Toxicokinetic modeling and its applications in chemical risk assessment

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Abstract

In recent years physiologically based pharmacokinetic (PBPK) modeling has found frequent application in risk assessments where PBPK models serve as important adjuncts to studies on modes of action of xenobiotics. In this regard, studies on mode of action provide insight into both the sites/mechanisms of action and the form of the xenobiotic associated with toxic responses. Validated PBPK models permit calculation of tissue doses of xenobiotics and metabolites for a variety of conditions, i.e. at low-doses, in different animal species, and in different members of a human population. In this manner, these PBPK models support the low-dose and interspecies extrapolations that are important components of current risk assessment methodologies. PBPK models are sometimes referred to as physiological toxicokinetic (PT) models to emphasize their application with compounds causing toxic responses. Pharmacokinetic (PK) modeling in general has a rich history. Data-based PK compartmental models were developed in the 1930’s when only primitive tools were available for solving sets of differential equations. These models were expanded in the 1960’s and 1970’s to accommodate new observations on dose-dependent elimination and flow-limited metabolism. The application of clearance concepts brought many new insights about the disposition of drugs in the body. In the 1970’s PBPK/PT models were developed to evaluate metabolism of volatile compounds of occupational importance, and, for the first time, dose-dependent processes in toxicology were included in PBPK models in order to assess the conditions under which saturation of metabolic and elimination processes lead to non-linear dose response relationships. In the 1980’s insights from chemical engineers and occupational toxicology were combined to develop PBPK/PT models to support risk assessment with methylene chloride and other solvents. The 1990’s witnessed explosive growth in risk assessment applications of PBPK/PT models and in applying sensitivity and variability methods to evaluate model performance. Some of the compounds examined in detail include butadiene, styrene, glycol ethers, dioxins and organic esters/aids. This paper outlines the history of PBPK/PT modeling, emphasizes more recent applications of PBPK/TK models in health risk assessment, and discusses the risk assessment perspective provided by modern uses of these modeling approaches.

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1. Introduction

The goal of most toxicity research is to infer expected human health risks from the results in test animals. Animals are commonly exposed to extremely high concentrations of test chemicals, by unusual routes of administration, for their entire lifetime. The challenge is to extrapolate from the animal results to predict effects in human populations that have very different exposure patterns. This process entails at least four types of extrapolations: from high-doses to much lower doses; from the test species to humans; from one route of administration to routes more relevant to human exposure; and from constant concentration, daily dosing regimens in the animal tests to the discontinuous exposures common in human populations. A key to successful extrapolation is the reliability with which target tissue dose can be predicted in different animal species under a variety of exposure conditions. Pharmacokinetic (PK) models have the potential to estimate time-course concentrations of parent compounds and metabolites for different exposure conditions.

Much of the earlier work with PK modeling in toxicology was based on determining the time-course of chemical concentrations in various tissues after different doses (Fig. 1). These time-course curves were then analyzed with so-called data-based compartmental PK models to estimate the dose-dependence of kinetic parameters. The compartments in these models do not correspond directly to the anatomy, physiology, or biochemistry of the animal itself (cf., Gibaldi and Perrier, 1982). Despite the relative simplicity of these descriptions, these data-based compartmental PK approaches were instrumental in uncovering dose dependencies of PKs and examining the influence of dose-dependent kinetics on dosimetry in target tissues. However, this approach to PK analysis was primarily of use for interpolation between doses or for limited extrapolation in the test animal. Interpolation is not adequate for most risk assessment needs that extrapolate to very low-doses and from animals to humans.

A PK modeling process referred to as physiologically based pharmacokinetic (PBPK) modeling is particularly well-suited to calculation of tissue doses of chemicals and their metabolites over a wide range of exposure conditions in different species (Dedrick, 1973; Clewell and Andersen, 1985; Leung, 1991). In PBPK models (Fig. 2), the compartments correspond to discrete tissues or groups of tissues with appropriate volumes, blood

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**Fig. 1.** Data-based PK modeling: in this common approach to PK modeling, time-course data are collected (left-most panel) and fit to specified models that describe the body as a series of compartments (middle panel). These compartments have no direct physiological correspondence with specific anatomical structures within the body. The best fit of the model to the data provides estimates of the various micro-constants ($k_{12}$, $k_{21}$, $k_1$, $k_2$) in the model.
flow rates, and pathways of metabolism of the test chemical (Bischoff and Brown, 1966; Andersen, 1991). In PBPK models, pertinent biochemical and physical chemical constants for metabolism and tissue solubility are incorporated directly in the descriptions of each tissue compartment. Routes of administration are included in their proper relationship to the overall physiological structure and exposure scenario differences are accounted for in the time sequence of the dose input terms. Each compartment (i.e. tissue) in the model is described by a mass-balance differential equation, and the set of equations is solved by numerical integration to predict tissue time-course concentrations of the test chemical and its metabolites. PBPK models have been developed for a variety of chemicals (Himmelstein and Lutz, 1979; Germanowski and Jain, 1983; Clewell and Andersen, 1987) and are finding increasing use in chemical risk assessment (Andersen et al., 1987; NRC, 1987; Leung, 1991). Both in Canada and in Europe, PBPK modeling is commonly referred to as physiological toxicokinetic (PT) modeling to emphasize the applications with compounds that have adverse, toxic responses at high-doses. This article traces the history of PBPK/PT modeling and describes some recent applications of PBPK/PT modeling to improve risk assessments with a variety of compounds.

