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Physiologically Based Pharmacokinetics and Cancer Risk Assessment

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Physiologically based pharmacokinetic (PBPK) modeling involves mathematically describing the complex interplay of the critical physicochemical and biological determinants involved in the disposition of chemicals. In this approach, the body is divided into a number of biologically relevant tissue compartments, arranged in an anatomically accurate manner, and defined with appropriate physiological characteristics. The extrapolation of pharmacokinetic behavior of chemicals from high dose to low dose for various exposure routes and species is possible with this approach because these models are developed by integrating quantitative information on the critical determinants of chemical disposition under a biological modeling framework. The principal application of PBPK models is in the prediction of tissue dosimetry of toxic moiety (e.g., parent chemical, reactive metabolite, macromolecular adduct) of a chemical. Such an application has been demonstrated with dichloromethane, a liver and lung carcinogen in the B6C3F$_1$mouse. The PBPK model-based risk assessment approach estimated a cancer risk to people of 3.7×10^{-8} for a lifetime inhalation exposure of 1 μg/m^3, which is lower by more than two orders of magnitude than that calculated by the U.S. Environmental Protection Agency using the linearized multistage model (for low-dose extrapolation) and body surface correction factor (for interspecies scaling). The capability of predicting the target tissue exposure to toxic moiety in people with PBPK models should help reduce the uncertainty associated with the extrapolation procedures adopted in conventional dose-response assessment.

Introduction

The process of risk assessment for chemical carcinogens is conducted in four parts: hazard identification, dose–response assessment, exposure assessment, and risk characterization (*1*). Dose–response assessment entails both high-dose to low-dose and interspecies extrapolation of the tissue response. These extrapolations are usually conducted with "mandated" models, a linearized multistage (LMS) cancer model for the low dose, and a body surface or body weight correction for interspecies extrapolation (*2*). In the LMS model, the independent variable, dose, is most usually regarded simply as administered dose or inhaled concentration during the bioassay exposure period. Low-dose extrapolation activities consist of the extrapolation of both tissue dosimetry and response. Nonlinearities in either or both of these processes can influence the tumor outcome depending on whether the mechanism of tumor induction is dose–invariant. The assessment of risk associated with exposure to chemicals should be based on all the biologically relevant mechanistic data, and not simply on the administered dose, thus enabling a more accurate estimation of actual risk. This paper discusses the methodological aspects of extrapolating tissue dosimetry with the use of physiological pharmacokinetic models and presents an example of use of such a model to improve the assessment of tumorigenic risk associated with human exposure to dichloromethane.

Physiologically Based Pharmacokinetic Modeling

Physiologically based pharmacokinetic (PBPK) modeling involves the computer simulation of the uptake and disposition of chemicals based on their blood and tissue solubility characteristics, metabolism and protein binding in various tissues, and physiology of the organism. The tissue compartments in these models are interpretable in biological terms, thus enabling interspecies scaling by substitution of parameter estimates with appropriate values for any species of interest. Once formulated by integrating information on these critical biological determinants of disposition, the PBPK models can be used to simulate the kinetic behavior of a chemical in the test species. Model simulations of percent dose exhaled, amount of metabolites produced, level of hepatic and extrahepatic glutathione depletion, tissue and blood concentrations of parent chemical and its metabolites etc., can be generated for exposure scenarios of interest. When the model adequately predicts the pharmacokinetic behavior of a chemical over a variety of exposure situations,it is considered to be "validated" and used for high-dose to low-dose and exposure-route extrapolation of chemical disposition in the test species. The animal PBPK model can then be used for interspecies extrapolation of pharmacokinetic behavior of a chemical by scaling the physiological

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parameters and determining the biochemical parameters in the species of interest. Limited validation studies are necessary to verify the adequacy of the model description for the species of interest.

Failure of a model to accurately predict the pharmacokinetic behavior of a chemical indicates incomplete understanding of the critical processes involved in its uptake, distribution, metabolism, and elimination. In such cases, further experimentation to obtain information of a specific nature to refine and validate the model might be required (*3*). The steps involved in the development of PBPK models and their use in interspecies scaling and risk assessment are schematically presented in Figures 1 and 2.

