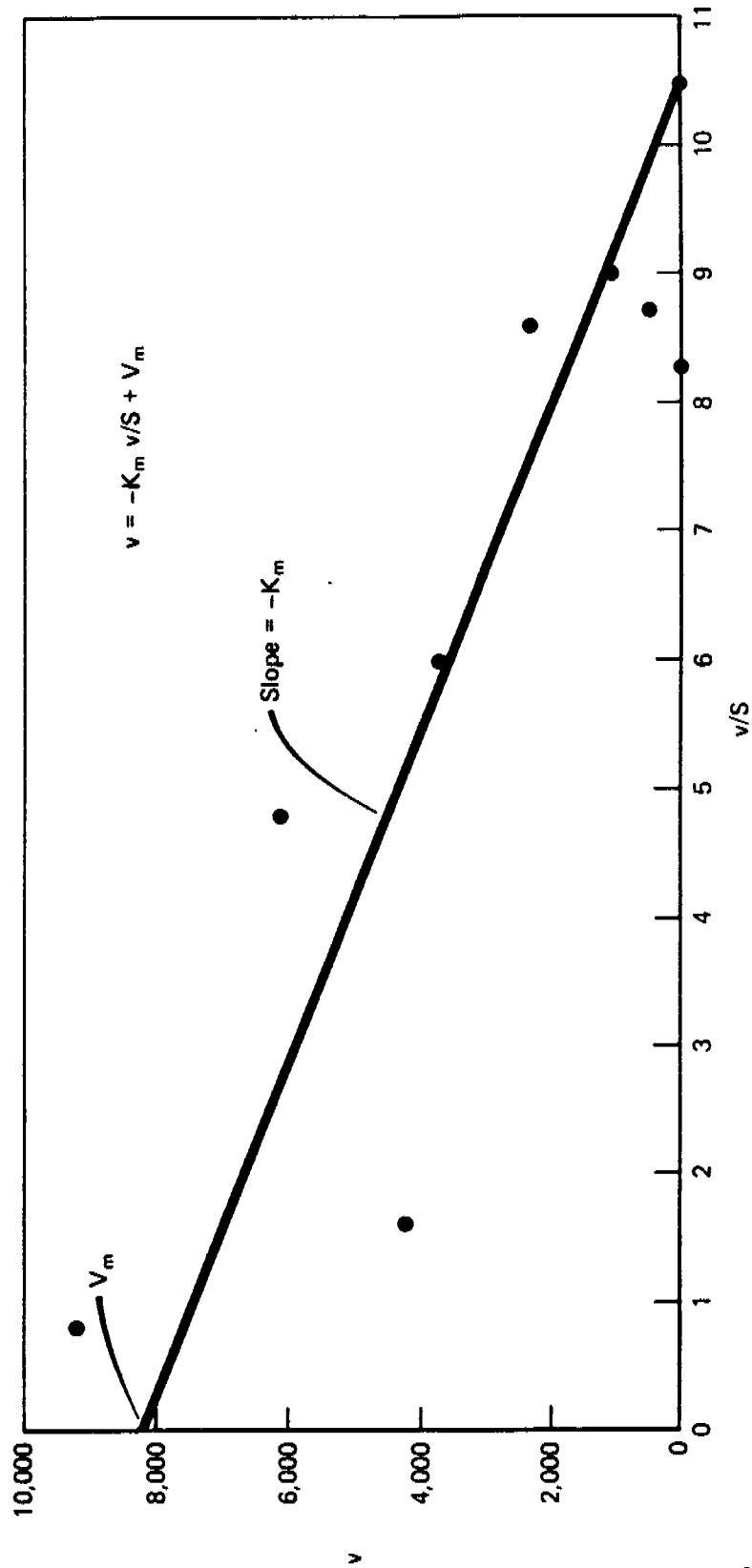
LEGENDS

Figure 1. Metabolism of vinyl chloride analyzed in accordance with the Woolf-Augustinson-Hofstee linearized form of the Michaelis-Menten equation. Values of v and v/S were taken from Table 1. The line was fit by linear regression analysis. The correlation coefficient, R , was 0.88.

Figure 2. (a) Metabolism of vinyl chloride expressed as $\log v$ ($\frac{\mu\text{g VC metabolized}}{4 \text{ hr}}$) versus percent incidence of hepatic angiosarcoma (probability scale). (b) Exposure concentration expressed as $\log S$ (ppm) versus the percent incidence of hepatic angiosarcoma. The probit equivalents of the percent incidence are shown on the right hand ordinant. The solid line is the best fit for experimentally observed responses while the dashed line represents extrapolation below those doses producing an observable response assuming no threshold.

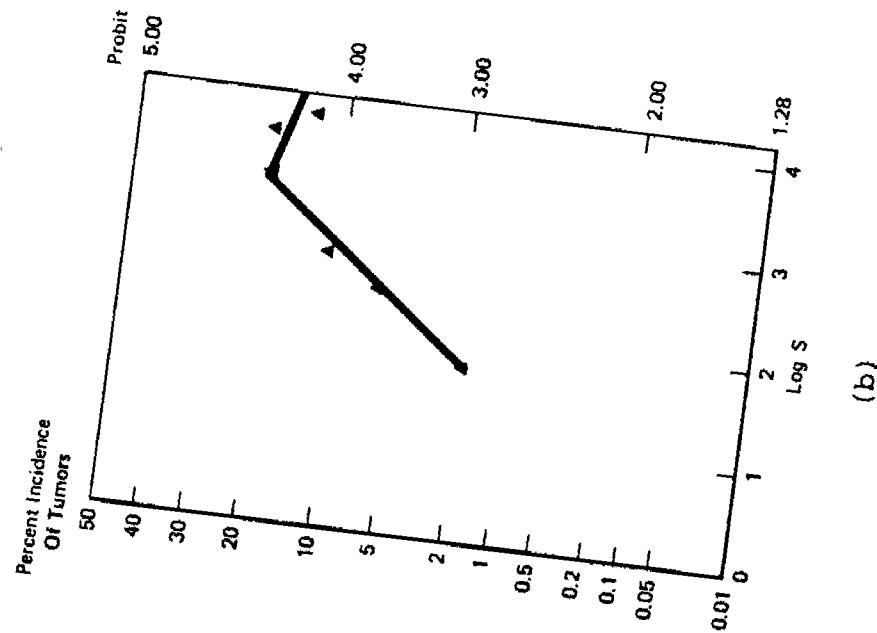
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FIGURE 1



