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PBPK MODELLING OF INTER-INDIVIDUAL VARIABILITY IN THE PHARMACOKINETICS OF ENVIRONMENTAL CHEMICALS

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Abbreviations

AAG:	α_1-acid glycoprotein
ABCB1:	Adenosine triphosphate binding cassette member B1
ABCC2:	Adenosine triphosphate binding cassette member C2
ABCG2:	Adenosine triphosphate binding cassette member G2
ACAT model:	Advanced Compartmental Absorption and Transit model
ADAM model:	Advanced Dissolution, Absorption and Metabolism model
ADME:	Absorption, distribution, metabolism, excretion
AUC:	Area under the curve

BCRP:	Breast cancer resistance protein
CAT model:	Compartmental Absorption and Transit model
CYP:	Cytochrome P450
GFR:	Glomerular filtration rate
HPGL:	Hepatocellularity
IPA:	Isopropanol
MCMC:	Markov Chain Monte Carlo
MDR1:	Multi-drug resistance protein
MRP2:	Multidrug-resistance protein 2
PBPK:	Physiologically Based Pharmacokinetic Model
P-gp:	P-glycoprotein

ABSTRACT

Generic PBPK models, applicable to a large number of substances, coupled to parameter databases and QSAR modules, are now available for predictive modelling of inter-individual variability in the absorption, distribution, metabolism and excretion of environmental chemicals. When needed, Markov chain Monte Carlo methods and multilevel population models can be jointly used for a Bayesian calibration of a PBPK model, to improve our understanding of the determinants of population heterogeneity and differential susceptibility. This article reviews those developments and illustrates them with recent applications to environmentally relevant questions.

KEYWORDS

Drug-drug interactions, Markov chain Monte Carlo, Monte Carlo simulations, PBPK models, Pharmacokinetics, Population models, Susceptible populations, Toxic interactions, Toxicokinetics, Variably assessment.

1. INTRODUCTION

PBPK models are now commonly used in drug development and regulatory toxicology to predict the kinetics and metabolism of substances in the body, with a focus on the effective dose at the expected target site (Barton et al. 2007; Bouvier d'Yvoire et al. 2007; Edginton et al. 2008; Loizou et al. 2008).

The physiological basis of PBPK models makes them especially suited to explore, understand and predict the determinants of inter- or intra-individual variability in pharmacokinetics. Those translate into variability of target doses and can have direct consequences for therapeutic safety and the likelihood of toxicity, especially for compounds with narrow therapeutic windows or a steep dose-response for toxicity. Therefore, simulation of inter-individual variability has become an integral part of the assessment of pharmacokinetics in humans (Bois 2001; Rostami-Hodjegan and Tucker 2007). The mechanistic framework of PBPK models provides the capacity of predicting inter-individual variability in pharmacokinetics when the required information is adequately incorporated. This short review presents the state of the art on this question and illustrates the approach with two recent applications of the Simcyp software to environmentally relevant questions.

2. PBPK MODELING

When a chemical substance penetrates an animal body (following intentional administration or unintentional exposure), it is usually distributed to various tissues and organs by blood flow (Gerlowski and Jain 1983; Nestorov 2007). Following its distribution to tissues, the substance can bind to various proteins and receptors, undergo metabolism, or can be eliminated unchanged. The concentration *versus* time profiles of the xenobiotic in different tissues, or the amount of metabolites formed, are often used as surrogate markers of its internal dose or biological activity (Andersen 1995).

Mathematical models can be used to interpolate and extrapolate (predict) such concentration-time profiles from data. Reported models range from simple compartmental (Gibaldi and Perrier 1982) to very sophisticated (Jamei et al. 2009a; Luecke et al. 2008). PBPK models are evolved compartmental models which tend to use realistic biological descriptions of the determinants that regulate the disposition of drugs in the body (Andersen et al. 2005). Those models describe the body as a set of compartments corresponding to specific organs or tissues (*e.g.*, adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, and spleen, *etc.*). Between compartments, the transport of substances is dictated by various physiological flows (blood, bile, pulmonary ventilation, *etc.*) or by diffusion (Bois and Paxman 1992; Gerlowski and Jain 1983). Perfusion-rate-limited kinetics applies when the tissue membrane presents no barrier to distribution. Generally, this condition is likely to be met by small lipophilic substances. In contrast, permeability-rate kinetics applies when the distribution of the substance to a tissue is rate-limited by the drug's permeability across the tissue membrane. That condition is more common with polar compounds and large molecular structures. Consequently, the related PBPK models may exhibit different degrees of complexity. In the simplest and most commonly applied form (see Figure 1), each tissue is considered to be a well-stirred compartment in which the substance distribution is simply limited by blood flow. In such a model, any of the tissues can be a site of elimination. However, in Figure 1, it is assumed that the gut, liver and kidney are the only metabolising tissues and that excretion only happens in the kidney.

Building a PBPK model requires gathering a considerable amount of data which can be categorised in three groups: namely, the system's data (physiological, anatomical, biochemical data); drug-specific data; and the model structure, which refers to the arrangement of tissues and organs included in the model (Rowland et al. 2004). In a sense, PBPK modelling is an integrated systems approach to both understanding the pharmacokinetic behaviour of compounds and predicting concentration-time profiles in plasma and tissues. Additional details on PBPK modelling can be found elsewhere (Gerlowski and Jain 1983; Nestorov 2003; Rowland et al.

2004). Indeed, such a description of the body is approximate, if not rough, but a balance has to be found between precision (which implies complexity) and simplicity (for ease of use). Yet, the generic structure of a PBPK facilitates its application to any mammalian species as long as the related system data are used. Therefore, the same structural model can approximately be used for a human, a rat or a mouse.

The model structure can be described by a set of differential equations, with parameters representing blood flow rates, organ volumes *etc*., for which information is available in the published scientific literature or may be obtained *in vitro* (Parrott et al. 2005; Woodruff and Bois 1993). Numerical integration of that differential system computes the quantity and concentration of the drug considered in each compartment, as a function of time and exposure dose.

3. VARIABILITY *VS.* UNCERTAINTY

Inter-individual differences ("variability"), in the anatomical and physiological characteristics of humans or animal are glaring and a common experience. Such differences affect, for example, organ volumes and blood flow and translate quite naturally into variability in the pharmacokinetics of drugs and chemical substances from one individual to the next. Since pharmacokinetics determine, in part, effective dose and ensuing effects, pharmacokinetic variability has an impact on individual susceptibility. Two major, complementary, modelling approaches have been developed to understand, evaluate and predict that variability: *A priori* and *a posteriori* modelling.

A priori (bottom-up), purely predictive, modelling of variability can be undertaken either through deterministic descriptions of the determinants of variability or through stochastic simulations (Monte Carlo methods) (Bois et al. 1990; Clewell and Andersen 1996; Clewell et al. 2004; Rostami-Hodjegan and Tucker 2007; Willmann et al. 2009; Willmann et al. 2007). Both approaches can also be combined. Deterministic modelling motivations stem, partly, from the

fact that some of the differences between individuals are due to growth and other age-related changes, or sex, *etc.* Such changes can be explicitly modelled in life-time PBPK models (Clewell et al. 2004; Edginton et al. 2006). Monte Carlo methods acknowledge (some may say: hide intellectual laziness behind) the fact that part of the differences between individuals seem to occur by chance or cannot be ascribed simply to easily modelled determinants such as age *etc.* In that case, those differences are considered as purely random and parameter values are described by statistical distributions, rather than point estimates or simple functions of time, sex or other covariates.