2. PBPK/PT modeling

2.1. A historical perspective

The disciplines of inhalation anesthesia, compartmental PKs, chemical engineering, and computer sciences have each contributed to the maturation of PBPK/PT modeling as a tool for estimating tissue dosimetry. The intellectual tradition in inhalation anesthesia has maintained a strong emphasis on the role of breathing rates, blood flow rates, and chemical solubility on the uptake and distribution of volatile anesthetic agents. In the 1920’s Haggard (1924a,b) quantitatively described the importance of these physiolo-
gical factors for the uptake of diethyl ether during the first few breaths. More complete kinetic models of uptake and elimination of volatile anesthetics were developed by Kety (1951), Mapleson (1963), Riggs (1963). In these descriptions, various body tissues were lumped together based on blood flow rates and tissue partitioning. In addition, all kinetic processes were considered to be linear with respect to the tissue concentrations of these anesthetics. Mapleson (1963) used an electrical analog—consisting of resistors, capacitors, etc.—to simulate anesthetic kinetics. In a series of contributions, Fiserova-Bergerova (1975), Fiserova-Bergerova and Holaday (1979), Fiserova-Bergerova et al. (1980) extended the electric analog model to include metabolism of the inhaled vapors in systemic organs. This extension was particularly important for toxicology because most inhaled vapors are metabolized in various visceral organs, such as liver and kidney. Frequently, the metabolites are more toxic than the parent volatile chemical. Thus, the earlier development of PK models for anesthetics provided a rich background and intellectual tradition that pointed the way forward for using these approaches with volatile compounds of concerns as inhalation risks in occupational settings.

Most therapeutically important drugs are not volatile. Another discipline, data-based compartmental PKs, developed to study the time-course behavior of drug concentrations in the body. In the 1930’s, Teorell (1937a,b) very thoroughly described the physical, chemical, and physiological factors that govern the uptake, distribution, and elimination of any chemical in the body, and actually provided the first physiologically based model for drug disposition. Because computational methods did not exist to solve the sets of equations by numerical methods, it was impossible to solve the resulting sets of mass-balance equations except in certain limiting cases. Exact solutions could only be obtained for very much simplified models in which the body was reduced to a small number of compartments. These simplifications led to the development of the data-based compartmental PK models with small numbers of compartments whose characteristics were inferred from fitting the models to specific data sets. The parameters in this type of model lack correspondence with specific physiological or anatomical parameters of the organism and statistical considerations are paramount in establishing good fits of the model to experimental data.

Over the next 30 years, data-based compartmental PKs became more mathematically oriented and was more often the study of those compartmental models with exact analytical solutions than the study of the biological and chemical basis of drug disposition in test animals and people. This situation was more a reflection of the lack of appropriate mathematical tools than an unwillingness to provide physiologically realistic approaches. The discipline of chemical kinetics was similarly stymied by deficiencies of available mathematical approaches, as eloquently noted by Edelson (1981).

In the 1960’s and 1970’s, it became apparent that many physiological processes important for PK behavior, especially chemical metabolism and excretion, were not simple linear relationships with respect to dose. Two examples, the elimination of ethanol (Lundquist and Wolthers, 1958) and elimination of aspirin (Levy, 1965, 1968) were extensively studied. The potential for toxicity increased markedly with these drugs at dosages that caused metabolism to be saturated. Perry Gehring and his colleagues at Dow Chemical in Midland, MI in the United States (Gehring et al., 1976, 1977) applied non-linear compartmental models to problems in toxicology. The work of these investigators unveiled the extensive nonlinear behaviors associated with saturation of metabolism that frequently occurred at high-doses used in toxicology studies in test animals. Among the important classes of chemicals studied by these investigators were herbicides (Sauerhoff et al., 1976, 1977), solvents (McKenna et al., 1982), industrial monomers (McKenna et al., 1978a,b), and hydrocarbons (Young et al., 1979; Ramsey et al., 1980). This work generally took the form of careful, detailed collection of biological data on time-course blood levels or excretion patterns at various dosage levels. Time-course data were examined with nonlinear models for metabolism to estimate relative kinetic constants for various exposure conditions. Such studies accounted for
the disproportionate increase in tissue exposure to dioxane associated with saturation of dioxane metabolism (Young et al., 1977, 1978). Another important behavior, a limiting rate of metabolism with increasing exposure concentration, became evident in their classic work on vinyl chloride metabolism and its relationship to production of hemangiosarcoma in exposed rodents (Watanabe et al., 1976; Gehring et al., 1978). Despite the elegance of this work, the compartments in these models lacked realistic anatomic detail and were difficult to use for extrapolation to other routes and exposure scenarios, or to other species.

