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Incorporation of Pharmacokinetic and Pharmacodynamic Data into Risk Assessments

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Risk assessment methodologies are being updated to allow the inclusion of numerical values for variance in pharmacokinetic (PK) measures and pharmacodynamic (PD) processes related to toxicity. The key PK measures and PD processes are identified from the results of carefully conducted and adequately reported studies. In some instances, studies with humans are not possible, and so the development of data useful for human PK evaluations and on PD processes in vitro or in silico represent an alternative. These results can be integrated under physiologic, anatomic, and biochemical constraints of the intact body through physiologically based pharmacokinetic (PBPK) modeling. This manuscript presents the rational for and key considerations related to the inclusion of quantitative PK and PD data in assessing chemical risks.

Keywords Chemical-Specific Adjustment Factors (CSAF), Enzyme Kinetics, Human Variability, In Silico Predictions, In Vitro Studies, Interspecies Differences, Metabolism, Risk Assessment, Toxicokinetics, Toxicodynamics, Uncertainty Factors

The establishment of health-protective short-term and long-term exposure limits depends upon an adequate characterization and assessment of the likelihood of an adverse event following human exposure to the chemical. In research animals and humans, the expression of toxicity is dependent upon contact between the biologically-active form of the chemical and the ultimate receptor, as well as the response elicited by that contact. These two events broadly represent the areas of pharmacokinetics (PK—how and in what form a chemical travels through the body) and pharmacodynamics (PD—how the chemical produces its biological response). The extrapolation of risk from the results of animal toxicity studies to humans involves an initial animal-to-human extrapolation and an extrapolation within the human species to cover those who may be more sensitive than others. As practiced by the U. S. Environmental Protection Agency (U.S. EPA), these two steps are addressed in non-cancer risk assessment and their respective uncertainty factors are referred to as UF-A and UF-H, respectively. Recently, these uncertainty factors have sometimes been further divided into their

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respective PK and PD components. Occasionally, PK data in animals and humans allows risk assessors to argue for the reduction of the PK default uncertainty value to a value represented by quantified animal-to-human differences in the risk–relevant PK outcome (also called the dose metric). Data of this type may also argue for a value above the default value. However, no Agency-endorsed guidance exists to inform a fully systematic approach to this process at present[1]

Increased generation of relevant data and an understanding of the mode of action provide opportunity to use chemical-specific PK and PD data in humans and relevant mammalian test species to better inform risk assessment. As illustrated in this paper, research is being conducted in a number of areas related to approaches for incorporating pharmacokinetic and pharmacodynamic data into risk assessments. Much of this work is being framed by an international effort led by the International Programme on Chemical Safety (IPCS) to develop guidance for the adequacy of chemical-specific data for replacing default uncertainty factors (IPCS 2001), as described in further detail below. This guidance is being used (Meek et al. 1999, 2001) in the development of chemical-specific adjustment factors (CSAFs), and suggests new avenues of research and improved experimental protocols for evaluating interspecies and intraspecies variability.

Several common themes were evident in the analyses summarized in the rest of this paper. First identification of the chemical's mode of toxic action, including identification of the active form of the chemical, is essential for quantitative incorporation of pharmacokinetic data in risk assessment. Second, it is important to consider physiological limits when considering total variability. For example, when metabolism of a chemical is limited by blood flow to the liver, variability in tissue dose is determined primarily by variability in tissue flow, rather than variability in metabolic capacity (unless the administered dose is very high). This suggests the third point—that variation in tissue dose resulting from a given administered amount is often less than might be expected from naïve consideration of variation in individual factors influencing tissue dose. This principle results both because variability in one parameter may not drive the overall variability, and because it is unlikely for one individual to be at the extreme of variability for multiple unrelated parameters. Finally, PBPK modeling is a useful approach for evaluating how variability in parameters affects overall variability. PBPK modeling is a particularly useful tool, because a sensitivity analysis of its parameters and constants can determine which of these biologic, anatomic, physiologic or biochemical factors are most important in determining tissue dose, and this information can be used in combination with Monte Carlo modeling to simultaneously evaluate variability in multiple parameters, and for predictive if/then exercises to evaluate a potential basis for susceptibility.

[1] An intermediate approach that takes into account some aspects of PK differences between animals and humans, is used in EPA's approach for developing reference concentrations (RfCs) [U.S. EPA 1994]. This approach uses a particle deposition model to compare tissue doses for animals and humans following inhalation exposure to particles; species differences in clearance from the respiratory tract are not taken into account in this model. The U.S. EPA [1994] approach also calculates differences in tissue dose between experimental animals and humans for gas effects in various regions of the respiratory tract and systemic effects of gases. When the particle or gas dosimetric adjustments are used, practice is to use a reduced factor of 3 for animal to human extrapolation.

CHEMICAL SPECIFIC ADJUSTMENT FACTORS (CSAF) IN DOSE/CONCENTRATION RESPONSE ASSESSMENT—GUIDANCE FOR ADEQUACY OF DATA

This brief overview addresses guidance for the consideration of kinetic and dynamic data as a basis for replacement of default values for interspecies differences and human variability in dose-response analyses through the application of chemical specific adjustment factors (CSAFs). CSAFs represent part of a broader continuum of approaches which incorporate increasing amounts of data to reduce uncertainty, ranging from default ("presumed protective") to more "biologically-based predictive" (Meek 2001). This guidance has been developed in one of the projects of the initiative of the International Programme on Chemical Safety (IPCS) on *Harmonisation of Approaches to the Assessment of Risk from Exposure to Chemicals* (IPCS, 2001).

Framework for Development of CSAFs

Renwick (1993) proposed a framework to address kinetics and dynamics separately in considering uncertainty related to interspecies differences and interindividual variability in the development of reference or tolerable concentrations/doses. Quantitation of this subdivision is supported by data on kinetic parameters and pharmacokinetic-pharmacodynamic (PKPD) modeling for a range of pharmacological and therapeutic responses to pharmaceutical agents (Renwick 1993; Renwick and Lazarus 1998). This framework allows the incorporation of quantitative chemical-specific data, relating to either toxicokinetics or toxicodynamics, to replace part of the usual default uncertainty factor for consideration of both interspecies differences and interindividual variability, but collapses back to the usual 100-fold default in the absence of appropriate information (Fig. 1).

Chemical-Specific Toxicokinetic Adjustment Factors—[AK_{AF}, HK_{AF}]

The chemical-specific adjustment factors for the toxicokinetic components of interspecies differences and interindividual variability are ratios of measurable metrics for internal exposure to the active compound such as Area under the Curve (AUC), Cmax or clearance. For interspecies differences, this is generally determined on the basis of comparison of the results of in vivo kinetic studies with the active compound in animals and a representative sample of the healthy human population.