A posteriori (top-down) modelling of inter-individual variability is data-based, and was originally developed in pharmacokinetics, where human data were routinely generated through clinical trials. The aim here is to assess, for example, how across-subject variability of blood concentration for a substance can be explained by variability in renal excretion rate, bioavailability, metabolic rate, *etc.* That is best achieved through inverse simulation in the framework of multilevel statistical models ("population pharmacokinetic models") (Bois 2001; Sheiner 1984). That approach was first used with classical (minimal) compartmental models (Beal and Sheiner 1980, 1982), but the added ingredients of MCMC simulations and Bayesian inference make it amenable to the treatment of PBPK models (Bois et al. 1996a; Bois et al. 1996b; Gelman et al. 1996). After unrolling the determinants of variability, forward, predictive, simulations can be performed to assess their impact in various exposure and risk or clinical treatment scenarios.

Uncertainty is different from variability, although their effects may be confounded and compounded. Uncertainty is essentially due to lack of knowledge and may have various sources. For example, measurements are made only with finite precision; so when data are used to fit toxicokinetic or toxicodynamic models, there is always some uncertainty ("noise") about the estimated parameters values. Also, studying a limited population sample introduces an element

of randomness when attempting to extrapolate the results to the whole population. Finally, our inability to describe or model precisely a system may result from our lack of understanding and simplifications or misspecifications of the fitted models translate into parametric uncertainty (Bois 2001; Bois and Diack 2005). While variability is by essence irreducible and unavoidable, since it is a state of Nature, uncertainty can be reduced by new experiments, or by a better understanding of the biology and better models. In a further twist, the fact that uncertainty is almost always present hampers a precise assessment of variability. Statistical "population" models are able to disentangle partly uncertainty from variability. These models will be reviewed in section 7, below.

4. *A PRIORI* PREDICTION OF VARIABILITY USING PBPK MODELING

Predictive variability assessment with PBPK models proceeds nowadays with a combination of mechanism and data based refinements of the model structure (*e.g.*, using age-dependent compartment volumes) (Clewell et al. 2004; Luecke et al. 2008) and stochastic modelling of the remaining random components of inter-individual differences. Numerical Monte Carlo methods (Clewell et al. 1999; Price et al. 2003; Spear et al. 1991; Woodruff et al. 1992), eventually hierarchical (Bois 1999; Bois et al. 1996a; Bois et al. 1996b), are the most widely used for the latter task. Those are best suited for complex models with many parameters. The basic assumption made by Monte Carlo simulations is that the randomness of the model state variables (*e.g.*, blood concentration) is simply due to the randomness in the model parameter values. It is then enough to define statistical distributions for the parameters supposed to vary randomly in the population, and to sample the model parameter values from those distributions. The model is then run with the needed inputs and its outputs of interest are recorded. That sampling-running step constitutes a basic Monte Carlo iteration. As many iterations as needed can be performed to

characterise precisely the statistical distribution of the recorded outputs. Additional details on Monte Carlo methods can be found in Ripley (1987).

Early attempts to use Monte Carlo methods and to simulate pharmacokinetic behaviour in "virtual populations" date back to the mid-1980s. Jackson *et al.* assessed the robustness of different experimental *in vivo* indices to detect and display genetic polymorphisms in human drug-metabolising activity (Jackson and Tucker 1990; Jackson et al. 1986). Monte Carlo analyses of PBPK models were also described at that time (Bois et al. 1990; Portier and Kaplan 1989). These simulations were later expanded to show the effect of variability in absorption, distribution, metabolism and excretion (ADME) parameters on the power of single time point estimates for the assessment of metabolic activity (Jackson et al. 1991), power analyses of bioequivalence measures (Bois et al. 1994a, b), the differentiation of parent drug and metabolite data in bioequivalence assessment (Rostami-Hodjegan et al. 1994), the discriminatory power of different indices of *in vivo* enzyme activity and the optimisation of sampling to assess such activity (Rostami-Hodjegan et al. 1996). Coupled with Monte Carlo methods, PBPK modelling has been used to assess the quantitative impact of physiological and environmental factors on human variability in toxicokinetics and pharmacokinetics in other publications (Bois et al. 1991; Clewell and Andersen 1996; Jamei et al. 2009a; Nestorov 2001; Sato 1991).

5. SOURCES OF VARIABILITY IN PHARMACOKINETICS

The overall inter-individual variability in pharmacokinetics can be simulated by considering the variability in key system parameters in PBPK modelling (Jamei et al. 2009a) (see Figure 2 for examples of clearance covariates). Details on the prediction of inter-individual variability in pharmacokinetics are discussed in detail below.

5.1. ABSORPTION

There are many routes for xenobiotics to enter into the body which can generally be divided into topical, enteral and parenteral categories. Some of these routes (oral, infusion, intravenous, intramuscular, transdermal and inhalation) are routinely used to administrate drugs. The oral, transdermal and inhalation routes, which are most relevant to environmental exposures, are briefly discussed in this section.

Oral administration is the most common and convenient route of drug administration. Yet, it is often associated with low bioavailability and high inter-individual variability. Oral bioavailability (F_{oral}) is defined as:

$$F_{oral} = f_a \cdot F_G \cdot F_H \tag{1}$$

where f_a is the net fraction of dose absorbed from the intestinal tract, F_G is the fraction of dose that escapes intestinal first-pass metabolism in the enterocytes, and F_H is the fraction of dose that escapes hepatic first-pass metabolism. f_a is discussed in this section and F_G and F_H will be discussed later.

Various factors can affect oral drug absorption. They can be divided into two categories: system (physiological and biological) factors and drug-related (physicochemical and pharmaceutical) factors. These factors can all contribute to the overall rate and extent of absorption, but mainly the system factors determine the inter-individual variability in absorption.

5.1.1. Oral Absorption

Key physiochemical properties, such as solubility and permeability are used in empirical methods to estimate the absorption potential of a drug (Artursson and Karlsson 1991; Dressman et al. 1985). Physiological models, such as the CAT model, have been developed to simulate mechanistically drug absorption (Yu and Amidon 1999). The CAT model has been further

developed into the ACAT (Agoram et al. 2001) and the ADAM (Jamei et al. 2009b) models, which are respectively implemented within software Gastroplus (Simulation Plus Inc, California, USA, http://www.simulationsplus.com) and Simcyp Population-based ADME Simulator (Simcyp Ltd, Sheffield, UK, http://www.simcyp.com). In brief, these absorption models consist of physiologically based compartments corresponding to different segments of the gastrointestinal tract.

Gastric Emptying Time

The residence time of a drug in the stomach is an important factor determining the initiation of oral drug absorption. Variability in gastric emptying rates results in variable absorption rates and sometimes even variable absorption extents.

