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Relevance of Experimental Studies to Human Risk

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Confidence in the extrapolation of animal toxicity data to humans can be enhanced by the application of pharmacokinetic concepts integrated with chronic toxicity data and knowledge of a chemical's mechanism(s) of toxicity. Basic pharmacokinetic concepts (including dose-dependent or Michaelis-Menten kinetics) and their relationship to the risk estimation process are discussed using vinyl chloride and styrene as specific examples. Species differences in metabolic rates must be considered in order to arrive at realistic estimates of human risk to vinyl chloride-induced liver angiosarcomas utilizing vinyl chloride toxicity data observed in rats. Because small animal species generally metabolize chemicals more rapidly than larger species on a body surface area basis, small animals should be more sensitive to chemicals (such as vinyl chloride) that exert their toxicities via the metabolic formation of toxic products. Inhaled styrene is a chemical whose clearance from the blood at low exposure levels in both rats and humans follows first-order kinetics. However, at higher exposure levels, the pharmacokinetic fate of styrene in rats is dose-dependent, suggesting a saturation of styrene metabolism. These data indicate that any extrapolation of observable toxicity at elevated exposure levels in rats to anticipated responses at lower levels in either rats or humans may be invalid. An integration of the foregoing concepts provides a sound scientific basis for the use of experimental animal data to predict the risk to humans from chemical exposure.

Introduction

Chemically induced carcinogenicity is one type of toxic response that has received primary attention in recent years. The potential lethality of cancer, combined with its generally irreversible nature and long latent period are all characteristics that have placed carcinogenesis in the forefront of public concern. Many chemicals shown to be mutagenic in short-term *in vitro* studies and/or carcinogenic in long-term animal studies have consequently been considered as potential human carcinogens. This conclusion is typically based on studies of animal models performed under tightly controlled experimental conditions in which potentially interfering variables are kept to a minimum. Although a basic toxicological goal is to evaluate the risk to man associated with exposure to potentially harmful substances, interspecies variation in response may often preclude a simple extrapolation of animal toxicity to that anticipated in man. The purpose of this presentation is to review and emphasize the importance of animal pharmacokinetic studies in properly implementing animal toxicity data to predict human risk from chemical exposure.

Pharmacokinetic Concepts and Dynamics of Toxicity

Pharmacokinetics is a study of the dynamics of absorption, distribution, metabolism and excretion of a chemical within the body. Pharmacokinetic studies of a chemical as a function of administered dose often provide valuable information on how a chemical's overall biological fate may change in response to different amounts within the body. Since many toxic responses to chemical exposure are not only dependent on the amount of a chemical that reaches a target site but also on how long a sensitive site might be exposed, a comparison of the pharmacokinetic fate of a chemical between different species of

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animals can provide data about differential susceptibility relating to interspecies extrapolation of toxicity data.

A classical approach to pharmacokinetic analysis depicts the body as consisting of a system of compartments. An individual compartment generally refers to all tissues, organs, cells and/or fluids within the body for which the rate of uptake and loss of a chemical is sufficiently similar as to preclude further kinetic resolution. Gehring et al. (*1*) have provided a detailed description of pharmacokinetic compartments and their use in evaluating toxicity data, and consequently no detailed discussion will be included here. While compartments do not always have direct physiological or anatomical counterparts when analyzed kinetically, they constitute a basic tool by which quantitative expressions describing the fate of a chemical within the body can be derived.

Dose-Independent Pharmacokinetics

Over a range of selected dose levels, many chemicals exhibit first-order kinetics which can be referred to as being "linear." For these chemicals, the rates of absorption, distribution, metabolism and elimination from the body are proportional to the concentration or amount of the chemical within the body. As a consequence of this proportionality, the rate constants of all these processes are thus independent of the administered dose. In a simplified fashion, first-order kinetics may be expressed by the equation:

$$\text{rate} = -dC/dt = kC$$

in which C is the concentration of the chemical in the body at time t and k is the rate constant for the given process. As long as first-order kinetics apply and thus the rate constants for all processes responsible for a chemical's pharmacokinetic fate are independent of administered dose, tissue concentration and consequent toxicity should also be proportional to administered dose.

Dose-Dependent Pharmacokinetics

In actuality, many reactions that influence a chemical's pharmacokinetic fate are not independent of administered dose, but are instead dose-dependent. In this situation, saturable active transport systems or metabolic reactions that often play key roles in the prevention or enhancement of chemical toxicity are not adequately described by first-order kinetics. As a consequence, the administration of high dose levels, as frequently done in long-term animal bioassays, may overwhelm these processes and result in a disproportionate increase in blood and/or tissue concentration and possibly elicit a toxic response.