For interindividual variability, while this adjustment factor could potentially be addressed on the basis of in vivo kinetic studies in a sufficiently broad range of subgroups of healthy and potentially susceptible populations to adequately define the population distribution, this may not be practicable or even

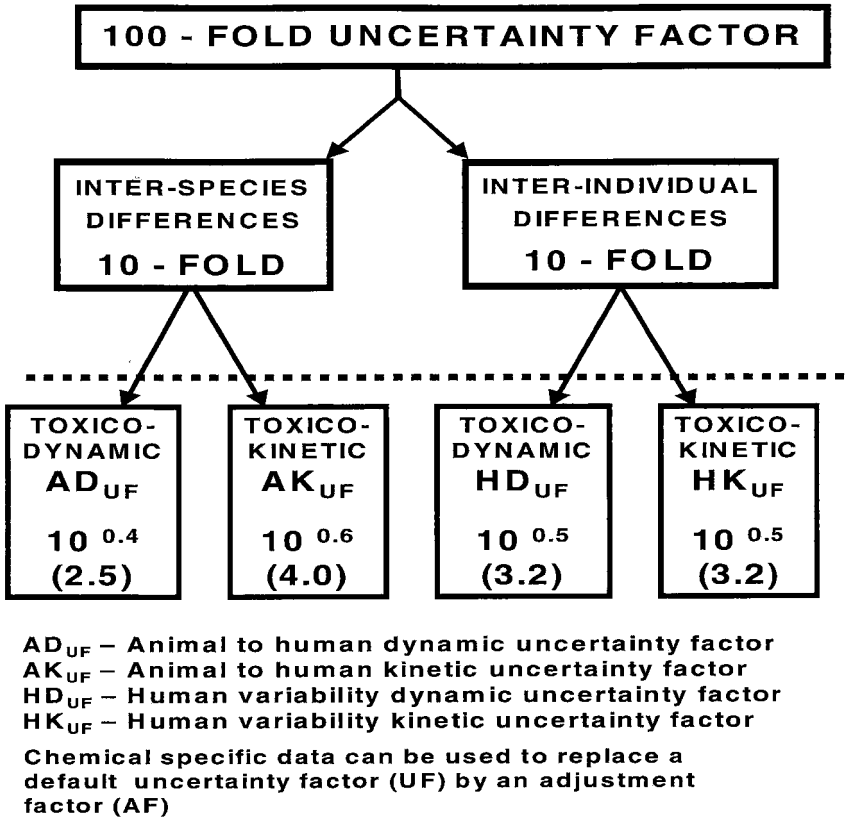

FIG. 1. The sub-divison of the 100-fold fold uncertainty factor to allow chemical-specific data to replace part of the default factor. Both the uncertainty factor for animal to human extrapolation and the factor for extrapolation among humans is divided into PK and PD components. With appropriate information, the default values for these four components can be replaced with quantitative measures of variability.

possible. More often, factors responsible for the clearance mechanisms are identified (renal clearance, CYP-specific metabolism, etc.) and a chemical-specific adjustment factor is derived based on measured or PBPK modelled human variability in the relevant physiological and biochemical parameters. The population distribution for the relevant metric (e.g., AUC, Cmax, renal clearance) for the active entity is analyzed and the CSAF (HK_{AF}) calculated as the difference between the central values for the main group and given percentiles (such as 95th, 97.5th and 99th) for the whole population (Fig. 2). These differences are analyzed separately for any potentially susceptible sub-group (Fig. 2).

Chemical-Specific Toxicodynamic Adjustment Factors [AD_{AF}, HD_{AF}]

While information that informs the development of these factors include kinetic-dynamic link models, the chemical-specific adjustment factors for the toxicodynamic components are most simply, ratios of the doses which induce the critical toxic effect or a measurable related response in vitro in relevant

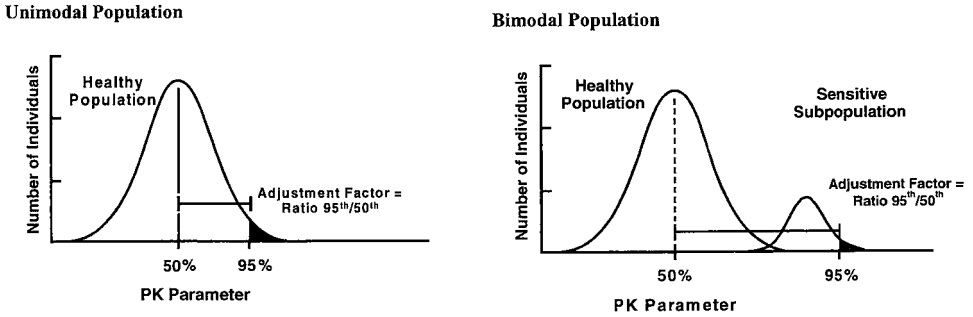

FIG. 2. Development of CSAFs for interindividual variability. A key decision in the methodology is whether the sensitive subpopulation is a discrete group or a fraction of the general population (Adapted from Naumann et al. 2001).

tissues of animals and a representative sample of the healthy human population (interspecies differences) or in average versus sensitive humans (interindividual variability). At its simplest, then, replacement of the dynamic component of the default factor for inter-species differences is the ratio of the effective concentrations in critical tissues of animals versus humans (e.g., $EC_{10\ animal}/EC_{10\ human}$) for interspecies differences and in healthy human and susceptible subpopulations for interindividual variability (e.g., the $EC_{10\ average}/EC_{10\ sensitive}$).

Guidance for Development of CSAF

IPCS (2001) provides guidance on several aspects of the development of CSAF, which are only briefly outlined here. For example, data for application in the four components of the framework must relate to the active form of the chemical. For the components of the framework addressing toxicokinetics [AK_{AF}] and [HK_{AF}], choice of the appropriate metric is also an essential first step. The time-weighted concentration of biologically active metabolite in kidney tissue might be such a metric among species as well as between humans.

Choice of the appropriate endpoint is critical for the components addressing toxicodynamics [AD_{AF}] and [HD_{AF}]. The selected measured endpoint must either be the critical effect itself or intimately linked thereto (with similar concentration-response and temporal relationships) based on an understanding of mode of action.

In addition, the metric for toxicokinetics or the measure of effects for toxicodynamics as a basis for CSAF needs careful consideration in relation to the delivery of the chemical to the target organ. Measures of various endpoints in vivo may represent only toxicokinetics, or toxicokinetics and part or all of the toxicodynamic processes, as defined based on the subdivision of defaults. This necessitates consideration of the impact of specific data to replace the toxicokinetic and potentially a proportion or all of the toxicodynamic components of the default uncertainty factors.

For data that serve as the basis for all components, relevance of the population, the route of exposure, dose/concentration and adequacy of numbers of subjects/samples must also be considered and the potential impact on the validity of the calculated ratio addressed. For example, for in vitro studies which inform primarily dynamic components [AD_{AF}] [HD_{AF}], the quality of the samples should be considered, and evidence provided that they are representative of the target population, e.g. viability, specific content or activity of marker enzymes.

Conclusions

Consideration of relevant data in the context of a framework that addresses kinetic and dynamic aspects, explicitly, should result in greater understanding of contributing components and transparency in risk assessment. It is also hoped that consideration in this context will lead to clearer delineation and better common understanding of the nature of specific data required which would permit development of more informative measures of dose response.