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FIGURE 2



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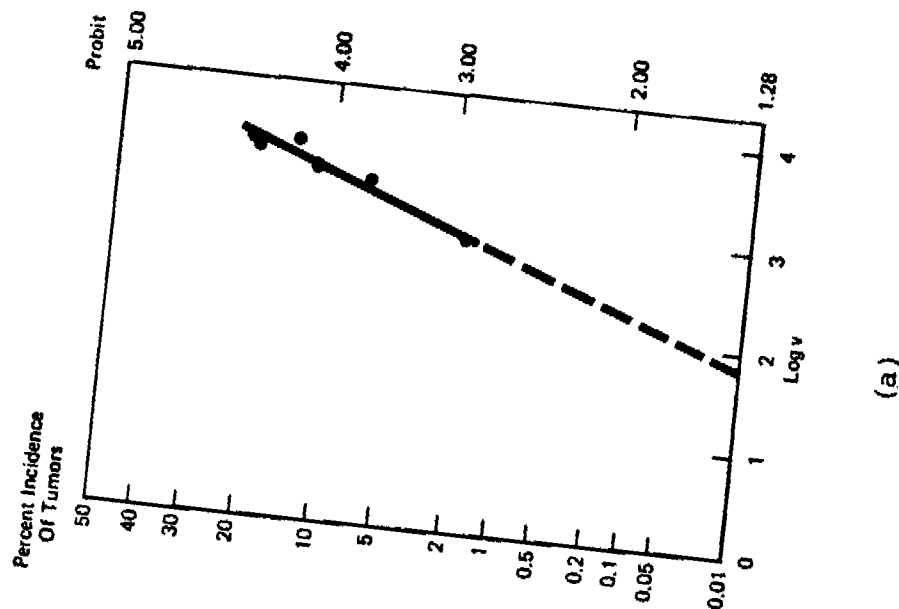


TABLE 1

Parameters for Describing the Metabolism of Inhaled Vinyl Chloride (VC) Using Michaelis-Menten Kinetics

Exposure Concentration <u>S (ppm VC)</u>	<u>S (μg VC/l air)^a</u>	<u>v μg VC metabolized^b 6 hr</u>	<u>v/S</u>
1.4	3.6	30±3 ^c	8.33
9	23.0	242±26	10.52
25	64.0	557±42	8.70
51	130.6	1181±93	9.04
109	279.0	2406±173	8.62
250	640.0	3826±345	5.98
511	1308.2	6263±355	4.79
1020	2611.2	4257±765	1.63
4600	11776.0	9255±1467	0.79

^a 1 ppm VC = 2.56 μg VC/l air

^b Determined from the total radioactivity in the carcass

^c Mean ± standard deviation

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TABLE 2

Correlation Between Exposure Concentration of Vinyl Chloride,
Metabolism and Induction of Hepatic Angiosarcoma in Rats

Exposure Concentration S (ppm VC)	S ($\mu\text{g VC/l air}$) ^a	$v \frac{\mu\text{g VC metabolized}}{4 \text{ hr}}$ ^b	log v	Percent Incidence of Hepatic Angiosarcoma ^c
10,000	25,600	5,521	3.742	15
6,000	15,360	5,403	3.733	22
2,500	6,400	5,030	3.702	22
500	1,280	3,413	3.533	12
250	640	2,435	3.386	7
50	128	739	2.869	2

^a 1 ppm VC = $2.56 \left(\frac{\mu\text{g VC}}{\text{l air}} \right)$

^b V_m corrected for 4 hour exposure, 8558 $\left(\frac{\mu\text{g VC metabolized}}{6 \text{ hr}} \right) \cdot 4/6 = 5706 \left(\frac{\mu\text{g VC metabolized}}{4 \text{ hr}} \right)$

$$v = \frac{5706 \left(\frac{\mu\text{g VC}}{4 \text{ hr}} \right) \cdot S(\mu\text{g/l})}{860(\mu\text{g/l}) + S(\mu\text{g/l})}$$

^c From Maltoni and Lefemine (1975).

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TABLE 3

Theoretical Amounts of Reactive Product Formed From VC By a 70 kg Man Exposed Continuously For 8 Hours and the Corresponding Expected Incidence of Angiosarcoma as Predicted From Data Collected in Rats Assuming No Threshold

Exposure Concentration		v μg VC metabolized ^b 8 hr	Log v	Probit Response ^c	Theoretical Percent Incidence of Angiosarcoma ^c
ppm	$\mu\text{g}/\ell$ ^a				
200	512	625	2.79	2.68	1.02
50	128	217	2.34	1.98	0.11
5	12.8	24.6	1.39	0.52	3.74×10^{-4}
1	2.56	4.97	0.70	-0.54	1.5×10^{-6}

^a 1 ppm = 2.56 $\mu\text{g}/\ell$.

^b v has been calculated using the Michaelis-Menten equation after calculating the V_{max} for man from the V_{max} determined for rats. This calculation was made by assuming V_{max} is proportional to body surface area and subsequently adjusting it to a mass equivalent to that of rats. See text.

^c The expected probit response was calculated from probit equation 3 (text). Subsequently the theoretical percent incidence of angiosarcoma was determined from the respective probit.

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SUMMARY OF THE STUDIES CONDUCTED ON THE PHARMACOKINETICS/
METABOLISM OF VINYL CHLORIDE IN RATS

By:

P. G. Watanabe, R. E. Hefner, Jr., J. A. Zempel,
D. G. Pegg, C. N. Park, and P. J. Gehring

May 31, 1977

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These studies were funded by companies supporting the vinyl
chloride projects being administered by The Manufacturing
Chemists Association, Washington, D.C.

SUMMARY OF THE STUDIES CONDUCTED ON THE PHARMACOKINETICS/
METABOLISM OF VINYL CHLORIDE IN RATS

Preliminary studies on the fate of inhaled vinyl chloride (VC) indicated two important points: 1) VC was metabolized extensively in vivo; and 2) the metabolism of VC was saturated at high exposure concentrations. Since it appeared that VC was biotransformed to a reactive metabolite which was responsible for carcinogenesis, studies on the pharmacokinetics and metabolism of VC were pursued. These studies confirmed that following large doses via oral administration (100 mg/kg) or high exposure concentrations via inhalation (1000 ppm) the metabolism of VC approached saturation. Furthermore, urinary metabolites of VC were identified to be conjugates of cysteine indicating that the reactive metabolite(s) are detoxified primarily by conjugation with hepatic glutathione (GSH).

