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Toxicokinetics and Physiologically Based Toxicokinetics in Toxicology and Risk Assessment

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TOXICOKINETICS AND PHYSIOLOGICALLY BASED TOXICOKINETICS IN TOXICOLOGY AND RISK ASSESSMENT

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Toxicokinetics is the study of kinetics of absorption, distribution, metabolism, and excretion of a xenobiotic under the conditions of toxicity evaluation. Conventional toxicokinetics uses the hypothetical compartments, and the model is composed of rate equations that describe the time course of drug and chemical disposition. The utility of toxicokinetics in toxicity evaluation and interpretation of animal toxicology data is emerging as an important tool in product discovery and development. With implementation of the International Conference on Harmonization (ICH) guidelines on systemic exposure and dose selection, toxicokinetics have been integrated in routine toxicity evaluations. Although traditional compartmental/noncompartmental models are generally adequate for assessing systemic exposure, they are unable to the predict time course of drug disposition in target tissues and often fail to relate systemic drug levels to a biological response. Physiologically based toxicokinetic (PB-TK) models address this deficiency of traditional compartmental models. PB-TK models are the kinetic models of the uptake and disposition of chemicals based on rates of biochemical reactions, physiological and anatomical characteristics. These models, when developed appropriately, can predict target organ drug distribution in different species under variety of conditions. This minireview discusses the basic principles, and applications of traditional compartmental toxicokinetic and physiologically based toxicokinetics (PB-TK) models in drug development and risk assessment. Special emphasis will be placed on discussion related to interpretation of the ICH guidelines related to toxicokinetics and the utility of toxicokinetics data in dose selection for toxicity and carcinogenicity studies. The utility of PB-TK models in risk assessment of methylene chloride, vinyl chloride, retinoic acid, dioxin, and inhaled organic esters is discussed.

Preclinical safety evaluation of chemicals, including pharmaceutical agents, is conducted in animals to predict the safety of investigational products in humans under a variety of exposure conditions. Toxicokinetics-related investigations

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are basic to safety evaluation since the vast majority of animal toxicity data cannot be extrapolated to assure human safety without an understanding of kinetics of absorption, distribution, metabolism, and excretion in preclinical species and humans. It is almost certain that doses used in toxicity tests will be greater than clinical pharmacological doses in humans by orders of magnitude; therefore, it is likely, but not always, that nonlinear or saturable kinetics may occur with saturation of absorption, and/or clearance in preclinical toxicology studies. Greater than dose proportional increase in systemic exposure at high doses may lead to unexpected dose and/or species-specific toxicities. While blood/plasma-based toxicokinetics evaluations are often sufficient to meet the objectives of systemic exposure assessment, they are too simplistic to relate biochemical and physiological processes to kinetics of drug absorption, distribution, metabolism and excretion. Physiologically based toxicokinetics (PB-TK) models have been developed to improve and correct some of the deficiencies of conventional compartmental models. PB-TK models can provide kinetics of tissue distribution of drug under a variety of exposure and disease conditions.

This review is a synopsis of a continuing education course offered at 39th Annual Meeting of Society of Toxicology in March 2000. The synopsis is intended as a minireview of compartmental (traditional) and physiologically based toxicokinetics, and it should not be regarded as a complete or exhaustive review of these topics. In this review, basic principles of compartmental and PB-TK models and the approaches to integration of toxicokinetics in a safety assessment program are discussed. The International Conference on Harmonization (ICH) guidelines on toxicokinetics and dose selection and their application in safety evaluation are presented. With appropriate examples, the applications and limitations of compartmental and physiologically based toxicokinetic models are discussed.

BASIC TOXICOKINETICS PRINCIPLES

Toxicokinetics is the use of mathematical models to quantitate the time course of drug and chemical absorption and disposition in man and animals. A toxicokinetic model is in reality an artificial mathematical link to the underlying interaction of a chemical with an animal's physiology. Models are composed of rate equations defined using differential calculus. Toxicokinetics differs from pharmacokinetics in many important ways. To support clinical phase 1 studies in human volunteers, pharmacokinetic investigations in animals are often conducted at low pharmacological doses where kinetic processes can be described as linear. Additionally, low pharmacological doses are often not susceptible to solubility-related dosing problems leading to drug precipitation in intestinal tract or a different pattern of absorption in toxicity studies. The information regarding bioavailability, half-life, volume of distribution, and clearance obtained in early low-dose pharmacokinetics studies cannot be easily extrapolated for high doses used in toxicology studies. In contrast, toxicokinetic studies utilize often very high doses that are susceptible to drug solubility problems, and the

kinetic processes at these high doses are generally nonlinear. These differences in pharmacokinetics and toxicokinetics must be considered in designing toxicokinetic studies when conducted alone or in conjunction with toxicity investigations.

A toxicokinetic model is only a tool to estimate chemical concentrations and generate parameters that are useful for further analyses and quantitating the biological processes under investigation. Models are neither correct nor incorrect, but should be judged only as to how accurately chemical concentrations are predicted under new exposure conditions. When two competing models are equivalent in this respect, then other criteria would include parsimony and mechanistic reality. Toxicokinetics is a bridge for extrapolating chemical concentrations across different species, from in vitro to in vivo studies, from preclinical studies to clinical trials, from chemical exposure to systemic dose in risk assessment, or as a link between physiology or genetics and chemical disposition in populations of animals and humans.

There are a number of modeling approaches that have been employed in this area, including compartmental, noncompartmental or stochastic, physiologically based, and population or mixed effect. They are differentiated primarily on the assumptions made relative to the nature of their links to physiology. Many are designed to predict chemical concentrations and do not portend a link to biological reality. Others are used to explain sources of variability in chemical disposition. Finally, approaches such as physiologically based modeling, which are fully described later in this article, are designed to be based on solid biological and biochemical mechanisms. However, there are a number of underlying mathematical constructs that are common to all modeling endeavors and will be briefly reviewed. One should consult the suggested reading list for complete reviews of these other modeling approaches that are beyond the intended scope of this brief introduction (Bourne, 1995; Caines, 1988; Gabrielsson & Weiner, 1997; Gelman et al., 1996; Gerlowski & Jain, 1983; Gibaldi & Perrier, 1982; Riviere, 1999; Rowland & Tozer, 1995).

Toxicokinetic models are often defined on the nature of the rate processes they describe. The process can be absorption across the gastrointestinal tract, metabolism by the liver or elimination by the kidney. The *order* of a rate process is defined as the exponent (n) in the general differential rate equation:

$$dX/dt = KX^n$$

where dX/dt is the rate of change of the amount of a chemical versus time.

A first-order process is defined with $n=1$, making the rate equation reduce to KX. This is termed a linear model and K is the fractional rate constant expressed in units of 1/time. The overall rate of a first-order process is dependent upon the mass of chemical (X) present. However, the rate constant describing this process is fixed and independent of the quantity of chemical available.

A zero-order or nonlinear rate process is defined with $n = 0$, which reduces to K. In this case, the constant is expressed in terms of mass/time and is fixed. The overall rate of the process is thus independent of the amount of chemical available. If more chemical is available than this process can handle, saturation occurs and chemical will accumulate. In contrast, first-order processes can always "process" a constant fraction of chemical. If the amount of chemical available for action by a process is termed the dose, then in a first-order or linear process, the rate of a process is directly related to the available dose. In contrast, in nonlinear or zero-order processes, the rate of the process is fixed and independent of the dose.

The rate of a process can easily be determined by assessing its behavior after administration of multiple doses. Since a first-order process is fixed, it can also be described by a half-life ($T_{1/2}$), which is the length of time it takes for 50% of the available dose to be acted on by a process. Thus if the process is elimination, the half-life is the time it takes for half of a dose to be eliminated. Half-life can be calculated from the first-order rate constant (K) as (ln 2)/K. A zero-order process does not have a half-life over a large number of doses.

For example, you determine that the elimination $T_{1/2}$ of a chemical is 8 h after administration of either 10 or 1000 mg of a chemical to an animal. This similarity suggests first-order kinetics is operative. The difference is that after 8 h, 5 mg will be left after the 10-mg dose, but 500 mg will be left 8 h after administering the 1000-mg dose; 50% of the chemical will have been eliminated after both doses. After $5T_{1/2}$, 97% of a chemical will have been eliminated. In fact, after $5T_{1/2}$, 97% of any first-order kinetic process will have been completed.

There are two pharmacokinetic parameters that are useful to describe rate processes and subsequent disposition of a chemical in the body. The first is a proportionality constant termed the volume of distribution (V_d), which relates the mass of chemical and its concentrations in biological fluids and the body:

$$\text{Volume of distribution} = \text{mass/concentration}$$

The V_d describes the volume of fluid that would be required to contain the mass of chemical under consideration at the concentration observed. Its calculation is very dependent upon the type of pharmacokinetic model employed. The second fundamental parameter is the clearance (Cl) of chemical from the system and is the preferred parameter to quantitate chemical elimination from the body. It is defined as the volume of distribution cleared of a substance per unit of time, or alternatively as the rate of drug excretion relative to its plasma concentration. It is calculated as (V_d)(K) or Dose/AUC where AUC is the area under the blood concentration versus time profile. The clearance from the body is a sum of the clearances from all of the organs of elimination (e.g., kidney, liver). These physiological relationships are instructive as they indicate that the rate constant of elimination of a first-order process (K) is equal to Cl/V_d. Thus, the rate of elimination from the body is a function of both its elimination clearance and the extent of it distribution within the body.

One must appreciate that these relationships are often defined by the nature of the pharmacokinetic model required to accurately describe the process being studied. Many assumptions are made that result in limitations of model applications. Some of these details applied to specific models are described later in this article.

Even with this relatively basic knowledge of pharmacokinetics, a number of important insights can be made. It is clear that first-order pharmacokinetics is simpler to describe since the parameters such as K, $T_{1/2}$, V_d, and Cl are constant and independent of dose. That is, a model that holds at one dose can be used to predict behavior at another. This behavior can be used to design experiments to determine the dose range that this linear behavior is operative by simply determining the value of a parameter after administration of different doses. If the parameter is constant, first-order kinetics is operative.

These simple parameters may also be used to explore the disposition of a chemical in different animal species by determining values for Cl and V_d. This provides an assessment of how chemicals are handled in preparation for dose scale-up or risk assessment purposes. Since the rate of many metabolic functions is proportional to basal metabolic rate (BMR), which indirectly is proportional to body surface area and not body weight, pharmacokinetics parameters such as Cl and half-life are also expected to be proportional to BMR and thus surface area. This approach is termed allometric scaling and is widely used in extrapolating chemical doses between animal species. Allometry works well for drugs that are metabolized in the liver by flow-limited processes, but breaks down when capacity-limited hepatic metabolism is present or if one animal species eliminates or metabolizes a compound by a species-specific process or route of elimination.

Often the value of a pharmacokinetic parameter can be correlated to a physiological parameter to probe the relationship of altered physiology (e.g., renal clearance) secondary to disease or a toxin itself, to the value of clearance determined in a pharmacokinetic study. This approach, termed mixed-effect modeling or population pharmacokinetics, has been adopted in pharmacology to use physiological parameters to help reduce variability in pharmacokinetic studies by simultaneously measuring clinical values such as serum creatinine to reduce the error in estimating the renal clearance of a compound. This reduces the number of samples needed to solve a pharmacokinetic model and allows inferences to be made over larger populations of humans. This approach has yet to be adopted in toxicology.

Overall, this brief introduction was intended to describe fundamental toxicokinetic principles and is not intended to be a comprehensive review. Authors recommend that readers consult the following references (in parentheses) for an in-depth understanding of first-order and zero-order pharmacokinetics and an understanding of certain toxicokinetic parameters such as clearance, half-life, volume of distribution, and others (Gibaldi & Perrier, 1982; Gerlowski & Jain, 1983; Smith, 1990; Voisin et al., 1990; Welling & de la Iglesia, 1993; Monro, 1994; Renwick, 1994; Bourne, 1995; Rowland & Tozer,

1995; Gelman et al., 1996; Gabrielsson & Weiner, 1997; Caines, 1998; Mahmood & Balian, 1999; Riviere, 1999).

The next section provides a brief overview of the applied toxicokinetics in nonclinical safety assessment studies and the authors' approaches to the study design dose selection, and the interpretation of the regulatory guidelines.

INTEGRATION OF TOXICOKINETICS IN SAFETY ASSESSMENT

The main objective of nonclinical safety assessment studies is to provide information regarding the safety and potential target organ toxicity of an investigational drug prior to and during its clinical testing for efficacy and safety in humans. To ensure the human safety of an investigational drug in several phases of clinical trials for efficacy and safety, it is desirable to know both short-term and long-term toxic effects of drug in at least one rodent and one nonrodent species, and whether or not the drug is genotoxic or has any reproductive and developmental toxicity potential. If a drug is to be given chronically, it is also important to know its carcinogenic potential in two rodent species after a 2-yr exposure generally prior to marketing approval. The extrapolation of nonclinical animal safety assessment data to predict human safety is complicated by species differences in absorption, distribution, metabolism, and excretion processes, as well as pharmacodynamics. With a better understanding of toxicokinetics and mechanisms of biological activity, it is possible to reduce the uncertainty regarding the extrapolation of animal toxicology data to predict human safety. The main objectives of nonclinical toxicokinetics studies are to provide information on systemic exposure and its relationship to dose levels and toxicity. The toxicokinetics data are also used to justify the choice of species/strain, study design, dose selection for subsequent studies, and estimation of safety margins based on the ratio systemic exposure between animal species and humans.

Given the very general nature of the discussion related to toxicokinetics, one should consult the suggested reading list for comprehensive reviews (and references therein) related to the approaches described below which are beyond the intended scope of this review (Smith, 1990; Voisin et al., 1990; Monro, 1994; Renwick, 1994; Welling & de la Iglesia, 1993; Mahmood & Balian, 1999).