IN SILICO APPROACHES FOR PBPK MODELING AND ESTIMATION OF INTERINDIVIDUAL VARIANCE

The development of physiologically-based pharmacokinetic (PBPK) models requires the knowledge of several physiological (tissue volumes, blood flow rates, cardiac output, alveolar ventilation rate), physicochemical (blood:air partition coefficients [PC], tissue:blood partition coefficients, absorption rate constants, permeability coefficients), and biochemical (maximal velocity, Michaelis affinity constant) parameters (Krishnan and Andersen 2001). The PBPK models, once developed, can be used for assessing the impact of inter-individual variability in input parameters on the appropriate dose surrogate (e.g., parent chemical concentration in target tissue, amount of metabolites formed) (Gentry et al. 2002; Lipscomb et al. 2003a). PBPK modeling approaches to assess inter-individual variability on tissue dose continue to evolve and may prove useful in quantifying variance in the risk-relevant PK outcome between individuals at the mean of the general population and those individuals in whom the value of the PK outcome predisposes risk. Fundamentally, PBPK models can be constructed using individual-specific parameter values and then simulations of dose surrogate in each individual may be performed (Fig. 3). Alternatively, distributions of input parameter values representing the nature of their variability in the population can be specified in conducting PBPK simulations of dose surrogates. The knowledge of individual-specific or population-specific PBPK parameter values (in the context of this presentation, partition coefficients and metabolic constants) is often the limiting factor for conducting simulations of individual differences in pharmacokinetics. The challenge of estimating metabolic constants and partition coefficients for human populations may be dealt with, by using in silico approaches. There are at least two in silico approaches that are useful in this context: (i) quantitative structure-property relationship (QSPR) approach and (ii) biologically-based algorithms. The conceptual basis, equations and examples of parameter estimation using these two in silico approaches are provided in the following paragraphs.

QSPRs

QSPRs involve the use of available data for various PBPK parameters in order to develop equations that associate characteristics of chemicals to the magnitude of the parameters. There are a number of QSPR algorithms in the literature, which have been specifically developed with human data. These approaches relate the magnitude of the human PBPK model parameters to properties of chemicals. Table 1 presents several QSPRs for estimating human blood:air partition coefficients, muscle:air partition coefficients, liver:air partition coefficients and metabolic constants. The use of these QSPRs is often limited by the class and type of chemicals used in developing them. An alternative in silico approach, which continues to evolve, is the development of biologically-based algorithms, which relate certain biological and chemical determinants to PBPK model parameters.

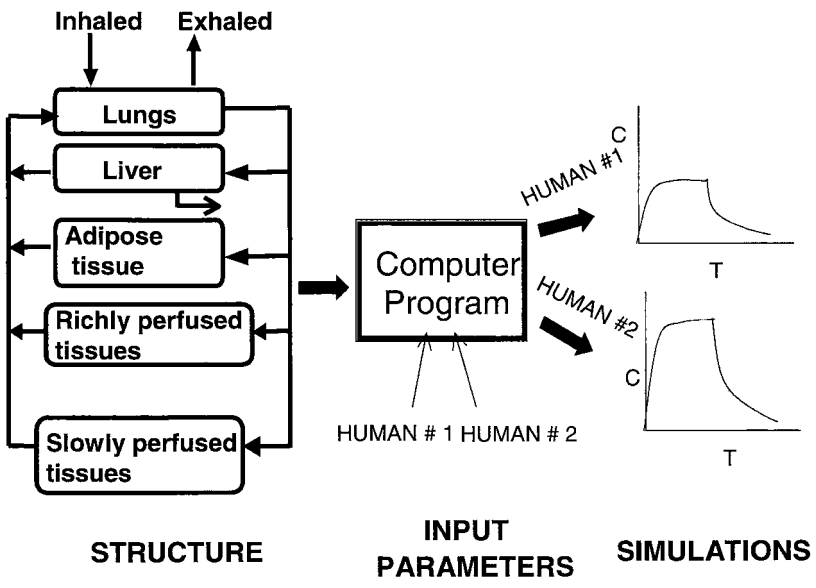

FIG. 3. Application of PBPK Modeling to assess the impact of subject-specific parameters on pharmacokinetic outcomes in humans. The PBPK model structure is determined and populated with basal parameters and data; subject-specific input parameters like PC values and metabolic rate constants are derived and incorporated into the simulation software; and the model is exercised to develop subject-specific predictions of the risk-relevant PK outcome.

Biologically-Based Algorithms for Chemical Partitioning

Biologically-based algorithms either predict individual-specific parameter values as a function of the individual-specific values of biological determinants, or provide estimates of the lower and upper bounds of parameters based on knowledge of such values for biological determinants.

The following (Equation 1) is the biologically-based algorithm developed by Poulin and Krishnan (1996) for predicting tissue:air partition coefficients ($P_{t:a}$) of volatile organic chemicals (VOCs):

$$P_{t:a} = [P_{o:w}P_{w:a}(V_{nt} + 0.3V_{pt})] + [P_{w:a}(V_{wt} + 0.7V_{pt})] \quad [1]$$

where $P_{o:w}$ = n-octanol:water partition coefficient, $P_{w:a}$ = water:air partition coefficent, V_{nt} = volume fraction of neutral lipid in tissue, V_{pt} = volume fraction of phospholipid in tissue, and V_{wt} = volume fraction of water in tissue.

In order to solve equation (1), $P_{o:w}$ (also written as $K_{O:W}$) and $P_{w:a}$ can be estimated directly from molecular structure information of chemicals (i.e., by the freeware, KOWwin and HENRYwin, respectively; US EPA 2003) whereas the other parameters (neutral lipid, phospholipid, and water content of tissues) can be set to reflect the individual-specific or population-specific values.

Similarly, Poulin and Krishnan (1996) proposed the following algorithm (Equation 2) for predicting blood:air partition coefficients ($P_{b:a}$) of VOCs:

$$P_{b:a} = [P_{o:w}P_{w:a}(V_{nb} + 0.3V_{pb})] + [P_{w:a}(V_{wb} + 0.7V_{pb})] \quad [2]$$

where V_{nb} = volume fraction of neutral lipid in blood, V_{pb} = volume fraction of phospholipid in blood, and V_{wt} = volume fraction of water in blood.

The tissue:blood partition coefficients (input parameters for PBPK models) can be computed (Equation 3) by dividing the above two equations, yielding the following algorithm:

$$P_{t:b} = \frac{P_{o:w}(V_{nt} + 0.3V_{pt}) + (V_{wt} + 0.7V_{pt})}{P_{o:w}(V_{nb} + 0.3V_{pb}) + (V_{wb} + 0.7V_{pb})} \quad [3]$$

Using individual-specific values of blood and tissue levels of lipids and water in the above equation, the interindividual variability in tissue:blood and blood:air partition coefficients can be assessed. Table 2 presents some data on lipid and water content in human tissues obtained from reference literature. Similar ranges for lipid and water content were used by Pelekis et al. (1999) to assess the lower and upper limits of partition coefficients for specification in human PBPK models (Table 3).

Metabolism

Chemical metabolism, in contrast to tissue partitioning, is an active process, and itself relies on both active and passive processes. Since the liver is the primary site of metabolism for ingested chemicals, the following discussion focuses on metabolism by the liver. Substrate is delivered to the liver via the blood. The rate at which substrate becomes available for metabolism in the liver depends on the rate of hepatic blood flow and the ability of the chemical to partition from blood into the liver. Once the chemical is delivered to the liver, enzyme activity depends on cofactor levels, and maintenance of cofactors depends on many variables, not the least of which is the maintenance of cellular redox potentials—an energy-requiring process.