The parallel development of improved computational resources and of modeling expertise in other disciplines, especially chemical engineering, brought to PK modeling the versatility needed to conduct physiological modeling of tissue dosimetry for a wide variety of chemicals. With improved computational methods, it was straightforward to include multiple physiologically defined compartments with non-linear disposition processes into PK models, paving the way development of PBPK/PT models in pharmacology and toxicology. Today even very complicated PBPK/PT models can be rapidly solved using desktop personal computers with any of a variety of simulation languages. In another case of parallel development of new methods, scientists trained in chemical engineering began to develop more detailed PBPK models for the PK behavior of various drugs (Bischoff et al., 1971). Especially detailed contributions were made in the study of anti-neoplastic drugs, such as methotrexate (MTX) (Bischoff et al., 1971), 5-fluorouracil (Collins et al., 1982); and cisplatin (Farris et al., 1988). These studies showed the ease with which realistic descriptions of physiology and of the relevant pathways of metabolism could be combined into PBPK descriptions of chemical disposition and paved the way for more extensive use of physiological modeling of tissue dosimetry, both in toxicology and in extrapolating tissue dosimetry from test animals to humans. Ramsey and Andersen (1984) examined the kinetics of inhaled styrene in rats and humans using a PBPK modeling approach. Their work, which relied heavily on these advances in inhalation anesthesia, compartmental PKs, chemical engineering, and computer sciences, emphasized the ability to extrapolate beyond the test conditions of the inhalation experiments, by conducting low-dose, dose route, and interspecies extrapolations. Based on the PBPK model for styrene in the rat, they were able to predict blood styrene time-course behavior for oral and intravenous dosing in the rat and for inhalation exposure of human volunteers.

2.2. Predictive PBPK models for tissue dosimetry—a continuing goal

The advent of biologically structured PBPK models had a dramatic influence on the nature of the experiments conducted to determine PK behavior and to estimate tissue dosimetry. In PBPK descriptions, time-course behavior is not an intrinsic property of the system, accessible only by direct experimentation. It is instead a composite behavior, governed by more fundamental physiological and biochemical processes. More importantly, these fundamental processes can be studied directly to obtain the necessary model parameters in experiments separate from collection of time-course concentration curves. Based on these constants, tissue dosimetry can be predicted by computer simulation with PBPK models (Clewell and Andersen, 1988). Once more volatile chemicals provided a good platform for examining this approach to PBPK modeling. The disposition of volatiles in the body is related to breathing rates, tissue volumes, tissue blood flow rates, tissue partition coefficients, and the kinetic constants for metabolism of the chemical in a particular tissue. Values for the physiological parameters can be found in the biomedical literature (for a recent compilation see Brown et al., 1997). Partition coefficients are measured by equilibrating tissue homogenates in a vial with an atmosphere containing the test chemical (Sato and Nakajima, 1979a). Metabolic rates are determined in an in vitro system in which tissue homogenates are supplemented with reactants to promote metabolic reactions (Sato and Nakajima, 1979b) or by putting small numbers of live animals into a closed chamber to measure the rate of loss of chemical at a variety of chamber concentrations (Filser and
Bolt, 1979; Gargas et al., 1986). These experiments provided all the parameters necessary for constructing a PBPK model for the parent chemical and time-course behavior is now ‘predictable’, based on results of these ancillary studies. In general, the development of PBPK models requires fewer animals than would be needed for the detailed time-course curves necessary for conventional compartmental approaches. Some in vivo experimentation will always be required to test the accuracy of the predicted behavior, but this limited work requires fewer animals than do conventional approaches for assessing PK behavior (Clewell and Andersen, 1988). Other approaches for developing predictive PBPK models include using structure activity relationships to estimate model parameters for classes of compounds (Gargas et al., 1988; Parham et al., 1997; Parham and Portier, 1998; Poulin and Krishnan, 1996, 1999).

Predictive PBPK models have a very useful property. They can be wrong! Intellectually, the ability to predict a particular outcome is a powerful tool for enhancing the information content of an experiment. In effect, PBPK models make predictions that become testable (Fig. 2). Trans-1,2-dichloroethylene (trans-DCE) provided a good example of a fairly spectacular, but enlightening, failure of a PBPK/PT model. A simple PBPK model structure had worked well in predicting the disappearance of a diverse group of volatile chemicals from a closed chamber (Gargas et al., 1986, 1990). This model, with metabolism in the liver and time-invariant biochemical constants, regarded the chamber atmosphere as another compartment (Filser and Bolt, 1979). When applied to analyzing the loss of trans-DCE from the chamber, the PBPK model was unable to fit the uptake curves (Gargas and Andersen, 1988; Gargas et al., 1990). This failure led to a search for the biological basis of the poor fit. It turned out that trans-DCE, or more accurately, metabolites of trans-DCE, rapidly react with and inactivate the enzyme(s) responsible for trans-DCE metabolism (Lilly et al., 1999). The successful PBPK description for trans-DCE accounted for loss of trans-DCE metabolizing activity over time, an additional complexity that was easily accommodated by virtue of the versatility of a physiological modeling framework. Earlier PK models that simply fit the time-course data for chamber loss without a physiological structure failed to uncover the suicide inhibition of trans-DCE metabolism (Filser and Bolt, 1979).