Intestinal Residence Time

Intestinal residence time can greatly affect oral drug absorption; particularly for drugs with low permeability. Yu and co-workers analyzed published data for low intestinal residence time in over 400 subjects and reported a mean value of 199 min with ranges of about 1 and 6 hours (Yu et al. 1996). Intestinal residence time appears to be relatively less dependent upon the nature of the dosage form (liquid *vs.* solid) than gastric residence time (Davis et al. 1986). The presence of food appears not to influence intestinal transit (Davis et al. 1986; Fadda et al. 2009).

Gastrointestinal pH

The regional pH in the gastrointestinal tract can influence drug solubility and hence the dissolution of solid dosage forms. Gastrointestinal pH may also affect drug permeability by influencing the balance between ionised and non-ionised moieties. Fallingborg and co-workers measured pH profiles along the gastrointestinal tract in 39 healthy volunteers and observed a

range of values up to two pH units at the same site in different subjects (Fallingborg et al. 1989). In addition, the presence of food in the gastrointestinal tract can raise the pH in the stomach and the proximal part of the small intestine, due to the buffering capacity of proteins.

Transporters

Various transporters are expressed in the apical and basolateral membranes of intestinal epithelial cells (Hilgendorf et al. 2007; Koepsell 1998; Koepsell et al. 2007; Murakami and Takano 2008; Tsuji and Tamai 1996). Much attention has been given to the efflux transporters (*e.g.*, P-gp, MDR1 (ABCB1), MRP2 (ABCC2) and BCRP (ABCG2)) at the apical (brush-border) membrane of the intestine, as they can limit the intestinal absorption of drugs administered orally. Von Richter et al. (2004) measured P-gp in the human small intestine and reported a marked inter-individual variation in the intestinal P-gp expression. Additional data were reported by several later publications (Canaparo et al. 2007; Mouly and Paine 2003). Available data demonstrate that the expression levels of transporters vary along the gastrointestinal tract. Mouly and Paine (2003) found that relative P-gp levels increase progressively from the proximal to distal region of the small intestine. Other intestinal transporters may or may not follow the same pattern.

5.1.2. Inhalation

The large absorptive surface area, limited metabolic enzyme activity and active transporters in the pulmonary system make inhalation a favourable delivery strategy for systemic drugs with low bioavailability. In addition, many chemicals present in the air can get into body via the respiratory system. As for other routes of administration, the absorption kinetics in the lung tissues depend on both drug and system related parameters. Although inhalation is an established delivery strategy, the relationship between drug physicochemical properties and drug absorption kinetics in the lung has not been extensively investigated. In contrast to oral drug absorption

little attention has been devoted to the question of how to optimize local drug absorption and retention in the lung (Yu and Rosania 2010). The state of the art on this question is somewhat more advanced for occupational exposures to volatile substances (Johanson 1990; Löf and Johanson 1998). Different aspects of lung physiology and formulation composition that influence the systemic delivery of inhaled therapeutics and recent advancement in inhaled drug delivery are reviewed in Patton and Byron (2007).

5.1.2. Dermal Absorption

The skin, the largest interface between the body and the environment, protects our body against chemical, physical, and microbial injury, loss of water, and other endogenous substances. It is also involved in the thermoregulation of the body and serves as an excretory organ (Schaefer et al. 2008). Understanding skin absorption processes enables us to assess the safety aspects of chemicals, xenobiotics, and cosmetic formulations as well as optimally utilizing dermal drug delivery. Permeation of drug molecules across the skin occurs by passive diffusion according to the activity gradient (Cleek and Bunge 1993; Krüse et al. 2007; McCarley and Bunge 2001; Potts and Guy 1992). The outer skin layer, stratum corneum, forms a rate-controlling barrier for diffusion of most compounds. The predominant diffusional path for a molecule crossing the stratum corneum appears to be intercellular (Hadgraft and Guty 2002). However this path is not exclusive and probably most molecules will pass through the stratum corneum by a combination of intercellular lipid domains, transcellular route and via the appendages (hair follicles, *etc.*) (Farahmand and Maibach 2009b).

Farahmand *et al.* investigated dermato-pharmacokinetic parameters of 12 transdermal patches and concluded that the serum concentration profile for transdermal therapeutic systems was affected by the physiological parameters, drug absorption and elimination. Therefore, in order to understand the variability in serum concentration it is necessary to take into account variability of each process involved (Farahmand and Maibach 2009a).

5.2 DISTRIBUTION

The distribution process refers to the reversible transfer of drug from one location to another within the body. Factors that determine the distribution pattern of a drug with time include delivery of drug to tissue by blood, ability to cross tissue membranes, binding within blood and tissues, partitioning into fat, and tissue uptake (Rowland and Tozer 1995). The traditional description of the volume of distribution at steady state (V_{ss}) corresponds to the sum of the products of each tissue to plasma partition coefficient ($K_{p:t}$) and the respective tissue volume in addition to the plasma volume (Sawada et al. 1984):

$$V_{ss} = V_p + V_e \times (E:P) + \sum V_t \times K_{p:t} \qquad (2)$$

where V_p, V_e and V_t are the volumes of plasma, erythrocyte and tissue, respectively, $E:P$ is erythrocyte-to-plasma coefficient. Physiological factors affecting drug distribution include tissue volumes, tissue composition, blood perfusion rates to the tissues, plasma protein concentrations, hematocrit, and the expression of transporter proteins. Drug-specific factors determining the distribution behaviour of a drug include its ionisation, ability to cross membranes, bind to plasma proteins, partition into red blood cells and fat, and its specific affinity to influx or efflux transporter proteins.

Direct determination of $K_{p:t}$ usually involves intravenous constant infusions to animals followed by an extraction and quantification of drugs from tissue homogenates (Lin et al. 1982a, b; Sawada et al. 1984), which is costly and time consuming. It is, therefore, of interest to predict $K_{p:t}$ values without conducting *in vivo* animal studies.

Several mechanistic equations have been proposed to predict the tissue affinities of volatile organic compounds (Fiserova-Bergerova 1983; Fiserova-Bergerova and Diaz 1986; Fiserova-Bergerova et al. 1980). Poulin and co-workers also developed mechanistic equations to predict the affinity of drugs for various tissues and organs (Poulin et al. 2001; Poulin and Theil 2000)

and subsequently V_{ss} predictions (Poulin and Theil 2002a) using Eq. 2. The species-specific tissue composition parameters can be found in the literature and have been summarised by Poulin and Theil (2002b). Corrections on these equations were later made by Berezhkovskiy (2004). More recently, Rodgers and co-workers extended and improved these equations by considering drug ionisation and incorporating more details on drug distribution inside tissues (Rodgers et al. 2005; Rodgers and Rowland 2006). Briefly, those equations are based on the assumption that all drugs will dissolve in intra- and extracellular tissue water and partition into the neutral lipids and neutral phospholipids located within tissue cells.

5.2.1 Tissue Volumes and Tissue Blood Flows

Tissue volumes and blood flows are essential components of a PBPK model. Early publications reported representative physiological parameters values but did not indicate the biological variability associated with those data (Davies and Morris 1993; Williams and Leggett 1989). Inter-individual variability on tissue volumes and tissue blood flows has been reported by later publications (de la Grandmaison et al. 2001; Price et al. 2003).