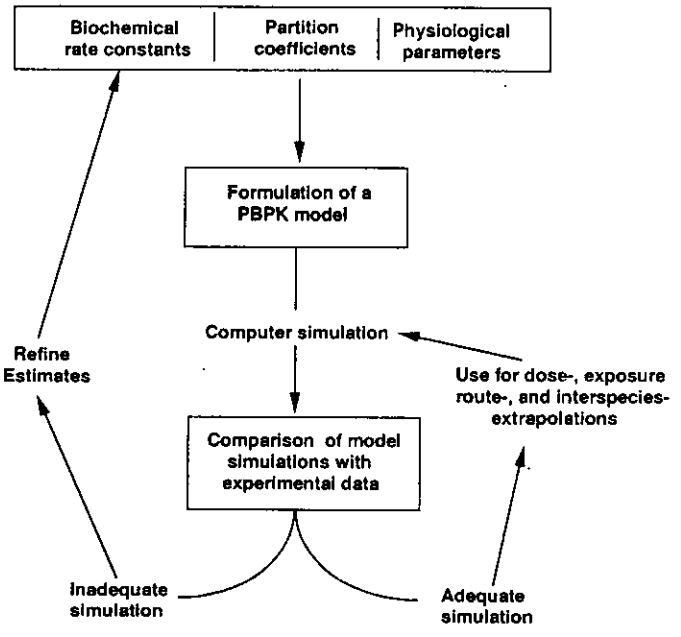

FIGURE 1. Schematic representation of the development of physiologically based pharmacokinetic (PBPK) models for chemical disposition in a test species.

The principal application of PBPK models is to predict the tissue dosimetry of the toxic moiety (i.e., parent chemical, active metabolite, macromolecular adduct, etc.). Quantitative information on the dose of the active form of a chemical in target tissues provides a better basis for extrapolation. Because PBPK models allow the prediction of target tissue dosimetry in people based on physiological and mechanistic considerations, they can also help reduce the uncertainty of extrapolation procedures adopted in conventional risk assessment approaches. Such an application has already been demonstrated with dichloromethane (*4*).

PBPK Models In Cancer Risk Assessment: Dichloromethane

Dichroromethane (DCM; methylene chloride; CH_2Cl_2) causes significant increases in the incidence of liver and lung tumors in $B6C3F_1$ mice after inhalation of 2000 or 4000 ppm for 6 hr/day, 5 days/week for 2 years (*5*). The toxic moiety responsible for DCM tumorigenicity has not been identified; however, it is known that potentially reactive intermediates are produced by two major metabolic pathways (*6–8*). DCM is metabolized in both target organs by a cytochrome P-450-mediated oxidative pathway that yields formyl chloride and by conjugation with glutathione (GSH) yielding chloromethyl glutathione. Using the PBPK modeling approach, information on tissue dosimetry of parent chemical and its metabolites in the most sensitive test species (i.e., mouse) was obtained (*4*). Target-tissue exposure to an appropriate dose surrogate was related to the tumor levels seen in the National Toxicology Program (NTP) bioassay to derive the acceptable target dose and external exposure concentration for humans. These predictions were then compared to those obtained with the conventional risk assessment approach adopted by the U.S. Environmental Protection Agency (U.S. EPA).

Model Development

The PBPK model for DCM consisted of the following tissue compartments: liver, lung, fat, slowly perfused tissues, and richly perfused tissues. The rate of change in the amount of DCM in the tissue compartments (dA_i/dt) was described by a series of mass-balance differential equations of the following form:

$$dA_i/dt = Q_i(C_a - C_{vi}) - dA_{met}/dt$$

where Q_i is the rate of blood flow to tissue i (L/hr); C_a is the concentration of DCM in arterial blood (mg/L); C_{vi} is the concentration of DCM in the venous blood leaving the tissue (mg/L); dA_{met}/dt is the rate of the amount of DCM metabolized (mg/hr).