Study Design Considerations

The extent of toxicokinetics depends upon the nature of toxicity observed in early safety assessment studies. When a test compound is well tolerated and does not show frank toxicity, an extensive toxicokinetics program may be necessary to justify dose selection. For compounds that produce well-defined target organ toxicity, the plasma toxicokinetics phase of toxicity studies is conducted mainly to obtain information for interpretation of toxicity findings, and determination of exposure margins for human safety. However, in the absence of good correlation between plasma exposure and toxicity, target organ toxicokinetic studies

may be necessary to interpret organ-specific toxicity, and these studies provide valuable data to understand the mechanisms of toxicity. In designing the toxicokinetic phase of toxicity study one needs to consider the following: (1) whether the drug is being administered as a dietary admixture or by oral gavage, (2) age of animals and feeding conditions, (3) dosing regimen—once a day or multiple doses per day—and (4) satellite animals versus main toxicity study animals (to assess variability in toxicity response). All these conditions may impact on estimation of systemic exposure. In the authors' opinion, toxicokinetics data should be collected under conditions of toxicity studies utilizing routes and schedule of dose administration that are similar to those used in humans.

Assessment of Toxicokinetics Parameters and Selection of Time Points

Because of the administration of large doses and other constraints (small number of animals, limited sampling, etc.) in toxicity study design, the scope of toxicokinetics in safety assessment studies is often limited to systemic exposure assessment. In authors' opinion, detailed information on pharmacokinetics is neither necessary nor easily obtained from integrated toxicity–toxicokinetics studies (due to limited blood sampling); however, it is important to accurately assess at least the following parameters: C_{max} (maximal plasma concentration), T_{max} (time at which maximal plasma concentration occurred), and AUC_{0-24h} (area under the plasma concentration versus time curve for 0 to 24 h). All toxicokinetic-related calculations are generally model independent and can be easily determined from the plasma concentration versus time data, and the details of the AUC calculations are outlined by Renwick (1993). The area under the plasma concentration versus time curve (AUC_{0-24h}) is the most ideal parameter for assessment of exposure; however, if a drug is very rapidly eliminated and AUC_{0-24h} cannot be accurately determined, the C_{max} or the concentration at an appropriate timepoint may also be used to monitor exposure. The rate and extent of absorption may be assessed by T_{max}, C_{max} and the plasma concentration versus time profile, Because of the small number of time points used for toxicokinetic sampling, it is often difficult to determine a half-life in toxicity studies. In absence of half-life data, the ratio of C_{min} (trough concentration) and C_{max} may provide a subjective estimate of the rate of plasma drug elimination.

The amount of blood and frequency of blood sampling allowed for a given species often limit the selection of time points for blood sampling and the extent of toxicokinetic data. Because in toxicology studies blood samples are taken at regular intervals to monitor hematology and clinical chemistry changes, excessive blood sampling for toxicokinetics may undermine important toxicological evaluations. In authors' experience, typically six to eight time points representing the majority of absorption, distribution, and elimination phases are often sufficient to estimate systemic exposure. In authors' experience, in typical toxicokinetic studies time points are not taken between 8 and 24 h postdosing. This is done to avoid excessive blood sampling and also to not

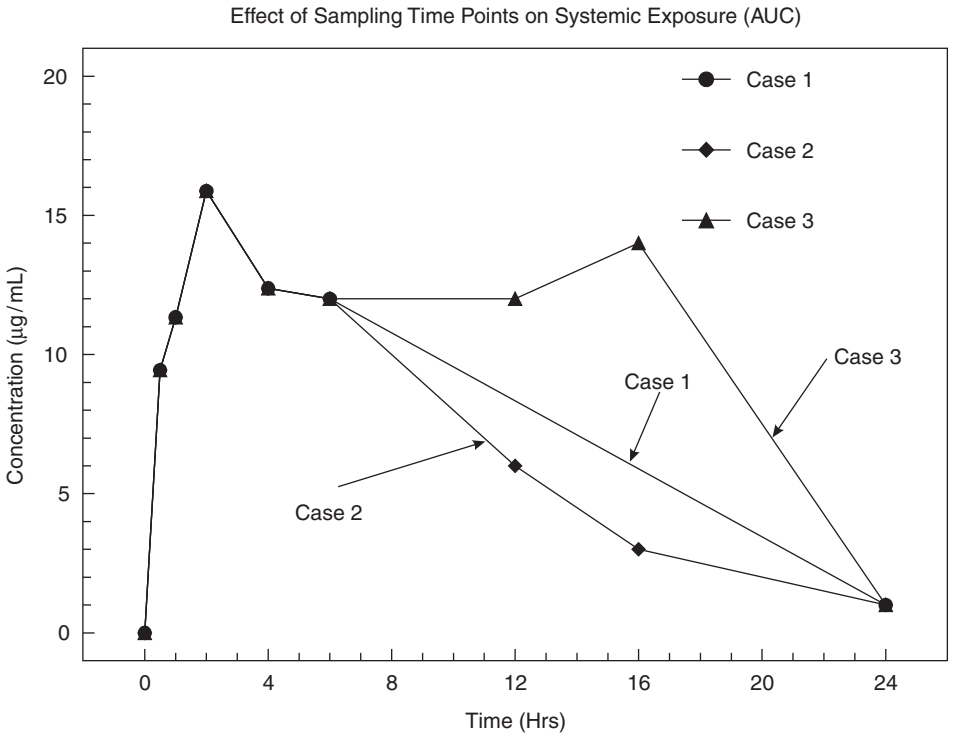

FIGURE 1. Effect of time points on plasma drug AUC. Case 1: Four male rats/time point were bled at 0.5, 1, 2, 4, 6, and 24 h postdosing. The estimated AUC was 192 µg·h/ml. Cases 2 and 3: Because the actual elimination may be either faster (case 2) or slower (case 3) than the expected rate of elimination in case 1, two hypothetical time points at 12 and 16 h were added for toxicokinetic blood sampling. The estimated AUC values were 192, 163, and 259 µg·h/ml for the cases 1, 2, and 3, respectively. The AUC for the case 1 was overestimated by approximately 18% when compared to the hypothetical case 2; the AUC for the case 1 was underestimated by about 40% when compared to the hypothetical case 3. The example illustrates the need of late timepoints (12 and 16 h) for drugs with an unpredictable elimination rate especially at higher doses.

disturb the light–dark cycle of animals. For the vast majority of compounds that are rapidly eliminated (e.g., monophasic decay), the exclusion of late time points has no significant impact on exposure assessment. However, when the absorption and elimination processes are different than expected from earlier pharmacokinetic studies or may change at higher doses, additional time points between 8 and 24 h postdosing may be needed to estimate systemic exposure. Figure 1 shows the impact of selection of timepoint on assessment of systemic exposure. Overall, it can be concluded that when the test compound is more rapidly eliminated (case 2) than expected (case 1), the systemic exposure is not greatly impacted by late time points (e.g., AUC in case 2 is only 18% lower than case 1). However, when the test compound is more slowly eliminated (case 3) than the expected rate of elimination (case 1), the systemic exposure

is significantly underestimated (approximately 40%) by the exclusion of late timepoint. Given the wide range of doses used in toxicity studies, it is likely that high doses may show a very different plasma concentration versus time profile than the low doses and may exhibit dose-dependent changes in absorption and clearance. It is imperative that special consideration be given to the possibility of slow absorption, slow elimination, saturation of absorption and clearance, and enterohepatic circulation at very high doses used in toxicity studies. If metabolite(s) are to be quantified, additional time points may be necessary to quantify metabolite(s) exposure, since the rate of formation and elimination of metabolites is likely to be different than the absorption and elimination of the parent drug. For dietary admixture studies, it is our recommendation that blood sampling match closely the feeding patterns of rodents. Blood sampling every 4 h during the light cycle and every 2 h during the dark cycle is appropriate in most cases to estimate the toxicokinetic parameters of interest. In designing continuous infusion studies to assess the steady state, one needs to know the clearance and projected steady-state concentration of drug. The product of the projected steady-state concentration and clearance provides the infusion rate of dose. It is our recommendation that plasma samples be frequently taken prior to achieving projected steady-state concentration followed by less frequent blood sampling to monitor the maintenance of steady state.

ICH GUIDELINES ON TOXICOKINETICS AND DOSE SELECTION

The International Conference on Harmonization of technical Requirements for the Registration of Pharmaceutical for the Human Use (ICH) was created in 1990 as a joint regulatory and private pharmaceutical industry project. The goals of ICH were to improve, through global harmonization, the efficiency of the process for developing and registering new medicinal products in Europe, Japan, and the United States. The six parties to ICH represent regulatory agency and pharmaceutical manufactures in three major geographical areas of the world, Europe, Japan, and the United States, where the vast majority of new medicines are developed. In the last decade, the ICH process has been successful in implementing a large number of guidelines. The ICH safety guidelines are available via the Internet (http://www.fda.gov/cder/guidance/index.htm). With global implementation of ICH guidelines for pharmaceuticals (ICH Guidelines, 1995a, 1995b, 1995c), the toxicokinetics have become an integral part of most toxicity and carcinogenicity studies. The following guidelines apply to toxicokinetics studies: (a) Guideline on Assessment of Systemic Exposure in Toxicity Studies (ICH, 1995a), (b) Guideline on Dose Selection for Carcinogenicity Studies of Pharmaceuticals (ICH, 1995b), and (c) Guideline on Repeated Dose Tissue Distribution Studies (ICH, 1995c). The discussions that follow are limited to very general interpretation of these ICH guidelines, and readers should consult the original ICH Guidelines S1C, S1C(R), and S1 for details (http://www.fda.gov/cder/guidance/index.htm).

Contribution of Toxicokinetics to Selection of Dose Levels for General Toxicity Studies (ICH, 1995a)

It is expected by regulatory agencies that animal studies demonstrate some toxicity at the dose levels used in toxicity studies. However, there are compounds that show very little or no toxicity even at high multiples (≥25-fold) of human clinical dose or systemic exposure in animals. For compounds that show no or little toxicity, toxicokinetics may play a major role in supporting the study design and selection of appropriate dose levels. Although selection of dose levels in toxicity studies is largely a function of toxicity findings, systemic exposure data can assist in dose selection. It is preferred that the low dose selected in toxicity study achieve exposure that ideally is equal to or exceeds maximal exposure (known or expected to be attained) in patients at appropriate clinical doses. The middle dose is expected to provide appropriate multiples of the low dose or an appropriate fraction of the high dose. The high dose levels should provide appropriate (e.g., ≥10-fold) multiple of maximal systemic exposure in patients. Since rodent and nonrodent species generally clear drugs faster than humans (Bachmann et al., 1996), high multiples of exposures relative to human exposure may not be achievable in preclinical animal species. When there are no toxicity constraints, the high dose selected may be the dose that limits systemic exposure to parent drug and its metabolites due to dissolution-limited saturable absorption. Additionally, it is important to consider the following for dose selection: (a) linear versus nonlinear (saturable) toxicokinetics, (b) dose response for expected toxicities, (c) mechanisms of toxicity (e.g., cell proliferation, apoptosis, increase in oxidative stress), (d), age- and/or chronic exposure-related toxicity, and (e) pharmacodynamics.

Overall, a sound justification is necessary for all dose levels for conducting nonclinical/preclinical safety assessment studies. In our experience, ideals regarding exposure margins in preclinical animal species are not always achievable; therefore, doses should be selected to maximize the possibility of identifying target organs of toxicity. It is worth noticing that in the U.S. Food and Drug Administration (FDA) database, approximately one-third of all drugs tested at the maximally tolerated dose in rodents provided systemic exposures which were equal to or less than observed in patients at maximally effective clinical dose. (Contrera et al., 1995). It is to be emphasized that the lack of exposure margin does not necessarily mean the lack of safety, and it is important to justify the lack of safety margins on the basis of species differences in toxicokinetics and toxicodynamics (mechanisms of toxicity and pharmacological activity) between animals and humans.

Dose Selection for Carcinogenicity Studies of Pharmaceuticals

The selection of appropriate dose levels remains the major challenge in the design of 2-yr carcinogenicity studies, since inappropriate dose levels may compromise the validity of a study. To assist in selection of dose levels, the

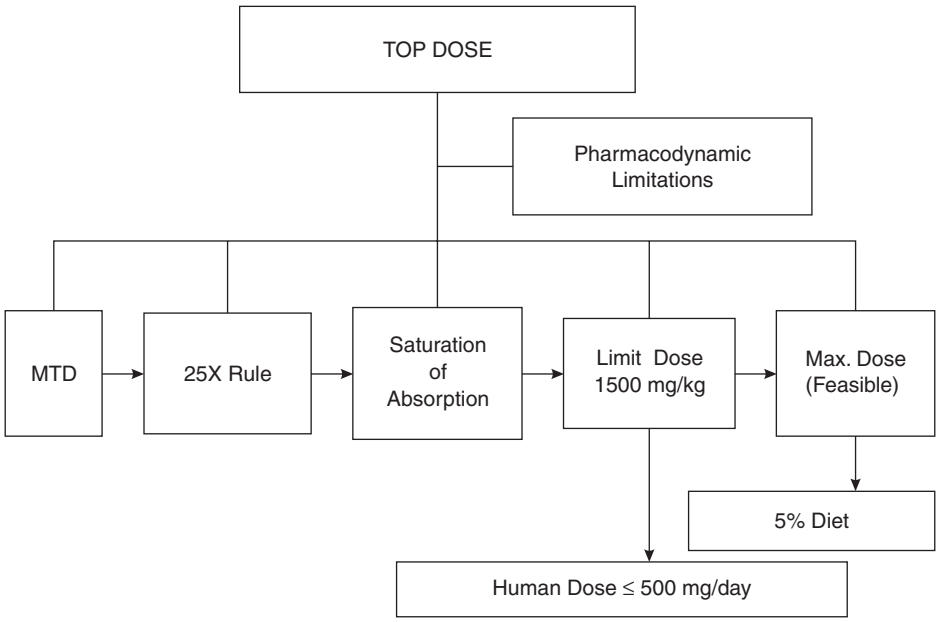

FIGURE 2. Toxicity and toxicokinetics-based approaches to top dose selection. Adapted from ICH (1995a, 1995b, 1995c).

ICH has provided guidelines on Dose Selection for Carcinogenicity Studies of Pharmaceuticals (ICH, 1995b). It has been recognized that for nongenotoxic carcinogens a threshold may exist, and positive findings in carcinogenicity studies may result from the use of very high doses often in great excess of clinical doses. Ideally, the doses selected for carcinogenicity bioassays should provide an adequate margin of safety over clinical exposure and should be compatible with long-term survival of animals. In selection of top doses, both toxicity and toxicokinetics-based end points should be considered. Figure 2 lists five different approaches for top dose selection.