Unlike partition coefficients, for which in silico approaches can provide useful estimates, in silico approaches are not

TABLE 1

Some examples of structure property relationships derived to estimate tissue:air PC values, blood:tissue PC values and metabolic parameters in humans

	Structure property relationship	Chemical class[c]	References
Blood:Air PC values[a,b]	$P_{b:a} = 0.89P_{w:a} + 0.011$	Esters; alcohols	Kaneko et al. 1994
	$P_{b:a} = 0.08^{e0.0308TB}$	Aliphatic hydrocarbons	Perbellini et al. 1985
	$LogP_{b:a} = -0.003LogP_{w:a} + 1.47$	VOCs	Laass 1987
	$LogP_{b:a} = P_{w:a}*\{V_{lb}P_{o/w}^{0.85} + V_{prb}(86.2/P_{o:w} + 3.70) + V_{wb}\}$	LMWVOCs	Connell et al. 1993
	$LogP_{b:a} = 1,269 + 0.612R_2 + 0.916\pi_2 + 3.614\alpha_2 + 3.381\beta_2 + 0.362LogP_{he:a}$	Inert gases; LMWVOCs	Abraham and Weathersby 1994
	$LogP_{b:a} = 0.48LogS_w + 0.75Log1000/P + 1.67LogV_o - 2.77$	VOCs	Laass 1987
	$LogP_{b:a} = 1.21LogV_o - 0.17$	VOCs	Laass 1987
	$LogP_{b:a} = 8.90LogV_w - 33.40$	VOCs	Laass 1987
	$LogP_{b:a} = -3.922 + 1.369R_G$	Inert gases; LMWVOCs	Abraham et al. 1985
Tissue:Blood PC values[d]	$P_{br:b} = [(V_{lt}P_{o:w}^{A1} + V_{wt})/(V_{lt}P_{o:w}^{A2} + V_{wt})] + B$	LMWVOCs	DeJongh et al. 1997
	$LogP_{br:b} = 0.39LogP_{o:w} + 0.68$	Drugs, hormones	Seydel and Schaper 1982
	$LogP_{br:b} = 0.476 + 0.541LogP_{o:w} - 0.00794MW$	H_2-R antagonists	Kalizan and Markuszewski 1996
	$LogP_{br:b} = 0.054G^o + 0.43$	H_2-R antagonists; LMWVOCs	Lombardo et al. 1996
	$P_{l:b} = [(V_{lt}P_{o:w}^{A1} + V_{wt})/(V_{lb}P_{o:w}^{A2} + V_{wb})] + B$	LMWVOCs	DeJongh et al. 1997
	$P_{f:b} = [(V_{lt}P_{o:w}^{A1} + V_{wt})/(V_{lb}P_{o:w}^{A2} + V_{wb})] + B$	LMWVOCs	DeJongh et al. 1997
	$P_{f:b} = 1.9988 - 0.5004UNS + 0.1793NPL + 0.05931DIFF^2$	PCBs	Parham et al. 1997
Metabolic parameters[e]	$Log\ K_m = -0.42LogP_{u:i} + 0.14pK_a - 2.89$	Sulfonamides	Seydel and Schaper 1982
	$Log\ 1/_{Km} = 0.46LogP_{o:w} + 0.63\sigma^- + 2.62$	X-C6H4N(CH3)2	Hansch and Leo 1995
	$Log\ 1/_{Km} = 0.92LogP_{o:w} - 1.48MR_4 - 0.64MR_3 + 1.04MR_2 + 0.67\sigma^- + 4.01$	Phenols	Hansch and Leo 1995
	$Log\ 1/_{Km} = 2.93F_2 + 1.16\pi_2 + 0.91\pi_3 + 0.82MR_2 - 0.59I_{\beta OH} + 1.29I_{ET} + 2.59$	Phenols	Hansch and Leo 1995

[a] $P_{b:a}$ = blood:air partition coefficient; $P_{br:b}$ = brain:blood partition coefficient; $P_{w:a}$ = water:air partition coefficient; $P_{l:b}$ = liver:blood partition coefficient; $P_{f:b}$ = fat:blood partition coefficient; $P_{o:w}$ = octanol:water partition coefficient; $P_{he:a}$ = hexadecane: air partition coefficient; $P_{u:i}$ = n-octanol:water partition coefficient for the nonionized form;

[b] P = vapour pressure; V_{lb} = volume fraction of lipids in blood; V_{prb} = volume of farction of proteins in blood; V_{wb} = volume fraction of water in plasma; Vw = heat released due to evaporation of the substance at boiling temperature; V_o = surface tension; S_W = solubility in water; TB = boinling point, R2 = excess molar refraction; R_G = parameters relative to the solvent; π_2 = dipolarity/polarizability; α_2 = overall hydrogen-bond acidity; β_2 = overall hydrogen-bond basicity.

[c] VOC = volatile organic coumpound; LMWVOC = low molecular weight VOC; PCB = polychlorobiphenyls.

[d] A1, A2 = Collander-type coefficient; B = correction factor; V_{lt} = volume fraction of lipids in tissue; V_{wt} = volume fraction of water in tissue; G° = Gibbs free energy related to the solvation of the substance in water; UNS = variable dependant on the number of atoms in the molecule that are not chlorides; NPL = variable dependant on the number of chloride atoms in the molecule in ortho position; DIFF = variable dependant on the number of chloride atoms in the molecule in the aromatic cycle; MW = molecular weight;

[e] Km = Michealis-Menten affinity constant; pK_a = log dissociation constant of an acid in water; $\sigma-$ = Hamelet constant; $MR_{2,3,4}$ = molar refractory indices; π, π_2, π_3 = molecular hydrophobicity constants; $I_{\beta OH}+$ = variable dependant on the number of β OH groups in the molecule; $I_{ET}+$ = variable dependant on the family of the substance.

available for estimating metabolism constants. Instead, in vitro approaches probably represent the best possible way of assessing the interindividual differences in affinity and velocity of metabolism of xenobiotics. The kinetic constraints on chemical metabolism are the same in vivo as in vitro, and are less difficult to measure in vitro. Metabolism in PBPK models is represented using Vmax and Km values, or alternatively using clearance, which is the product of the organ extraction ratio (E) and tissue

TABLE 2
Water and lipid content of human tissues, from reference man (ICRP, 1975)

Tissue	Age	Water content percent wet weight	Lipid content percent wet weight
Blood	Newborn	78.9 – 80.8[a]	NR
	Adult	80.5–80.8	0.65[b]
Liver	Newborn	73–80	3.6
	Adult	63.6–73.9	6.9
Kidney	Newborn	NR	2.73
	Adult	76 (range 70.6–81)	5
Skeletal muscle	Newborn	80.4 (range 79.3–81.2)	2 (range 0.67–2.2)[b]
	Adult	79 (range 68.9–80.3)	2.2 male; 2.9 female (range 2.2–9.4)[b]
Adipose tissue	Newborn	47.5[c]	35.5[b]
	Adult	15 (range 10.9–21)	62–91[b]

[a]Reported for children.
[b]Reported as fat content.
[c]During the first year of life.
NR = not reported.

