Parameters for Pesticide QSAR and PBPK/PD Models to inform Human Risk Assessments

M.R. Goldsmith,[a] J.C. Johnson,[b] D.T. Chang,[a] R. Tornero-Velez,[a] J.B. Knaak,[c] Curtis C. Dary[b]*

[a]National Exposure Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC 27711, USA

[b]National Exposure Research Laboratory, US Environmental Protection Agency, Las Vegas, NV 89119, USA

[c] Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, SUNY at Buffalo, Buffalo, NY 14214, USA

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*dary.curtis@epa.gov

Abstract

Physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling has emerged as an important computational approach supporting quantitative risk assessment of agrochemicals. However, before complete regulatory acceptance of this tool, an assessment of assets and liabilities is in order. Good modeling practices (GMP) serve to sort the assets from the liabilities under the conditions of model structure accuracy, precision, representativeness, completeness, comparability and reasonableness. PBPK/PD models may be seen as dynamic platforms to test these GMP strictures through the parameter calibration process. Inherent in this process, is the sorting and vetting of parameters from quantitative structure activity relationships (QSAR) to the gathering of "in vitro" and "in vivo" study data. "Good" parameters are assets that anchor the model as data is gleaned from the literature or experimentally produced. Faulty or suspect parameters are revealed and excised to strengthen the model structure to fit the intent, regulatory, exploratory or heuristic. It is from these considerations, that a symposium was formed to address parameter requirements for exposure/dose PBPK/PD modeling of agrochemicals. We offer this introduction as primer to more in depth discussion advanced within.

Introduction

A group of researchers was assembled to update the scientific community on pesticide toxicology parameters required for PBPK/PD model development and application in exposure and risk assessment (1). PBPK/PD modeling (2) has been demonstrated to

be an important tool for relating exposure to the disposition of pesticide active ingredient and metabolites in tissues and predicting health and risk outcomes (3). Several models have been created and published that address the risk of exposure to organophosphorus (4-6), carbamate (7-9) and pyrethroid (10-12) insecticides. In many cases, these modeling efforts did not lead to complete and total acceptance of exposure-dose modeling in the risk assessment process. The reasons for this lack of acceptance are mainly related to the technical aspects of model development such as: 1) parameter calibration (i.e., physiological/ biochemical constants and pharmacodynamics) and 2) model simulation/validation. Many biochemical parameter values (metabolism, AChE inhibition, etc) have been obtained for pesticides but in most cases these values have not been used in PBPK models to describe the chain of events (pharmacokinetics/pharmacodynamics) occurring after controlled dosing or exposure. The events are usually described as a series of observed interactions (decrease in LD_{50}'s, muscle spasms, increase in AChE inhibition, etc) and not as output from models involving rates of absorption, metabolism, inhibition, and elimination (ADME). This treatise offers a look at research to obtain kinetic parameters important in skin and gastrointestinal absorption, distribution (tissue/blood partition), metabolism (metabolic pathways, V_{max}, K_m for CYPs, carboxylesterases, OP-oxonases, etc), and pharmacodynamics involving target enzymes and neurotoxicity (electrophysiology: modified ion channels).

Toward PBPK/PD Modeling

The environmental fate and toxicology of agricultural chemicals has been studied for many years (13-22) and the results of these studies have been published and used to support federal pesticide guidelines (23). Traditionally, risk assessment has

followed a weight-of-evidence paradigm (24). Initially, the hazard must be identified according to dose response and exposure assessment criteria to arrive at a risk characterization. Hazard assessments are made from standard guidelines studies. As observed by Metzger (25), hazard characterization informs us about the toxicity of a chemical, and when this information is combined with information about exposure, a risk assessment can reliably be articulated. To arrive at an informed and reasonable risk assessment, substantial and representative data must be amassed and gleaned.

This "weight-of-evidence" process would also hold for source-exposure-dose modeling which can be perceived as a detective story (26). Computational modeling would not offer a short cut to the risk assessment. Indeed, modeling would simply offer an alternative with all of the same data requirements. Exposure modeling combined with PBPK/PD modeling has been demonstrated (27) to address elements of the risk assessment paradigm. At issue is the depth and breadth of the assessment needed to arrive at a risk calculation, such as average daily dose (ADD), reference dose (RfD) and margin of exposure (MOE), as shown in Figure 1.