Toxicity-Based Dose Selection Within the scope of the toxicity-based endpoints, the maximally tolerated dose (MTD) has been traditionally used as the top dose. Ideally, the MTD is the minimally toxic dose derived from 3-mo dose range-finding studies in rodents. Consideration should be given to interpretation of minimal toxicity in repeated-dose rodent studies. The minimal toxic effects should be limited to no more than 10% decrease in body weight gain, and the MTD must not induce dose-limiting clinical signs of toxicity, clinically relevant hematology and/or clinical chemistry changes, and/or significant target organ toxicity (ICH, 1995b). In our experience, the following endpoints have not been generally accepted by regulatory agencies as a possible criteria for MTD selection: small (≤10%) and inconsistent decrease in body weight gain, biologically insignificant changes in organ weights (e.g., liver weight), increases

in hepatic microsomal drug-metabolizing enzymes, hormonal changes, increased cell proliferation, apoptosis, and/or increased oxidative stress. However, depending upon the chemical administered and the severity of these changes, these endpoints could affect the long-term survival of animals and may decrease the sensitivity to detect true-treatment related effects. The doses that may cause excessive toxicity and reduce animals' life span must be avoided. Overall, assurance must be sought that the toxicity-based endpoints used to justify MTD are severe enough to impact the long-term survival of animals in rodent bioassays.

The use of MTD in carcinogenicity bioassays remains highly controversial (McConnell, 1989; Ames & Gold, 1990; Abelson, 1990; Counts & Goodman, 1995). The MTD has been supported by the fact that a retrospective analysis of the rodent carcinogenicity bioassays by the National Toxicology Program (NTP) indicated that a dose equivalent to an MTD was necessary in most rodent bioassays to produce a positive neoplastic response (Haseman & Lockhart, 1994). In our analysis of the NTP database, more than two-thirds of all rodent carcinogens would not have been missed even if doses less than MTD were used in carcinogenicity studies. Additionally, no efforts were made by the NTP to distinguish between genotoxic and nongenotoxic carcinogens. High background rate and great variability in spontaneous tumors further complicate the rodent bioassays. The sensitivity of the bioassays is further compromised by the use of a small number of animals (50/sex/group). This was nicely shown in the "megamouse" (ED-1) study conducted at the National Center for Toxicological Research (Littlefield et al., 1979). The "megamouse" study demonstrated that genotoxin 2-acetylaminofluorene (2-AAF) was carcinogenic to the urinary bladder, and this target organ would have been missed if the dose group were limited to 50 animals/sex/group only. Although the inclusion of MTD may increase the sensitivity of bioassays for certain compounds to detect a positive response, biological relevance of these results may be highly questionable when MTD is excessive. The major disadvantage of MTD is that when a compound is well tolerated and has a low order of toxicity, the selected MTD may be excessively high relative to potential human dose or systemic exposure. Additionally, in practice, many studies conducted for product approval utilize high doses, which often exceed the intended MTD and invariably affect biological processes and homeostasis, resulting in long-term health and survival problems. To alleviate the problem of very high MTD for compounds with low degree of toxicity, non-toxicity-based endpoints for dose selection have been proposed and are discussed later.

Toxicokinetics-Based Approaches to Top Dose Selection For compounds that exhibit a low degree of toxicity and are nongenotoxic in a standard battery of genotoxic tests, it may be appropriate to use toxicokinetics-based endpoints (ICH, 1995b) for top dose selection. The following approaches have been recommended by the ICH Guidelines.

25-Fold multiple of human systemic exposure Scientists at the U.S. Food and Drug Administration (FDA) conducted a retrospective analysis of carcinogenicity studies of 35 pharmaceuticals representing wide range of therapeutic categories

(Contrera et al., 1995). Their analysis showed that about 67% of drugs at MTD had a margin of exposure of less than 10 [systemic exposure in rats (top dose) divided by systemic exposure in human (maximally clinically effective dose)] and about 33% of drugs at MTD had a ratio of less than or equal to 1. When the analysis was expanded to include margins of safety based on the ratio body surface area between rats and humans, it was concluded that about 25% of 123 drugs at MTD attained a margin of safety of less than 1 and about 75% drugs had a margin of safety of less than 10. Based on the criteria established by the International Agency for Research on Cancer (IARC), two IARC 2A compounds (probable human carcinogens) phenacetin and 8-methoxypsoralen achieved a systemic exposure margin of greater than 10 (Contrera et al., 1995, and references therein). It was concluded that a systemic exposure ratio of 25 represents an adequate margin of safety for detecting a positive neoplastic response in animal studies. In considering the dose that provides a 25-fold margin of systemic exposure, the following conditions must be met:

1. The drug must be nongenotoxic with low degree of toxicity (lack of target organ toxicity),
2. The drug must be metabolized at least qualitatively similar in both rodents and humans,
3. The systemic exposure should be corrected for protein binding especially when the plasma protein binding is significant (e.g., approximately >80%) and is greater in humans than in animals,
4. The systemic exposure must be based on either parent drug, parent drug plus major metabolite(s) or solely on metabolites,
5. Human systemic exposure must be determined at the maximum recommended human daily dose for clinical practice.

Saturation of absorption For compounds that are poorly absorbed, the systemic exposure may reach a plateau due to saturable absorption of drug that is typically used to describe dose-limiting systemic exposure. *For the vast majority of chemicals absorption occurs via a passive diffusion process, and therefore in most cases absorption is not a saturable process*. Given a small number of animals used coupled with often large interanimal variability in plasma drug concentrations, it is important to evaluate dose versus systemic exposure using a wide range of doses, generally up to 1500 mg/kg/d (ICH-recommended limit dose) or the maximal feasible dose. Based on the U.S. FDA bioequivalence criteria applied to qualify generic drugs, it is the authors' opinion that a plateau in exposure suggestive of dose-limiting absorption is demonstrated when there is 20% or less increase in systemic exposure at the next high dose. To demonstrate a frank plateau in systemic exposure, it is imperative that the systemic exposure remain similar across many doses (doses below and above the dose providing the maximal exposure). When there is a large variability in

plasma drug concentrations due to variable absorption and or elimination, an appropriate statistical test (e.g., comparison of mean values and confidence interval or trend test) may be necessary to interpret the plateau in systemic exposure over various doses. When the increase in systemic exposure is limited by absorption, the lowest dose level, which provides the maximum systemic exposure, should be considered as the top dose. It should also be established that limitations in systemic drug exposure are not related to the increased metabolic clearance of drug with increasing doses. In the vast majority of cases, the increased clearance of drug is related to increased metabolism. Therefore, it is important to show that limitations in systemic drug exposure are not related to increased metabolism resulting in increased exposure to major metabolites. Overall, the lowest dose that provides maximal systemic exposure to parent drug and major metabolites should be considered as the top dose. Figure 3 shows an example for dose-limited absorption based top dose selection.

Implications of toxicokinetics-based high-dose selection Overall, for compounds with a low degree of toxicity the toxicokinetics-based top dose selection for carcinogenicity studies provides an alternate approach to MTD; however, compounds that show significant species difference in metabolism

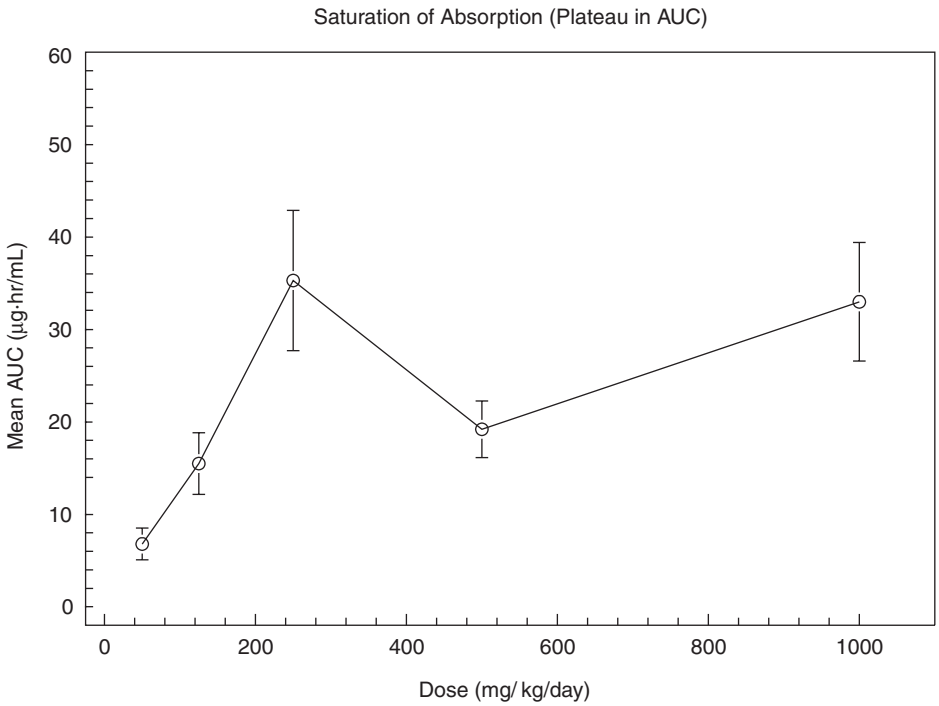

FIGURE 3. Effect of increasing doses on systemic drug exposure to compound X. The data show that systemic exposure to compound X plateaued at the dose of 240 mg/kg/d, suggesting saturable drug absorption.

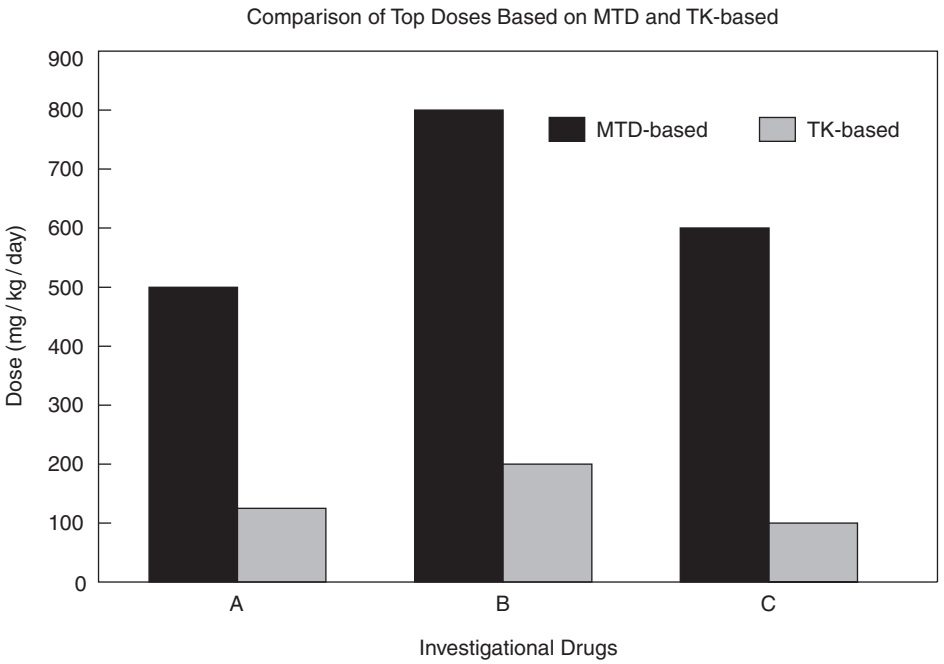

FIGURE 4. Comparison of top doses based on MTD and TK for rat carcinogenicity studies: Compound A in a 3-mo toxicity study showed an MTD of 500 mg/kg, while the selected top dose of 125 mg/kg/d provided a systemic exposure margin (relative to human systemic exposure at the maximal recommended dose). Compounds B and C in a 3-mo toxicity study had an estimated MTD of 800 and 600 mg/kg/d, respectively; however, saturable drug absorption (plateau in exposure to drug and its major human metabolites) was observed at the doses of 200 and 100 mg/kg/d, respectively.

(lack of major systemic human metabolites in rodents) or show a high degree of toxicity or genotoxicity are poor candidates for toxicokinetics-based high dose selection. Figure 4 shows an example of comparison of top doses based on an estimated MTD and systemic exposure margins. The results show that the toxicokinetics-based dose selection may be a better approach to dose selection for compounds with low degree of toxicity since this approach significantly reduces the top dose while providing an adequate safety margin (margins of systemic exposure between animals and humans).

Pharmacodynamic Endpoints Top dose selection based on pharmacodynamic endpoint may be appropriate for compounds that show a dose-limiting pharmacological response (e.g., hypotension, inhibition of blood clotting, and depression and somnolence affecting food intake). For example, if an investigational drug induces somnolence and depression in food intake, long-term exposure may result in significant weight loss that may be incompatible with the long-term survival of rodents. In such a case, the high dose selected may be based on the pharmacological activity. The dose-limiting pharmacological effects are generally very compound specific, and assurance must be sought that high

doses can not be administered due to dose-limiting pharmacological effects and that these adverse effects will not be compatible with long-term well being and survival of animals in 2-yr carcinogenicity studies.

Limit Dose and Maximal Feasible Dose Based on 900 carcinogenicity studies in FDA's carcinogenicity data base (ICH, 1995b; http://www.fda.cder/guidance/index/htm), it was concluded that a dose of 1500 mg/kg will be considered as the limit dose provided that the maximal clinical dose is 500 mg/d or less. Additionally, it is expected that the limit dose of 1500 mg/kg provides a margin of systemic exposure (AUC) that exceeds the human systemic exposure obtained at the maximally recommended human dose by greater than an order of magnitude. This can be demonstrated by providing data that show that the lower confidence limit for rodent AUC is at least 10 times higher than the AUC in humans.

For compounds where the clinical dose may exceed the total dose of 500 mg/d, the top dose should be the maximal feasible dose. For dietary administration, the maximal feasible dose is 5% of diet. For nondietary routes, the maximum feasible dose may be based on solubility (maximum solubility and dose volume limitation) and palatability (local tolerance) limitations of the test drug (ICH, 1995b; http://www.fda.cder/guidance/index/htm).