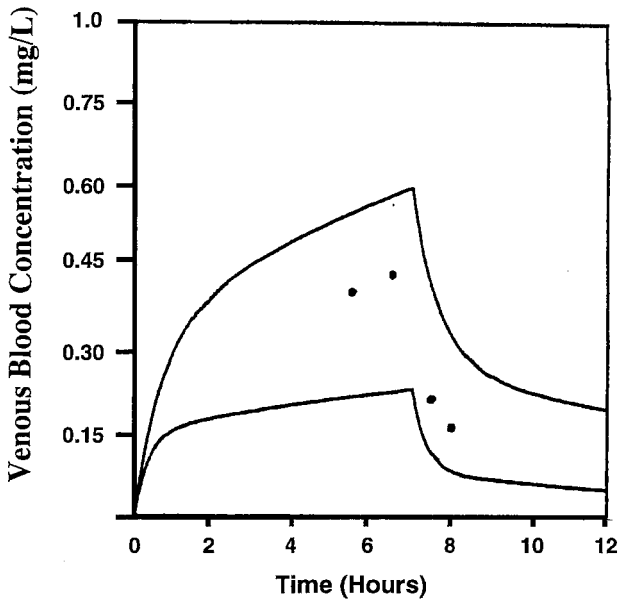

FIG. 4. Physiological limits of hepatic clearance and pharmacokinetics of volatile organic chemicals in humans. This figure demonstrates the constraints of a pharmacokinetic outcome introduced by the biological limits of hepatic blood flow. The symbols represent actual data, the upper line represents model predictions of the concentration of agent in blood when hepatic extraction ratio is set to zero and the lower line represents the same when a hepatic extraction ratio value of 1.0 is employed.

blood perfusion (Qt). For the liver, tissue blood perfusion becomes the liver blood perfusion (Ql) a known quantity. Because of the metabolic enzyme specificity of compounds and the complexity of the mechanisms behind metabolism, QSARs relating metabolic parameters and structure are few and have mainly focused on selected families of chemicals, mostly pharmaceutical agents (Table 1). These empirical QSARs do not permit the evaluation or prediction of interindividual differences in the metabolic constants. To date, there are no mechanistic algorithms either, to facilitate the prediction of metabolism rates of chemicals. However, it is possible to use the physiological limits of clearance in order to estimate the range of blood (or tissue) concentration possible in an individual (or population). For example, in the case of chemicals metabolized in the liver, the rate of amount metabolized (RAM) can be calculated by equation (4) and would be equal to:

$$RAM = Ca^{*}CLh \quad [4]$$

where Ca = concentration of the chemical in arterial blood, and CLh = hepatic clearance.

Because CLh = Ql*E and E cannot be lower than 0 or higher than 1, the envelope of possible concentrations is obtained by setting CLh in the above equation to its physiological limits (i.e., Ql or 0). This approach can provide a credible first-cut estimate of the range of blood concentration profiles of chemicals in a population, as a result of interindividual differences in metabolism rates (Fig. 4).

TABLE 3
Bounds of human tissue composition data used for assessing the upper and lower limits of partition coefficients of dichloromethane in an adult population (Pelekis et al. 1995)

	Neutral lipid		Water	
Tissue	Low	High	Low	High
Liver	0.02	0.10	0.67	0.83
Muscle	0.03	0.08	0.70	0.82

Conclusions

Overall, in silico approaches to estimating PBPK model parameters have mainly centered on empirical, linear-free-energy (LFE)-type QSPRs and mechanistically-based equations. While LFE QSPRs have the advantage of being easily derived, they are limited to the chemical class for which they were developed. Furthermore, resulting parameter estimates cannot be extrapolated across species or among individuals. There is also growing concern regarding the mechanistic relevance of some of the structural descriptors used in these types of equations. The emerging mechanistically-based approaches offer the advantage of being relevant regardless of the chemical family, and being capable of extrapolation across species and between individuals. The applicability of these approaches has been verified largely with

inhaled VOCs, for the determination of partition coefficients, a parameter determined largely by the water and lipid content of the tissue. Even though these approaches are conceptually applicable to nonvolatile organics as well, it becomes more challenging to predict the other PBPK model parameters required for modeling the kinetics of these chemicals (i.e., metabolic constants, tissue diffusion coefficients, tissue binding association constants, oral absorption rates, and dermal permeability coefficients). However, bounding estimates of the extraction ratio, together with standard toxicokinetic relationships can be used to describe the physiological limits of hepatic clearance. As our understanding of the mechanistic determinants of each of these parameters improves, in silico approaches can be developed to provide a priori predictions of these parameters to assess interindividual differences in pharmacokinetics for risk assessment purposes.

IN VITRO TO IN VIVO EXTRAPOLATION OF METABOLIC RATE CONSTANTS AND THEIR USE IN PBPK MODELING

In Vitro Biotransformation Systems

In vitro systems offer a number of benefits for determination of the kinetic parameters for xenobiotic biotransformation compared to in vivo systems. In vitro systems allow isolation of the critical aspects of the experimental question in a simpler setting than the whole animal. Inhibitors can be used with specificity in vitro whereas in vivo these agents often have diverse pharmacological activities in addition to enzyme inhibition. Tissues from both experimental animals and humans can be used to prepare in vitro systems, avoiding the problems of human experimentation and allowing direct comparison of human and animal xenobiotic metabolism. However, the in vitro system used to study xenobiotic biotransformation must reflect the appropriate biochemistry and physiology to have relevance to the in vivo situation.

In vitro tissue preparations can be used to determine the organ-specific biotransformation of xenobiotics. Liver is the predominant biotransforming organ for xenobiotics but other organs such as the kidney, intestine, lung, and nasal epithelium can be important depending upon the nature of the substance under study. For example, peptides predominantly undergo hydrolysis in the gut while organic chemicals are frequently oxidized by cytochromes P450 in the liver. While the liver is the predominant eliminating organ for many toxicants, local bioactivation of toxicants in target tissues is often a crucial event leading to the expression of target organ toxicity.

There are a number of in vitro systems available for prediction of xenobiotic metabolism and pharmacokinetics. Our discussion will focus on the liver, but the concepts are applicable to other organs as well. Each in vitro system has distinct advantages and disadvantages and the choice of which system to use depends upon the experimental question being asked. The most physiologically integrated and complex in vitro system is the isolated perfused liver. However, this system is only practical for small experimental animals. Precision-cut liver slices retain the architecture of the liver and the interactions between different cell types, but problems with diffusion of substrates and nutrients into the slices make in vivo extrapolation of the in vitro data problematic (Ekins et al. 1995; Worboys et al. 1996). Isolated hepatocytes have been shown to be the system of choice for in vitro prediction of pharmacokinetics (Kedderis et al. 1993; Houston 1994) since the cells maintain a biochemical homeostasis of cofactors and enzymes similar to the intact liver. While freshly isolated hepatocytes suspended in a nutritive medium catalyze xenobiotic biotransformation reactions similarly to the liver in vivo (Billings et al. 1977), hepatocytes in monolayer culture rapidly differentiate and decrease xenobiotic metabolism capabilities (Sirica and Pitot 1980). Similarly, immortalized liver cell lines have low and variable xenobiotic metabolism capabilities that depend upon the culture conditions and are not appropriate model systems for predicting the disposition of xenobiotics in vivo. Subcellular fractions such as hepatic microsomes have been used successfully to predict pharmacokinetics (Houston 1994) and can be stored frozen for years with little loss of enzyme activity. With all of the in vitro systems, knowledge of the actual substrate concentration in the incubations (as opposed to the nominal concentration added) through partition coefficient determination is crucial to the accurate determination of the kinetic parameters for biotransformation.