The rate of the amount of DCM metabolized per unit time in the liver and lung was described by accounting for both microsomal oxidation, a saturable process, and GSH conjugation, a first-order process, at all exposure concentrations used in the NTP cancer bioassay:

$$dA_{met}/dt = V_{max}C_{vi}/(K_m + C_{vi}) + K_f C_{vi} V_i$$

where V_{max} is the maximum enzymatic reaction rate (mg/hr); K_m is the Michaelis constant for enzyme reaction (mg/L); K_f is the first-order rate constant for GSH conjugation (hr^{-1}); V_i is the volume of the tissue (L).

The physiological parameters required for the PBPK model (i.e., alveolar ventilation rate, blood flow rates, tissue volumes) were obtained from the literature (*9,10*). The blood:air and tissue:air partition coefficients for DCM were determined by vial equilibration techniques (*11,12*). The tissue:blood partition coefficients required for the model were determined by dividing tissue:air values by the blood:air value. The rate constants for DCM metabolism were determined by apportioning the whole-body metabolic capacity between lung and liver by assuming that the distribution of enzyme activities metabolizing DCM was the same as the distribution of enzyme activities acting on two model substrates, 7-ethoxycoumarin for microsomal oxidation, and 2,5-dinitrochlorobenzene for GSH conjugation (*13*).

The mouse PBPK description, once validated by comparing model predictions with observed pharmacokinetic data, was scaled to predict the tissue dosimetry of DCM and its metabolites in humans. This was accomplished by scaling the physiological parameters of the model and determining chemical-specific parameters for humans. Thus, the tissue: blood partition coef-

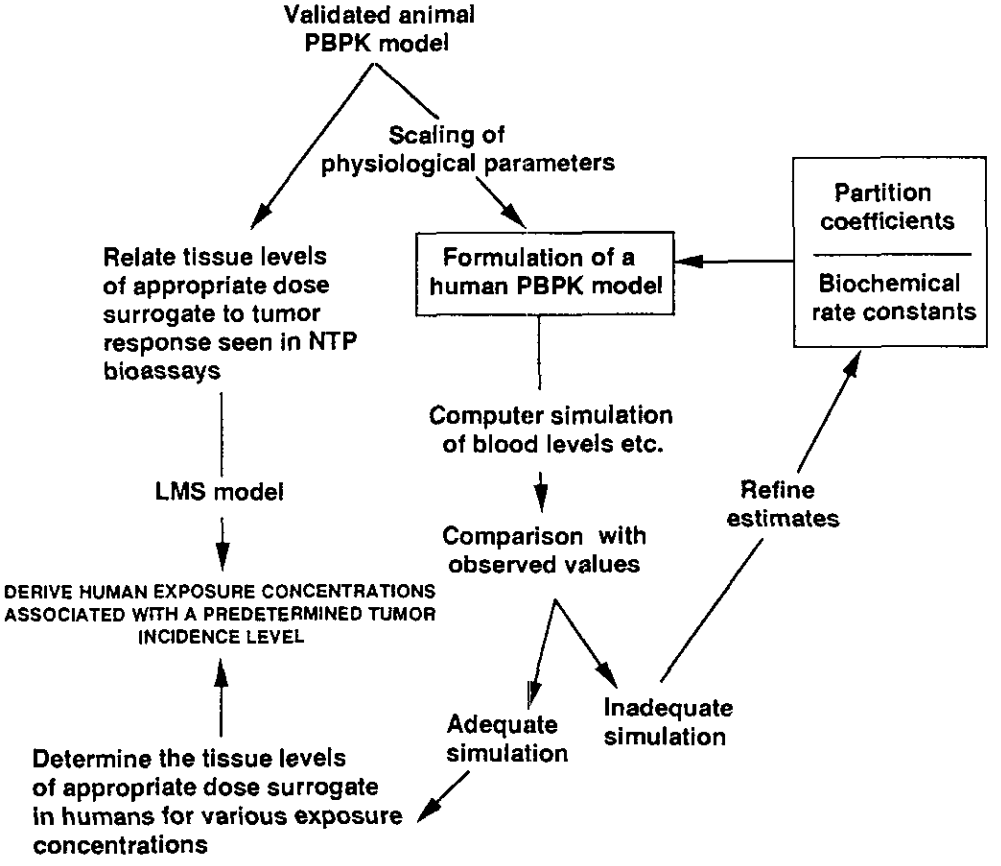

FIGURE 2. Schematic representation of the use of physiologically based pharmacokinetic (PBPK) modeling in interspecies extrapolation and risk assessment.

ficients for humans were calculated by dividing mouse tissue:air partition coefficients by human blood:air partition coefficient. Further, the metabolic rate constants for humans were estimated from volunteer human exposure studies, in which levels of DCM and carboxyhemoglobin in blood were determined during and following a 6-hr exposure to 100 and 350 ppm DCM (*14*). The glutathione S-transferase activity (GST) in humans was set equal to the highest activity reported in rodents.