The closed chamber model has about 20 constants. An oft-repeated maxim says that any curve (or family of curves) can be fit with a model that has a sufficiently large number of variables. This maxim is clearly true when all the parameters can be freely varied. With PBPK models, however, most of these parameters are constrained within fairly narrow limits by prior knowledge of physiology, biochemistry and secondary information developed from studies distinct from the curve-fitting exercises. In the unsuccessful fitting attempt with trans-DCE, physiologic constants and tissue partitioning were fixed to measured values. Only the metabolic parameters constants could be freely varied. No single set of time-invariant metabolic parameters could successfully describe the full set of curves. With trans-DCE, several good fits were obtained when the models were fit to a broad range of kinetic parameters. However, many fitted parameters were completely unrealistic in terms of Vmax and Km values for similar values. In estimating metabolic parameters, it is best to have data from ancillary studies available to provide bounds for their values. Ancillary studies might include in vitro estimations of kinetic constants or specific in vivo studies that are uniquely sensitive to metabolic clearances, such as gas uptake methods for assessing metabolic rates for inhaled volatiles (Gargas et al., 1990).

2.3. A PBPK model for dioxin—early approaches

Volatile chemicals are by no means the only chemicals that can be examined with techniques of PBPK modeling. Several research groups have developed PBPK models for the disposition of 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) beginning with work of Leung et al. (1988). Dioxin exerts a wide range of biological effects, all of which appear to require the initial interaction of dioxin with a cytosolic protein, the Ah receptor. This dioxin–Ah receptor complex moves from the cytoplasm to the nucleus, where it binds to specific
regions of DNA and regulates the expression of specific genes. Dioxin dosimetry, then, is related to its high affinity binding to a cellular protein, in this case, the Ah receptor, and the interaction of this dioxin–Ah receptor complex with DNA binding sites. This section outlines the early efforts to construct a PBPK model for dioxin; a later section outlines some uses of PBPK models in risk assessments for this compound. With dioxin, the challenge in constructing the PBPK model was to provide a biologically realistic description of the binding of this molecule with hepatic proteins and the biological consequences of Ah receptor binding.

Earlier PBPK models for chemotherapeutic agents also included avid protein binding. The efficacy of MTX as a chemotherapeutic drug is also related to its high avidity binding to a cellular protein, dihydrofolate reductase (DHFR). By binding DHFR, MTX disturbs single carbon metabolism necessary for synthesis of the building block constituents of cellular DNA. With this drug, tissue dosimetry is related to the extent of MTX binding to DHFR and binding is a measure both of chemotherapeutic efficacy and of expected cellular toxicity. Elegant physiological modeling of tissue dosimetry has been conducted with MTX (Bischoff et al., 1971; Morrison and Allegra, 1987). The earlier body of work with MTX formed a starting point for developing physiological models that included tissue binding for dioxin. Unlike MTX, dioxin is highly lipophilic. However, dioxin is often found in higher concentrations in the liver than in the fat of dosed animals.

In earlier physiological models developed to examine the kinetics of dioxin-like materials, chemicals were apportioned to tissues based on an apparent partition coefficient determined from tissue concentrations observed in vivo (King et al., 1983; Tuey and Matthews, 1980). Thus, the liver:blood partition coefficient was regarded to be larger than the fat:blood partition coefficient, despite the high degree of lipophilicity of these chemicals. Leung et al. (1988) used an alternative approach to examine liver and fat dioxin concentrations in mice. They defined the intrinsic solubility of the liver for dioxin to be approximately equal to that of the kidney, based on the similar lipophilicity of these tissues. The ability of the liver to further concentrate dioxin, then, must be due to the presence of specific dioxin-binding proteins. Quantitative physiological models were used to examine the role of the Ah receptor in accounting for the accumulation of dioxin in the liver (Leung et al., 1989a,b). The results were clear-cut. Despite its very high affinity, the Ah receptor binding could not account for dioxin sequestration in the liver at dosages used in the earlier studies. The physiological modeling approach indicated the existence of important non-Ah receptor binding sites and estimated both the concentration and affinity of these secondary dioxin-binding sites. Subsequent work showed that these binding sites are microsomal proteins with binding constants in the nanomolar range (Poland et al., 1989a,b). An additional complication arises because the microsomal binding protein was inducible by dioxin to about eightfold its level in untreated mice. This induction, which produces dose-dependent alterations in dioxin disposition, was readily incorporated into the PBPK model by having the dioxin–Ah receptor complex induce the binding protein, now known to be cytochrome P450 1A2.