Basis for Extrapolation of In Vitro Data

Most of the enzymes involved in xenobiotic biotransformation follow Michaelis-Menten saturation kinetics according to equation (5):

$$v = Vmax * [S]/Km + [S] \quad [5]$$

where v is the initial velocity of an enzyme catalyzed reaction, Vmax is the maximal velocity at infinite substrate concentration, [S] is the substrate concentration, and Km is the Michaelis constant, defined as the substrate concentration that yields one-half Vmax (Kedderis 1997a). The Michaelis-Menten equation (5) indicates that the initial velocity of the reaction will increase hyperbolically as a function of substrate concentration (Fig. 5). The Vmax is a horizontal tangent to the top (saturated) part of the curve, while the tangent to the initial linear portion of the hyperbolic curve is the initial rate of the reaction, V/K. The V/K is the pseudo-first-order rate constant for the reaction at low substrate concentrations. The point where these two tangents intersect corresponds to the Km (Northrop 1983).

The basis for extrapolation of in vitro biotransformation data to whole animals is that the overall rate of enzyme-catalyzed reactions is directly proportional to the total amount of enzyme present in the system (Kedderis 1997b). Therefore data generated with subcellular fractions such as microsomes or cytosols can be extrapolated to in vivo based on protein content (Snawder and Lipscomb 2000; Lipscomb et al. 2003a, b). Data from intact cellular systems such as hepatocytes can be extrapolated to

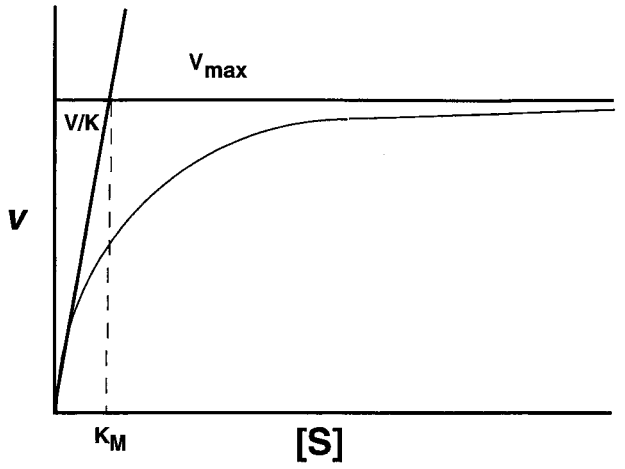

FIG. 5. The initial velocity (v) curve for an enzyme-catalyzed reaction following Michaelis-Menten saturation kinetics as a function of substrate concentration ([S]). Adapted from Northrop (1983) and Kedderis (1997a).

in vivo systems based on cell number. There are approximately 130×10^6 hepatocytes per gram of mammalian liver (Arias et al. 1982). The liver is approximately 4% of rat body weight, 5.5% of mouse body weight, and 2.6% of human body weight (Arms and Travis 1988).

In order to extrapolate in vitro kinetic data to whole animals or humans, the overall enzyme kinetic mechanism must be known (or assumed) and a compartmental pharmacokinetic model of the organism must be used. The data-based pharmacokinetic models that are widely used in the analysis of clinical pharmacokinetic data generally describe individual data sets and cannot usually extrapolate between dose routes or species. This type of analysis of in vivo or in vitro kinetic data is of limited value in understanding species differences in the biotransformation of toxicants. In contrast, physiologically based models are based on the physiology and anatomy of the organism and can describe chemical pharmacokinetics in a wide variety of exposure scenarios (Clewell and Andersen 1994). The goal of physiologically based pharmacokinetic modeling is to describe the behavior of a chemical in an animal, incorporating the necessary degrees of mechanistic detail to ultimately define one set of parameters to describe chemical behavior. Physiologically based models contain physiological parameters from the literature and chemical-specific parameters for tissue solubility, biotransformation, and protein binding (Clewell and Andersen 1994). Thus physiologically based models can extrapolate across dose routes and species. Several examples will illustrate the utility of in vitro kinetic studies coupled with physiologically based pharmacokinetic models.

Examples of In Vitro to In Vivo Extrapolation

In vitro kinetic data from hepatocytes were used to develop PBPK models for furan in rats (Kedderis et al. 1993), mice, and humans (Kedderis and Held 1996). Simulation of inhalation exposure to 10 ppm furan for 4 h indicated that the absorbed dose of furan (mg/kg; inhaled minus exhaled divided by body weight) and the integrated exposure of the liver to the toxic metabolite were approximately 3.5-fold and 10-fold greater in rats and mice, respectively, than in humans. This is because the volatile toxicant furan is metered into the blood stream via the breathing rate and distributed throughout the organism at rates that are a function of body size. These results clearly indicate that the inhalation exposure concentration of a toxicant is not an appropriate measure of the dose to the organism and internal dosimeters such as the concentration of the toxic metabolite in the target tissue should be used. The initial rates of furan oxidation for rats, mice, and humans were approximately 13- to 37-fold higher than the rate of furan delivery to the liver via blood flow (Kedderis and Held 1996), indicating that furan bioactivation is limited by hepatic blood flow. One important consequence of the hepatic blood flow limitation of bioactivation is that enzyme induction will have little or no effect on the amount of the toxic metabolite formed in the liver (Kedderis 1997b).

The contribution of variance in cytochrome P450 2E1 content and activity on the risk of hepatotoxic injury in human adults from oxidative metabolites of trichloroethylene was investigated using 60 human liver samples (Lipscomb et al. 2003a). The increased availability of human liver tissue enabled the construction of a bank of frozen human liver samples that were analyzed for protein content, cytochrome P450 2E1 content (Lipscomb et al. 2000; Snawder and Lipscomb 2000; Lipscomb et al. 2003b), and oxidative activity toward trichloroethylene (Lipscomb et al. 1997). The data were log-normally distributed and the 5th and 95th percentiles of the distribution of trichloroethylene oxidized per min per gram liver differed by approximately 6-fold. The significance of the variability was investigated using a human PBPK model for trichloroethylene. Simulations of 8 hr inhalation exposure to 50 ppm (the TLV) and oral exposure to 5 μg trichloroethylene/L in 2L drinking water (the MCL) using the human PBPK model showed that the amount of trichloroethylene oxidized in the liver of humans varied by 2% or less even though the distribution of metabolic capacity (enzyme content or activity) varied 6-fold. These results indicate that differences in cytochrome P450 2E1 expression among the central 90% of the adult human population account for only approximately 2% of the variance in the risk-relevant pharmacokinetic outcome for trichloroethylene-mediated liver injury (amount oxidized in the liver) and that physiological processes such as hepatic blood flow limit the full impact of the differences in cytochrome P450 activity mediating the formation of toxic metabolites (Lipscomb et al. 2003a). These results underscore the conclusion that the significance of in vitro data must be evaluated in the context of the intact animal. A framework describing this process has been recently published (Lipscomb and Kedderis 2002).

