

## Letters to the Editor

Dear Sir:

The recent article by McKenna et al. (1) on pharmacokinetics of vinylidene chloride (VDC) in the rat has evoked a number of thoughts. These relate both to one detail of data presentation from the excellent experimental studies and to the general role of pharmacokinetics in supporting risk assessment from high dose studies in mammals.

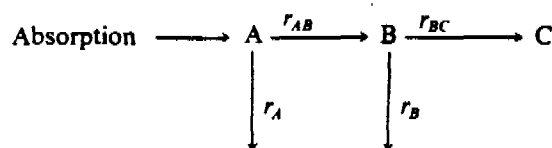
The detail concerns Figure 1a (Figure 2 of the McKenna article) in which they plotted covalent binding to liver macromolecules versus the logarithm of the exposure concentration of VDC. They concluded that the "dose-dependent or saturable character of VDC detoxification is apparent, indicating an initial deviation from first-order or linear kinetics at about 50 ppm." A linear-log plot such as this does not display underlying linear processes as a straight line but rather as a curved line such as they show. The data have been replotted on a linear scale in Figure 1b to illustrate this fact. The straight line is simply a reference through zero and the higher points. Quite clearly, the amount of radioactivity derived from VDC which remains associated with liver macromolecules is very nearly proportional to concentration at all concentrations studied. No simple relationship with nonprotein sulfhydryl (NPSH) levels is apparent from this data set alone.

My general comment concerns extrapolation of irreversible toxicity such as a cancer from an observable range to a lower range which is of social

concern but statistically inaccessible in an experiment which can be done. A theoretical rationale for extrapolation could be established by measurement of a biochemical effect which correlates with carcinogenicity in an observable range. One candidate which should be examined for such a correlation is covalent binding to DNA or other molecules.

It is well established that a foreign chemical may undergo metabolism to a more toxic form. The kinetic implications and consequences have been thoroughly discussed by Gillette (2, 3) and extended to include numerical simulations involving nonlinear transient reactions by Gehring (4).

The diagram shows a simplified reaction scheme following the approach of Gillette (3); much more complex biochemical networks are possible.



The parent chemical is represented by A, and the reactive intermediate is represented by B. The various reaction rates are indicated by  $r$  as follows:  $r_A$  and  $r_B$  represent the summation of all processes for elimination of A and B or chemical conversion to nontoxic products;  $r_{AB}$  is the reaction rate for con-

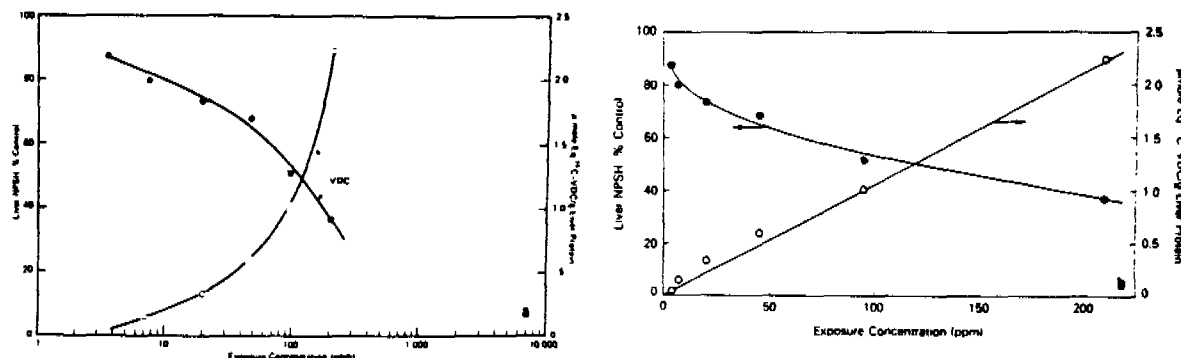


FIGURE 1a, b. Dose-response relationship for (●) hepatic NPSH depletion and (○) covalent binding of <sup>14</sup>C-VDC metabolites to liver protein following inhalation exposure of rats (1).

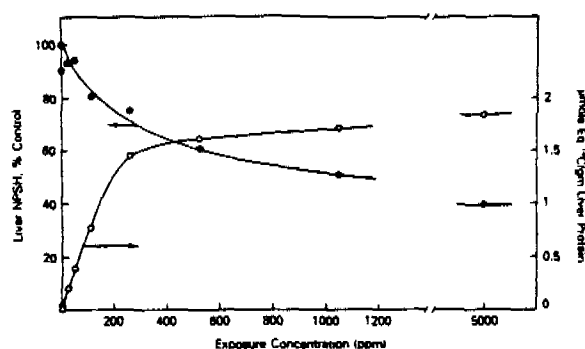


FIGURE 2. Dose-response relationship for hepatic NPSH depletion and covalently bound  $^{14}\text{C}$ -activity in the liver following  $^{14}\text{C}$ -VC exposure of rats (1).