Choice of Dose Surrogate

The mouse PBPK model for DCM, formulated by integrating information on mouse physiology, DCM solubility characteristics, and metabolic rate constants, was successfully used to describe the disposition of DCM (*4*). The mouse PBPK model was then used to calculate the tissue dose of metabolites and parent chemical arising from exposure scenarios comparable to those of the NTP bioassay studies. Their relationship to the observed tumor incidence was examined. Because DCM is very unreactive, it is unlikely to be directly involved in its tumorigenicity. Hence the relationship between the tissue exposure to its metabolites and tumor incidence was examined (Table 1). Whereas the dose surrogate based on the oxidative pathway did not vary between DCM exposure concentrations of 2000 and 4000 ppm, the flux through the GSH conjugation pathway did correspond well with the degree of DCM-induced cancer at these exposure concentrations. These observations are consistent with a role for the metabolite(s) arising from the GSH conjugation pathway in DCM-induced lung and liver cancer. The GSH conjugation of DCM, reported to be mediated by a new class of glutathione *S*-transferase enzymes (*15*), yields formaldehyde as a metabolite. Recently, Casanova et al. (*16*) have reported DNA–formaldehyde–protein crosslinks from DCM exposure, further strengthening the case for the GSH conjugation as the pathway leading to potentially carcinogenic metabolites. Therefore, the high-dose to low-dose extrapolation and interspecies extrapolation of DCM-induced cancer risk were conducted with the tissue dose of the GSH-pathway metabolite predicted by the PBPK model.

Table 1. Tumor incidence and calculated tissue dose of dichloromethane (DCM) metabolites following inhalation exposures of DCM in female mice.

Tissue	DCM exposure, ppm	Tumor incidence, %	Tissue dose, mg/L tissue/day GSH pathway	Oxidative pathway
Liver	0	6	—	—
	2000	33	851	3575
	4000	83	1800	3701
Lung	0	6	—	—
	2000	63	123	1531
	4000	85	256	1583

GSH, glutathione. Adapted from Anderson et al. (4.)

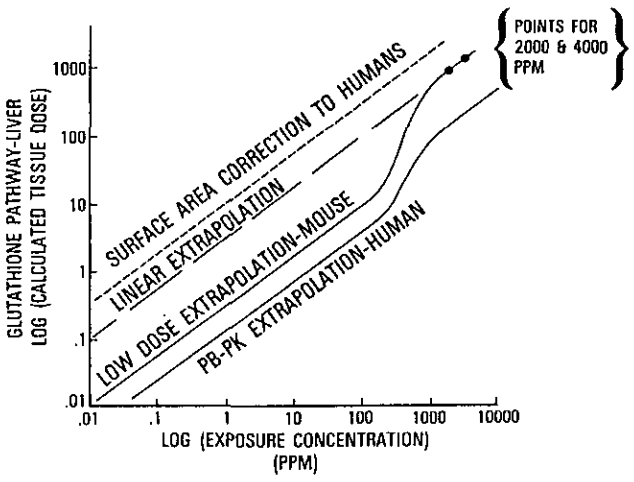

FIGURE 3. Dichloromethane tissue-dose assessment: low-dose and interspecies extrapolation. Estimation of liver tissue dose for dichloromethane glutathione conjugates with a physiologically based pharmacokinetic model. Reproduced with permission from *Applied Industrial Hygiene* (*32*).

