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. 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

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.

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

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.

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 metabolized.

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:

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

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 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.

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:

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

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:

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