Toxicokinetics in Various Phases of Drug Safety Assessment Program (ICH Guideline, 1995a)

Single-Dose Toxicokinetic Studies The goal of single-dose toxicokinetic studies is to provide systemic exposure data over a wide range of doses and to assess if dose-limiting absorption or nonlinear toxicokinetics may occur at high doses. Although single-dose toxicokinetic studies are normally not required by the regulatory agencies to support acute toxicity studies, they are often valuable in providing data for dose selection for subsequent repeat dose toxicity studies. These data become critical for compounds that are expected to produce low degree of toxicity in animals. The single-dose studies are also useful in assessing which formulation may provide the maximal exposure and justify the dosing regimen (single versus multiple dosing per day). One approach might consider that single-dose studies utilize a wide range of doses up to the limit dose of 1500 mg/kg, provided that the projected human dose not to exceed 500 mg total up to the maximally feasible dose (limit of solubility or palatability). In the absence of any dome-limiting toxicity, a plateau in exposure may suggest a dose-limiting absorption, which can be used to justify the selection of top dose for subsequent repeated dose toxicity studies. A nonlinear TK with greater than dose proportional increase in systemic exposure may suggest saturable clearance and may aid in selection of the middle dose.

Repeated Dose Toxicity–Toxicokinetics Study The goal of toxicokinetics in repeated-dose toxicity studies is to assess dose and time (relative to the first dose) related changes in systemic exposure. In the author's experience in large

animals, toxicokinetics are usually conducted both after single dose and repeated doses (toward the end of study); however, in rodents the toxicokinetics evaluations are generally limited to one time period, such as, at the end of study (to avoid excessive blood sampling). Comparison of repeated-dose and single-dose systemic exposure data is often helpful in determining if there is an induction or inhibition of systemic drug clearance. Additionally, the repeated-dose exposure data help an assessment of steady-state toxicokinetics and systemic exposure margins (relative to actual or expected human systemic exposure) at various dose levels. The systemic exposure data may also be useful in interpreting dose response in toxicity; however, in certain cases target organ exposure may be more valuable than systemic exposure in interpreting target organ toxicity.

Monitoring of Systemic Exposure in Carcinogenicity Studies The objective of systemic exposure monitoring in carcinogenicity studies is to ensure that the steady-state toxicokinetic profile (plasma drug concentration versus time profile) in the carcinogenicity study is consistent with the toxicokinetic profile in carcinogenicity dose range-finding studies. Pharmacokinetically, the steady state is used to describe attainment of equilibrium conditions related to the processes of absorption, distribution, metabolism, and excretion (ADME). Since systemic exposure data are generally available at doses used in carcinogenicity studies, in our experience it is normally not necessary to reassess AUC (systemic exposure) in carcinogenicity studies. However, it is important to monitor toxicokinetic profile generally at one to three time points, including at previously known T_{max} (time to reach peak concentration) and T_{min} (time to reach trough concentration) time points. In our opinion, it is also important to monitor toxicokinetics at regular intervals during the first 6 mo of study only (ICH, 1995a). Steady-state toxicokinetics would be achieved within a week of repeated dosing because the vast majority of drugs have half-lives of less than 24 h in rodents (Bachmann et al., 1996), and steady-state plasma toxicokinetics is achieved after a drug has passed through approximately 6 half-lives. One approach might consider monitoring exposure in rats at the end of 3 mo and 6 mo in rats and at the end of 1 mo and 6 mo of dosing in mice. A similarity in limited plasma concentration (maximum and trough concentrations) versus time profiles between the two time periods would be indicative of steady-state toxicokinetics and would demonstrate consistency in steady-state systemic drug exposure in carcinogenicity bioassays. Figure 5 shows an example of steady-state toxicokinetics profile in carcinogenicity studies and demonstrates that equilibrium conditions with regard to ADME processes have been established.

Toxicokinetics to Support Negative In Vivo Genotoxicity Assays

Systemic exposure assessment is normally unnecessary for in vivo genotoxicity studies; however, in our experience the negative in vivo genotoxicity studies may need to be supported by the evidence that the negative results are not due to lack of systemic drug exposure or lack of drug exposure in the genotoxicity-relevant target tissue. As mentioned before, for drugs with a low degree of toxicity,

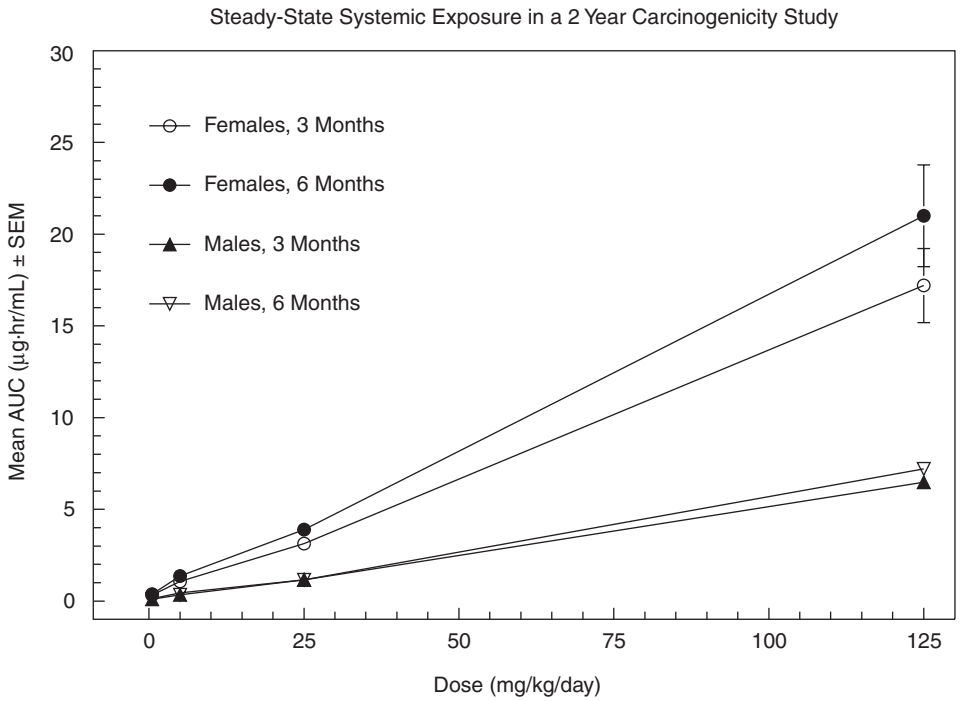

FIGURE 5. An example of steady-state systemic exposure for a 2-yr carcinogenicity study in rats. Data show that systemic exposure (plasma drug AUC) did not change between 3 and 6 mo, indicating steady-state exposure to drug in a carcinogenicity study.

the top dose for in vivo genotoxicity studies may be based on a dose-limited plateau in systemic exposure (see discussion of saturable absorption). This will avoid the use of excessive doses that may produce excessive toxicity or mortality and may compromise the validity of genotoxicity studies.

Tissue Distribution of Drug

Tissue distribution of drug to support the preclinical safety of drug is normally not required for all compounds; however, tissue distribution data are often highly valuable in interpretation of target organ toxicity. The tissue distribution data are needed to support the mass balance of an investigational drug in rodents. From preclinical safety assessment perspective, the conditions that may require the conduct of tissue distribution studies to support target organ toxicity are these:

1. When target tissue half-life exceeds the dosing interval by at least twofold (target tissue half-life of 48 h for once-a-day drug) in single-dose tissue studies. The data regarding the target tissue half-life may be obtained from the mass balance studies conducted by the drug metabolism scientists to support the Investigational New Drug Application (IND).

2. When the half-life of the parent drug and/or metabolite(s) in repeated-dose toxicity studies is significantly greater than expected from single-dose plasma toxicokinetic study.
3. Unanticipated (from short-term toxicity study) target organ toxicity (e.g., histopathological lesions) is suspected to result from the tissue sequestration of drug in target organs of toxicity.
4. When drugs are intended for tissue-specific delivery.

The guideline requires that the selection of dose levels and species/strain for the repeated dose tissue distribution studies should be based on all available information from the repeated-dose toxicity studies in which target organ toxicity was identified. Duration of study should be long enough to monitor steady-state drug concentration at critical time points (T_{max}, T_{min}, etc.), and for the vast majority of drugs the duration of study would be from 1 to 3 wk.

Toxicokinetics to Support Developmental and Reproductive Toxicity Studies (ICH, 1995a)

The systemic exposure data and information about similarity in metabolism between selected animal species and humans are often necessary to justify the choice of species, study design, and dosing schedules. The need to assess systemic exposure in developmental and reproductive toxicity (DART) studies is dependent on the extent of observed toxicity in dose range-finding studies. The lack of toxicity or low degree of toxicity in early dose range-finding studies may justify the assessment of exposure in reproductive toxicity studies to support dose selection, and assurance must be sought that the lack of reproductive and developmental toxicity is not due to lack of systemic exposure.

Toxicokinetic studies in pregnant and lactating rats may be needed to support developmental toxicity findings. If differences in toxicity between nonpregnant and pregnant animals are observed, it is important to know if this is due to differences in toxicokinetics profile. Although dose selection for developmental toxicity is mostly based on maternal toxicity findings, excessive doses with little or no relevance for human systemic exposure may be selected for compounds with a low degree of toxicity. Under the conditions of low toxicity, toxicokinetics data may be helpful in selecting doses based on either dose-limiting saturation of absorption or high systemic exposure margins (e.g., at least 25-fold). Information on the placental transfer of drug and exposure of embryos and fetus to drug is often necessary to interpret developmental toxicity findings. The extent of toxicokinetics in pregnant animals is dependent on the type of information desired. If compound has no biologically relevant developmental toxicity, in our experience it may be sufficient to assess maternal exposure (full profile) and placental transfer of drug by monitoring fetal exposure to drug at one to three time points, generally at the end of the gestation period. Because of species differences in placental transfer between animals and humans, the lack of placental transfer in DART studies may pose special problem in risk assessment and the validation of species/strain used for

developmental toxicity. This problem is best illustrated with the example of indinavir, an HIV protease inhibitor. While pregnant rats showed a moderate placental transfer of indinavir, there was lack of placental transfer of drug in rabbits, accompanied by absence of any significant maternal and developmental toxicity. The lack of placental transfer of drug in rabbits was not due to poor maternal drug exposure, since it was comparable to exposure observed in rats. This necessitated the evaluation of placental transfer of drug in other nonrodent species, including dogs and monkeys. Placental transfer studies in monkeys were complicated by poor maternal drug exposure. Pregnant dogs showed an approximately 50% placental transfer of drug, and this attribute was used as an appropriate nonrodent model to conduct developmental toxicity studies of indinavir in pregnant dogs. For compounds that show significant gestation-day-specific developmental toxicity, a detailed toxicokinetics evaluation (full profile to assess toxicokinetics parameters) in maternal plasma, placenta, embryo, and/or fetus at the critical period of susceptibility may be necessary to interpret developmental toxicity.

Information about the milk secretion of a drug is needed to understand the role of milk in overall exposure of neonates to the drug. In our experience, if there are no significant neonatal toxicity concerns, the milk transfer of drug may be determined by comparing milk and maternal plasma concentration of drug in rats on lactation day 14 at a selected time point (typically at maternal plasma drug T_{max}). When there is significant neonatal toxicity in suckling animals, it may be necessary to measure both milk concentration as well as neonatal plasma drug exposures. This may help in interpretation of neonatal toxicity and its relationship to drug exposure through milk.

Assessment of Metabolites (ICH, 1995a, 1995b)

The similarity in metabolic profiles between preclinical species and humans provides assurance regarding the validity of species for human health risk assessment. The qualitative similarity in metabolites can be demonstrated by comparing in vitro metabolic profile using liver slices, hepatocytes, and hepatic microsomes; however, confirmation must be provided by comparing plasma metabolites profile in preclinical species and humans. Although qualitative species differences in metabolism are uncommon, certain preclinical animal species may not form primate-specific metabolites. Table 1 lists the metabolic reactions that are generally limited to primate species, and if a test drug is metabolized through these metabolic reactions, the selected rodent species may not show exposure to human- or primate-specific metabolites. If exposure to the human specific metabolites can not be demonstrated, efforts should be made to provide separate safety data (e.g., limited genotoxicity and subchronic toxicity data in one rodent and one nonrodent species) on human-specific metabolites. Because of the lack of specific guidelines related to safety testing of unique human metabolites, appropriate regulatory agencies should be consulted to support human safety of metabolites. Unlike qualitative metabolism, species differences in quantitative metabolism are fairly common.

TABLE 1. Examples of Primate-Specific Metabolic Reactions

- Carbamate acyl glucuronidation
- Quarternization by glucuronidation of tertiary amines
- C-Glucuronidation of pyrazolones
- N^1-Glucuronidation of sulfadimethoxine
- O-Methylation of 4-hydroxy-3,5-diiodobenzoic acid
- Glutamine conjugation of arylacetic and aryloxyacetic acids
- Aromatization of quinic acid

Note. Adapted from Caldwell (1992).

Quantitation of metabolites in safety assessment studies is becoming increasingly common, and the following points may be useful when deciding about the quantitation of metabolites to support the safety of drug-related substances:

1. When a pro-drug is converted (nonenzymatically or enzymatically) to bio active metabolites, both pro-drug and active metabolite may be quantified, since there may be species differences in conversion of pro-drug to active metabolite.
2. When the drug is metabolized to highly potent pharmacologically and/or suspected toxicologically active metabolites, the quantitation of these metabolites may be critical for the assessment of safety of metabolites.
3. When metabolites constitute the predominant circulating drug-related moieties, the systemic exposure to these metabolites may be assessed.

While model-independent toxicokinetic evaluations are often sufficient to meet the needs of safety assessment studies, they are too simplistic to relate biochemical and physiological processes to drug toxicity. In absence of target organ(s) tissue concentrations, it is often difficult to interpret toxicokinetic-toxicodynamic (target organ toxicity) relationships. To improve the conventional compartmental/noncompartmental based toxicokinetic models, physiologically based toxicokinetics (PB-TK) models have been developed. If the mechanisms of toxicity are known, PB-TK models can provide valuable information on kinetics of tissue distribution of drug and its metabolites under a variety of exposure and disease conditions and may greatly assist in human health risk assessment of animal toxicology data. The discussion that follows provides a brief overview of PB-TK models and their application in human health risk assessment.