High-Dose to Low-Dose Extrapolation

The model prediction of the target tissue dose of the DCM-GSH conjugate resulting from 6-hr inhalation exposures of 1–4000 ppm of DCM is presented in Figure 3. The estimation of target tissue dose of DCM-GSH conjugate by linear back-extrapolation gives rise to a 21-fold higher estimate than that obtained by the PBPK modeling approach. This discrepancy arises from the nonlinear behavior of DCM metabolism at high exposure concentrations. At exposure concentrations exceeding 300 ppm, the cytochrome P-450-mediated oxidation pathway is saturated, giving rise to a corresponding disproportionate increase in the flux through the GSH conjugation pathway.

Interspecies Extrapolation

The interspecies extrapolation of DCM disposition behavior was possible because the critical biological determinants of disposition were first identified in the test species, the mouse. Thus, the physiological parameters were scaled allometrically, the metabolic parameters were determined experimentally and the tissue:air partitioning of DCM was assumed to be species-invariant. The PBPK model adequately simulated the blood levels of DCM observed in humans after a 6-hr inhalation exposure to 100 or 350 ppm DCM (Fig. 4). The target tissue dose for humans was estimated to be some 2.7 times lower than that for the mouse. Considering these data, the human tissue dose of DCM–GSH conjugate for a 6-hr exposure to 1 ppm DCM is expected to be some 57 times lower than that expected by linear extrapolation of its behavior at high doses, such as the doses used in the mouse bioassay (*4*).

Risk Assessment

The cancer risk assessment for DCM was conducted using the LMS model to relate tissue dose of DCM–GSH metabolite (rather than DCM exposure concentration) to the observed

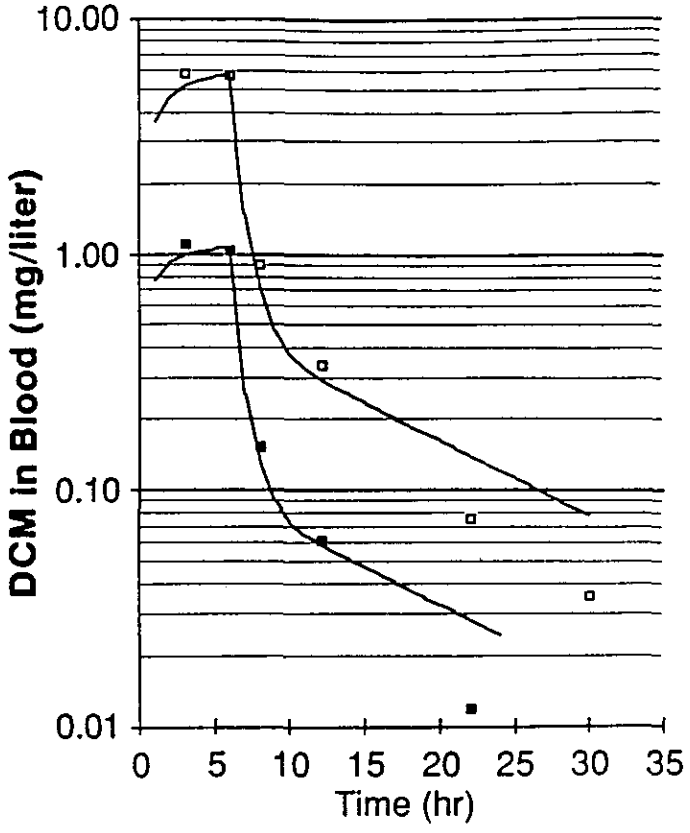

FIGURE 4. Comparison of model simulations (solid lines) with the observed data on blood dichloromethane levels in humans during and after 6-hr inhalation exposures of 100 ppm (■) or 350 ppm (□). Reproduced with permission from *Toxicology and Applied Pharmacology* (*4*).

tumor incidence rates at high exposure concentrations in the mouse. In assessing the tumorigenic risks associated with human exposure to this chemical, it was assumed that humans are as sensitive as the most sensitive target species. Therefore, equal target tissue doses are expected to produce similar tumor incidence regardless of the species. This conclusion is in contrast to that obtained by the EPA, which estimates that people are more sensitive than mice, based on the use of a surface-area scaling approach (*17*). In the human DCM risk assessment based on the PBPK model using the GST pathway dose, the predicted human low-lose cancer risk was about 100- to 200-fold less than that estimated by the EPA using their standard default assumptions (*4*). With further refinement of the model with the estimation of the metabolite rate constants for humans *in vitro*, Reitz et al. (*18*), using the delivered dose calculated with the PBPK model, predicted a cancer risk of 3.7×10^{-8} for a lifetime inhalation exposure of 1 $\mu g/m^3$. This risk estimate is still lower, by more than two orders of magnitude, than that calculated by the EPA (4.1×10^{-6}) using the default assumptions and exposure concentration of DCM. The EPA has amended its original risk assessment for DCM (*19*) and incorporated some, but not all, of the concepts used in the physiological pharmacokinetics-based risk assessment approach outlined here.