The example of physiological modeling with dioxin shows the continuum of processes involved in determining tissue dosimetry. The chemical form of dioxin responsible for its toxicity is its free tissue concentration in target tissues. The next step is the interaction of free dioxin with biological structures, in which dioxin binds the Ah receptor in the cytoplasm. This interaction is the initial element of biologically relevant tissue dose and is included directly in the modeling conducted by Leung et al. (1988, 1989a,b). Physiological modeling need not stop at this level. The Ah–dioxin complex interacts with DNA to cause induction or repression of gene expression. All these time-dependent processes, including synthesis of specific messenger-RNA or specific proteins, can be modeled with approaches that realistically incorporate our knowledge of the chemistry and biology of the overall response of the tissue or organism. More recent PBPK models for TCDD (Kohn et al., 1993; Andersen et al., 1993, 1997) have elaborated on this initial model to include increasing biological detail in relation to DNA.
binding, mRNA induction and protein synthesis for the induced gene products.

At some point, physiological modeling strategies move from tissue dosimetry (PKs) to tissue response (pharmacodynamics (PDs)). The ability to confidently extrapolate from test animal responses to predict outcome in humans for any chemical will largely be dependent on the development of physiologically realistic, mechanistic models for both tissue dosimetry and tissue response. In this way, steps from exposure to response become more completely defined mechanistically, avoiding the need to make empirical correlations between elements of tissue dose and the ultimate biological response. The physiological models for tissue dosimetry are more completely developed than are the models for biological response. A particularly good risk assessment example of the extension of PBPK modeling to include a more PD portion is pH control models in respiratory tract epithelial tissues following exposures to vinyl acetate (VA) (Plowchalk et al., 1997; Bogdanffy et al., 1999).

2.4. Current status of PBPK/PT models

Reviews of advances in PBPK/PT modeling have appeared (Gerlowski and Jain, 1983; Leung, 1991). A book chapter by Krishnan and Andersen (2001) outlines current practices in model development. Recently a group of us at Colorado State University have begun preparation of a monograph on the current status/applications of these models in relation to toxicology and risk assessment applications. The literature through the end of 2001 has upwards of 700 papers that we identified that would be included in such a review! The contributions are worldwide from Asia, Europe and North America. This workshop highlights several of the contributions from Europe (Green et al., 2001; Filser and Csanady, in press, Johanson, in press). The group of Krishnan in Quebec, Canada (see for instance, the Poulin references at the end of this paper) is a major contributor to current advances in PBPK/PT modeling. In Japan, Sugiyama and colleagues have used PBPK modeling with pharmaceuticals to establish in vivo model parameters from in vitro methods (cf., Iwatsubo et al., 1997). This high level of activity is a sign of the health of the field, the intense current interest in these methods, and the potential application in risk and safety assessment arenas.

3. PBPK/PT models in risk and safety assessments

Contemporary risk assessments for chemicals, illustrated in the proposed carcinogen guidelines developed by the US 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 (areas-under-the-curve). Dose measures can be based on parent chemical or metabolites; they could include consequences of interactions such as receptor binding, macromolecular adduct formation, or depletion of necessary biological molecules, such as glutathione (GSH). 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 dosimetry are qualitative, or at best semi-quantitative, concepts. They are implemented in a more quantitative manner in PK and PD models. The new US EPA cancer guidelines also talk about the use of biologically based dose response models 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 and 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 PBPK/TK predictions of target tissue dose metrics than on the basis of atmospheric exposure concentration or administered dose. Finally, these PBPK/PT have been helpful in assessing mechanisms of toxicity by providing a refined understanding of relationships of dose metrics with specific responses. Recent development and applications of PBPK/PT models with methylene chloride, vinyl chloride, organic esters, retinoic acid and dioxin, discussed briefly below, provide clear examples of the uses of these PBPK/PT models to support various extrapolations. In these examples, the emphasis is neither on the detailed descriptions of the compartmental structures of these models, nor on the steps required to provide accurate parameterization of the models. Instead, they focus on the breadth of risk assessments that have applied PBPK/TK approaches and the main characteristics that were modeled with these compounds.

3.1. Methylene chloride

The first application of a PBPK/PT model in a risk assessment was with CH$_2$Cl$_2$. A PBPK/PT model for CH$_2$Cl$_2$ was initially developed to explore causality between various dose metrics and carcinogenicity (Andersen et al., 1987). This solvent caused tumors in lung and liver in mice by inhalation at 2000 and 4000 ppm, but was not carcinogenic by the oral route. It is metabolized to carbon monoxide by oxidation and to carbon dioxide by a GSH-conjugation pathway. Each pathway has reactive compounds that are present transiently. Oxidation produces formyl chloride; GSH-conjugation produces chloromethylglutathione. Either of these two pathways could produce a potentially mutagenic metabolite. One difference is that the two pathways have different kinetic parameters. Oxidation is low capacity and high affinity. GSH-conjugation is high capacity with low affinity. The PBPK/PT 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 PBPK/PT model, it was possible to calculate the expected tissue exposures to the two reactive metabolites for different exposure conditions in liver and in lung for mice and humans.