PHYSIOLOGICALLY BASED TOXICOKINETICS (PB-TK): PRINCIPLES AND METHODOLOGY

The PB-TK models are mathematical descriptions of the uptake and disposition of chemicals in biota based on quantitative interrelationships among critical determinants of these processes. The critical determinants of the toxicokinetic behavior of chemicals include physiological characteristics of

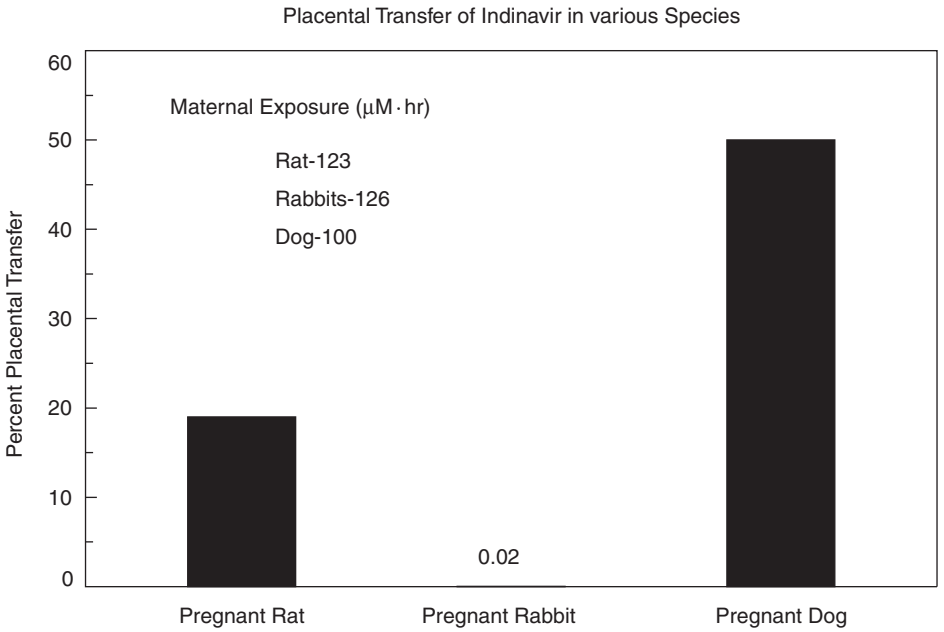

FIGURE 6. Placental transfer of indinavir in various preclinical species. The placental transfer was determined by dividing the fetal drug concentration by maternal drug concentration.

the animal, physicochemical properties of the chemical, and the rates of biochemical reactions in tissues. The PB-TK models are essentially compartmental models in which the compartments represent either individual tissues or tissue groups that are physically, physiologically, and biochemically characterized.

The classical compartmental models and PB-TK models differ in the way they are constructed parameterized and used. The construction of the classical model involves a fitting exercise for which time course on the pharmacokinetics of chemicals is required to begin with, contrary to the PB-TK approach in which data on tissue compartments and other parameters are used along with mechanism-based equations to predict the time-course data. Further, unlike the PB-TK approach, the type of equation chosen to fit to the data determines the number, behavior, and volumes of the compartments of the classical models, and not the physiological characteristics of the organism in which the blood concentration data were acquired. The parameters of PB-TK models correspond to compartment-specific mechanistic determinants (e.g., liver: blood partition coefficient, blood flow rate to a specific tissue), whereas those of the classical models are essentially a result of a lumping analysis (Cl, V_d) that does not reflect the contribution and role of specific mechanistic determinants. The numerical values of these parameters are the result of model fitting to experimental time-course data, whereas in the PB-TK approach independent measures of the model parameters are obtained to the extent possible. Although

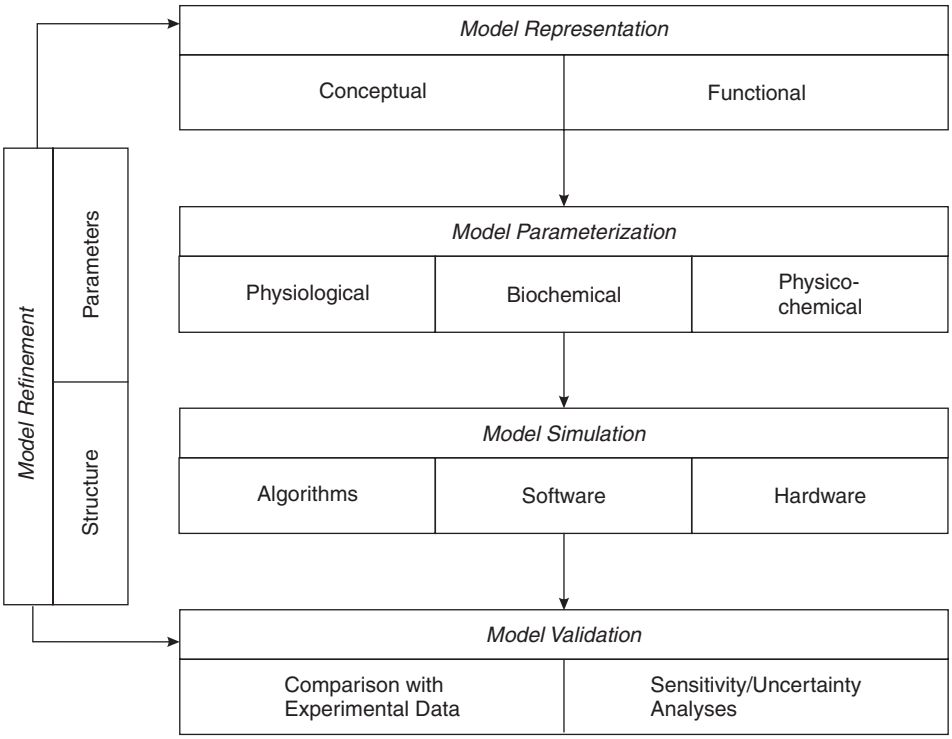

FIGURE 7. Schematic of the steps involved in the development of physiologically based toxicokinetic models.

the classical compartmental models are useful for conducting interpolations, they should not be used for extrapolation outside the range of doses, exposure routes, scenarios, and species used to generate the data for constructing such models. Due to the lack of biological realism, these models are not particularly useful for conducting interspecies extrapolations of the pharmacokinetic behavior of chemicals. Such extrapolations, essential for the conduct of dose-response assessment of chemicals, can be performed more confidently with the use of PB-TK models.

The steps involved in the development of PB-TK models are depicted in Figure 7. The first step, model representation, involves the development of conceptual and mathematical representations of the absorption, distribution, metabolism, and excretion of the chemical in the organism. In developing PB-TK model structures, thought should be given to the objective and proposed end use of the model. The second step involves obtaining the numerical values of the various parameters (physiological, biochemical, physicochemical) included in the mathematical descriptions of the PB-TK model. Once a structure is proposed, equations are written, and parameter values are known, then these

are integrated within a computer program and simulations of the pharmacokinetic profiles of chemicals in exposed animals obtained. The model simulation step requires the use of integration algorithms and appropriate software and hardware. The model validation step involves the comparison of a priori predictions of the PB-TK model with experimental data to refute, validate or refine the model description. The validated PB-TK models are then used to conduct extrapolations of the toxicokinetic behavior and tissue dose of chemicals from one exposure route/scenario to another, from high dose to low dose and from one species to another. PB-TK model-derived estimates of target tissue dose have been instrumental in resolving behavior that appears complex at the administered dose level (e.g., Andersen et al., 1987). The following paragraphs provide brief descriptions of the various steps of PB-TK modeling.

Model Representation

Model representation in this context refers to the diagrammatic representation of the relevant anatomical and physiological features of the organism in relation to the toxicokinetics and target organ of the chemical. The animal is represented as a set of individual organs or groups of organs interconnected by systemic circulation, whereas absorption, metabolism, distribution, and excretion pathways of chemical are represented by adding appropriate arrows to the tissue compartments (Figure 8). The rate of change in the amount of chemical in each compartment is described with a series of clearance terms accounting for uptake and metabolism (Krishnan & Andersen, 2001). The uptake is described on the basis of Fick's law of simple diffusion, whereas metabolism is often described as a saturable process using a Michaelis–Menten equation. A sample of the set of differential equations used for calculating the rate of change in the amount of chemical and algebraic equations for calculating the tissue concentration and effluent tissue venous concentrations of chemicals is included in Figure 8.

Model Parameterization

Several parameters are included in the equations constituting the PB-TK model. These are frequently physiological, physicochemical, and biochemical in nature. A list of specific parameters belonging to these classes is given in Table 2. Several compilations of physiological parameters are available or these parameters can be estimated as deemed appropriate (Arms & Travis, 1988; Davies & Morris, 1993; Brown et al., 1997). The biochemical and physicochemical parameters can be estimated in vivo, in vitro, or using animal-replacement algorithms.

The physicochemical parameters commonly used in PB-TK models are the partition coefficients. Partition coefficients represent the relative distribution of a chemical between two matrices (e.g., tissue and blood, blood and air) at steady state. A common in vivo method for estimating tissue:blood partition coefficient (P_{tb}) involves the administration and measurement of the parent

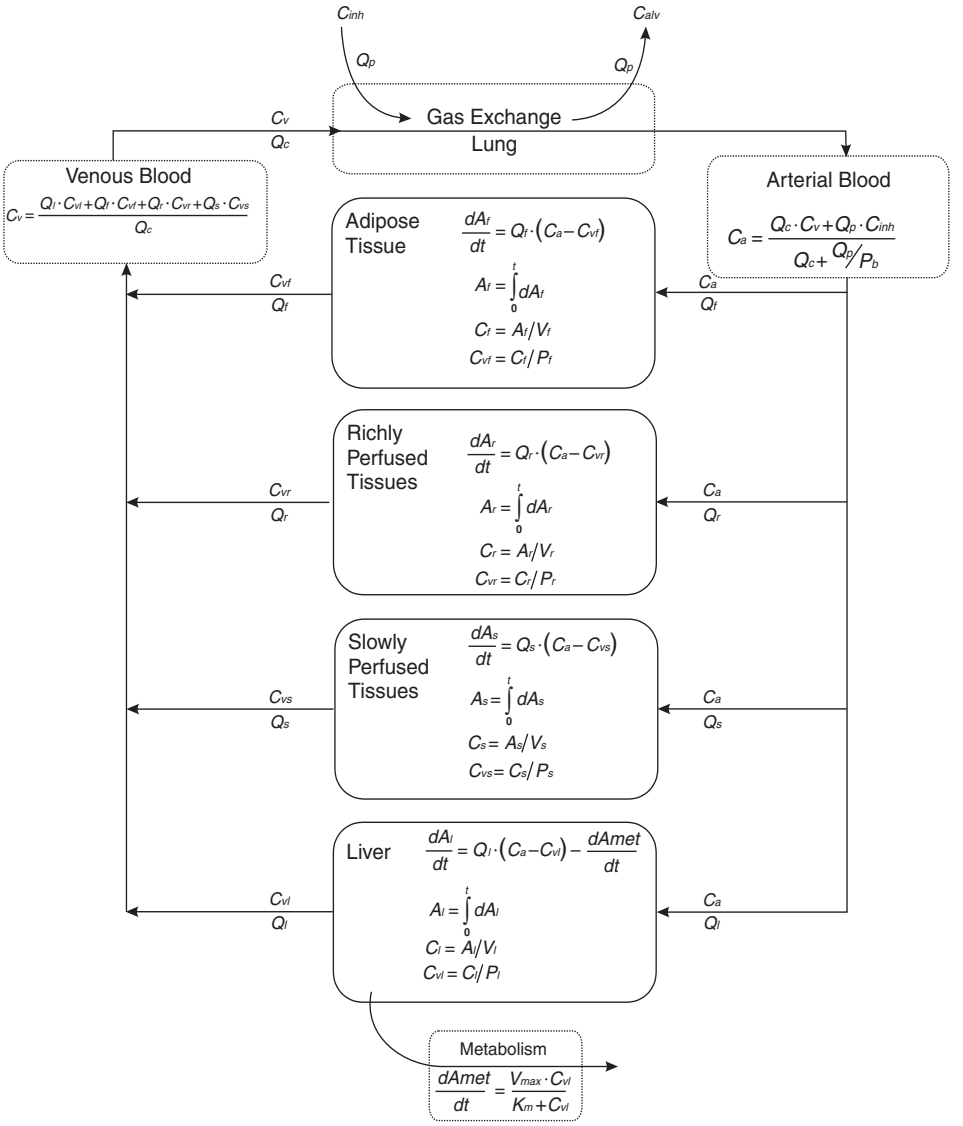

FIGURE 8. A schematic of the PBTK model for styrene. In this model, the rat is represented as a four-compartment system interconnected by systemic circulation. The input for the system is the product of the inhaled concentration of styrene times the alveolar ventilation rate. The resulting arterial blood concentration is in turn provided as input to the tissue compartments, the effluent venous blood concentrations of which are provided as input for the calculation of mixed venous concentration. C_{inh}, inhaled chemical concentration; C_{alv}, alveolar air concentration; C_a, arterial blood concentration; C_v, venous blood concentration; C_i, concentration in tissue i; C_{vi}, concentration of chemical leaving tissue i; A_i, amount of chemical in tissue i; *Amet*, amount of metabolized chemical; Q_p, alveolar ventilation rate; Q_c, cardiac output; Q_i, blood flow to tissue i; V_i, volume of tissue i; P_b, blood:air partition coefficient; P_i, tissue:blood partition coefficient for tissue i; V_{max}, maximal velocity of metabolism; K_m affinity constant; and i, adipose tissue (f), richly perfused tissues (r), slowly perfused tissues (s), and liver (l).

TABLE 2. Examples of PBTK Model Parameters

Physiological	Physicochemical	Biochemical
Cardiac output	Tissue:blood partition coefficient	Maximal velocity of metabolism
Alveolar ventilation rate	Blood:air partition coefficient	Michaelis–Menten affinity constant
Tissue blood flow		First-order elimination constant
Tissue volume		Hepatic extraction ratio

chemical in tissues and blood at steady-state condition. The in vitro methods for measuring P_{tb} include the vial equilibration, equilibrium dialysis, and ultrafiltration methods (Krishnan & Andersen, 2001). The animal-replacement approaches for predicting P_{tb}, based on the consideration of the solubility of chemicals in the lipid and water components of tissues and blood, are of the following general form (e.g., Poulin & Krishnan, 1996):

$$P_{tb} = \frac{P_{o:w} \cdot F_{lt} + F_{wt}}{P_{o:w} \cdot F_{lb} + F_{wb}}$$

where P_{ow}, F_{lt}, F_{wt}, L_b, and F_{wb} refer to oil-water partition coefficient, volume fraction of neutral lipid in tissue, volume fraction of aqueous component in tissues, volume fraction of neutral lipid in blood, and volume fraction of water in blood.

