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

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

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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.

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

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.

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.

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.

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:

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

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 µg 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),

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

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 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

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 :

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

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:

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

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:

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

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

Using equation 3, the concentration of exposure to VC needed to give this value for v is 11.66 µg/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.

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.

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.

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;

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.

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 S (ppm VC)	S (µg VC/ℓ air)[a]	v $\frac{\mu g \text{ VC metabolized}}{6 \text{ hr}}$[b]	v/S
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

TABLE 2

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

Exposure Concentration S (ppm VC)	S (µg VC/ℓ air)[a]	v $\frac{\mu g\ VC\ metabolized}{4\ hr}$[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 = $2.56\left(\frac{\mu g\ VC}{\ell\ air}\right)$

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

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

[c] From Maltoni and Lefemine (1975).

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 ($\frac{\mu g \text{ VC metabolized}}{4 \text{ hr}}$) 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.

FIGURE 1

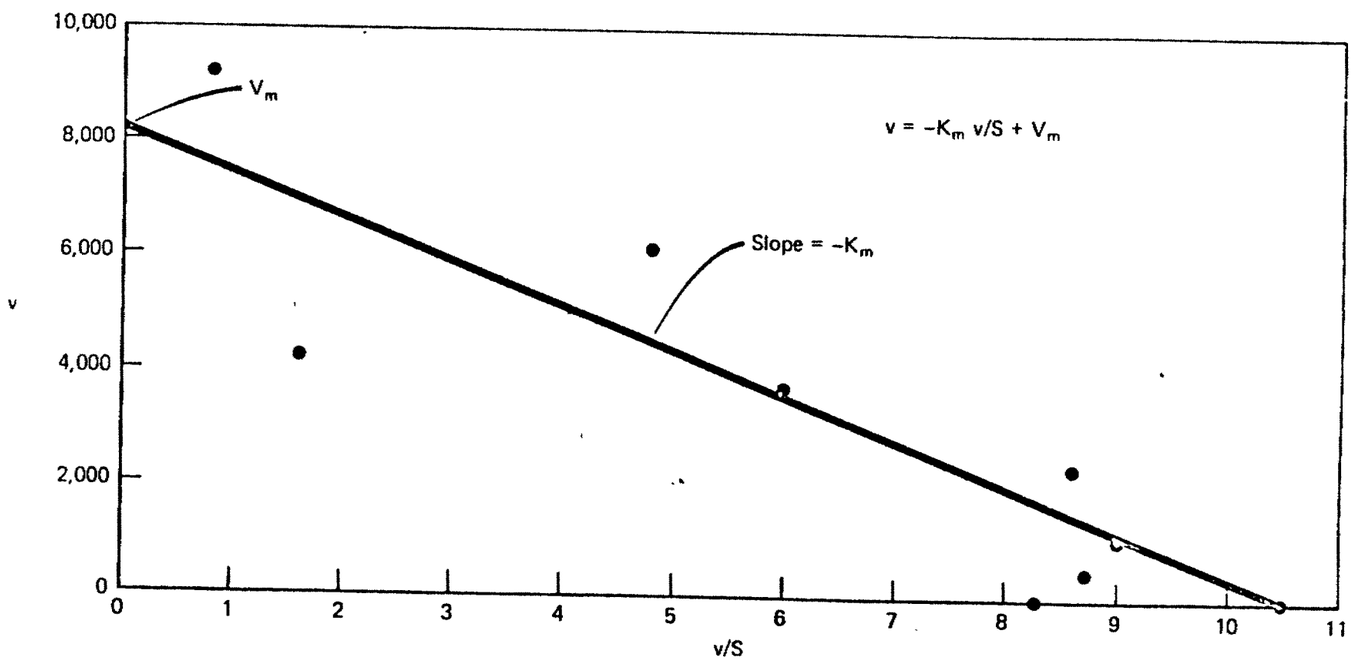

FIGURE 2