5.2.2 Tissue Composition

Hematocrit refers to the percentage of total blood volume composed of red blood cells. It is influenced by factors including age, sex, seasonal influence, and habits of physical activity (Morse et al. 1947a; Morse et al. 1947b; Thirup 2003). Compared with men, women on average have lower hematocrit. Hematocrit ranges between 40%−54% in males and 38%−47% in females.

Drug protein binding is the reversible interaction of drugs with plasma proteins. The extent of protein binding is a function of drug and protein concentrations, the affinity constant for the drug-protein binding and the number of protein binding sites (Grandison and Boudinot 2000).

The major drug binding proteins in plasma are: albumin, AAG and lipoproteins. Albumin levels are generally decreased with age, whereas AAG levels are not significantly affected by age.

5.2.3 Transporters

Numerous drug transporters are found on the membranes of various tissues. These transporters can influence drug distribution into the tissues, particularly for drugs with low passive permeability. There is now increasing evidence to suggest that transporters may affect the volumes of distribution of certain drugs (Grover and Benet 2009). For most drugs, however, transporters may not significantly influence the volume of distribution, but may still influence the local kinetics in certain tissues (e.g., brain, liver, etc) and cause pharmacological or toxicological consequences. Polymorphism has been identified in transporters, as reviewed by Ho and Kim (2005).

5.3 METABOLISM

Drug metabolism reactions are generally grouped into 2 phases. Phase I metabolism includes oxidation, reduction, hydrolysis and hydration reactions. Phase II reactions use an endogenous compound, such as glucuronic acid, glutathione, or sulphate, for conjugation to the drug or its phase I-derived metabolite to produce a more polar end product that can be more readily excreted in bile or urine.

Although drug metabolism can take place in many organs, the liver has been long-recognised as the major site of metabolism for most drugs. More recently, the role of gut metabolism in first-pass metabolism has been increasingly recognised. The intestinal tissue is endowed with phase I and II enzymes, although at lower levels than those for the liver (Pang 2003). Several CYP enzymes have been detected in the human small intestine, including CYP1A2, CYP2D6, CYP2E1, CYP2C8, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 (Paine et al. 2006). Among

them, CYP3A4 is the most prominent enzyme present in the human intestine (Paine et al. 2006; Paine et al. 1997).

5.3.1 Hepatic Metabolism

Rane *et al.* (1977) successfully predicted *in vivo* hepatic metabolic clearance in rats based on *in vitro* data obtained from rat liver microsomes, taking into consideration the hepatic blood flow rate and the unbound fraction in blood. Since then, significant progresses have been achieved on predicting human hepatic metabolic clearance from a variety of *in vitro* systems, such as human liver microsomes, recombinant enzymes, and hepatocytes (Galetin et al. 2004; Houston 1994; Howgate et al. 2006; Iwatsubo et al. 1997; Obach 1999; Riley et al. 2005).

The unbound total hepatic intrinsic clearance ($CLu_{int,H}$) can be extrapolated from *in vitro* clearance determined in a variety of *in vitro* systems using scaling factors as described in Barter (2007) and according to the procedure described by Rostami-Hodjegan and Tucker (2007):

Recombinantly expressed enzymes:

$$\text{CLu}_{int,H} = \left[\sum_{j=1}^{n} \left(\sum_{i=1}^{n} ISEF_{ji} \times \frac{V\max_i(rhEnz_i) \times Enz_i\, abundance}{Km_i(rhEnZ_i)} \right) \right] \times MPPGL \times Liver\_weight \qquad (3)$$

where there are *i* metabolic pathways for each of *j* enzymes; '*rh*' indicates recombinantly expressed enzyme; *Vmax* is the maximum rate of metabolism by an individual enzyme; *Km* is the Michaelis constant; *MPPGL* is the amount of microsomal protein per gram of liver; and *ISEF* is a scaling factor that compensates for any difference in the activity per unit of enzyme between recombinant systems and hepatic enzymes (Proctor et al. 2004).

Human liver microsomes:

$$\text{CLu}_{int,H} = \text{CLu}_{int}(per\_mg\_microsomes) \times MPPGL \times Liver\_weight \qquad (4)$$

Human hepatocytes:

$$\mathrm{CLu}_{\mathrm{int,H}} = \mathrm{CLu}_{\mathrm{int}}(per\_millions\_hepatocytes) \times HPGL \times Liver\_weight \tag{5}$$

where HPGL refers to hepatocellularity (millions of hepatocytes per gram of liver).

$CLu_{int,H}$ is then combined with other determinants to obtain total hepatic intrinsic clearance, using a liver model. Several hepatic clearance models have been developed to quantify the effects of hepatic blood flow, fraction unbound in blood, and hepatic intrinsic clearance on hepatic clearance (Wilkinson 1987). Among these models the well-stirred model (Eqs. 6 and 7) has been widely used for its mathematical simplicity and practicality, as shown below:

$$CL_{H,B} = \frac{Q_{H,B} \cdot fu_B \cdot CLu_{\mathrm{int},H}}{Q_{H,B} + fu_B \cdot CLu_{\mathrm{int},H}} \tag{6}$$

$$F_H = \frac{Q_{H,B}}{Q_{H,B} + fu_B \cdot CLu_{\mathrm{int},H}} \tag{7}$$

where $CL_{H,B}$ is hepatic drug clearance based on whole blood drug concentration, $Q_{H,B}$ is hepatic blood flow, fu_B is the free fraction of drug in blood.

As indicated by Eq. 6, inter-individual variability in $CL_{H,B}$ is influenced by the variability in three key parameters: $Q_{H,B}$, fu_B and $CLu_{int,H}$.

5.3.2 Gut Metabolism

An operational model has been developed to predict first-pass metabolism in the gut. The "Q_{Gut}" model (Eq. 8) (Rostami-Hodjegan and Tucker 2002; Yang et al. 2007; Yang et al. 2001) retains the form of the "well-stirred" model but the flow term (Q_{Gut}) is a hybrid of both permeability through the enterocyte membrane and villous blood flow.

$$F_G = \frac{Q_{Gut}}{Q_{Gut} + fu_G \cdot CLu_{\mathrm{int},G}} \tag{8}$$

where F_G is intestinal availability (fraction of dose that escapes intestinal first-pass metabolism in the enterocytes), fu_G is the fraction of drug unbound in the enterocyte and its value is close to

1 in most cases (Yang et al. 2007), $CLu_{int,G}$ is the unbound total gut intrinsic clearance, and Q_{Gut} is a hybrid of both permeability through the enterocyte membrane and villous blood flow:

$$Q_{Gut} = \frac{Q_{villi} \cdot CL_{perm}}{Q_{villi} + CL_{perm}} \tag{9}$$

where CL_{perm} is a clearance term defining permeability through the enterocyte and Q_{villi} is villous blood flow (Yang et al. 2007).

5.4 EXCRETION

Excretion is the irreversible loss of the chemically unchanged drug. For most drugs, excretion occurs predominantly via the kidneys. However, some drugs and their metabolites are extensively excreted via the bile. Drug excretion can also happen via saliva, sweat, breast milk, and lungs, although their contributions to overall drug elimination are often small.