The biochemical parameters such as the rates of absorption, metabolism, macromolecular binding, and excretion can be determined by conducting specific time-course analysis in vivo and in vitro. One strategy involves conducting in vivo experiments and analysis of data using PB-TK models with one or two dominant but unknown biochemical parameters, thereby deriving estimates of these parameters (Krishnan & Andersen, 2001). Biochemical constants, specifically metabolism rate constants, may be obtained in vitro using subcellular fractions, postmitochondrial preparations, isolated cells, tissue slices, or isolated perfused organs. The relevance of rate constants determined in vitro to the intact animal is not clear in all cases. However, several studies using microsomes, postmitochondrial preparations, and hepatocytes have demonstrated the usefulness of these systems to provide metabolic rate constants for direct incorporation within PB-TK models (e.g., Krishnan et al., 1993; Kedderis & Held, 1996; Iwatsubo et al., 1997; Lipscomb et al., 1998; Mortensen & Nilsen, 1998). Mechanistic animal-alternative systems for providing a priori predictions of biochemical parameters are not available yet. At present, however, the hepatic extraction can be assumed to be complete or negligible in PB-TK models, in order to generate simulations. Accordingly, the numerical values of E (the hepatic extraction ratio) in the hepatic clearance equation should be set to 0 or 1 during model simulations. The region

encompassed by the simulated lines obtained with $E=0$ and $E=1$ will generally contain the experimental data for that particular exposure scenario (Poulin & Krishnan, 1999).

Model Simulation

Simulation, in the context of PB-TK modeling, refers to the prediction of the kinetic profiles of chemicals in blood and tissues, by solving the set of mass balance differential equations. Some of the commonly used algorithms for this purpose are Euler, Gear, Runge-Kutta routines, and predictor-corrector methods. The general principle underlying these algorithms used for solving first-order ordinary differential equations is represented as follows:

$$\text{New value} = \text{old value} + (\text{slope} \times dt)$$

where dt is the integration interval.
For a tissue compartment in PB-TK model,

$$A_{t,1} = A_{t,0} + (dA_t/dt) \times dt$$

where A is the amount of chemical, and t,0 and t,1 refer to the time at the beginning and end of an integration.

The PB-TK model equations, along with the integration algorithms, are written and solved using programming languages, simulation software, or spreadsheets. In the first two cases, the style of computational representation of the model is determined by the grammatical and precedence rules of the programming language (e.g., FORTRAN, BASIC) or simulation language to be used (e.g., Simulsolv, ACSL, MATLAB, Mathematica, ScoP). The choice of a particular programming or simulation software is up to the individual as long as the software package provides the framework for creating and solving the type of equations used in PB-TK modeling. The spreadsheet approach, on the other hand, is recommended for individuals who do not have sufficient knowledge of numerical integration algorithms and simulation software (Haddad et al., 1996). Once a beginner understands how the PB-TK models work using the spreadsheet-based methodology, he or she can then move on to use advanced techniques and specialized simulation languages offering flexibility, speed, and additional features (e.g., sensitivity analysis, uncertainty analysis, optimization routes).

Model Validation, Refinement, and Applications

Model validation refers to the evaluation of the adequacy of the conceptual and mathematical representations of the system under study in specific use conditions. The purpose of the validation process is to determine whether all major determinants/process that are essential for describing the system behavior have been adequately identified and characterized. The approaches

used for testing the adequacy of PB-TK models are inspection approach, discrepancy measures, and statistical tests (Krishnan & Andersen, 2001). The most commonly used approach is the inspection approach, which involves visual comparison of the plots of simulation data (usually continuous and represented by solid lines) with experimental data (usually discrete, and represented by symbols) against a common independent variable (usually time). When the experimental data and a priori simulations do not match satisfactorily, it reflects incorrect representation of the system or failure to include specific mechanistic determinants or biochemical processes. Further experimentation in such cases results in significant improvement of the biological understanding of the chemical under study (Clewell & Andersen, 1987; Haddad et al., 1998). The validated PB-TK models can then be applied to predict the behavior of chemicals administered by various exposure routes, at different doses, and in several animal species. These extrapolations are particularly important since human health risk assessment is based on responses seen in animal toxicity studies in which the test chemical is administered at high doses by routes often different from anticipated human exposures. The next section provides examples of the application of PB-TK models in the conduct of extrapolations for enhancing the scientific and mechanistic basis of health risk assessments of specific chemicals.

TOXICOKINETICS AND PHYSIOLOGICALLY BASED MODELING: UTILITY IN CHEMICAL AND PHARMACEUTICAL RISK ASSESSMENT

Contemporary risk assessments for chemicals, illustrated in the proposed carcinogen guidelines developed by the U.S. EPA (1996), are organized around two basic tenets: mode of action and target-tissue dosimetry. Mode of action, in a broad sense, conveys the biological steps involved in the expression of adverse effects. Dosimetry defines the form of the chemical causally related to toxicity. The form of the chemical is frequently called the *dose metric*. A complete statement of the mode of action encompasses both the biological basis of the response and describes the dose metric. Dose metrics include measures of concentration or net tissue exposures (area under the curve); they can be based on parent chemical or metabolites; and they can include consequences of interactions such as receptor binding, macromolecular adduct formation, or depletion of necessary biological molecules, such as glutathione. As risk assessments become more solidly based on understanding of chemical interactions with biological systems, our definition of dose metrics will likely expand.

Mode of action and dosimetery are qualitative, or at best semiquantitative, concepts. They are implemented in a more quantitative manner in pharmacokinetic (PK) and pharmacodynamic (PD) models. The cancer guidelines also talk about the use of biologically based dose response (BBDR) models in assessing the relationship of dose and response under various conditions. As discussed previously, physiologically based pharmacokinetic (PB-PK) or

physiologically based toxicokinetic (PB-TK) models describe the time course disposition of chemical within the body in relation to blood flows, tissue volumes, specific routes of administration, biotransformation pathways, tissue interactions, and so on (Andersen, 1991).

PB-PK/TK MODELS AND CHEMICAL RISK/SAFETY ASSESSMENTS

The past 20 years has witnessed extensive development of PB-PK/TK modeling for studying various problems in toxicology and pharmacology (Gerlowski & Jain, 1983; Leung, 1991; Krishnan & Andersen, 2001). The impetus for model development has been the nature of the questions that can be more quantitatively examined with these models. The PB-PK/TK models have been utilized to better understand the biological determinants that govern kinetic behaviors for a wide variety of chemicals. They have provided better methods to calculate target tissue dose metrics in conducting risk assessments. Due to their biological basis, they are more amenable to extrapolation across dose routes, between species, from high to low doses, and across exposure scenarios (Clewell & Andersen, 1985). The cancer guidelines refer to low dose and interspecies extrapolations as an analysis outside the region of observation. This analysis is more confidently conducted on the basis of the PB-PK/TK predictions of target tissue dose metrics than on the basis of atmospheric exposure concentration or administered dose. Finally, these models have been helpful in assessing mechanisms of toxicity by providing a refined understanding of relationships of dose metrics with specific responses. This report highlighted the use of these models with five different compounds, focusing on insights derived from quantitative approaches for assessing target tissue dosimetry. The five examples were methylene chloride (CH_2Cl_2), vinyl chloride (VC), inhaled organic esters (IOE), retinoic acid (RA), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

Methylene Chloride

Probably the earliest application of a PB-PK/TK model in a risk assessment was with CH_2Cl_2. A PB-PK/TK model for CH_2Cl_2 was initially developed to explore causality between various dose metrics and its carcinogenicity (Andersen et al., 1987). This solvent caused tumors in lung and liver in mice by inhalation, but was not carcinogenic by the oral route. It is metabolized to carbon monoxide by oxidation and to carbon dioxide by a glutathione (GSH) conjugation pathway. Each pathway has reactive compounds that are present transiently. Oxidation produces formyl chloride; GSH conjugation produces chloromethyl-glutathione. Either of these two pathways could produce a potentially mutagenic metabolite. The two pathways have different kinetic parameters. Oxidation has low capacity and high affinity. GSH conjugation has high capacity with low affinity. The PB-PK/TK model contained tissue clearance by both pathways in liver and kidney; accounted for dosing by inhalation or drinking water; and

allowed simulation of expected tissue dose metrics in mice and humans. With the model, it was possible to calculate the expected tissue exposures to the two reactive metabolites for different exposure conditions.

The dose metrics chosen for analyzing the tumor responses were related to the integrated intensity of tissue exposure to reactive intermediates, that is, the rate of metabolism through a specific pathway/volume of tissue/time. The carcinogenic responses in both tissues were closely correlated with the GSH pathway metabolism, not with oxidation. In addition, the PB-PK/TK model indicated that drinking water, because of slower intake and first-pass clearance in liver, would produce very low tissue exposures to the GSH pathway metabolites. Thus, route-to-route differences with CH_2Cl_2 also were consistent with the GSH pathway being responsible for the carcinogenic responses. The work with CH_2Cl_2 provided the first use of a PB-PK/TK model for extrapolation based on tissue dose metrics. This extrapolation used human specific parameters for tissue volumes, breathing rates, distribution of enzymes involved in oxidation and conjugation, and so on in the model structure. The estimation from this dose-metric-based analysis was that the risks were actually lower than estimated by the 1985 U.S. EPA default procedures by two orders of magnitude. The extrapolation assumed that mouse and human tissues would be equally responsive to equivalent tissue exposures to the reactive GSH pathway intermediates. This PB-PK/TK model has been cited and used in risk assessments by Health Canada (1993) and by the Occupational Safety and Health Administration (OSHA) in the United States (OSHA, 1997).

Vinyl Chloride

This commercially important plastics monomer causes liver hemangiosarcoma in multiple species, including humans. It is metabolized to a DNA-reactive epoxide. The epoxide has several fates. It can react with GSH or react via other pathways to yield carbon dioxide. Both of these are detoxification pathways. Alternatively, the epoxide can react with DNA to form promutagenic adducts. As with CH_2Cl_2, the dose metric with vinyl chloride (VC) was the production of the reactive metabolite with terms for consumption of the epoxide by detoxification pathways. PB-PK/TK models for VC of this general nature were described by several groups (Chen & Blancato, 1989; Reitz et al., 1996; Clewell et al., 1995, 2001). These models provide estimates of VC metabolite dose for different routes of administration and rodents and humans. As with CH_2Cl_2, the PB-PK/TK model could be used for extrapolation outside the range of analysis. Because of the observations of cancer in rodents and in people, the model can also be used to assess the consistency in risk estimates made for rodent and humans. The interspecies risk comparison with the PB-PK/TK model derived tissue dose described next is from the work of Clewell et al. (1995, 2001).

The risk estimates were conducted by correlating tumor outcome in rodent studies with the tissue dose metric for the putative concentration of the short-lived reactive epoxide metabolite. The 95% upper confidence limits (UCLs) on the human risk estimate for lifetime exposure to 1 ppb were calculated on

the basis of each of the sets of bioassay data, using the LMS model. Inhalation and corn oil gavage studies (Maltoni et al., 1981, 1984) and rat dietary studies (Feron et al., 1981) were available for this analysis. Dose metrics were first derived for the rodents; extrapolations in the range of extrapolation were conducted based on these measures of tissue dose; a human PB-PK/TK model was used to back-calculate the exposure concentrations associated with a particular tissue dose metric. The range of human risks estimated from these rodent assays for male and female animals was 1.10 to 5.17/million exposed individuals/ppb. The corn oil gavage studies gave slightly higher risks, 8.68 for males and 15.70 for females. Provision of high levels of corn oil in the diet is likely to act as a confounding factor altering the response of the tissue to the epoxide metabolite.

Are these dose-metric-based risks estimated for humans from the rodent bioassay data plausible? To assess this question, the PB-PK/TK model was run for human parameters for the human exposure scenario appropriate to each of the cohorts. The resulting liver dose metrics were multiplied by the appropriate duration to obtain the cumulative internal liver dose. This estimate was the input into a linear relative risk model for hemangiosarcoma, along with the observed and expected cancer deaths, in order to derive an estimate of carcinogenic potency in the population. The last step is to compare tissue dose metrics in humans exposed to 1 ppb to the potency based on the tissue dose of reactive metabolite. This step is accomplished by running the human PB-PK/TK model at 1 ppb for continuous exposure to determine the average daily tissue dose. The range of risk estimated in humans by this analysis for three epidemiological cohorts (Fox & Collier, 1977; Jones et al., 1988; Simonato et al., 1991) ranged from 0.4 to 4.22/million/ppb, a remarkably good agreement with the estimates from the animal bioassay. When the risks were based on biologically appropriate dose metrics, interspecies scaling of lifetime cancer risks with VC were successfully performed on the basis of lifetime average daily tissue dose, without applying a surface area adjustment.

Inhaled Organic Esters

Various organic esters, including both vinyl acetate (VA) and methylmethacrylate (MMA), cause nasal olfactory degeneration following inhalation exposures. PB-PK/TK models developed for these esters (Plowchalk et al., 1997; Bogdanffy et al., 1999; Andersen et al., 1999) calculate tissue dose metrics in the epithelial tissue compartments of the nose in support of estimations of cancer risks (with VA) or estimations of reference concentrations (with both VA and MMA). The target tissue compartments in the ester models differ from the liver compartment with the models for CH_2Cl_2 and VC. The liver was described as single homogeneous compartment with enzyme distributed uniformly throughout the organ. Blood entering the tissue was assumed to freely distribute into the entire liver. This behavior is referred to as blood-flow-limited uptake (Andersen, 1991). The geometry of the nasal airways is complex, and the esters reaches target cells by diffusion through mucus and

epithelial tissues rather than via the blood stream. The inhaled ester models divide the nasal lumen into a series of discrete airway regions. The underlying tissue in each of these is then divided into a series of layers. With VA, each epithelial tissue layer is 10 μm thick to allow modeling with experimentally measured diffusion coefficients. This tissue compartmentalization structure leads to five to seven tissue compartments, depending on the total thickness of the tissues in any region (Plowchalk et al., 1997). With MMA (Andersen et al., 1999), tissue thickness was estimated for the three main barriers—the mucus, epithelial cell, and submucosal regions. Each tissue compartment has clearance due to diffusion or metabolism. The submucosal region is perfused with blood and chemical absorbed into this region can be carried away in the venous drainage.