Conclusions

In vitro systems are useful for the prediction of xenobiotic biotransformation and pharmacokinetics. Isolated hepatocytes

and hepatic microsomes have been shown to be the best systems for prediction of pharmacokinetics from in vitro data (Houston 1994; Kedderis 1997b). In vivo extrapolation of in vitro data is based on cell number or protein content, from the basic principle that the rate of enzyme catalyzed reactions is directly proportional to the total enzyme in the system (Kedderis 1997b). The overall enzyme kinetic mechanism for the biotransformation reaction must be known (or assumed) and a compartmental pharmacokinetic model of the organism must be used. Physiologically based pharmacokinetic models have the ability to extrapolate among dose routes and species and have the predictive power needed in risk assessment. In vitro data need to be interpreted in the context of physiology to understand the significance of the data in vivo. This is important for enzyme expression data as well as kinetic data. The bioactivation of rapidly metabolized substances can be limited by hepatic blood flow delivery to the liver (Kedderis 1997b). The hepatic blood flow limitation dampens or eliminates the effects of interindividual differences in enzyme expression due to differences in genetics (polymorphisms) or enzyme induction. The combined application of human in vitro systems and physiologically based pharmacokinetic analysis of the data can provide useful insights for the development of human health risk assessments.

USE OF PBPK MODELING TO EVALUATE THE IMPACT OF HUMAN VARIABILITY ON RISK

One of the more challenging issues that must be considered in performing a human health risk assessment is the heterogeneity among humans. This heterogeneity is produced by interindividual variations in physiology, biochemistry, and molecular biology, reflecting both genetic and environmental factors, and results in differences among individuals in the biologically effective tissue dose associated with a given environmental exposure (pharmacokinetics) as well as in the response to a given tissue dose (pharmacodynamics). Because the parameters in a PBPK model have a direct biological correspondence, they provide a useful framework for determining the impact of observed variations in physiological and biochemical factors on the population variability in dosimetry within the context of a risk assessment for a particular chemical (Clewell and Andersen 1996).

It is useful to consider the total variability among humans in terms of three contributing sources: (1) the variation across a population of "normal" individuals at the same age (e.g., young adults) (2) the variation across the population resulting from their different ages, e.g., infants or the elderly; and (3) the variation resulting from the existence of subpopulations that differ in some way from the "normal" population (e.g., due to genetic polymorphisms). A fourth source of variability, health status, should also be considered, although it is frequently disregarded in environmental risk assessment. To the extent that the variation in physiological and biochemical parameters across these population dimensions can be elucidated, PBPK models can be used together with Monte Carlo methods to integrate their effects on the in vivo kinetics of a chemical exposure and predict the resulting impact on the distribution of risks (as represented by target tissue doses) across the population.

Determinants of Impact

There has sometimes been a tendency in risk assessments to use information on the variability of a specific parameter, such as inhalation rate or the in vitro activity of a particular enzyme, as the basis for expectations regarding the variability in dosimetry for in vivo exposures. However, whether or not the variation in a particular physiological or biochemical parameter will have a significant impact on in vivo dosimetry is a complex function of interacting factors. In particular, the structures of physiological and biochemical systems frequently involve parallel processes (e.g., blood flows, metabolic pathways, excretion processes), leading to compensation for the variation in a single factor. Moreover, physiological constraints may limit the in vivo impact of variability observed in vitro. For instance, high affinity intrinsic clearance can result in essentially complete metabolism of all the chemical reaching the liver in the blood; under these conditions, variability in amount metabolized in vivo would be more a function of variability in liver blood flow than variability in metabolism in vitro. Thus it is often true that the whole (the in vivo variability in dosimetry) is less than the sum of its parts (the variability in each of the pharmacokinetic factors).

The dosimetric impact of variations in physiological factors also depends on the nature of the chemical causing the toxicity, including such physicochemical properties as reactivity, lipophilicity, water solubility, and volatility. For example, variations in inhalation rate will tend to have more impact on the uptake of a water soluble chemical such as isopropanol than on a relatively water insoluble chemical such as vinyl chloride. In addition, the impact of a particular factor on dosimetry also depends on the mode of action of the chemical; that is, how the chemical causes the effect of concern. Of particular importance is whether the toxicity results from exposure to the chemical itself, one of its stable, circulating metabolites, or a reactive intermediate produced during its metabolism.

Another key issue is whether the toxicity results from direct reaction with tissue constituents, from binding to a receptor, or from physical (e.g., solvent) effects on the tissue. To illustrate these considerations, one can contrast the acute neurotoxicity of many solvents (a physical effect of the chemicals themselves) with their chronic hepatotoxicity (produced by products of their metabolism). The most important pharmacokinetic factor in the acute toxicity of volatile solvents is the blood:air partition coefficient, and increasing metabolic clearance typically decreases toxicity. In contrast, the most important pharmacokinetic factors in the chronic toxicity are liver blood flow and metabolism, and increasing metabolic clearance typically increases toxicity.

Example: Age-Dependent Variability

The following example illustrates the use of PBPK modeling to investigate the impact of pharmacokinetic variability on risk

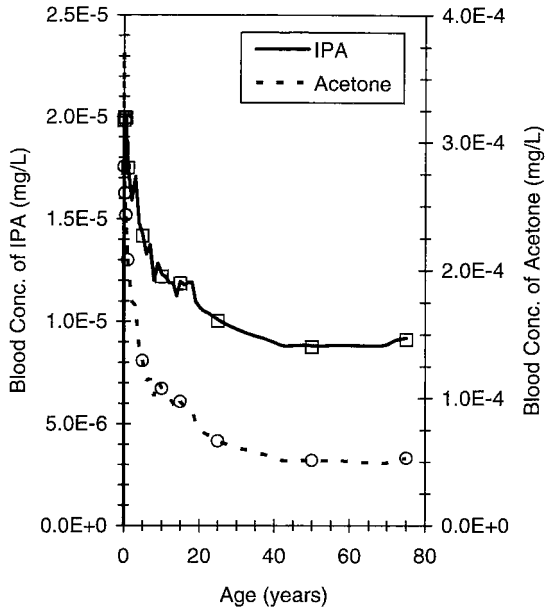

FIG. 6. Blood concentrations of isopropanol (IPA) and its metabolite acetone as a function of age for continuous inhalation exposure at 1 ppb.

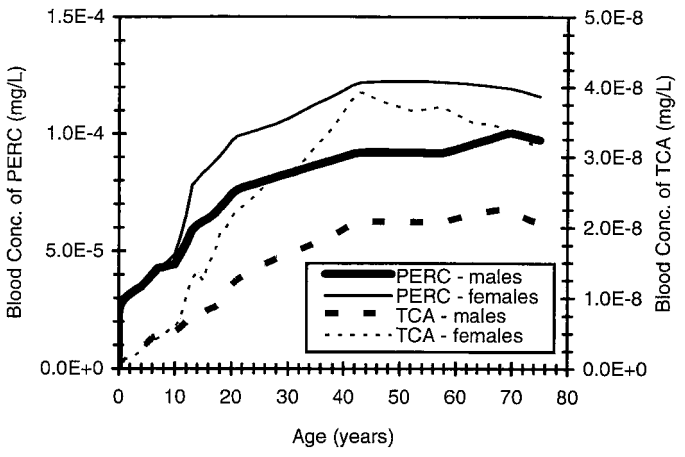

FIG. 7. Blood concentrations of perchloroethylene (PERC) and its primary metabolite trichloroacetic acid (TCA) as a function of age for continuous oral exposure at 1 μg/kg/day.

for the case of age-dependent pharmacokinetics. Specifically, the question being evaluated in this example is how normal changes in pharmacokinetic parameters from birth, through childhood, and across adulthood effect the dosimetry for environmental exposures to chemicals. To this end, a previously developed PBPK model for isopropanol and its metabolite acetone (Clewell et al. 2001) was adapted to simulate the physiological and biochemical changes in humans associated with growth and aging (Sarangapani et al. 2003; Clewell et al. 2004). In the age-dependent model, all physiological and biochemical parameters change with time based on data from the literature (Clewell et al. 2002).