Since the conjugation of chemicals with hepatic GSH has been shown to be a saturable process, studies were conducted to determine the effect of increasing exposure to VC on the depression of GSH in the liver. Exposure for 7 hours to concentrations of VC ranging from 150-2000 ppm caused a dose-related depression of GSH. Exposure to 50 ppm caused an inconsistent depression and exposure to 10 ppm caused no

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significant depression of hepatic GSH. These results along with the pharmacokinetic data suggested that reactive metabolites formed from exposure to low levels of VC (50 ppm) are detoxified readily by conjugation with GSH. However as the exposure concentration is increased detoxification will be impaired by the reduction of GSH. This will lead to an increased level of reactive metabolite at high exposure concentrations resulting in induction of cancer. The dose-related increase of hepatic angiosarcoma in rats exposed to VC concentrations ranging from 50-500 ppm (Maltoni data) correlate well with the dose-related depression of GSH.

Chemical carcinogenesis has been attributed to the reaction of electrophilic metabolites with intracellular macromolecules. The saturation of GSH dependent detoxification of VC with increasing exposure suggested that this would result in a disproportionate increase in the formation of reactive metabolite and subsequent reaction with intracellular macromolecules. Therefore, studies were conducted to assess the interaction of VC with intracellular macromolecules including nucleic acids.

The results showed that the total amount of radioactivity bound to macromolecules in the liver did not increase proportionately with the increase in the exposure concentration of

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VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized, and the amount of VC metabolized indicated evidence of saturation as the exposure concentration increased. There was no indication of a threshold for the reaction of VC metabolites with total intracellular macromolecules. However, at exposure concentrations exceeding 50 ppm the covalent binding of VC metabolites to macromolecules correlated well with the percent incidence of hepatic angiosarcoma in rats. The toxicologic significance of the covalent binding of VC to cellular components below 50 ppm was not clear. Deviation in covalent binding from the log-linear relationship at higher levels suggested that the carcinogenic response of the population may be changed at lower level exposures.

The majority of studies on the fate of VC have been conducted following single exposure. Since cancer is induced by repeated exposure a study was conducted to determine if the fate of VC in rats is altered with repeated exposure. The results indicated that repeated exposure to VC (6 hours/day, 5 days/week for 7-8 weeks) does not induce its biotransformation or alter the major routes or rates of elimination.

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However, covalent binding of VC metabolites to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. This increase in binding indicated that repeated exposure augments the reaction of electrophilic metabolites of VC with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

The results of all of the studies conducted by our laboratory thus far indicate that: 1) the metabolism of VC to a reactive metabolite which is ultimately responsible for carcinogenicity is a saturable process; and 2) the detoxification of VC at exposure levels below 10 ppm is more efficient than at higher levels and this diminished ability to detoxify VC at higher levels correlates with the induction of hepatic angiosarcoma. However, it has not been possible to associate definitive evidence for a threshold in the reaction of VC with hepatic macromolecules which can subsequently be correlated with induction of cancer in rats. More and more evidence is accumulating which suggests that carcinogenesis is associated with reaction of chemicals at specific sites on DNA rather than total reaction with DNA and other macromolecules. Since our studies to date have measured reaction of VC with total intracellular macromolecules or nucleic acids, this may be an explanation why it has not been possible to observe definitive evidence for a threshold. Studying the dose-

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response relationship between interaction of specific sites of DNA with VC may be a worthwhile endeavor for future studies.

The final report submitted with this summary illustrates the concept of relating the carcinogenicity of VC to the amount of VC metabolized rather than the exposure concentration. This concept is exceedingly important for chemicals such as VC where the metabolism to a toxic species is a saturable process. In such cases the increase in toxicity becomes diminishingly smaller with increasing dose or exposure because activation of the chemical follows apparent Michaelis-Menten (saturable) kinetics. Extrapolation of the VC data in this manner (assuming no threshold for carcinogenesis) indicated that an incidence of .01% hepatic angiosarcoma in rats may be expected from a daily exposure to 4.6 ppm VC. Failure to consider this concept leads to unrealistic estimates of risk.

Individual abstracts from all of the studies conducted in our laboratory on the pharmacokinetic/metabolism of VC are attached.

The secondary objective of the protocol for continued studies on the metabolism of vinyl chloride (Feb. 5, 1976) involving in vitro metabolism and identification of reactive metabolites was not completed. Problems were encountered on devising an

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in vitro system to metabolize satisfactorily sufficient amounts of VC, therefore it was not possible to pursue this aspect.

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ABSTRACTS

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PRELIMINARY STUDIES ON THE FATE OF INHALED VINYL CHLORIDE
MONOMER (VCM) IN RATS.

R. E. Hefner, Jr., P. G. Watanabe, and P. J. Gehring

ABSTRACT

Rats were exposed to vinyl chloride monomer gas (VCM) in a closed recirculating system. The rate at which VCM was removed from the system via metabolism was determined for rats exposed to initial concentrations of VCM ranging from 50 to 1167 ppm. Upon exposure to initial concentrations of 50 to 105 ppm, the rate of metabolism was $8.04 \pm 3.04 \times 10^{-3} \text{ min}^{-1}$. Upon exposure to initial concentrations ranging from 220 to 1167 ppm, the rate constants were less; the mean value being $2.65 \pm 1.35 \times 10^{-3} \text{ min}^{-1}$. Regardless of concentration, the disappearance followed apparent first order kinetics.

Pretreatment of rats with pyrazole prior to exposure to initial concentrations of 65 and 1234 ppm VCM caused 71 and 87% reductions in the rate of metabolism. Ethanol caused 96% and 83% reductions in the rate of VCM metabolism by rats exposed to 56 and 97 ppm VCM, respectively. Ethanol was less effective in blocking the rate of metabolism by rats exposed to high concentrations of VCM; 46 and 36% in rats exposed to 1025 and 1034 ppm VCM. In rats exposed to an initial concentration of 65 ppm VCM, SKF-525-A administration caused no inhibition of the rate of VCM metabolism; however, a 19% inhibition was seen in rats exposed to 1038 ppm.

The nonprotein sulfhydryl content of the liver (glutathione and cysteine) of rats exposed to VCM concentrations ranging from 50 to 15,000 ppm VCM is reduced without a relationship to dose. With repeated daily exposure the degree of reduction is reduced. Preliminary results indicate that the primary metabolites of VCM

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react with the nonprotein sulfhydryls. Final metabolic products excreted in the urine appear to be S-(2-hydroxyethyl) cysteine and S-(2-carboxymethyl) cysteine and the respective N-acetyl derivatives. Monochloroacetic acid was identified as another potential metabolite.