5.4.1. Renal Excretion

The kidney is the major site of drug excretion. Net renal drug excretion is a combination of three processes – glomerular filtration, tubular secretion and tubular reabsorption, as described by Eq. 10. Glomerular filtration of a drug is a passive process that is dependent upon the unbound fraction of a drug in plasma (*fu*) and renal blood flow available for filtration, as described by Eq. 11. Tubular secretion occurs predominantly in the proximal tubule, and is mediated by several families of transporters. Tubular reabsorption of a drug can be a passive or an active transport process. Passive reabsorption may occur throughout the nephron. Active reabsorption occurs in the proximal tubule and, similar to tubular secretion, is energy-dependent, saturable, stereospecific and also likely to be associated with competitive drug interactions (Tett et al. 2003).

$$\text{Rate of Excretion} = (\text{Rate of Filtration} + \text{Rate of Active Secretion})(1 - F_R) \tag{10}$$

$$\text{Rate of Filtration} = fu \times GFR \times C \qquad (11)$$

where F_R is the fraction of drug reabsorbed from tubule lumen; fu is the fraction unbound in plasma; GFR is the glomerular filtration rate; and C is drug concentration in plasma.

The primary determinants of renal excretion include renal blood flow, plasma protein binding, urine flow, urine pH, and renal transporters (Garrett 1978; Tucker 1981).

Renal transporters play key roles in the secretion and reabsorption of many drugs and can significantly contribute to the variability in renal excretion of these compounds (Lee and Kim 2004). Organic anion and organic cation transport systems are two major drug transport systems in the human kidney (Dresser et al. 2001), and the effects of genetic variations in transporters on renal clearance have been investigated recently (Wang et al. 2008).

5.4.2 Biliary Excretion

Biliary excretion is one of the primary elimination routes for xenobiotics and the conjugate metabolites (Arias et al. 1993). Biliary excretion requires active secretory transport because drugs are transported across the biliary epithelium against a concentration gradient. Often drugs excreted into the bile undergo some degree of reabsorption along the intestine (enterohepatic circulation).

Ghibellini and co-workers used *in vitro* data obtained from sandwich-cultured human hepatocytes to predict the biliary clearance for three drugs, and the predicted values were significantly lower than *in vivo* data (Ghibellini et al. 2007). Biliary excretion is mediated by transporters in the canalicular membrane. Therefore, genetic variation in these transporters contributes to the inter-individual variability in biliary excretion. Several recent reviews have summarised the key transporters involved in hepatobiliary disposition of drugs (Chandra and Brouwer 2004; Ghibellini et al. 2006).

5.5 CO-EXPOSURES

Part of the variability in metabolism observed in humans may also be due to uncontrolled co-exposures to naturally occurring food-borne substances, environmental contaminants, therapeutic drugs, or chemical substances in the workplace. Drug-drug interactions are a well-known problem falling under that umbrella (Jamei et al. 2009a; Rostami-Hodjegan and Tucker 2007), but its generalisation to many-substance exposure remains to be better explored. The necessary tools are becoming available under the auspices of systems biology (Bois 2010).

6. EXAMPLES OF APPLICATION OF *A PRIORI* MODELLING TO CHEMICAL RISK ASSESSMENT

Risk assessments are performed to estimate the conditions under which individuals or populations may be harmed by exposure to environmental or occupational chemicals. In the absence of quantitative data in the human, this process is often dependent upon the use of animal and *in vitro* data to estimate human response. To reduce the uncertainty inherent in such extrapolations, there has been considerable interest in the development of PBPK models of toxic chemicals for application in quantitative risk assessments. PBPK models are effective tools for integrating internal dose assessment with diverse dose-response and mechanistic data in order to more accurately predict human risk (Andersen et al. 1987). 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 inter-individual 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).

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* (Johanson et al. 1999). 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 part (the variability in each of the pharmacokinetic factors). 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; Price et al. 2003).

It is important at this point to remember the distinction made above between uncertainty and variability. Early attempts to distinguish the contributions of uncertainty and variability can be found in Bogen and Spear (1987) or Allen *et al.* (1996). Several studies have attempted to estimate the impact of parameter variability in PBPK models on risk assessment predictions using the Monte Carlo approach (Allen et al. 1996; Clewell and Andersen 1996; Clewell et al. 1999; Clewell and Jarnot 1994). As will be discussed in the next section, the use of a hierarchical Bayesian approach and Markov chain Monte Carlo simulations makes it possible to refine prior estimates of parameter variability on the basis of experimental data. The hierarchical Bayesian

approach is increasingly being used to characterize both the uncertainty and variability in PBPK model predictions (Bois 2000; Gelman et al. 1996; Hack et al. 2006; Jonsson et al. 2001b; Jonsson and Johanson 2001a; Qiu et al. 2010).

It is useful in that context 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. The following examples illustrate the application of PBPK models to inform population variability of the three types described above.

Example 1: Population Variability

Acceptable exposures to environmental contaminants are typically defined using a single value, such as the USEPA's Reference Dose (RfD), which represents a daily ingestion rate considered to be without harm for most individuals. In the case of methylmercury, a PBPK model was used in a Monte Carlo analysis to provide information on the distribution of acceptable ingestion rates across the population (Clewell et al. 2000; Clewell et al. 1999). That is in contrast to the regulatory approach based on a single point estimate obtained using conservative assumptions. In that analysis, the maternal hair concentration associated with neurological effects in the offspring from an epidemiological study was converted to an expected distribution of daily ingestion rates across a population of U.S. women of childbearing age. The resulting distribution

of acceptable daily ingestion rates (RfDs) ranged from approximately 0.3 to 1.1 μg/kg/day, with a population median (50th percentile) of 0.5 μg/kg/day. This population distribution was used to inform risk-benefit analysis for alternative risk management options for contaminated sediment. In essence, the question here was: "How conservative are default conservative assumptions?". The answer, given by Monte Carlo derived uncertainty and variability estimates, was in that case: "Very conservative".

Example 2: Age-Dependent Variability

The following example illustrates the use of PBPK modelling to investigate the impact of pharmacokinetic variability on risk 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 affect 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. In the age-dependent model, all physiological and biochemical parameters change with time based on data from the literature (Clewell et al. 2004).

Figure 3 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 (Clewell et al. 2004). 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. Obviously, these are only model predictions, but while waiting for a real-life epidemiological validation (which would be very costly and difficult to perform), they form a reasonable and transparent basis for immediate decision making.

Example 3: Genetic Polymorphism

The next example demonstrates the use of PBPK modelling, 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. The PBPK model used in the analysis (Gearhart et al. 1993) 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 4 displays the predicted distribution for the time-integrated (area under the curve) blood concentrations of paraoxon (mg-hr/L) across the sensitive population (dark bars), as compared to the "normal" population, following exposure to parathion at a dose of 0.033 mg/kg (Gentry et al. 2002). The impact of genetic polymorphism is strongly dampened by rate-limiting pharmacokinetic effects, similarly to what was observed for methyl chloride (Johanson et al. 1999). The calculation performed here lead to internal dose levels very different from those which would be obtained using naive "all or nothing" guessed estimates based solely on qualitative genotype considerations.