The anatomical and biochemical parameters required for running the model simulations include surface areas, tissue thickness, airflow distribution within the nasal cavity, and diffusion coefficients. Morris et al. (1993) first organized many of these parameters in a nasal PB-PK/TK model. Esterase activities and distribution in the nasal epithelium and submucosal regions were measured by Plowchalk et al. (1997). The dose metrics calculated differed for these two esters. They were the amount of MMA metabolized per time in the tissue compartments and the pH changes that occur in the tissues due to acetic acid production from VA hydrolysis and metabolism of the alcohol hydrolysis products to acetaldehyde and acetic acid. For pH calculations with VA, the tissue dosimetry model includes proton pumps that control the internal proton concentration in cells. With both MMA and VA, the application of these dosimetry models indicated that current default dosimetry models included in the U.S. EPA reference concentration (RfC) documentation (1994) tend to overestimate the tissue dose of ester metabolites in the nose. Another advantage of the explicit construction of these models was the ability to conduct sensitivity analyses to assess the relative importance of the various model parameters in controlling achieved tissue doses. The sensitivity analysis was especially useful with VA (Plowchalk et al., 1997), where the model is highly nonlinear due to several saturable processes, including two distinct esterase pathways, proton pumping, and the sequential arrangement of compartments within the nose. These nasal dosimetry models will likely play an important role in RfC estimations for a variety of chemicals of interest in the IRIS (Integrated Risk Information System) Pilot Project at the U.S. EPA (http://www.epa.gov/iris).

All-*Trans*-Retinoic Acid

The first three examples are about the use of PB-PK/TK models for risk assessments for commercially important volatile compounds. An interesting example of a safety assessment for a pharmaceutical conducted with the aid of a PB-PK/TK dosimetry model occurred with all-*trans*-retinoic acid (ATRA). ATRA was being considered for use as a topical treatment for improving the appearance and health of skin. It improved skin tone and reduced the number

of small wrinkles. The benefits of a cosmetic improvement in skin appearance have to be weighed against the potential risks associated with retinoic acids. ATRA and related metabolites are potent teratogens at doses in the range of just a few milligrams per kilogram. The approach taken to assess this risk quantitatively was to develop a PB-PK/TK model that tracked the blood and fetal compartment concentrations of ATRA and other active retinoids formed from ATRA. The other active retinoids are 4-oxo-ATRA, 13-*cis*-RA, and 4-oxo-13-*cis*-RA. The results of this safety assessment have been published (Clewell et al., 1997) and are summarized here.

The dosimetry model calculated several dose metrics—primarily concentrations and areas under the curves for ATRA or for total active retinoids in the fetal compartment. The metabolism and interconversion of the various active retinoids is complex. Multiple metabolic steps have to be included: oxidation, glucuronidation, isomerization in intestinal tissue, enterohepatic recirculation, and so on. The set of data for analysis was large and diverse, with dosing in various species by a variety of routes of administration. The breadth of the set of data makes model testing an adventure where all available data are described with a single set of model parameters. Change in any one parameter necessitates reanalysis of the entire data set.

With the completed dosimetry model, a reality check was first performed to estimate the AUC for total active retinoids at minimal teratogenic dosages in rat, mouse, and monkey. The values were all within a factor of 2, with an absolute value near 10,000 ng-h/ml. Clinical doses of 1.1 mg/kg are used in treatment of certain types of leukemia. The expected human AUC was 1100 for this dosage with ATRA and 7000 ng-h/ml for 13-*cis*-RA. These therapeutic dosages for life-threatening cancer lead to relatively high tissue dose metrics for fetal exposure. For ATRA skin treatment, two scenarios were evaluated. The first was therapy that followed instructions provided with the skin cream; the second was a situation where the patient deliberately applies the cream to larger areas of skin more frequently. Even with the abuse situation, the AUC in the fetus for total active retinoids was less than 0.5 ng-h/ml. Thus, the margin of safety (MOS) for abuse conditions with use on face, chest, and arms was 10,000/0.5 or about 20,000. The MOS for facial use only as advised in packaging inserts would be 10,000/0.013 or about 770,000.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Various hepatic tumor promoters induce cytochrome P-450 enzyme isoforms in the liver in rodents. Most of these inducers act transcriptionally with regulatory proteins to alter the concentrations of specific messenger RNAs and their protein products. One of the concerns for risk assessment has been to decipher the dose-response behavior at low doses, that is, in the region of extrapolation, and to understand the molecular processes that lead to induction in these cells. A surprising characteristic is that induction is not uniform in the liver. At low doses, centrilobular regions become induced. As dose increases,

the areas of induction move outward from the centrilobular areas toward the mid-zonal regions, and at high doses all regions become induced (Tritscher et al., 1992). It appears as if cells are either at a control level or 100% induced, instead of a more gradual induction with increasing dose. Thus, the liver has to be defined with multiple regions, each with differing sensitivities for induction, if these regional characteristics of induction are to be successfully modeled. Early PB-PK models for protein induction by TCDD neglected the nonuniform induction (Andersen et al., 1993; Kohn et al., 1993).

In a more recent PB-PK/TK model for TCDD-mediated protein induction (Andersen et al., 1997), the liver was divided into five subcompartments arranged serially from the periportal through centrilobular regions. Induction in each was modeled with TCDD binding to its cognate receptor, the Ah receptor. The binding of the Ah receptor-TCDD complex to promoter sites on DNA enhanced a rate of gene transcription. The analysis focused on the question of how large a Hill coefficient was required in the induction equations, and the difference in DNA–Ah receptor–TCDD dissociation constant required between adjacent regions to match the overall patterns of induction. An adequate fit to a large body of data, including regional immunohistochemical staining patterns, low-dose induction of mRNA for cytochrome P-450 1A1, and overall induction, required high *n* values (4 to 5) in each region and threefold differences in binding affinity between adjacent regions. The total difference in affinity between the centrilobular and periportal regions would be 81-fold.

With TCDD, the combination of the PB-PK/TK model and the requirement to simulate both a quantitative measure of induction and the distribution of induced cells within the liver produced a model with interesting characteristics. The dioxin PB-PK/TK induction model had very steep responses in each zone and had highly nonlinear responses in the region of extrapolation. PB-PK/TK models that have ignored regional distribution predict a more nearly linear extrapolation for the region of extrapolation. While not adopted for regulatory use, this regional induction model does make some interesting conclusions about low-dose responses for various tumor promoters that should be more carefully examined by specific experiments in vivo and with isolated hepatocytes in vitro. The "switching" of hepatocytes from a basal to an "induced" state over a narrow range of tissue dose may be an important component of the control of "genetic circuitry" with a variety of receptor-mediated toxicants.

This overview of the use of PB-PK/TK dosimetry models in risk assessments has focused on three model applications. *Exploratory evaluations* of proposed modes of action are possible by comparing responses with various measures of dose, as done with contributions from the two pathways with CH_2Cl_2 or in assessing the relationship of nasal toxicity with different dose metrics for VA. Another example is with VC in assessing the correspondence between risk projections from rodent and human exposures. *Interpretive evaluations* occur in applying estimated dose metrics to assess acceptable exposure levels for

RfCs, cancer risk assessments, MOSs, and so on. In this use, the experimental exposures are first converted to internal dose metrics; the estimates of no-observed-adverse-effect levels (NOAELs) or benchmarks are then calculated in terms of this tissue dose metrics. Next, uncertainty factors are applied to reduce the target tissue dose metric, and finally a human PB-PK/TK model is used to estimate the ambient exposure level that would give rise to this target tissue dose metric. This process was more explicitly described in *Drinking Water and Health* (NRC, 1986). Third, *mechanistic evaluations* are intended to characterize the relationship of tissue dose and response and determine consistency with specific hypotheses regarding toxicity/biochemical responses. The evaluation of the Hill coefficients and "switching" phenomenon with TCDD exemplifies a mechanistic evaluation of a mode of action and hypothesis with a PB-PK/TK model.

Since the first proposal to use a PB-PK/TK model in risk assessment with CH_2Cl_2 in 1987, the field of PB-PK/TK modeling in relation to risk modeling has grown steadily. In more recent years, there has been increasing acceptance of the use of these dosimetry models in a variety of risk assessments. The U.S. EPA reference concentration (RfC) documentation explicitly includes routine application of interspecies differences in dosimetry in assessing RfCs (U.S. EPA, 1994). The recent RfC documentation for VC in IRIS (U.S. EPA, 2000a) specifically describes and uses a PB-PK/TK model for standard setting and dose route extrapolations. A recent risk assessment with acrylic acid (Andersen et al., 2000) applies a PB-PK model linked to computational fluid dynamic calculations of nasal airflow (Frederick et al., 1998) to derive an RfC for this compound. The Hazardous Air Pollutants (HAPS) Test Rule (*Federal Register*, 1996) invited increased efficiency/efficacy in testing by providing possible substitution of certain oral toxicity tests instead of requiring new inhalation studies, for those instances where a validated PB-PK/TK model is available to conduct extrapolations across dose routes. Several case studies under the proposed cancer guidelines (U.S. EPA, 1996)—for example, those with formaldehyde (CIIT, 1999), chloroform (International Life Sciences Institute, 1977) and vinyl acetate (Bogdanffy et al., 1999)—have based analysis in the region of extrapolation on mode-of-action specific target tissue doses calculated with dosimetry models. In chapter 8 of the U.S. EPA dioxin reassessment (U.S. EPA, 2000b), various mechanistic protein induction models are evaluated to assess the predictions for low-dose risks for each of them.

The past 15 years has seen the emergence of PB-PK/TK models for use in dosimetry and risk assessment. This period has seen model development and applications, evaluation of statistical methods for parameter estimation, improvements in sensitivity and variability analysis, and training of individuals in these more quantitative areas. The stage is set for wider penetration of these approaches in the risk assessment process. The examples described in this review capture some of the promising areas of application of dosimetry models and point the way toward other more novel applications in hypothesis testing and experimental design.

CONCLUSIONS

Integration of toxicokinetics into product safety assessment studies has been greatly beneficial in interpretation of the preclinical toxicity data and its utility in human health risk assessment. With implementation of the ICH Guidelines on systemic exposure and dose selection, toxicokinetics data also have been useful in selecting the dose levels for short-term to long-term toxicity and carcinogenicity studies for compounds that show a low degree of toxicity. This is an important achievement and a great improvement in dose selection for carcinogenicity studies in rodents, since in the past extremely high and unrealistic doses with no relevance to human exposure were selected for well-tolerated compounds. The greatest utilities of preclinical toxicokinetics data have been in the interspecies comparison of product toxicity. It is now widely accepted that toxic effects can be better extrapolated from animals to humans when these comparisons are based on toxicokinetics and disposition (absorption, distribution, metabolism, and excretion) data in preclinical species and humans. In this context, safety margins based on ratio of animal AUC at the no-observed-effect or toxic-effect dose level to the human AUC at the maximal clinical dose are considered important predictors of human toxicity risk, and it is generally accepted that the larger is the AUC ratio, the smaller is the expected risk of toxicity in humans. Although model-independent or compartment-based plasma/blood toxicokinetics have served well as a practical means of assessing systemic exposure, they provide no information on time course of exposure of target organs to drug or metabolites. It has been shown that for certain compounds with a high volume of distribution, tissue concentrations may exceed plasma concentrations by several orders of magnitude and the exposure based on plasma concentrations may underestimate overall exposure. To predict the time course of drug or metabolites transfers between organs and tissues, PB-TK models have been developed. PB-TK models are mathematical descriptions of the uptake and disposition of chemicals in biota based on quantitative interrelationships among critical determinants of these processes. There are many advantages of PB-TK models, and this review has provided some good examples. The PB-TK models are biologically realistic because the compartments are based are physiological and anatomical characteristics, regional blood flow rates, diffusion of drug between blood and tissues, and the affinity of drug to different organs. PB-TK models can predict tissue drug or metabolite concentrations, and true pharmacokinetics parameters values under a variety of conditions to reflect altered metabolism or organ function. On the negative side, PB-TK models are mathematically complex, require extensive drug disposition and physiological parameters-related data, and their validation in humans is challenging. Given these reasons and the rapid nature of pharmaceutical product development, the PB-TK models have found limited use in drug development. Despite these limitations, PB-TK models have been successfully used to predict time course of tissue drug disposition of many chemical compounds, and their

greatest utility is in reducing the uncertainty in human health risk assessment of animal toxicology data. Overall, toxicokinetics data have greatly enhanced our mechanistic understanding of interspecies differences in toxicity and improved the human health risk assessment of preclinical toxicology data.

REFERENCES

Abelson, P. H. 1990. Testing of carcinogens with rodents. *Science* 249:1387.

Ames, B. N., and Gold, L. S. 1990. Chemical carcinogenesis: Too many rodent carcinogens. *Proc. Natl. Acad. Sci. USA* 87:7772–7776.

Andersen, M. E. 1991. Physiological modelling of organic compounds. *Ann. Occup. Med*. 35:305–321.

Andersen, M. E., Clewell, H. J. III, Gargas, M. L., Smith, F. A., and Reitz, R. H. 1987. Physiologically-based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol*. 87:185–205.

Andersen, M. E., Mills, J. J., Gargas, M. L., Kedderis, L., Birnbaum, L. S., Neubert, D., and Greenlee, W. F. 1993. Modeling receptor-mediated processes with dioxin: Implications for pharmacokinetics and risk assessment. *J. Risk Anal*., 13:25–36.

Andersen, M. E., Birnbaum, L. S., Barton, H. A., and Eklund, C. 1997. Regional hepatic CYP1A1 and CYP1A2 induction with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin evaluated with a multi-compartment geometric model of hepatic zonation. *Toxicol. Appl. Pharmacol*. 144:145–155.

Andersen, M. E., Sarangapani, R., Frederick, C. B., and Kimbell, J. S. 1999. Dosimetric Adjustment factors for methylmethacrylate derived from a steady-state analysis of a physiologically based clearance-extraction model. *Inhal. Toxicol*. 11:899–926.

Andersen, M. E., Sarangapani, R., Gentry, P. R., Clewell, H. J. III, Covington, T. R., and Frederick, C. B. 2000. Application of a hybrid CFD-PBPK nasal dosimetry in an inhalation risk assessment: An example with acrylic acid. *Toxicol. Sci*. 57:312–325.