Considering the results in toto, it is hypothesized that VCM is readily and extensively metabolized. Metabolism via the primary pathway, postulated to involve alcohol dehydrogenase, is swamped by exposures to concentrations exceeding 220 ppm. In rats exposed to concentrations at and exceeding this level, metabolism occurs via a secondary pathway(s), postulated to be epoxidation and/or peroxidation. These results are considered pertinent in assessing the potential hazard at low level exposures to VCM. (Environmental Health Perspectives, (1975), 11, 85-95.

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FATE OF ^{14}C -VINYL CHLORIDE AFTER SINGLE ORAL ADMINISTRATION IN RATS

P. G. Watanabe, G. R. McGowan, and P. J. Gehring

ABSTRACT

Male rats were given single oral doses of 0.05, 1, and 100 mg/kg of ^{14}C -vinyl chloride (VC), and the routes and rates of elimination of ^{14}C activity followed for 72 hours. Following 0.05 and 1 mg/kg excretion in the urine as nonvolatile metabolites and as $^{14}\text{CO}_2$ in expired air accounted for 59-68% and 9-13%, respectively of the administered dose. Only 1-2% of the dose was expired by the lungs as VC. Conversely, after 100 mg/kg, 67% of the dose was eliminated by the lungs as VC, while urinary non-volatile metabolites and $^{14}\text{CO}_2$ comprised 11 and 3%, respectively. Pulmonary elimination after 100 mg/kg showed an apparent biphasic clearance with half-times ($t_{1/2}$) of 14.4 and 40.8 min for the respective fast and slow phases. Following 0.05 and 1 mg/kg the pulmonary clearance of VC was monophasic with $t_{1/2}$ of 53.3 and 57.8 min. The percentage of the dose remaining in the carcass after 72 hr was 10, 11 and 2% for the 0.05-, 1- and 100-mg/kg doses, respectively. The urinary radioactivity was separated by high pressure liquid chromatography into three major metabolites. Two of the three major urinary metabolites have been identified as N-acetyl-S(2-hydroxyethyl)-cysteine and thiodiglycolic acid by gas chromatography-mass spectrometry. The proportions of the urinary metabolites were not influenced by the dose. The fate of VC following an oral dose between 1 and 100 mg/kg was clearly dose-dependent. Consistent with our previous studies on the fate of VC following inhalation exposure in rats, the metabolism of VC appears to be a saturable process.

(Toxicology and Applied Pharmacology, (1976), 36, 339-352).

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FATE OF ^{14}C -VINYL CHLORIDE FOLLOWING INHALATION EXPOSURE IN RATS

P. G. Watanabe, G. R. McGowan, E. O. Madrid, and P. J. Gehring

ABSTRACT

Inhalation exposure to vinyl chloride (VC) has been shown to be carcinogenic in rats and man. It is important in assessing the toxicological potential of inhaled VC to understand the disposition of VC in the body. Therefore, the objective of the present study was to determine the fate of inhaled ^{14}C -VC at different exposure concentrations in rats. Male rats were exposed to 10 or 1000 ppm ^{14}C -VC for 6 hr and the routes and rates of elimination of ^{14}C -activity were followed for 72 hr after termination of exposure. Following exposure to 10 ppm of VC, urinary ^{14}C activity and expired VC comprised 68 and 2%, respectively, of the recovered radioactivity. After exposure to 1000 ppm of VC, the proportion of the radioactivity in the urine decreased while that expired as VC increased representing 56 and 12%, respectively. The pattern of pulmonary elimination of VC per se was described by similar apparent first-order kinetics following 10 or 1000 ppm with respective half-lives of 20.4 and 22.4 min. The elimination of ^{14}C activity in the urine occurred in accordance with a two-exponential equation; the half-lives for the initial phase of excretion were 4.6 and 4.1 hr following 10 and 1000 ppm, respectively. The percent of the recovered ^{14}C activity remaining in the carcass after 72 hr was 14 and 15% at the respective low and high exposure level. VC per se was not found in tissues. The urinary ^{14}C activity was separated by high pressure liquid chromatography into three major metabolites corresponding to N-acetyl-S-(2-hydroxyethyl)cysteine, thiodiglycolic acid, and a third unidentified metabolite. The proportions of the urinary metabolites were not markedly influenced by the exposure magnitude. The fate of inhaled ^{14}C -VC was shown to be dose-dependent; this is consistent with previous studies on the fate of VC following ingestion as well as inhalation.

(Toxicology and Applied Pharmacology, (1976), 37, 49-50.)

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

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

ABSTRACT

Rats were exposed by inhalation to 5000 ppm nonlabeled vinyl chloride (VC) 6 hours/day, 5 days/week for 7 weeks. On the last day of repeated exposure ^{14}C -labeled VC was used. The fate of the ^{14}C -VC was compared in the group of rats exposed repeatedly to a group exposed simultaneously for a single 6 hour period to 5000 ppm ^{14}C -VC. The routes and rates of excretion of ^{14}C -activity were the same for the two experimental groups. The activity of microsomal enzymes, as reflected by aniline hydroxylase and *p*-nitroanisole-*O*-demethylase of 9000 x g liver supernatants was essentially the same in rats exposed once, repeatedly or in nonexposed control rats. Covalent binding to hepatic macromolecules was greater in rats repeatedly exposed when compared to those subjected to a single exposure. These results indicate that repeated exposure to VC does not induce its biotransformation. However, the increase in hepatic macromolecular binding indicates that repeated exposure augments the reaction of electrophilic metabolites with macromolecules, and this may be expected to enhance potential toxicity including carcinogenicity.

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VINYL CHLORIDE-INDUCED DEPRESSION OF HEPATIC NON-PROTEIN
SULFHYDRYL CONTENT AND EFFECTS ON BROMOSULPHALEIN (BSP)
CLEARANCE IN RATS

P. G. Watanabe, R. E. Hefner, Jr., and P. J. Gehring

ABSTRACT

Rats were exposed to atmospheres of 2000, 250, 150, 50 and 10 ppm vinyl chloride (VC) for 1-7 hr to determine the effect of VC on the hepatic non-protein sulfhydryl content. Exposure to 2000, 1000, 250 and 150 ppm VC caused a progressive depression of the hepatic non-protein sulfhydryl content. Following exposure to 50 ppm VC for 7 hr the depression was inconsistent, and no depression was observed after 10 ppm VC for 7 hr. Also, exposure to 1000 ppm VC did not alter the serum clearance of bromosulphalein (BSP).