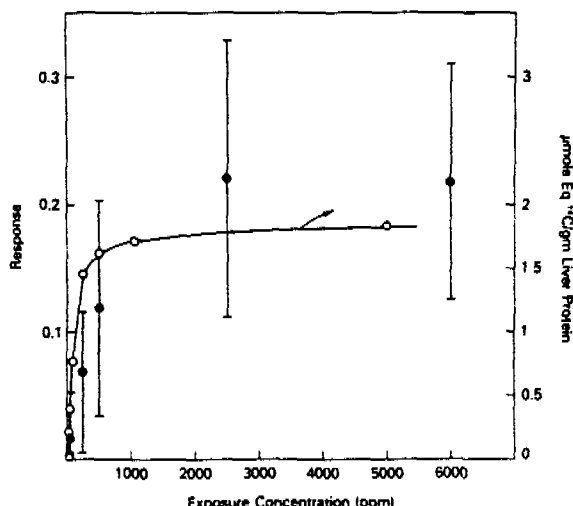


FIGURE 3. Covalent binding of  $^{14}\text{C}$ -activity from VC (1) compared with liver angiosarcoma response in rats. The error bars indicate 2 standard errors (5).

version of A to the reactive intermediate;  $r_{BC}$  represents the rate of irreversible reaction of the reactive intermediate with the site of toxicity, e.g., by covalent binding to RNA, DNA or protein molecules. Many of the various processes may be enzymatically mediated and kinetically saturable. While a one-compartment description of the body is implied by the diagram, the various chemical reactions and excretory processes do not necessarily occur at the same site. B may be formed in the liver, e.g., and, if sufficiently stable, could produce toxicity in other organs.

If all of the processes are linear, the concentration of the reactive intermediate B (and thus exposure to toxicity) will be proportional to the dose of A. All processes which are described by Michaelis-Menten kinetics appear linear at sufficiently low

concentration. At higher concentrations, saturation phenomena produce quite variable results depending upon the steps where they act. If the pathways  $r_A$  or  $r_B$  leading to nontoxic products and elimination start to saturate, then the production of C will increase more rapidly than the dose. This problem has been emphasized in the literature (4); however, there is no reason *a priori* to assume that pathways leading to detoxification will saturate first. If the pathway  $r_{AB}$  leading to B saturates first, then the production of C may increase less rapidly than dose.

Covalent binding of radioactivity derived from vinyl chloride provides a good example of a biochemical effect which is self-limiting with dose (1). Figure 2 is a replotting of the VC data (Fig. 3 of the McKenna article) on linear coordinates in the manner of Figure 1b to display this effect more clearly. It is apparent that covalent binding increases almost linearly up to an exposure concentration of about 200 ppm and then plateaus quite sharply with relatively little increase from 500 to 5000 ppm.

The covalent binding data for VC are plotted again in Figure 3 alongside the Maltoni data on liver angiosarcoma in the rat as cited by Guess et al. (5). The shapes of the curves are strikingly similar. While this does not prove that covalent binding will be proportional to tumor incidence at low dose, a relationship between tumor incidence and VC metabolism is consistent with these data as mentioned by McKenna. One disturbing consequence of a dose-response curve such as Figure 3 is that linear extrapolation from a single high dose will tend to underestimate the biochemical or pathologic effect over most or all of the range of lower doses.

It is not going to be possible to work out the qualitative and quantitative aspects of all biochemical reactions and physiologic processes which will determine dose-response relationships for every foreign substance in the environment. It may be possible to simulate mathematically the essential behavior of rather complex biochemical networks and make powerful generalizations about their dependence on dose and key kinetic parameters without quantitating or even knowing all details. This could be a very fruitful area for research which would involve both considerable mathematical sophistication as well as careful design of experiments *in vitro* and *in vivo* to obtain necessary parameters.

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Dear Sir:

Dr. Dedrick's perceptive comments regarding our paper on vinylidene chloride (1) are much appreciated. The discrepancy between the text and Figure 2 was an oversight in preparation of the manuscript. For clarification, Figure 1 shows the relationship between the total metabolism of VDC and depletion of hepatic glutathione in rats following a single 6 hr inhalation exposure. The nonlinear character of VDC metabolism is more appropriately shown in this figure. Since the majority of VDC metabolites are represented by glutathione conjugates the apparent dependence of VDC metabolism on the availability of GSH is not surprising. When viewed in the context of the covalent binding data shown in Figure 1b of Dr. Dedrick's letter, it is apparent that detoxification of VDC is saturable, whereas the production of a reactive intermediate capable of alkylating tissue macromolecules is a linear function of VDC exposure concentrations over the range examined. The data clearly indicate a saturation of detoxification pathways for VDC prior to an effect on pathways leading to alkylation of hepatic macromolecules.

Dr. Dedrick's thoughts on pharmacokinetics and risk assessment are certainly consistent with the

efforts of this laboratory over the recent past. The specific problem of vinyl chloride risk assessment and the role of pharmacokinetic considerations therein has been dealt with in detail in two recent publications from this laboratory (2, 3). Therefore I will make only one additional comment regarding the problems of extrapolation of dose-response relationships such as those shown in Figure 3 of Dr. Dedrick's letter.

Just as extrapolation from a single high dose tends to underestimate the effect of interest at lower doses, it can also be seen that extrapolation from two high doses on the upper portion of the curve would provide a gross overestimate of the effects at lower doses. The point to be made is simply that neither procedure provides an adequate risk assessment. Such considerations are important not only to the interpretation of hazard evaluation studies but also their design. In both instances the primary objective should be a well-defined dose-response relationship, which includes considerations of metabolic activation, thereby providing a sound data base for risk assessment.

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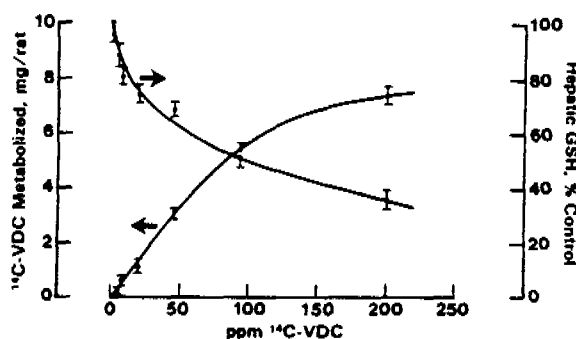


FIGURE 1.

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