7. *A POSTERIORI* ESTIMATION OF VARIABILITY USING BAYESIAN PBPK MODELING

Purely predictive modelling, as described above, can be cross-validated by confronting its predictions (*e.g.*, for plasma concentration of a substance) to data obtained of a sample of individuals. In the ideal case, data and predictions agree on average and in terms of variability

and no further model refinement is attempted. Yet, quite often, the predictions are less than "perfect" and may even be really poor (but publication bias reduces the visibility of that case). That may be the occasion to improve the model through some calibration or data integration procedure and learn something about the true determinants of variability in a population (Wakefield and Bennett 1996). However, some care should be taken to properly disentangle uncertainty from variability in that calibration process, while retaining the prior physiological knowledge afforded by PBPK modelling (Gelman et al. 1996). The "naive" approach consists in fitting individual subjects' data separately, collecting the resulting individual parameter estimates and forming their average *etc*. That approach does not work and is actually incorrect if very precise parameter estimates cannot be obtained from the data (Beal and Sheiner 1982; Smith and Wakefield 1994). For ethical, feasibility or cost reasons, the data on individuals tend to be sparse in clinical pharmacokinetics or toxicokinetics. Such data usually lead to fairly uncertain parameter estimates and the so-called population approaches should then be used (Beal and Sheiner 1980).

Population models, or multilevel models, were first introduced in the context of pharmacokinetic studies for drug development and evaluation (Sheiner 1984). Their objective is to obtain, from data on individuals, a quantitative description of the variability of the kinetics of a compound within a large population. The same structural (*e.g.*, PBPK) model is used describe the data for each subject, and that the model parameters differ randomly between subjects (see Figure 5) (Bois et al. 1996a; Bois et al. 1996b). Such randomness characterises variability can be described by a multivariate probability distribution. In population models, information of each subject is reinforced by "borrowing strength" from the other subjects' data and the overall estimation process is improved. Individuals' metabolic clearance, for example, can be assumed to be log-normally distributed around a "population mean", with a "population variance" which measures variability in the population. The population means and variances (one for each kinetic parameter supposed to vary between subjects) are aptly named "population" parameters. They

are estimated during the model calibration (data fitting) together with the parameters of each subject (Bois 2001).

A number of methods are available to calibrate population models (Beal and Sheiner 1982; Davidian and Gallant 1992; Mallet et al. 1988; Racine-Poon and Smith 1990). Bayesian approaches have emerged as the best suited for PBPK models, given the large amount of prior information they require (Bernillon and Bois 2000; Gelman et al. 1996). But as we have seen above, a large amount of that information is already encoded as prior distributions, and updating those distributions with system's level data, is simply a matter of using Bayesian numerical methods such as Markov chain Monte Carlo simulations (Gelman and Rubin 1996).

A number of applications of posterior Bayesian PBPK modelling have been published: on benzene (Bois et al. 1996b), butadiene (Mezzetti et al. 2003), carbaryl (Nong et al. 2008), chloroform (Lyons et al. 2008), dichloromethane (Bois 1999; David et al. 2006; Johanson et al. 1999; Jonsson et al. 2001b; Jonsson and Johanson 2001a, 2003; Marino et al. 2006; Marino and Starr 2007), methyl chloride (Jonsson et al. 2001a), methyl mercury (Allen et al. 2007), nanoparticles (Péry et al. 2009), tetrachloroethylene (Bois et al. 1996a; Chiu and Bois 2006; Covington et al. 2007), toluene (Jonsson and Johanson 2001b; Vicini et al. 1999), and trichloroethylene (Bois 2000). Extension to questions of optimal design (Bois et al. 1999), medical image analysis (Brochot et al. 2006), or exposure reconstruction (Allen et al. 2007) have also been proposed.

8. CONCLUSIONS

The state of the art on PBPK modelling of inter-individual variability has advanced to the point of being a mainstream commercial activity for drug development. We have shown how the concepts and tools now available (generic PBPK models, applicable to many substances, coupled to databases of parameter distributions and QSAR models; MCMC software routines for

Bayesian data integration) can foster predictive toxicokinetics for environmental or occupational contaminants. Computation time is not an issue anymore, and the approach is being extended to toxicodynamics through the use of biology-motivated effect models, toward a true predictive toxicology applicable to very large number of chemicals.

9. ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1: Structure of a generic PBPK model of the mammalian body.

Figure 2: The inter-correlation of covariates affecting drug clearance.

Figure 3: Blood concentrations of isopropanol (IPA) and its metabolite acetone as a function of age for continuous inhalation exposure at 1 ppb.

Figure 4: Paraoxon AUC distribution for the sensitive population compared to the AUC distribution for the "normal" population for a parathion dose of 0.033 mg/kg.