(Toxicology, (1976), 6, 1-8)

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EFFECT OF ETHANOL ON THE FATE OF VINYL CHLORIDE IN RATS

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

ABSTRACT

Ethanol pretreatment is known to alter the metabolism of many chemicals. Since it has been demonstrated previously that a single dose of ethanol inhibits the biotransformation of vinyl chloride (VC), the objective of this study was to investigate the effect of repeated and acute administration of ethanol on the fate of VC in rats. One group of rats was given 3.2 g/kg ethanol 0.5 hr prior to exposure of VC and another group was maintained on drinking water providing a daily dose of 11.4 g/kg ethanol for 22 days before exposure to VC. Subsequently, these rats and an untreated control group were exposed to an atmosphere containing 100 ppm ^{14}C -VC for 6 hours.

The rats pretreated repeatedly with ethanol showed a slight reduction in the total amount of VC metabolized (6%) and the degree of binding to hepatic macromolecules (26%) when compared to the group receiving no ethanol. In contrast, those pretreated acutely with ethanol showed a marked reduction in total metabolism (72%) and hepatic macromolecular binding (81%) when compared to controls. Similarly, repeated ethanol treatment did not affect markedly the routes or rates of excretion of ^{14}C -activity. However, associated with the reduction in overall metabolism of VC the acute ethanol treated rat excreted a larger proportion of the recovered radioactivity as exposed VC than the VC exposed control (13 versus 3%). It was concluded that repeated administration of ethanol for 22 consecutive days has little effect on the fate of VC in rats. In contrast, acute administration of ethanol markedly inhibits the metabolism of VC and subsequent covalent binding to hepatic macromolecules.

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HEPATIC MACROMOLECULAR BINDING FOLLOWING EXPOSURE TO VINYL CHLORIDE

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

ABSTRACT

Covalent binding of radioactivity to hepatic macromolecules in rats exposed to ^{14}C -labeled vinyl chloride (VC) was studied to determine if VC induced carcinogenesis may be related to electrophilic alkylation of macromolecules in vivo. Male Sprague-Dawley rats were exposed to 1, 10, 25, 50, 100, 250, 500, 1000 or 5000 ppm ^{14}C -VC for 6 hours. Following exposure radioactivity covalently bound to hepatic macromolecules and purified nucleic acids (RNA, DNA) were determined. The total amount of ^{14}C -VC metabolized and hepatic glutathione (GSH) content was also determined. The total amount of radioactivity bound to macromolecules in the liver did not increase proportionately with the increase in the exposure concentration of VC. A disproportionate decrease in macromolecular binding was observed as the concentration of VC increased. The covalent binding to hepatic macromolecules was related to the amount of VC metabolized. At exposures greater than 50 ppm, the amount of ^{14}C bound to macromolecules in the liver correlates with induction of hepatic angiosarcoma. There was no preferential binding of radioactivity to either DNA or RNA in the liver. Hepatic glutathione content was significantly depressed only at exposure concentrations greater than 100 ppm.

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RESOLUTION OF DOSE-RESPONSE TOXICITY DATA FOR CHEMICALS
REQUIRING METABOLIC ACTIVATION: EXAMPLE - VINYL CHLORIDE

P. J. Gehring, P. G. Watanabe, and C. N. Park

ABSTRACT

The toxicity of many chemicals result from exposure to biotransformation products formed from the chemical rather than to the chemical per se. In such cases, the incremental response may become diminishingly smaller with increasing dose or exposure because activation of the chemical to the toxic form follows apparent Michaelis-Menten rather than apparent first-order kinetics.

To illustrate this concept, rats were exposed to concentrations ranging from 1.4 to 4600 ppm vinyl chloride for 6 hours and the amount metabolized determined. The amount metabolized followed apparent Michaelis-Menten kinetics. Subsequently, it was found that the tumorigenic response to vinyl chloride was linear with respect to the amount of vinyl chloride metabolized rather than the concentration of vinyl chloride to which rats were exposed. Extrapolation of the data analyzed in this manner indicated that an incidence of 0.01% hepatic angiosarcoma may be expected from an exposure to 4.6 ppm vinyl chloride. The concepts presented herein are exceedingly important in designing and interpreting experiments for which the objective is to determine the dose-response to chemicals requiring metabolic activation to a toxic form.

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THE DOW CHEMICAL COMPANY

June 9, 1977

BENNETT BUILDING
2030 DOW CENTER
MIDLAND, MICHIGAN 48640

Mr. Joseph Seawell
Manufacturing Chemist Assoc.
1825 Connecticut Avenue, NW
Washington, DC 20009

IMPORTANT: RESPONSE MAY
BE REQUIRED

Dear Joe:

As indicated in my letter of April 5, 1977, the final portion of the metabolic studies have been completed and 10 copies of the final report dated May 28 are enclosed. In addition 10 copies of an overall summary report dated May 31 with abstracts are also enclosed as requested by the Task Group.

Since this completes the contract, MCA will under separate cover, be billed for the balance of the cost which is \$12,000. Again, to expedite the distribution of the report, I am sending copies of the report and the final summary to each of the Task Group members.

Since it is the wish of Drs. Watanabe and Gehring to present this information at an international Symposium on Industrial Toxicology at the University of Surrey in Surrey England on July 25-30, I ask that you and each of the Task Group members immediately inform me if they have reasons as to why these reports should not be discussed at that meeting. If we do not have your comments by July 15, I will inform Drs. Watanabe and Gehring that they may proceed with the presentation.

I apologize for the short time provided for your review and ask your cooperation. You may wish to call me (517)636-5197, Dr. Watanabe (517)636-1313 or Dr. Gehring (517)636-1089.

Sincerely yours,

T. R. Torkelson, ScD
Chairman, MCA Technical Task Group
on Vinyl Chloride Research

ebg

cc: MCA Technical Task Group
on Vinyl Chloride Research
(Metabolism Studies, Dow Chemical)
P. J. Gehring
P. G. Watanabe

Enclosures

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RESOLUTION OF DOSE-RESPONSE TOXICITY DATA FOR CHEMICALS
REQUIRING METABOLIC ACTIVATION: EXAMPLE - VINYL CHLORIDE

BY: P. J. Gehring, P. G. Watanabe and C. N. Park

May 28, 1977

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This study was funded by the companies supporting the vinyl chloride projects being administered by the Manufacturing Chemists Association, Washington, D.C.