The dose metrics chosen for analyzing the tumor responses were related to the integrated intensity of tissue exposure to reactive intermediates, i.e. the rate of metabolism through a specific pathway per volume of tissue per time. The carcinogenic responses in both tissues were closely correlated with the GSH-pathway metabolism, not with oxidation. In addition, the PBPK/PT model indicated that ingestion of CH$_2$Cl$_2$ drinking water because of slower intake and first-pass clearance in liver would produce very low tissue exposures to the GSH-pathway metabolites. Thus, it is not surprising based on target tissues doses of active toxicant that CH$_2$Cl$_2$ was not carcinogenic by the oral dose route. The work with CH$_2$Cl$_2$ also provided the first use of a PBPK/PT model for low-dose and interspecies 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, etc. in the model structure. The estimation from this dose metric based analysis was that the risks were actually lower than estimated by the 1985 US EPA default procedures by two-orders of magnitude (Fig. 3). The extrapolation assumed that mouse and human tissues would be equally responsive to equivalent tissue exposures to the reactive GSH-pathway intermediates. This PBPK/TK model has been cited and used in risk assessments by Health Canada (Health Canada, 1993) and by the Occupational Safety and Health Administration (OSHA) in the US (OSHA, 1997). The exercise with methylene chloride established a process for application of a PBPK/PT model in risk assessment that is still closely followed (Table 1). In addition, the PBPK/PT modeling spurred a variety of efforts to enhance variability and uncertainty analysis of the models and to conduct targeted research to confirm association of toxicity with GSH-pathway metabolites. The re-iteration of PBPK/PT modeling and targeted mechanistic studies was particularly useful in establishing the
GSH-pathway mode of action and increasing confidence in application of the PBPK model in risk assessment. This risk assessment also spurred a national Academy of Sciences workshop on PBPK methods in risk assessment that endorsed further development and application of these models with a broader suite of compounds (NRC, 1987).

3.2. 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$_2$Cl$_2$, the dose metric with VC was the production of the reactive metabolite with terms for consumption of the epoxide by detoxification pathways. Several groups had developed PBPK/PT models for VC (Chen and Blancato, 1989; Reitz et al., 1996; Clewell et al., 2001). These models provide estimates of VC-metabolite dose for different routes of administration and rodents and in humans. As with CH$_2$Cl$_2$, the PBPK/PT 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 PBPK/PT model derived tissue dose described below is from work of Clewell et al. (2001).

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 on the human risk estimate for lifetime exposure to 1 part per billion (ppb) were calculated on the basis of each of the sets of rodent bioassay data, using the linearized multi-stage model for cancer. 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; low-dose extrapolations were conducted based on these measures of tissue dose; and, a human PBPK/PT model was used to back calculate the exposure concentrations associated with a particular tissue dose metrics in humans. The range of human risks estimated from these rodent bioassays for male and female animals was 1.10–5.17/million exposed individuals/ppb. The corn oil gavage studies gave slightly higher risks 8.68 for males and 15.70 for females. High levels of corn oil in the diet likely acts 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 PBPK/PT model for vinyl chloride was run for human parameters for the human exposure scenario appropriate to each of the cancer cohorts. The resulting liver dose metrics were multiplied by the appropriate duration to obtain the cumulative internal liver dose of metabolites in human cohorts. 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 range of risk estimated in humans by this tissue dose-based analysis for three epidemiological cohorts (Fox and Collier, 1977; Jones et al., 1988; Simonato et al., 1991) ranged from 0.4 to 4.22 cases/million/ppb, a remarkably good agreement with the estimates from the animal bioassay. When the predicted human 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. In addition to providing a satisfactory explanation of potency across animal species and humans, the vinyl chloride PBPK/PT model was used for dose route extrapolations by using data from oral studies to assist in establishing inhalation reference concentrations (RfCs) for this compound (US EPA, 2000a).

3.3. Inhaled organic esters

A variety of organic esters, including VA and methylmethacrylate (MMA), cause nasal olfactory degeneration following inhalation exposures. PBPK/PT 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 RfCs (with both VA and MMA). The target tissue compartments in the ester models differ from the liver compartment with the models for CH$_2$Cl$_2$ and VCM. 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 reach 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 microns 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 tissue regions—the mucus, epithelial cell, and sub-mucosal regions. Each tissue compartment has clearance due to diffusion or metabolism. The sub-mucosal region is perfused with blood and chemical absorbed into this region can be carried away in the venous blood. Unlike the models for methylene chloride or vinyl chloride discussed earlier, the PBPK models for these reactive esters focused on airflow delivery rather than blood mediated delivery and the linkage of airflow, solubility and reactivity in determining tissue delivery from the airstream.