Figure 5: Graphical representation of a toxicokinetic population model. Unknown quantities are in circles, known quantities in squares. At the individual level, exposure (**E**), time (**t**) and specific parameters (θ) condition the data (**y**). The structural PBPK model, f, links **E**, **t**, θ and **y**. Individual parameter values are randomly distributed in the population with population means μ and variances $\sum^2$. Residual errors (measurement errors, modelling errors *etc*.) are lumped in the variance term σ^2.
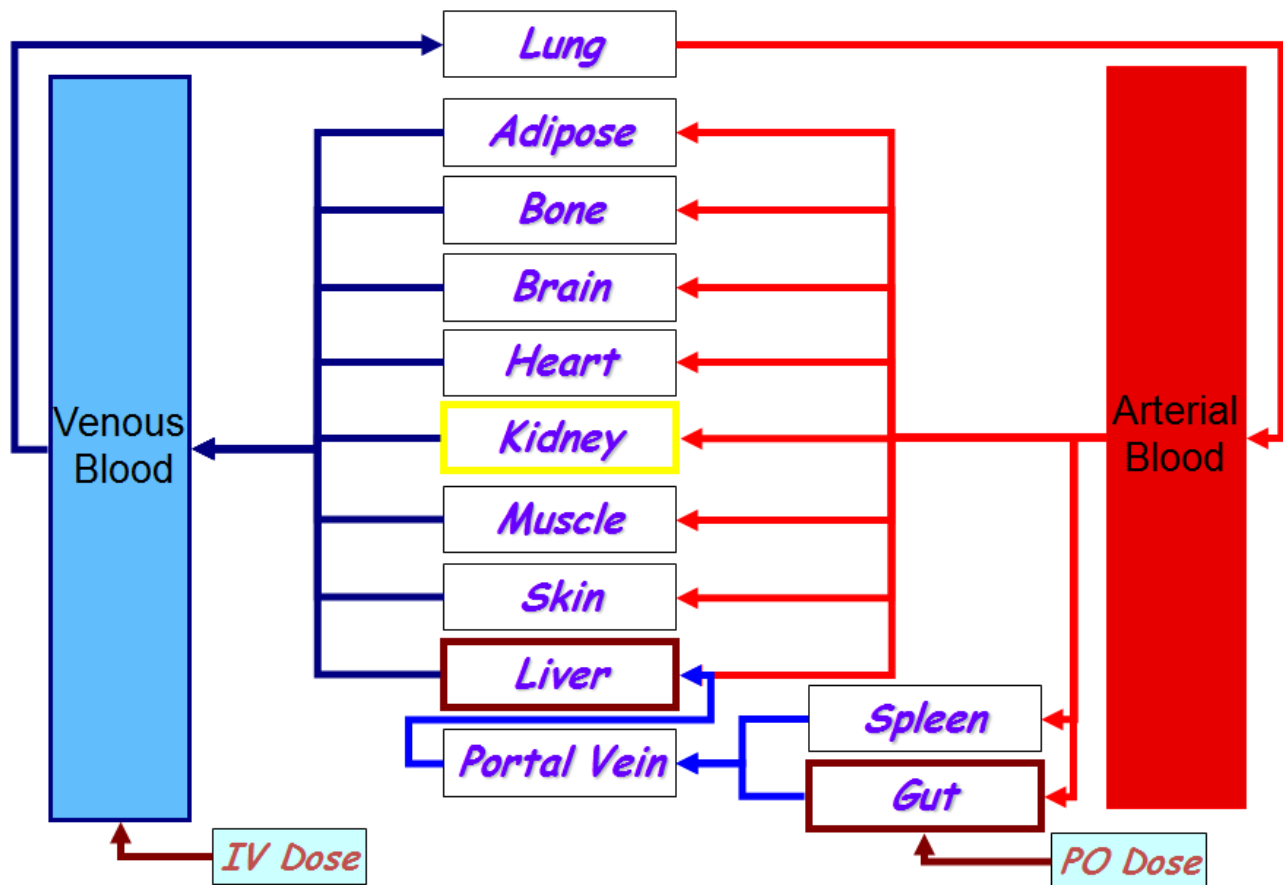
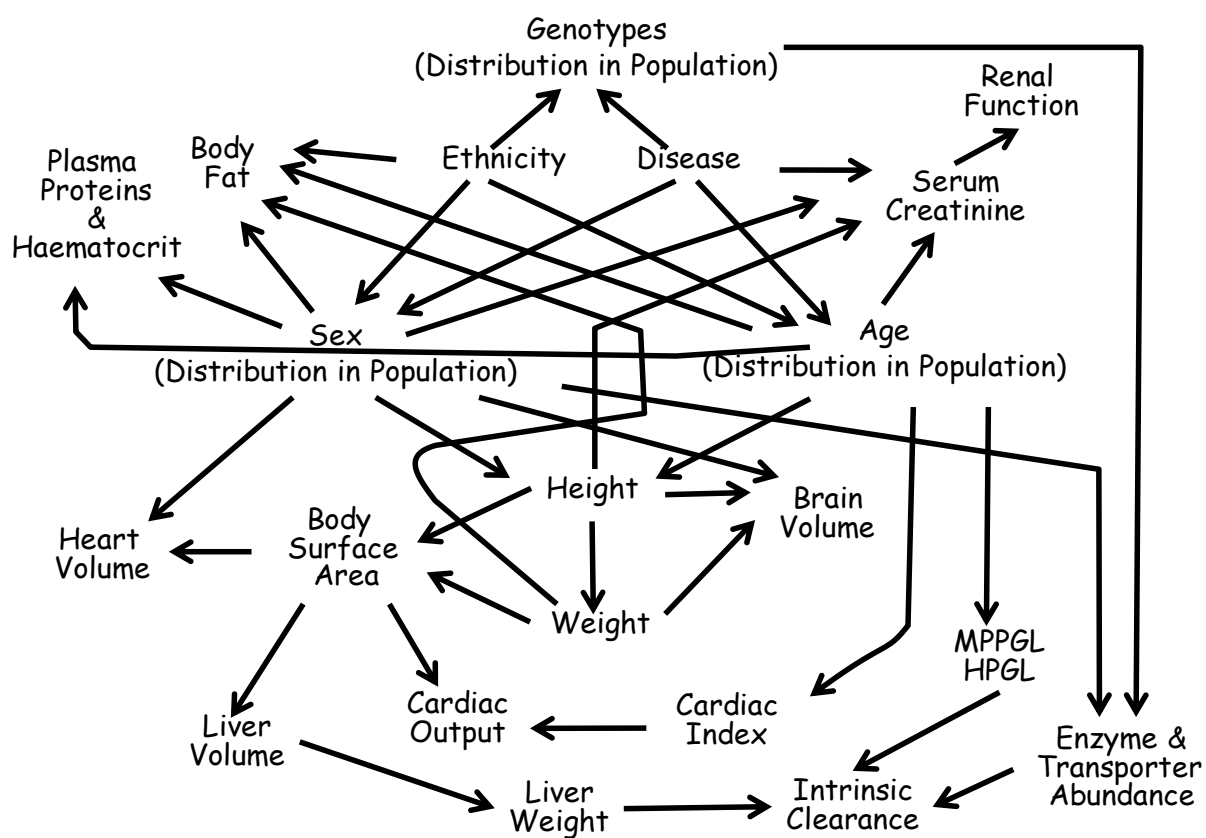**Figure 1**: Structure of a generic PBPK model of the mammalian body.