The rates of saturable processes are often described by Michaelis-Menten or dose-dependent kinetics according to the equation:

$$\text{rate} = -dC/dt = V_{max}C/K_m + C$$

In this equation, $-dC/dt$ is the rate of change in the concentration of the chemical's concentration at time t, V_{max} is the maximum velocity of the process and K_m is the Michaelis constant or that concentration at which the rate of the process is at a value of one-half V_{max}. There are two limiting situations to this equation. When the concentration (C) is much greater than K_m, the Michaelis-Menten equation approaches a limit of:

$$\text{rate} = -dC/dt \approx V_{max} \qquad C >> K_m$$

In this situation, the rate of the process is limited by the value V_{max}, and, as C increases, the rate of the reaction remains constant. It is in this concentration range $(C >> K_m)$ that the biological processes governed by this type of kinetic behavior have become overwhelmed and can be considered to be saturated.

Conversely, if the concentration C is much less than K_m, the rate of the processes described by the Michaelis-Menten equation can be approximated by:

$$\text{rate} = -dC/dt \approx kC \qquad C << K_m$$

where $k = V_{max}/K_m$. Under these conditions, the rate of the process remains proportional to the chemical's concentration and all of the previously described concepts for first-order kinetics regarding proportionality between blood and tissue concentration and toxicity apply.

Use of Animal Studies for Predicting Human Toxicity

Recent authors have suggested that there are at least five potential factors responsible for species variations in response to chemical exposure (*2*). Collectively, these factors include absorption, distribution, metabolism, site and mechanism of action and excretion of the chemical from the body. It is of interest to note that an analysis of the dynamics of many, if not all, of these factors as a function of administered dose level constitutes what has been previously described as a pharmacokinetic study.

In utilizing animal studies to predict possible human toxicity, it is important to determine if the toxicity resulting from chemical exposure is due to the parent chemical itself or rather to an

active metabolite generated via metabolic processes occurring within the animal. It is commonly accepted that metabolism can either lead to detoxification or activation, depending on the toxicological potential of the parent compound and/or its possible metabolites. Consideration of this information is important for the extrapolation of results from animal toxicity studies to man, since there may be relative species differences in the activity of enzymes responsible for chemical metabolism. As pointed out by Rall (*3*), it is often possible to estimate the relative sensitivity of different species to a chemical by using an approximation that the basal metabolic rate is roughly proportional to the body surface area. This suggests that if factors other than metabolism are ignored, a large animal species is more sensitive to a directly toxic agent than a small animal species (Table 1). In contrast, a large animal species should then be less sensitive to toxicity mediated by a metabolic activation than a smaller species (*4*). It should be emphasized, however, that for some types of metabolic activation the relative rates may not only be a simple function of body size. For example, the metabolic activation of 2-acetaminofluorene involves the formation of an active sulfate, and the species and organ sensitivity of the tumorigenicity of this agent correlates well with the level of sulfotransferase enzyme activity (*5*). Since the rat has a higher level of sulfotransferase than the mouse, it develops more tumors when exposed to an equivalent amount of 2-acetaminofluorene. In this instance, the larger animal species is more sensitive to the effects of the metabolically activated agent than the smaller species. Thus, the reliability of interspecies extrapolation depends to a large degree upon how much is known of the details of metabolism and how metabolism affects toxicity in the species of interest.

Pharmacokinetic Concepts and Risk Estimation

Many mathematical models are commonly used in extrapolating an observed carcinogenic response in animal bioassays at relatively high dose levels (*6*). While these models differ from each other by the rapidity in which a zero response is approached as the dose level approaches zero, they are similar in that they usually assume that a zero response occurs only when the dose level equals zero. Another feature common to these models is their assumption that the concentration of the carcinogenic entity is directly proportional to the dose level of the administered chemical.

Table 1. Predicted relative cancer risk from equivalent doses (mg/kg) calculated on the basis of (body weight).[a]

Species	Predicted relative cancer risk	
	Directly toxic agents	Metabolically activated agents
Man (70 kg)	1.00	1.00
Dog (20 kg)	0.66	1.52
Rabbit (3 kg)	0.35	2.85
Rat (0.5 kg)	0.18	5.58
Mouse (0.03 kg)	0.08	13.20

[a]After Rall (*3*).

This assumption applies whether the carcinogenic entity is produced via a metabolic activation of the parent chemical or whether the parent chemical itself is the primary toxicant. An important consequence of this assumption is that toxicity or carcinogenicity is also expected to be proportional to administered dose level. Recent studies of several chemicals, including vinyl chloride and styrene, illustrate how an understanding of the pharmacokinetic fate of these compounds as influenced by the magnitude of administered dose can be used to evaluate animal toxicity data in order to predict the hazard to man from exposure to these agents.