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ABSTRACT

The toxicity of many chemicals results from biotransformation products formed from the chemical rather than to the chemical per se. In such cases, the incremental response may become diminishingly smaller with increasing dose or exposure because activation of the chemical to the toxic form follows apparent Michaelis-Menten rather than apparent first-order kinetics.

To illustrate this concept, rats were exposed to concentrations ranging from 1.4 to 4600 ppm vinyl chloride for 6 hours and the total amount metabolized determined. The amount metabolized followed apparent Michaelis-Menten kinetics. Subsequently, it was found that the tumorigenic response to vinyl chloride was linear with respect to the amount of vinyl chloride metabolized rather than the concentration of vinyl chloride to which rats were exposed. Extrapolation of the data analyzed in this manner indicated that an incidence of 0.01% hepatic angiosarcoma may be expected from an exposure to 4.6 ppm vinyl chloride. The concepts presented herein are important in designing and interpreting experiments to determine the dose-response relationship for chemicals requiring metabolic activation to a toxic form.

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INTRODUCTION

There exists a great deal of uncertainty in predicting the potential response of exposure to chemicals at concentrations below those producing an experimentally discernible response. This is particularly true when the response to the chemical in question is tumorigenesis. It has been suggested that a logarithm probability plot (probit plot) of the incidence of the response versus dose may be projected to doses smaller than those producing a discernible response. For conservatism, Mantel and Bryan (1961) promoted using a slope of one. These procedures are strictly a descriptive exercise for the most part which are not applicable in all instances.

The use of high doses to reveal the chronic toxicity incurred with exposure to a chemical is a common, scientifically defensible practice if judgment and scientific rationale is used in designing the experiments and in assessing the resulting data. Such doses frequently overwhelm the enzymatic processes needed for activation of the chemical to the toxic form and the subsequent deactivation to an innocuous form. In this paper, it is demonstrated how the dose-dependent activation of vinyl chloride to a

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tumorigenic product must be considered in resolving the dose-response to vinyl chloride exposure in rats. This concept leads to a much more meaningful interpretation of the dose-response data and permits more meaningful extrapolations of that data.

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METHODS

Material.

Vinyl chloride (^{14}C -labeled) was synthesized from (1,2- ^{14}C) 1,2-dichloroethane (New England Nuclear, Lot #819-221 and 819-292, 5.0 and 4.8 mCi/mmole, respectively) directly prior to use (Wagner, et al., 1975). Non-labeled VC (Matheson Gas Products) of 99.9% purity was mixed with the ^{14}C -material to obtain the desired specific activity.

Animals.

Male, Sprague-Dawley rats (Spartan Research Laboratory) weighing 200-250 g were used throughout the study. Food and water were provided ad libitum except during the exposure. Exposures were conducted between 9:00 a.m. and 3:00 p.m. (EST). Groups of 3-6 rats were exposed to various concentrations of ^{14}C -VC for 6 hours.

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Exposure and Procedure.

The rats were exposed by inhalation under dynamic conditions in a 30 l glass inhalation chamber. The mean analytical concentrations of VC measured by gas chromatography were 1.4 ± 0.3 (SD), 9.3 ± 0.2 , 24.7 ± 1.4 , 51 ± 2 , 109 ± 23 , 250 ± 2 , 511 ± 11 , 1020 ± 13 , and 4600 ± 311 ppm. Details of this exposure and the method of analytical determinations have been reported previously (Watanabe, et al., (1976a). Immediately following

the 6-hour exposure to various concentrations of ^{14}C -VC (1.4-4600 ppm) the rats were killed by a blow to the head, and the carcass was analyzed for total radioactivity (Watanabe, et al., 1976b). Since radioactivity found in the carcass was non-volatile, this radioactivity represented the total amount of VC which was metabolized.

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RESULTS

Consistent with the results of previous studies (Watanabe, et al., 1976a and 1976b), the metabolism of VC does not increase proportionately with increasing concentrations of VC being inhaled (Table 1). The nonlinearity of the amount of VC metabolized during 6 hours of exposure to various concentrations of VC appeared to be in accordance with Michaelis-Menten kinetics as described by the equation:

$$v = \frac{V_m S}{K_m + S} \quad (1)$$

In this equation, v and V_m , are the velocity and maximum velocity respectively for the biotransformation of VC expressed as μg equivalents VC metabolized per 6 hours. S and K_m are the concentration of VC being inhaled and the Michaelis constant expressed as $\mu\text{g VC}/\ell$ air, respectively.

To ascertain whether Michaelis-Menten kinetics were applicable, the data in Table 1 were analyzed in accordance with the linear Woolf-Augustinson-Hofstee transformation of the Michaelis-Menten equation (Segel, 1976),

$$v = -K_m \frac{v}{S} + V_m \quad (2)$$

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It can be seen from the plot (Figure 1) that the data appear to lie along a straight line thus verifying, at least visually, the Michaelis-Menten model. V_m and K_m can be estimated by the ordinate intercept and the slope of the line or they can be estimated directly by fitting the nonlinear Michaelis-Menten model. Both procedures yield similar parameter estimates. The estimates derived by fitting the model directly are 8558 ± 11147 (SD) $\mu\text{g VC}$ metabolized and 860 ± 159 (SD) $\mu\text{g VC/l air}$ for V_m and K_m respectively.

Once a means is obtained to calculate the amount of VC metabolized as a function of exposure, it is then possible to relate the untoward effects associated with VC exposure to the amount biotransformed rather than the exposure concentration. Hence if the untoward effects of exposure are related to the formation of toxic metabolites of VC rather than VC per se, more meaningful predictions can be made.

Maltoni and Lefemine (1975) reported the incidence of hepatic angiosarcoma in rats exposed to different concentrations of VC, 4 hours/day, 5 days/week for 12 months and subsequently held for observation until death (Table 2). Before attempting to relate these data to the amount of VC biotransformed in accordance with the Michaelis-Menten equation using the previously determined values of V_m and K_m , the value for V_m must be adjusted for the shorter exposure duration used by

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Maltoni and Lefemine, 4 hours versus 6 hours. This adjustment is accomplished by multiplying V_m by 4/6. Thus, the amount of VC biotransformed daily by rats exposed to the various concentrations used in the experiment of Maltoni and Lefemine can be calculated from the equation :

$$v = \frac{5706 \left(\frac{\mu g \text{ VC}}{4 \text{ hr}} \right) \cdot S \left(\frac{\mu g}{g} \right)}{860 \left(\frac{\mu g}{g} \right) + S \left(\frac{\mu g}{g} \right)} \quad (3)$$

The resulting values for v are given in Table 2.