Arms, A. D., and Travis, C. C. 1998. *Physiological parameters in pharmacokinetic modelling*. EPA/600/6-88/004, 1-1-7.16. Washington, DC: Office of Health and Environmental Assessment, U.S. EPA.

Bachmann, K., Pardoe, D., and White, D. 1996. Scaling basic toxicokinetic parameters from rat to man. *Environ Health Perspect*. 104:400–407.

Benet, L. Z., and Sceiner, L. B., 1985. In *The pharmacological basis of therapeutics*, eds. A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, 7th ed. New York: Macmillan.

Bogdanffy, M. S., Sarangapani, R., Plowchalk, D. R., Jarabek, A., and Andersen, M. E. 1999. A biologically-based risk assessment for vinyl acetate-induced cancer and non-cancer inhalation toxicity. *Toxicol. Sci*. 51:19–35.

Bourne, D. W. A. 1995. *Mathematical modeling of pharmacokinetic data*. Lancaster, PA: Technomic.

Brown, R. P., Delp, M. D., Lindstedt, S. L., Rhomberg, L. R., and Beliles, R. P. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health* 13:407–484.

Caines, P. E. 1988. *Linear stochastic systems*. New York: Wiley and Sons.

Caldwell, J. 1992. Problems and opportunities in toxicity testing arising from species differences in xenobiotic metabolism. *Toxicol. Lett*. 64/65:651–659.

Chemical Industry Institute of Toxicology. 1999. Formaldehyde: Hazard characterization and dose-response assessment for carcinogenicity by the route on inhalation. Research Triangle Park, NC: CIIT.

Chen, C. W., and Blancato, J. N. 1989. Incorporation of biological information in cancer risk assessment: Example—Vinyl chloride. *Cell Biol. Toxicol*. 5:417–444.

Clewell, H. J. III, and Andersen, M. E. 1985. Risk assessment extrapolations and physiological modeling. *Toxicol. Ind. Health* 1:111–131.

Clewell, H. J. III, and Andersen, M. E. 1987. Dose, species and route extrapolation using physiologically based pharmacokinetic models. *Drinking Water Health* 8:159–182.

Clewell, H. J., Gentry, P. R., Gearhart, J. M., Allen, B. C., and Andersen, M. E. 1995. Considering pharmacokinetic and mechanistic information in cancer risk assessments for environmental contaminants: Examples with vinyl chloride and trichloroethylene. *Chemosphere* 31:2561–2578.

Clewell, H. J. III, Andersen, M. E., Wills, R. J. Wills, and Latriano, L. 1997. A physiologically based pharmacokinetic model for retinoic acid and its metabolites. *J. Am. Acad. Dermatol*. 36:S77–S85.

Clewell, H. J., Gentry, R., Gearhart, J. M., Allen, B. C., and Andersen, M. E. 2001. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. *Science of the Total Environment*. 274:37–66.

Contrera, J. F., Jacobs, A. C., Prasanna, H. R., Mehta, M., Schmidt, W. J., and De George, J. 1995. A systemic exposure based alternative to the maximum tolerated dose for carcinogenicity studies of human therapeutics. *J. Am. Coll. Toxicol*. 14:1–10.

Counts, J., and Goodman, J. 1995. Principles underlying dose selection for, and extrapolation from the carcinogen bioassay: Dose influences mechanism. *Regul. Toxicol. Pharmacol*. 21:418–421.

Davies, B., and Morris, T. 1993. Physiological parameters in laboratory animals and humans. *Pharm. Res*. 10:1093–1095.

DeGeorge, J. 1995. Food and Drug Administration's viewpoint on toxicokinetics. The view from review. *Toxicol. Pathology* 23:220–225.

Effect of dose and time in a long-term, low dose carcinogenicity study. *J. Environ Pathol. Toxicol*. 8:17–34.

Environmental Protection Agency (EPA), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, EPA/600/8-90/066F (Office of Health and Environmental Assessment, Washington, D.C., 1994).

Federal Register. 1996. Part II Environmental Protection Agency. 40 CFR 799. Proposed test rule for hazardous air pollutants; Proposed Rule. Fed. Reg., June 26, 1996. 33177–3320.

Feron, V. J., Hendriksen, C. F. M., Speek, A. J. 1981. Lifespan oral toxicity study of vinyl chloride in rats. *Food Cosmet. Toxicol*. 19:317–333.

Fox, A. J., and Collier, P. F. 1977. Mortality experience of workers exposure to vinyl chloride monomer in the manufacture of polyvinyl chloride in Great Britain. *Br. J. Ind. Med*. 34:1–10.

Frederick, C., Bush, M. L., Subramaniam, R. P., Black, K. A., Finch, L., Kimbell, J. S., Morgan, K. T., Subramaniam, R. P., Morris, J. B., and Ultman, J. S. 1998. Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. *Toxicol. Appl. Pharmacol*. 152:211–231.

Gabrielsson, J., and Weiner, D. 1997. *Pharmacokinetic/pharmacodynamic data analysis: Concepts and applications*, 2nd ed. Stockholm: Apotekarsocieteten.

Gelman, A., Bois, F., and Jiang, J. 1996. Physiological pharmacokinetic analysis using population modeling and informative prior distributions. *J. Am. Stat. Assoc*. 91:1400–1412.

Gerlowski, L. E., and Jain, R. K. 1983. Physiologically based pharmacokinetic modeling: Principles and applications. *J. Pharm. Sci*. 72:1103–1127.

Gibaldi, M., and Perrier, D. 1982. *Pharmacokinetics*, 2nd ed. New York: Marcel Dekker.

Haddad, S., Pelekis, M., and Krishnan, K. 1996. A methodology for solving physiologically based pharmacokinetic models without the use of simulation software. *Toxicol. Lett*. 85:113–126.

Haddad, S., Withey, J. R., Laparé, S., Law, F. C. P., and Krishnan, K. 1998. Physiologically-based pharmacokinetic modeling of pyrene in the rat. *Environ. Toxicol. Pharmacol*. 5:245–255.

Haseman, J. K., and Lockhart, A. 1994. The relationship between use of maximum tolerated dose and study sensitivity for detecting rodent carcinogenicity. *Fundam. Appl. Toxicol*. 22:382–391.

Health Canada, 1993. *Canadian Environmental Protection Act. Priority substances list assessment report. Dichloromethane*. Ottawa, Canada: Canada Communication Group.

ICH. 1995a. Guidelines on assessment of systemic exposure in toxicity studies. *Fed. Reg*. 60(40):11264–11268.

ICH. 1995b. Guidelines on dose selection for carcinogenicity studies of pharmaceuticals. *Fed. Reg*. 60(40):11278–11281.

ICH. 1995c. Guidelines on repeated dose tissue distribution studies. *Fed. Reg*. 60(40):11274–11275.

International Life Sciences Institute. 1977. *An evaluation of EPA's proposed guidelines for carcinogen risk assessment using chloroform and dichloroacetic acid as case studies: Report of an expert panel*. Washington, DC: International Life Sciences Institute, Health and Environmental Sciences Institute.

Iwatsubo, T., Suzuki, H., and Sugiyama, Y. 1997. Prediction of species differences (rat, dogs, humans) in the in vivo metabolic clearance of YM796 by the liver from in vitro data. *J. Pharmacol. Exp. Ther*. 283:462–469.

Jones, R. W., Smith, D. M., and Thomas, P. G. 1988. A mortality study of vinyl chloride monomer workers employed in the United Kingdom in 1940B1974. *Scand. J. Work Environ. Health* 14:153–160.

Kedderis, G. L., and Held, S. D. 1996. Prediction of furan pharmacokinetics from hepatocyte studies: comparison of bioactivation and hepatic dosimetry in rats, mice, and humans. *Toxicol. Appl. Pharmacol*. 140:124–130.

Kohn, M. C., Lucier, G. W., Clark, G. C., Sewall, C., Tritscher, A., and Portier, C. J. 1993. A mechanistic model of the effects of dioxin on gene expression in the rat liver. *Toxicol. Appl. Pharmacol*. 120:138–154.

Krishnan, K., and Andersen, M. E. 2001. Physiologically based pharmacokinetic modeling in toxicology. In *Principles and methods of toxicology*, ed. A. W. Hayes, pp. 193–241. Philadelphia: Taylor & Francis.

Krishnan, K., Gargas, M. L., and Andersen, M. E. 1993. In vitro toxicology and risk assessment. *Alt. Methods Toxicol*. 9:185–203.

Leung, H. W. 1991. Development and utilization of physiologically based pharmacokinetic models for toxicological applications. *J. Toxicol. Environ. Health* 32:247–267.

Littlefield, N. A., Farmer, J. H., and Gaylor, D. W. 1979. Effect of dose and time in a long-term, low dose carcinogenicity study. *J. Environ. Pathol. Toxicol*. 8:17–34.

Lipscomb, J. C., Fisher, J. W., Confer, P. D., and Byczkowski, J. Z. 1998. In vitro to in vivo extrapolation for trichloroethylene metabolism in humans. *Toxicol. Appl. Pharmacol*. 152:376–387.

Mahmood, I., and Balian. 1999. The pharmacokinetic principles behind scaling from preclinical results to phase I protocols. *Clin. Pharmacokinet*. 36:1–11.

Maltoni, C., Lefemine, G., Ciliberti, A., Cott, G., and Caretti, D. 1981. Carcinogenicity bioassay of vinyl chloride monomer: A model of risk assessment on an experimental basis. *Environ. Health Perspect*. 41:3–29.

Maltoni, C., Lefemine, G., Ciliberti, A., Cotti, G., and Caretti, D. 1984. Experimental research on vinyl chloride carcinogenesis. In *Archives of research on industrial carcinogenesis*, vol. 2, eds. C. Maltoni and M. A. Mehlman, pp. Princeton, NJ: Princeton Scientific.

McConnell, E. E. 1989. The maximum tolerated dose: The debate. *J. Am. Coll. Toxicol*. 8:1115–1120.

Morris, J. B., Hassett, D. N., and Blanchard, K. T. 1993. A physiologically based pharmacokinetic model for nasal uptake and metabolism of nonreactive vapors. *Toxicol. Appl. Pharmacol*. 123:120–129.

Monro, A. 1994. Drug toxicokinetics: Scope and limitations that arise from species differences in pharmacodynamic and carcinogenesis response. *J. Pharmacokinet. Biopharm*. 12:41–57.

Mortensen, B., and Nilsen, O. G. 1998. Allometric species comparison of toluene and *n*-hexane metabolism: Prediction of hepatic clearance in man from experiments with rodent liver S9 in a head space vial equilibration system. *Pharmacol. Toxicol*. 82:183–188.

National Research Council. 1986. *Drinking water and health*, vol. 6, chap. 6. Washington, DC: National Academy Press.

Occupational Safety and Health Administration. 1997. Occupational exposure to methylene chloride; final rule. *Fed. Reg*. 62(7):1493–1619.

Plowchalk, D. R., Andersen, M. E., and Bogdanffy, M. S. 1997. Physiologically-based modeling of vinyl acetate uptake, metabolism and intracellular pH changes in the rat nasal cavity. *Toxicol. Appl. Pharmacol*. 142:386–400.

Poulin, P., and Krishnan, K. 1996. Molecular structure-based prediction of the partition coefficients of organic chemicals for physiological pharmacokinetic models. *Toxicol. Methods* 6:117–137.

Poulin, P., and Krishnan, K. 1999. Molecular structure-based prediction of the toxicokinetics of inhaled vapors in humans. *Int. J. Toxicol*. 18:7–18.

Reitz, R. H., Gargas, M. L., Andersen, M. E., Provan, W. M., and Green, T. L. 1996. Predicting cancer risk from vinyl chloride exposure with a physiologically-based pharmacokinetic model. *Toxicol. Appl. Pharmacol*. 137:253–267.

Renwick, A. G., 1994. Toxicokinetics-pharmacokinetics. In *Priniciples and methods of toxicology*, 3rd edit., ed. A. W. Hayes, pp. 101–147. New York: Raven Press.

Riviere, J. E. 1999. *Comparative pharmacokinetics: Principles, techniques and applications*. Ames: Iowa State University Press.

Rowland, M., and Tozer, T. N. 1995. *Clinical pharmacokinetics: Concepts and applications*, 3rd ed. Philadelphia: Lea and Febiger.

Simonato, L., L'Abbe, K. A., Andersen, A., Belli, S., Comba, P., Engholm, G., Ferro, G., Hagmar, L., Langard, S., Lundberg, I., Pirastu, R., Thomas, P., Winkelmann, R., and Saracci, R. 1991. A collaborative study of cancer incidence and mortality among vinyl chloride workers. *Scand. J. Work Environ. Health* 17:159–169.

Smith, D. A. 1990. Design of toxicokinetic studies. *Xenobiotica* 20:1187–1199.

Tritscher, A. M., Goldstein, J. A., Portier, C. J., McCoy, Z., Clark, G. C., and Lucier, G. W. 1992. Dose-response relationships for chronic exposure to 2,3,7,8-tetrchlorodibenzo-*p*-dioxin in a rat tumor promotion model: Quantification and immunolocalization of CYP1A1 and CYP1A2 in the liver. *Cancer Res*. 52:3436–3442.

U.S. Environmental Protection Agency. 1994. *Methods for derivation of inhaled reference concentrations and application of inhalation dosimetry*. EPA/600/8-90/066F. Washington, DC: Office of Research and Development.

U.S. Environmental Protection Agency. 1996. *Proposed guidelines for carcinogen risk assessment*. EPA/600/P-92/003C. Washington, DC: Office of Research and Development.

U.S. Environmental Protection Agency. 2000a. *Toxicological review of vinyl chloride*. Appendices A–D. EPA/635R-00/004.

U.S. Environmental Protection Agency. 2000b. Exposure and human health reassessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds. Part II. In *Health assessment for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds*., chap. 8, *Dose-response modeling for 2,3,7,8-TCDD*. NCEA-1-0835. May. Science Advisory Board Review Draft.

Voisin, E., Ruthsatz, M., Collins, J. M., and Hoyle, P. C., 1990. Extrapolation of animal toxicity to humans: Interspecies comparisons in drug development. *Regul. Toxicol. Pharmacol*. 12:107–116.

Welling, P. G., and de la Igelsia, F. A., eds. 1993. *Drug toxicokinetics*. New York: Marcel Dekker.