The use of PBPK models in quantitative risk assessment does not always result in the estimation of lower risk than the conven-

tional approach adopted by the EPA. For example, if the test chemical acts directly, the PBPK approach could actually predict more risk to humans than to rodents because enzyme-mediated metabolic clearance (detoxification) is expected to be lower in the larger species. Similarly, if the toxicity of a chemical is mediated by reactive intermediate(s) resulting from a saturable metabolic process, then the high-dose to low-dose extrapolation conducted with the PBPK modeling approach would predict a greater risk at low doses than that predicted by the linear extrapolation procedure.

Issues Surrounding the Use of PBPK Models in Risk Assessment

The motivation for use of PBPK models in toxicology research is to uncover the biological determinants of tissue dosimetry. These models are part of a systematic approach to studying how chemicals gain entry to, distribute within, and are eliminated from the body. These models are complex with multiple parameters, but in this regard they simply reflect some of the obvious complexities of the biological system.

One strategy for accurately estimating specific parameters is to conduct kinetic studies under conditions where pharmacokinetic behavior of chemicals is related to one or two dominant factors and thereby derive estimates of the value of these parameters. An example is the estimation of metabolic parameters by gas uptake studies (*20*). Alternatively, biochemical and chemical-specific parameters may be directly estimated in some cases from studies with *in vitro* preparations (*18*) or obtained from the literature (*21*).

Parameter identifiability and model overspecification are problems inherent in these PBPK models or in any other multiparameter model. Direct measurement of model parameters by experimental methods, independent of analysis of tissue time-course curves, is the preferred approach. Nonetheless, limited numbers of parameters will often still have to be estimated by analysis of time-course data by curve-fitting techniques, under well-defined experimental conditions where the curves are particularly sensitive to the parameter of interest.

Other areas of concern relate to the adequacy of the model in biological terms. Are all important biological determinants of the uptake and disposition of the test chemical included in the model description? For risk assessment, some additional uncertainty surrounds the decision regarding which measure of tissue dose best correlates with tumor formation. For instance, is the GSH conjugation with DCM really the key determinant in DCM tumorigenesis? These are essentially biological, research-oriented issues whose answers rely on knowledge of mechanisms of toxicity and carcinogenicity of a particular chemical.

Another concern in the area of extrapolation to humans is the use of point estimates of model parameters instead of applying a distribution of parameter values to develop ranges of risk estimates (*22*). This issue deserves serious attention from a generic point of view, not solely as it applies to PBPK model-based assessments. Present interspecies scaling takes little account of variability in population characteristics to derive ranges of risk. The impact of variability in interspecies extrapolation needs to be considered for both default procedures (the LMS procedure, body surface correction, etc,) and for the case of PBPK model-based assessments such as the one proposed with DCM.

Despite these unresolved issues, PBPK models are becoming more widespread in many areas of toxicology research (*23,24*). We are beginning to see more examples of application of these models for the assessment of tumorigenic risk associated with human exposure to chemicals (*25–27*). The PBPK modeling addresses only the tissue dose aspect of the exposure–dose–response continuum. Detailed knowledge of all aspects of the continuum is required to improve risk assessment. The PBPK model-based risk assessments have used these models to estimate tissue dose but still rely on LMS approach as the response model. Biologically based response models are also being developed for use in risk assessment (*28,29*). Fully linked dosimetry–response simulation models promise to integrate a diversity of pharmacokinetic, mechanistic, and tumor progression studies into a unitary description of chemical carcinogenesis (*30,31*). These integrated biological models should greatly improve the scientific basis of low-dose and interspecies extrapolation of tissue dosimetry and response.

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