Figure 6 shows the results of using this age-dependent model to simulate continuous inhalation of isopropanol at 1 ppb, beginning at birth and continuing for 75 years. The model predicts that, for the same inhaled concentration, the blood concentrations achieved during early life are significantly higher than those achieved during adulthood. In the case of the metabolite acetone, however, it should be noted that production from isopropanol metabolism would be only a small fraction of endogenous production from ketogenesis.

Quite a different behavior is predicted for daily ingestion of perchloroethylene in drinking water. The exposure in this case is assumed to be a constant intake of perchloroethylene at a rate of 1 μg/kg/day throughout life. The chemical-specific parameters in the model are taken from Gearhart et al. (1993). As shown in Figure 7, predicted concentrations of perchloroethylene and its major metabolite, trichloroacetic acid, are much lower during early life than during adulthood. It is also interesting to note that for this lipophilic chemical, different behaviors are predicted in males and females due to sex-dependent differences in body fat content.

Example: Genetic Polymorphism

The next example demonstrates the use of PBPK modeling, together with Monte Carlo techniques, to evaluate the impact of a genetic polymorphism for metabolism. In the example described here, the polymorphism of interest is for the enzyme paraoxonase (Haber et al. 2002). The PBPK model used in the analysis (Gearhart et al. 1994) describes exposure to parathion, its metabolism to paraoxon, and the inhibition of acetylcholinesterase by paraoxon. Paraoxonase is one of the enzymes responsible for the metabolic clearance of paraoxon. In vitro data on the two human alleles of paraoxonase (low and high activity) were used to develop distributions for the metabolism parameters in the PBPK model (Gentry et al. 2002). Monte Carlo simulations were then performed to generate the resulting distribution of predicted blood concentrations of paraoxon across a population, considering the variability in other pharmacokinetic parameters. Figure 8 displays the predicted distribution for the time-integrated (area under the curve) blood concentrations of paraoxon (mg-h/L) across the total population (dark bars), as compared to the "normal" population (light bars, excluding individuals who are homozygous for the low activity allele), following exposure to parathion at a dose of 0.033 mg/kg. While the polymorphism does impact the distribution of blood concentrations, particularly at the higher internal exposures, the overall effect is relatively small when put in the perspective of the variability in other physiological and biochemical factors across the same population.

Conclusions

The overall pharmacokinetic variability across a population is a function of many chemical-specific, genetic, and physiological factors. Due to the complex interactions among these factors, speculation regarding the extent of population variability on the basis of the observed variation in a single factor can be highly misleading. Analysis using PBPK modeling and Monte

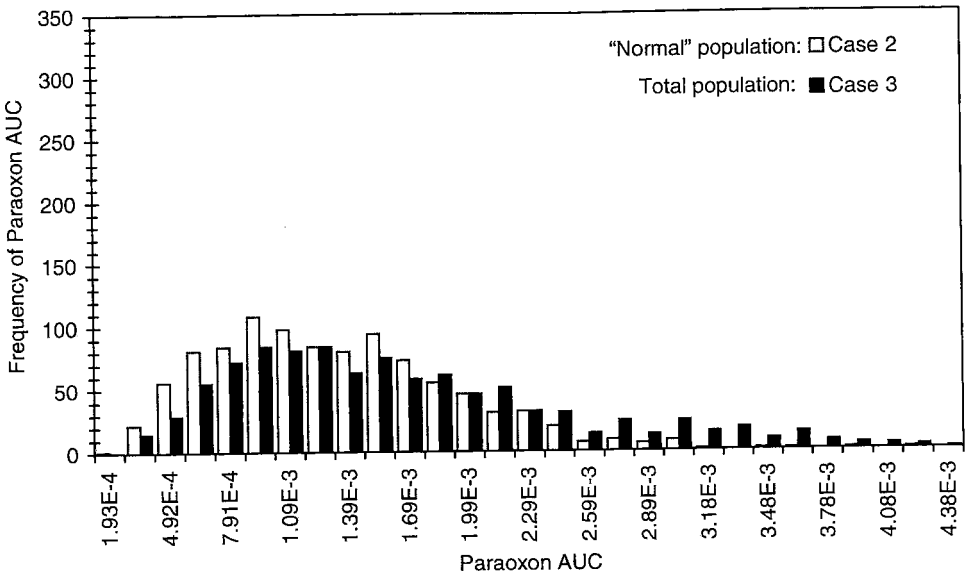

FIG. 8. Paraoxon AUC distribution for the total population (Case 3, including low activity allele) compared to the AUC distribution for the "normal" population (Case 2, using only high activity and heterozygous alleles) for a parathion dose of 0.033 mg/kg.

Carlo techniques provides a more reliable approach for estimating population pharmacokinetic variability. Analyses such as that described here for parathion can be used to develop quantitative Chemical-Specific Adjustment Factors to replace default uncertainty factors for human pharmacokinetic variability (Gentry et al. 2002). PBPK modeling can also be useful in a more qualitative sense, to determine whether there is reason for concern regarding a particular age-group that might be more sensitive due to pharmacokinetic differences, as illustrated by the example using the age-dependent model. Similar analyses can be performed to determine whether exposure during special life stages, such as gestation or lactation, represents a significant concern (Clewell and Gearhart 2002; Gentry et al. 2003). PBPK modeling of this nature, coupled with parameter estimation using quantitative structure activity relationship (QSAR) techniques and mechanistic information from genomic arrays, may prove particularly valuable in prioritizing testing requirements for new chemicals.

SUMMARY

Risk assessment methods are being modified to allow the increasing incorporation of data on PK and PD data both in the extrapolation from laboratory animals to humans and in the consideration of human variability and sensitive subpopulations. An international effort led by IPCS has developed guidance for the adequacy of chemical-specific data for replacing default uncertainty factors (IPCS 2001). This guidance breaks the interspecies and intraspecies uncertainty factors into adjustment factors for kinetics and dynamics. The IPCS guidance has framed much of the research and thinking on such applications, (http://www.ipcsharmonize.org), but there is no formal, quantitiative, technical guidance on this issue at U.S. EPA. A key first step in doing such assessments is identification of the chemical's mode of toxic action, including identification of the active form of the chemical. PBPK models (often combined with Monte Carlo modeling) provide perhaps the best approach to evaluating the implications of varying enzyme activity or other aspects of physiology. In silico computational methods can be used to estimate partition coefficients and metabolic parameters used in PBPK models when laboratory data on the chemical of interest are not available. In vitro systems, such as isolated hepatocytes and hepatic subcellular fractions, are useful for the prediction of xenobiotic biotransformation and pharmacokinetics. Use of PBPK models and consideration of physiological limits indicate that total variability is often less than the variability in specific parameters. For example, this occurs when an enzyme's metabolic capacity is high, so that metabolism is limited by hepatic blood flow, and large changes in enzyme activity result in minimal changes in total metabolism of the chemical. Thus, PBPK modeling can be used to both address whether specific populations are more sensitive than the general population, as well as to quantitate the population variability in tissue dose resulting from a given exposure.

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