Figure 2: The inter-correlation of covariates affecting a chemical's clearance from the body, updated after (Jamei et al. 2009a).
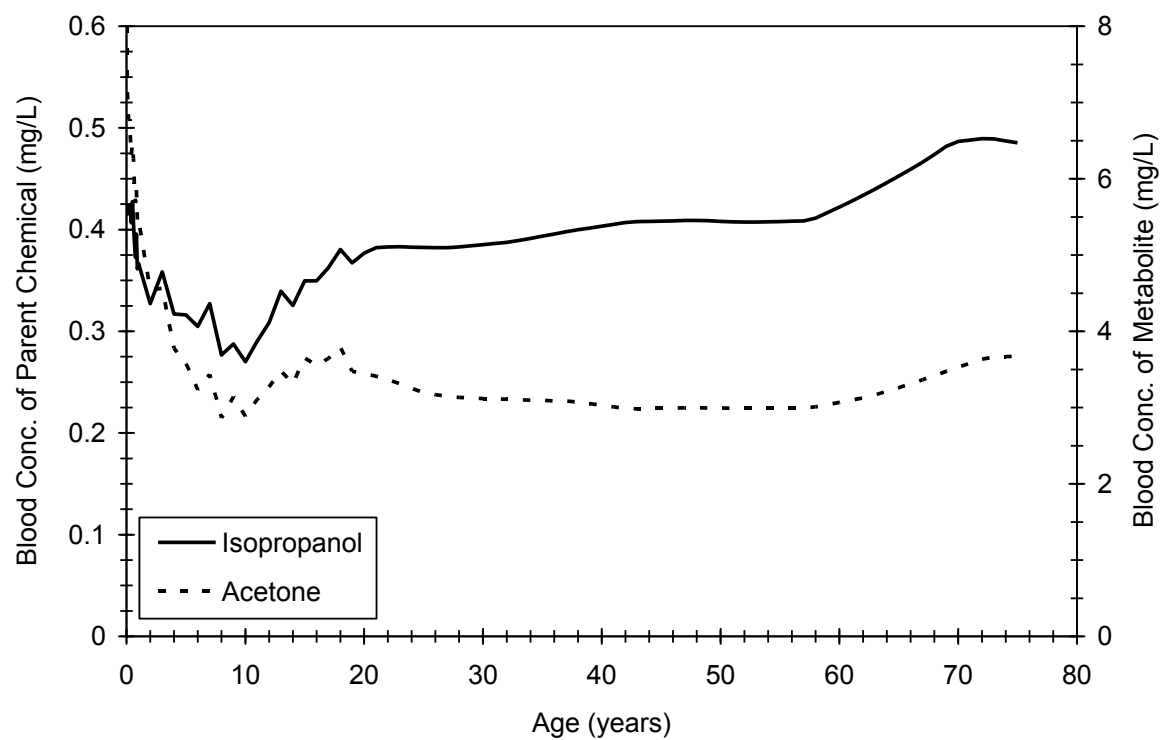
Figure 3: Blood concentrations of isopropanol (IPA) and its metabolite acetone as a function of
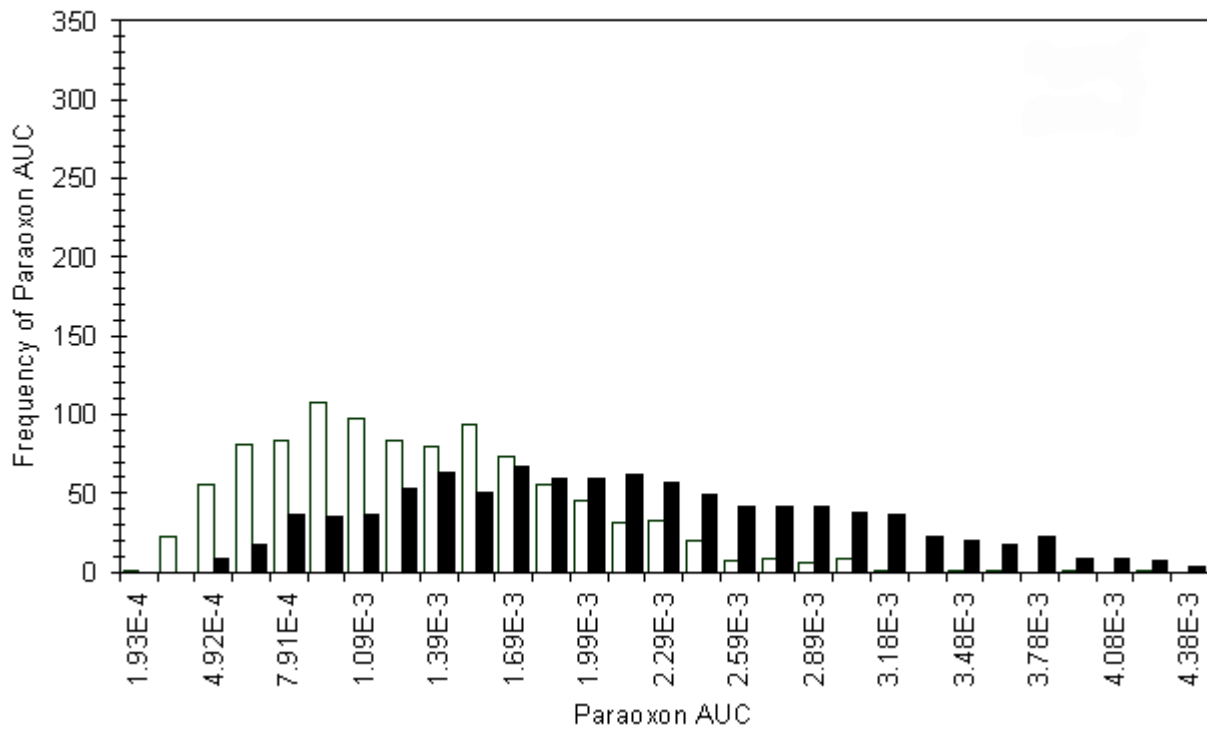
age for continuous inhalation exposure at 1 ppb.

Figure 4: Paraoxon AUC distribution for the sensitive population (black bars) compared to the AUC distribution for the "normal" population (white bars) in for a parathion dose of 0.033 mg/kg.
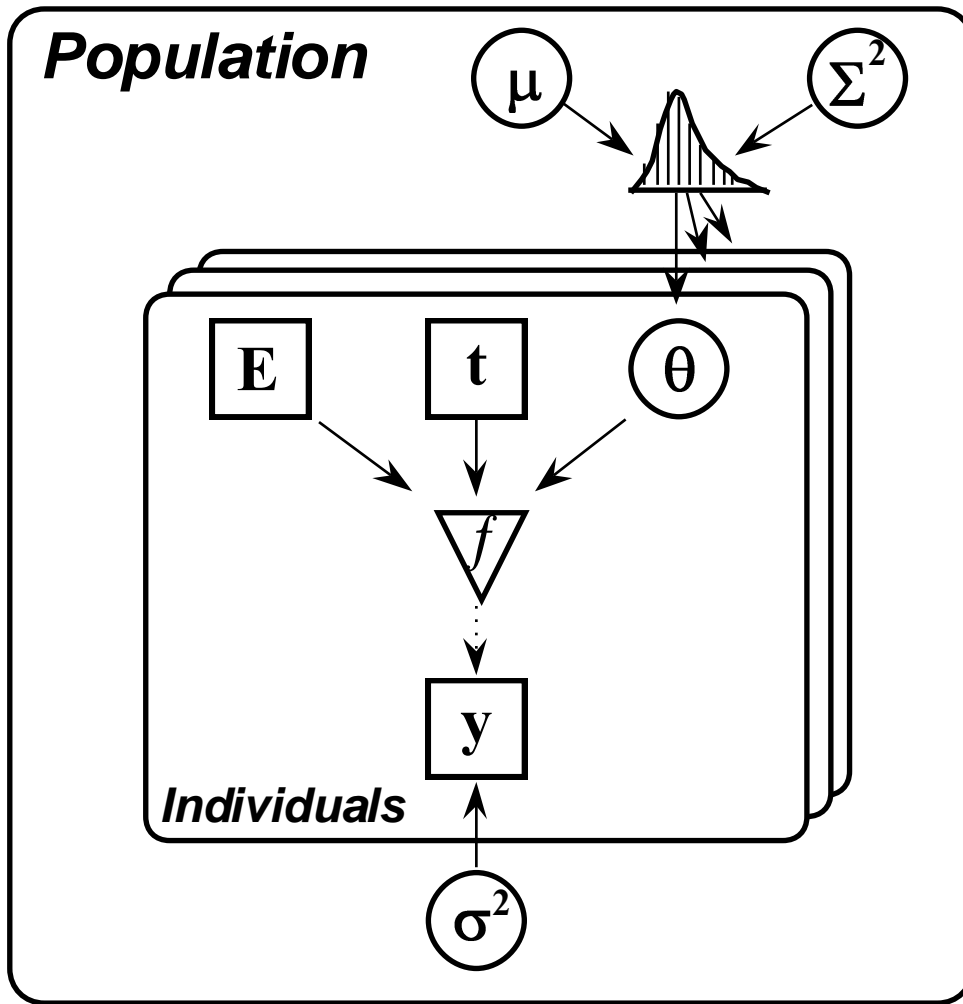
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