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) developed many of these parameters for a nasal PBPK/PT model. Plowchalk et al. (1997) measured esterase activities and their distribution in the nasal epithelium and sub-mucosal regions. The dose metrics calculated differed for these two esters. For MMA, the tissue dose metric was the amount metabolized (i.e. the amount of acid formed) per time in the total epithelial tissue compartments. For VA, the tissue dose metric was the pH changes predicted to occur in the uppermost epithelial tissue layer due to proton production from both production of acetic acid from VA hydrolysis and from metabolism of the acetaldehyde to acetic acid. For pH calculations with VA, the tissue dosimetry model included a proton pump that controls 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 US EPA RfC documentation (US EPA, 1994) tend to overestimate the tissue dose of ester metabolites expected in the human 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; Bogdanffy et al., 1999) where the model is highly non-linear due to a several potentially saturable processes, including two distinct esterase pathways, proton pumping, and the sequential arrangement of compartments within the nose. These nasal dosimetry models should play an important role in RfC estimations for a variety of chemicals of interest in the Integrated Risk Information System (IRIS) Pilot Project at the US EPA (www.epa.gov/iris/). Nasal epithelial toxicity is a very common response to during inhalation exposures in experimental animals and will form the critical effect for many RfC estimations in the future.

3.4. All-trans-retinoic acid

The first three examples discussed use of PBPK/PT 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 PBPK 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 PBPK model that tracked the blood and fetal compartment concentrations of ATRA and other active acid retinoids formed from ATRA. The other active retinoids are 4-oxo-RA, 13-Cis-RA, and 4-oxo-13-Cis-RA. The results of this safety assessment, (Clewell et al., 1997) are summarized here. This PBPK model was based on
a very broad range of PK data for retinoids in various species. It required parameterization for oxidation and isomerization reactions, along with a description of enterohepatic recirculation of glucuronide metabolites. Target tissues are the developing fetus at various times during gestation.

The dosimetry model calculated several dose metrics—primarily concentrations and areas-under-the-curve for ATRA or for total active retinoids in the fetal compartment. The metabolism and interconversion of the various active retinoids are complex. Multiple metabolic steps have to be included: oxidation, glucuronidation, isomerization in intestinal tissue, enterohepatic recirculation, etc. The data for analysis was large and diverse with dosing in various species by a variety of routes of administration. The diversity of the 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 re-analysis 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 a value near 10000 ng h/ml. Clinically 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 the fetus. 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 10000/0.5 or about 20000. The MOS for facial use only as advised in packaging inserts would be 10000/0.013 or about 770000. The PBPK/PT model was useful in this example in providing confidence that even in extreme abuse conditions in fertile women fetal exposures would be much lower than exposures associated with teratogenic responses.

3.5. 2,3,7,8-Tetrachlorodibenzo-p-dioxin—current modeling directions

A variety of hepatic tumor promoters induce cytochrome P450 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 the need to decipher the dose response behavior at low-doses and to understand the molecular processes that lead to induction in these cells. A surprising characteristic is that protein 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. The initial PBPK models for protein induction by TCDD, noted earlier in this paper, neglected the non-uniform induction (Leung et al., 1989a,b; Andersen et al., 1993; Kohn et al., 1993).

In a more recent PBPK/PT model for TCDD-mediated protein induction (Andersen et al., 1997), the liver was divided into 5 sub-compartments arranged serially from the periporal 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 the rate of gene transcription according to a Hill-equation relationship, as shown in the equation below. Here, $C$ represents the concentration of the Ah–TCDD complex binding to sites on DNA with affinity given by the dissociation constant $K_{\text{diss}}$. 

$$C = \frac{C_{\text{max}}}{1 + \left(\frac{C}{K_{\text{diss}}}\right)^n}$$
Rate of production

\[ \text{Maximal induced rate} \times \frac{C^n}{(C^n + K_{dna}^n)} \]

The analysis evaluated two factors: the Hill-coefficient required in the induction equations to capture the regional differences in induction and the difference in DNA-Ah receptor-TcDD dissociation constant \( K_{dna} \) between adjacent regions required 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 P450 1A1, and overall induction, required high \( n \) values (4–5) in each region and a threefold differences in binding affinity between adjacent regions.

With TCDD, the combination of the PBPK model and the requirement to simulate both a quantitative measure of induction and the distribution of induced cells within the liver produced a PBPK/PT model with interesting characteristics. The TCDD the PBPK-gene induction model had very steep responses in each zone and was highly non-linear responses in relation to low-dose extrapolation of the induction response. The earlier PBPK/PT models that ignored regional distribution predicted a more nearly linear extrapolation for the low-dose region. While not yet adopted for regulatory use, this regional PBPK/PT induction model does draw some interesting conclusions about low-dose responses for various tumor promoters that should be more carefully examined by specific experiments in vitro 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. A recent paper in Science (Xu et al., 2001) describes a transcriptional switch for a steroid hormone nuclear receptor-mediated by methylation of transcriptional co-factors. With TCDD, the basis of the observed switch for regional induction may also be associated with methylation of co-factors and histones that alter accessibility of groups of genes for transcriptional activation.