Figure 2 depicts a logarithm probability plot (probit plot) of the incidence of hepatic angiosarcoma versus the amount of VC biotransformed for 4 hours of exposure, v , or the exposure concentration, S . The incidence of hepatic angiosarcoma was linear with respect to $\log v$ but not $\log S$. The line drawn for $\log v$ versus tumor incidence (Figure 2) was determined by using a probit regression analysis program, and the equation relating the incidence of hepatic angiosarcoma to $\log v$ was:

$$\text{probit response} = -1.625 + 1.543 \log v \quad (4)$$

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Using the foregoing equation, the anticipated exposure concentration required to cause 1 angiosarcoma for 10,000 rats can be calculated. The probit percent representing an incidence of 0.01% is 1.28. Substitution of this value into the equation (4) yields:

$$\text{Log } v = 1.8827$$

$$\text{Antilog } v = 76.33 \text{ } \mu\text{g VC metabolized/4 hours}$$

Using equation 3, the concentration of exposure to VC needed to give this value for v is 11.66 $\mu\text{g/l}$ or 4.6 ppm (95% confidence limits are from .08-22.2 ppm). Hence, exposure of rats to 4.6 ppm VC for 4 hours daily, 5 days/week for 1 year can be expected to produce one angiosarcoma per 10,000 rats if the dose-response curve remains valid at exposures less than those producing a discernible experimental response.

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DISCUSSION

For many chemicals, toxicity may not be a function of exposure to the chemical per se, but rather to a biotransformation product of the chemical. Frequently, production of the toxic metabolite is dependent upon enzymatically mediated reactions which are classically described by Michaelis-Menten kinetics. Since enzymatically mediated reactions are concentration-dependent and saturable, toxicity resulting from exposures to chemicals requiring activation to a toxic form cannot be related directly to the magnitude of exposure or dose. In such a case, it is necessary to determine the amount of the chemical undergoing biotransformation as a function of dose or exposure before a meaningful dose-response relationship can be established.

There is considerable evidence that vinyl chloride requires bioactivation to produce tumors. Metabolic activation is required to induce mutations in bacteria exposed to VC (Bartsch, et al., 1975; Malavielle, et al., 1975; Rannug, et al., 1974). Covalent binding of ^{14}C to hepatic macromolecules in rats (Watanabe, et al., 1977) or in liver homogenates (Kappus, et al., 1976) exposed to ^{14}C -VC also requires bioactivation. Covalent binding of electrophiles to DNA has been associated with tumorigenesis.

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For vinyl chloride-induced hepatic angiosarcoma in rats, a logarithmic probability plot (probit plot) of the incidence versus the amount of vinyl chloride metabolized, v , over a range of exposures from 50 to 10,000 ppm VC gives a classical straight line, Figure 2. The dose-response relationship is not a straight line when plotted as a function of the exposure concentration, S . These results support further the conclusion that VC requires biotransformation to an active metabolite for tumorigenesis. Furthermore, a more reasonable evaluation of the dose-response data for vinyl chloride induced tumorigenesis requires knowledge of the amount of VC activated as a function of exposure.

Extrapolating the tumorigenicity data of Maltoni and Lefemine (1975), in the same manner as Schneiderman, et al., (1975) predicts that a concentration of 1 ppm VC will produce an incidence of 0.01% hepatic angiosarcoma compared to our prediction of 4.6 ppm VC for the same incidence. However, the Schneiderman, et al. prediction required arbitrary exclusion of data acquired from rats exposed to concentrations of VC exceeding 500 ppm. If all of the data had been used, a dose-response curve with an unrealistically shallow slope would have resulted and the predicted level causing 0.01% hepatic angiosarcoma would have been even smaller.

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The concepts developed herein allow use of all of the data presented by Maltoni and Lefemine (1975) to construct a dose-response curve on a scientifically defensible basis. Assuming that the resulting dose-response curve can be projected beyond the range of the experimentally discernible responses, the exposure concentration required to produce an incidence of 0.01% hepatic angiosarcoma is 4.6 ppm. Whether or not this statistical extrapolation below the range of discernible responses is reliable remains to be established. In this regard, there is evidence that detoxification of reactive electrophilic metabolites of VC may occur more efficiently in rats exposed to concentrations of VC below 50 ppm (Watanabe, et al., 1976c) in which case the actual concentration of VC required to produce an incidence of 0.01% hepatic angiosarcoma may be higher.

The concepts presented herein are exceedingly important in evaluating toxicity data obtained for chemicals requiring activation to a toxic form. Equally important are the inherent implications when designing an experiment to assess the toxicity, including carcinogenicity, of chemicals requiring bioactivation. For these chemicals, increasing the concentration above the apparent K_m will produce diminishingly smaller increments in the response;

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no increase in the response is to be expected when the exposure concentration is 2 or 3 times K_m . Since exposure is a function of time as well as concentration, it is important to determine the effect of exposure time on the response. As shown in Equation 3, the only parameter influenced by exposure time is V_m , which is increased linearly with time. Therefore, after the concentration to which the animals are exposed becomes 2 to 3 times K_m , the amount metabolized, v , will increase linearly with increasing exposure time. For this reason, the gradation of incidence of angiosarcoma in individuals exposed to high concentrations of VC will become a function of exposure time rather than concentration. This reasoning makes determination of exposure time as well as exposure concentration important in conducting epidemiological studies of people exposed to high concentrations of VC in the work environment.

Unless the dose-dependent, Michaelis-Menten type pharmacokinetic parameters are resolved prior to designing the experiment, the results may be useless for assessing the hazard incurred via exposure to the agent. Thus, the current approach using the maximum tolerated dose and fractions thereof may be scientifically tenuous if the objective is to assess the hazard of exposure to much lower doses or exposures.

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For some chemicals detoxification of reactive metabolites may also be dose-dependent and saturable leading to a build-up of toxic materials. In such cases, the incremental responses to increasing doses or exposures will become disproportionately larger rather than smaller (see Gehring and Blau, 1977). In conclusion, rational design of experiments to obtain dose-response data for chemicals requiring metabolic activation or deactivation and the subsequent rational interpretation of the results requires resolution of the pharmacokinetic parameters for the chemical.