Vinyl Chloride

An example of dose-dependent pharmacokinetics that directly relates to carcinogenic risk estimation in man is that of inhaled vinyl chloride (*7, 8*). Vinyl chloride has been demonstrated to induce hepatic angiosarcomas in rats at exposure levels ranging from 10 to 10,000 ppm, with an essentially flat dose-response curve at exposure levels from 1,000 to 10,000 ppm (*9*). Numerous studies have indicated that a reactive metabolite of vinyl chloride is likely to be the carcinogenic entity for this halogenated ethylene rather than the parent compound itself (*10–15*). Other studies have shown that the bioactivation of vinyl chloride in rats is a saturable process that follows Michaelis-Menten kinetics becoming overwhelmed at high exposure levels, thereby limiting the *in vivo* production of the toxic metabolite (*7, 16–19*). As a consequence of this saturable metabolic activation, Gehring et al. (*7*) have shown that the toxicity or carcinogenicity in rats resulting from vinyl chloride exposure is not directly proportional to all exposure concentrations. Alternatively, these authors found it was possible to relate the observed carcinogenicity in rats to the amount of vinyl chloride metabolized after pharmacokinetic parameters describing the

saturable bioactivation of vinyl chloride were determined.

In accordance with these observations of the kinetic behavior of vinyl chloride in rats, Gehring et al. (*7, 8*) utilized similar pharmacokinetic concepts to estimate the amount of vinyl chloride metabolized in man. Their prediction incorporated the concepts that vinyl chloride bioactivation in man was a saturable process as observed in laboratory animals and that the metabolic process responsible for vinyl chloride metabolism in mammals was related to differences in body surface area. Using this methodology, these authors predicted that man's rate of vinyl chloride bioactivation would be much less than that observed in rats, resulting in a decreased sensitivity of man to the tumorigenic effects of vinyl chloride (*7, 8*). Recent data from other laboratories on the rate of vinyl chloride metabolism have provided additional support for this conclusion. In studies of the pharmacokinetics of vinyl chloride in different species (including man), Buchter et al. (*20, 21*) and Filser and Bolt (*22*) have confirmed that a marked species variation in vinyl chloride metabolism does indeed exist (Table 2). These investigators found that mice and rats metabolized vinyl chloride at a rate approximately 5-12 times that for man. In contrast to the results observed in these rodent species, rhesus monkeys were found to metabolize vinyl chloride at a rate that closely paralleled that seen in man. Collectively, these results are significant in that they confirm the predictions reached by Gehring et al. (*7, 8*), who used pharmacokinetic concepts to predict differences in the relative rates of vinyl chloride metabolism in rats versus man.

Styrene

Styrene is another example of a chemical whose metabolic elimination in experimental animals is dose-dependent. In an analysis of the pharmacokinetic fate of styrene in rats following a 6-hr inhalation exposure to 80, 200, 600 or 1200 ppm, Ramsey and Young (*23*) observed that there was a marked dose dependency in the elimination of styrene from the blood. Figure 1 depicts a plot of the blood styrene concentration versus time data in animals exposed to 80 or 1200 ppm for 6 hr. Other animals were left in the exposure chambers for periods of up to 24 hr in order to establish whether plateau blood levels were achieved. Note that a disproportionality exists between maximum blood concentration and exposure level. At an exposure level of 80 ppm, the maximum styrene concentration was 0.8 μg/mL, while at 1200 ppm the maximum blood concentration reached a value of 64 μg/mL. Thus, as exposure concentration increased by 15-fold, the maximum blood concentration increased over 80-fold, indicating a dose dependency in the pharmacokinetic profile of styrene. As reviewed by the original authors (*23*), these and other data indicated that the capability of laboratory animals to metabolize styrene becomes overwhelmed at exposure concentrations somewhere between 200 and 600 ppm. These results indicate that any extrapolation of animal toxicity data observed at exposure levels of 600 ppm and above to anticipated responses in animals at lower levels may be invalid.

How do these results relate to the extrapolation of styrene toxicity data in laboratory animals to that in man? Such an extrapolation can be greatly facilitated by a direct pharmacokinetic comparison of the chemical in question between both species. Accordingly, Ramsey and Young

Table 2. First-order metabolic clearance rates for vinyl chloride in man versus other species.[a]

Species	Clearances, L/hr/kg body weight
Man	2.02
Monkey	3.55
Rat	11.00
Mouse	25.60

[a]Data from Buchter et al. (*20, 21*) and Filser and Bolt (*22*).