4. Discussion

In addition to the historical background for PBPK/PT models, this overview of the use of PBPK-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 evaluations of contributions from the two pathways with CH₂Cl₂ for carcinogenicity or in assessing the relationship of nasal toxicity with different dose metrics for VA. Another example with VC was in assessing the correspondence between risk projections from rodent and human exposures. Interpretative evaluations occur in applying estimated dose metrics to assess acceptable exposure levels for RfCs, cancer risk assessments, MOS’s, etc. In this use, the experimental exposures are first converted to internal dose metrics; the estimates of NOAELs or Benchmark Doses are then calculated in terms of these tissue dose metrics. Next, uncertainty factors are applied to reduce the target tissue dose metric and, finally, a human PBPK/TPT 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). Thirdly, mechanistic evaluations are intended to characterize the relationship of tissue dose, response and determine consistency with specific hypotheses regarding toxicity/biochemical responses. The evaluation of the Hill-coefficients and ‘switching’ phenomenon with TcDD exemplifies a quantitative evaluation of a hypothesis for mode of action with a PBPK/TK model.

Since the first proposal to use a PBPK/TK model in risk assessment with CH₂Cl₂ in 1987, the field of PBPK/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 US EPA’s RfC documentation explicitly includes routine application of interspecies differences in dosimetry in assessing RfCs (US EPA, 1994). The recent RfC documentation for VC in IRIS (US EPA, 2000a) specifically describes and uses a PBPK/PT model for standard setting.
and dose route extrapolations. A recent risk assessment with acrylic acid (Andersen et al., 2000) applies a PBPK 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 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 PBPK model is available to conduct extrapolations across dose-routes. Several case studies under the proposed cancer guidelines (US EPA, 1996), for example, those with formaldehyde (CIIT, 1999), chloroform (International Life Sciences Institute, 1977) and VA (Bogdanffy et al., 1999), base low-dose extrapolation on mode of action specific target tissue doses calculated with dosimetry models. In Chapter 8 of the US EPA’s dioxin reassessment (US EPA, 2000b), a variety of mechanistic protein induction models are evaluated to assess the predictions for low-dose risks for each of them. The discussion of these models includes a clear delineation of experiments that would be directly useful for establishing modes of action of the induction processes and for using the models to aid in risk assessment.

In closing, it seems appropriate to also note some important questions that arise in relation to uses of these models for health risk assessment. Here are four.

(1) Do current PBPK/PT modeling approaches follow good modeling practices? There have been several short papers related to good modeling practices (Yates, 1978; Andersen et al., 1995). The recommendations from such publications need to be re-visited to provide a more contemporary evaluation of the proper documentation of model structure and of model performance required in use of these models in health risk assessment. A consensus report on good modeling practices endorsed by multiple regulatory authorities could be helpful in standardizing model presentations and encouraging their use in risk assessments.

(2) Are estimates of ‘tissue dose’ derived from these various models plausible and reliable? This question becomes more important as the tissue dose metrics diverge substantially from directly measurable quantities, such as concentration or areas-under-a-curve—to rates of production of metabolites, pH changes, altered adduct or GSH levels in tissues. Continuing development in experimental methods for assessing tissue dose in vivo and in cells in vitro will improve the data available for assessing model validity for many substances.

(3) Are tissue dosimetry based extrapolations available from PBPK/PT models preferable to alternative default approaches? This question has bedeviled introduction of PBPK approaches in risk assessment since the methylene chloride risk assessment was completed. It is difficult to compare the PBPK approach with defaults that are not based on any specific understanding of modes of action and tissue dose metrics. With the PBPK approach, uncertainties in the model structure and knowledge of parameter values and their impact on risk estimates can be evaluated quantitatively. Similar evaluations are very difficult to interpret when applied to default approaches to risk assessment. It may be more important to carefully assess the ranges of risk possible from the PBPK approaches rather making inexact comparisons of PBPK modeling results with mechanistic, default approaches.

(4) How can investigators involved in developing PBPK/PT models in risk assessment most readily convey these models to regulatory scientists to aid in education, constructive dialog and improvement of current risk assessment interactions between regulated and regulatory communities? This last question is a practical issue in the process by which model developers aid end users of the technology. Advances here need to occur in determining the optimal way to convey a model, its results, and the extent to which the model has been tested and analyzed for uses in risk assessment. This area overlaps the good practices recommendation above. It also encompasses developing venues for education in both directions—from model developers about the process of modeling and from regulators to assure that the model development is properly suited to regulatory needs.
5. Summary

The past 15 years has seen the emergence of PBPK/PT 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 modeling disciplines. The stage is set for wider penetration of these approaches in the risk assessment process by a wide group of modeling practitioners throughout the world. The specific examples described in this review captured some of the promising areas of application of dosimetry models and should point the way towards other more novel applications of these modeling technologies in the near future. The final four questions are not intended as impediments to use of PBPK/PT applications in risk assessment. Rather, the solution to these cultural issues may accelerate acceptance of these modeling tools to both improve mechanistic studies in toxicology and integrate diverse data in current risk assessment practice.

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