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TABLE 1

Parameters for Describing the Metabolism of Inhaled Vinyl Chloride (VC) Using Michaelis-Menten Kinetics

Exposure Concentration <u>S (ppm VC)</u>	<u>S (µg VC/l air)^a</u>	<u>v µg VC metabolized^b</u> <u>6 hr</u>	<u>v/S</u>
1.4	3.6	30±3 ^c	8.33
9	23.0	242±26	10.52
25	64.0	557±42	8.70
51	130.6	1181±93	9.04
109	279.0	2406±173	8.62
250	640.0	3826±345	5.98
511	1308.2	6263±355	4.79
1020	2611.2	4257±765	1.63
4600	11776.0	9255±1467	0.79

^a 1 ppm VC = 2.56 µg VC/l air

^b Determined from the total radioactivity in the carcass

^c Mean ± standard deviation

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TABLE 2

Correlation Between Exposure Concentration of Vinyl Chloride,
Metabolism and Induction of Hepatic Angiosarcoma in Rats

Exposure Concentration S (ppm VC)	S ($\mu\text{g VC}/\ell$ air) ^a	v $\mu\text{g VC}$ metabolized ^b 4 hr	log v	Percent Incidence of Hepatic Angiosarcoma ^c
10,000	25,600	5,521	3.742	15
6,000	15,360	5,403	3.733	22
2,500	6,400	5,030	3.702	22
500	1,280	3,413	3.533	12
250	640	2,435	3.386	7
50	128	739	2.869	2

^a 1 ppm VC = $2.56 \left(\frac{\mu\text{g VC}}{\ell \text{ air}} \right)$

^b v_m corrected for 4 hour exposure, $8558 \left(\frac{\mu\text{g VC metabolized}}{6 \text{ hr}} \right) \cdot 4/6 = 5706 \left(\frac{\mu\text{g VC metabolized}}{4 \text{ hr}} \right)$

$$v = \frac{5706 \left(\frac{\mu\text{g VC}}{4 \text{ hr}} \right) \cdot S (\mu\text{g}/\ell)}{860 (\mu\text{g}/\ell) + S (\mu\text{g}/\ell)}$$

^c From Maltoni and Lefemine (1975).

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LEGENDS

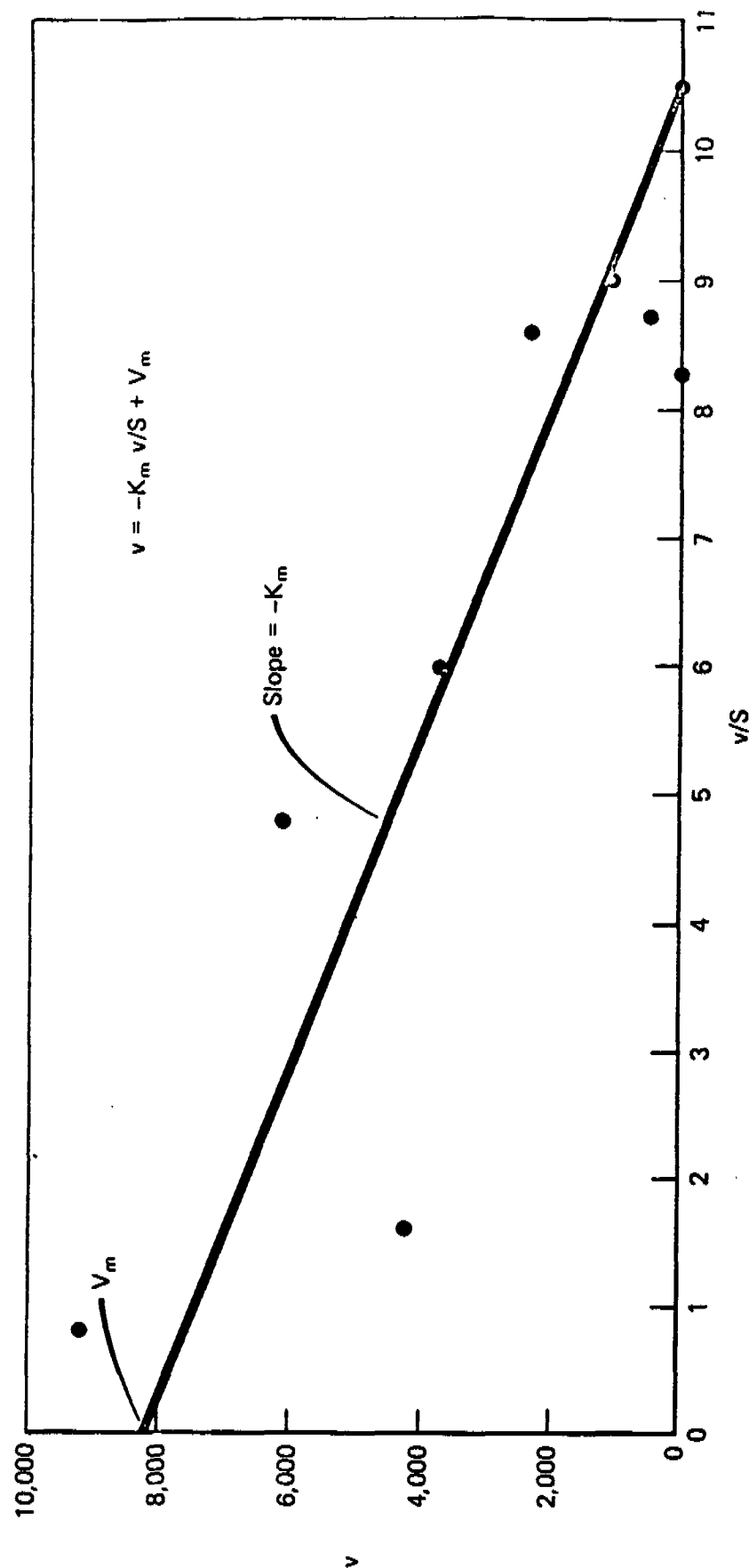
Figure 1. Metabolism of vinyl chloride analyzed in accordance with the Woolf-Augustinson-Hofstee linearized form of the Michaelis-Menten equation. Values of v and v/S were taken from Table 1. The line was fit by linear regression analysis. The correlation coefficient, R , was 0.88.

Figure 2. (a) Metabolism of vinyl chloride expressed as $\log v$ ($\frac{\mu\text{g VC metabolized}}{4 \text{ hr}}$) versus percent incidence of hepatic angiosarcoma (probability scale). (b) Exposure concentration expressed as $\log S$ (ppm) versus the percent incidence of hepatic angiosarcoma. The probit equivalents of the percent incidence are shown on the right hand ordinant.

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FIGURE 1



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FIGURE 2