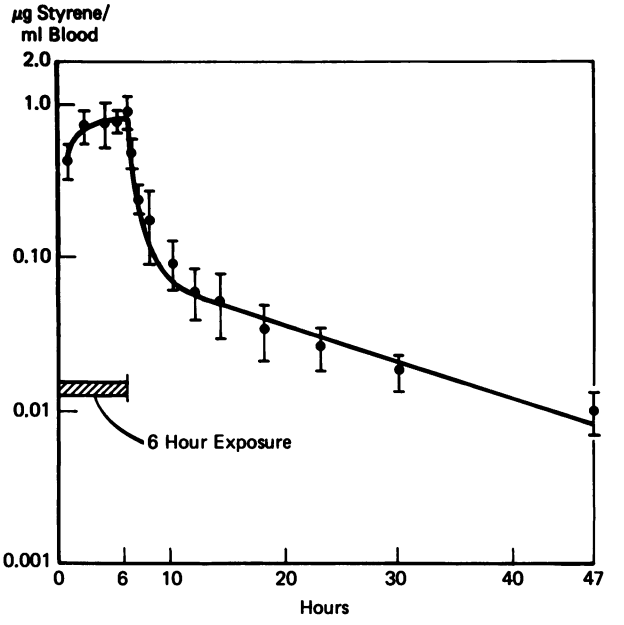

FIGURE 1. Blood styrene concentration in rats exposed to 80 or 1,200 ppm. Data from Ramsey and Young (*23*).

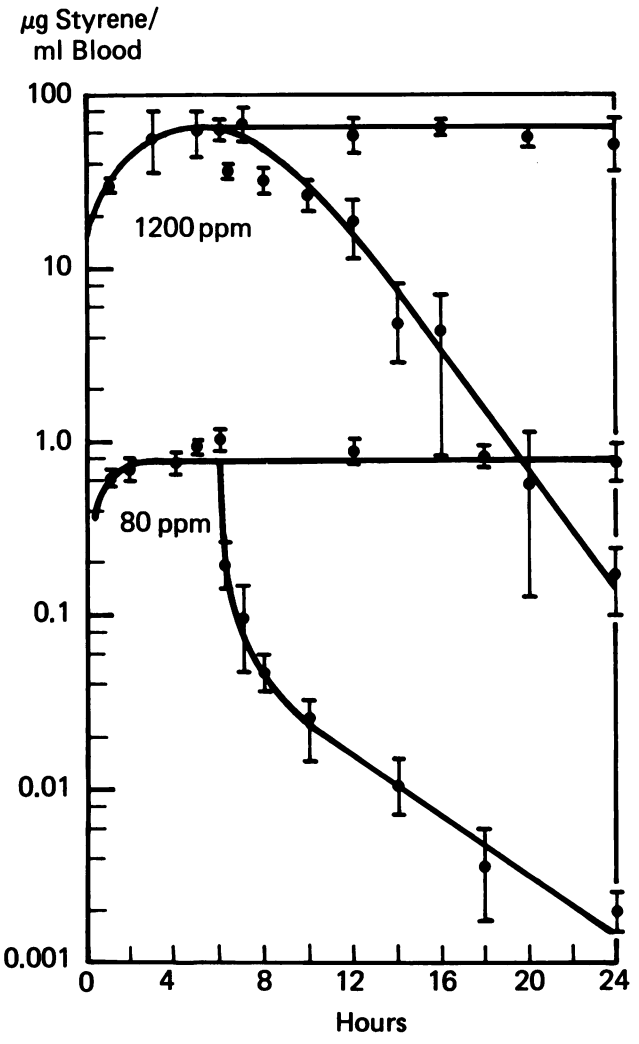

FIGURE 2. Blood styrene concentration in humans exposed to 80 ppm for 6 hr. Data from Ramsey and Young (*23*).

(*23*) conducted a pharmacokinetic study of inhaled styrene in human volunteers exposed to 80 ppm for 6 hr. Figure 2 depicts the blood styrene concentration during and after exposure in the four volunteers. Note that the blood styrene concentration rose to a maximum of 0.9 μg/mL at 6 hr and declined in a linear fashion. A comparison of these results with those of Figure 1 indicates a marked similarity between the pharmacokinetic fate of styrene in man and rats following exposure to 80 ppm. These authors concluded that this type of similarity lends confidence to the extrapolation of toxicity data observed in laboratory animals at levels below 80 ppm to that anticipated in man. In contrast, the demonstration of a saturable elimination of styrene from laboratory animals at higher exposure levels precludes the use of toxicity data obtained at high levels to predict possible risks to humans at lower exposure levels.

Conclusions

In summary, it should be noted that animal studies of pharmacokinetic behavior only represent one segment of the total data base of interrelated information required to make a rational extrapolation of toxicity data observed in laboratory animals to anticipated responses in man. Other authors have demonstrated that the risk estimation process is greatly enhanced when animal studies of pharmacokinetic behavior are integrated with observations of chronic toxicity and a knowledge of mechanisms of toxicity such as the production of active metabolites that interact with critical macromolecular sites. Collectively, an evaluation of the relationships between these parameters and toxicity in experimental animals will improve the estimation of relative degrees of risk to man associated with chemical exposure.

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