[Figure 1. Exposure-dose Paradigm]

Contributions along the source to exposure process can be shallow single point estimates or broader distributions. The key to the process is predicting responses from different pathways/routes or levels of exposure to the disposition of active forms of a substance in tissues and excreta in relation with time. The "depth" of understanding of the PK process within the test system essentially defines hazard characterization. Rapid single acute exposures related to the occupational use of a pesticide might be captured by single compartment models where the model structure and parameter requirements might be limited. It might be argued that a reasonably simple and direct acute exposure scenario would require fewer parameters and a less elaborate structure than an

aggregate exposure involving single or multiple chemicals. The complexity of the PK or PBPK model structure and the need for greater parameter representation would depend on whether these models have the same level of rigor and transparency as the weight of evidence approach.

Identifying the Parameters

We take the view that the exposure paradigm and all the connecting elements and underlying parameters and data are "a mile long and an inch deep". More is known about certain elements and the connections or "cusps" between these elements than others. We view the exposure-absorption cusp as the sine qua non between external and internal dose. In the case of dermal exposure, the depth of understanding must bridge/link exposure parameters in time and space (temporally and spatially) with the disposition, rate of loading (mass/time), dermal penetration and percutaneous absorption (flux) of a pesticide active ingredient with per time. As described by Ngo et al., (CHAPTER), the process of percutaneous disposition could involve as many as 15 potential steps. These steps may be arranged sequentially according to a perceived exposure, occupational or incidental (non-occupational), in an exposure-dose modeling framework, as depicted in Table I. Each element of this modeling framework would be a parameter requiring data. The depth of understanding attributed to each parameter would contribute to model veracity.

[Table I. Determinants of Percutaneous Disposition]

Both Ngo (CHAPTER) and Reifenrath (CHAPTER) explore factors that determine percutaneous absorption. Many of these factors (nature of the residue, formulation, volatility, and partition coefficient) would also have an impact on inhalation and ingestion (dietary and non-dietary) exposures. The nature of the residue (neat technical grade active ingredient, emulsifiable concentrate formulation, aqueous end-use product, dust, adhered to soil, as particles, aerosol, gas fumigant, solid bait, aged residues, and

residues in food, feed and filth and extraneous matter) in and on various media, e.g., hard surfaces, carpet and air and water, is expected to have a pivotal impact on mass transfer and subsequent dermal, tissue, and cellular absorption. Consequently, these exposure-absorption factors further influence other elements, distribution/metabolism, elimination and toxicity in the ADMET paradigm.

The ADMET process may be viewed schematically from the structure of a PBPK/PD model as provided by Hughes (CHAPTER) for an oral dosing and depicted herein for aggregate exposure, to include, dermal, oral and inhalation. These structures offer a glimpse of the tissue compartments considered important to the modeling purpose, regulatory, exploratory or heuristic. We find a workflow diagram (Figure 2) useful for identifying parameters for inclusion and deletion/rejection in our PBPK/PD models. This approach progresses in a step-wise fashion with the gleaning of information/data to support the selection of parameters. A quality assurance modeling plan (QAMP) is established a priori to set acceptance and rejection criteria for model structure, parameters and data (28-31).

[Figure 2. The Iterative Model Development Process]

For "canned" or generalized PBPK modeling platforms such as the exposure-related dose estimating model (ERDEM), parameter values can be entered using a graphic user interface (GUI) to populate the model structure. For practical purposes and since our research in the past has been with ERDEM development we briefly digress into the intricacies of our own model, though these features can be generalized to almost any GUI-enabled PBPK modeling platform or package available commercially or opensource. ERDEM is a PC-based modeling framework that allows for using existing models and for building new PBPK and PBPK/PD models (32). For the user, ERDEM requires no special software other than the basic Windows environment commonly used on PCs. ERDEM is comprised of the ERDEM Front End, the ERDEM Model and

the ACSL Viewer. The ERDEM Front End is a Windows based application which allows the user to enter exposure parameters and store them in a database for later use and export into ERDEM. The ERDEM pharmacokinetic modeling engine contains differential equations that use the physiological, biological, and pharmacodynamic modeling data that are entered via the ERDEM Front End. Computer screens are provided to instruct the user in the intricacies of the process. ERDEM consists of the following compartments: Arterial Blood, Brain, Carcass, Closed Chamber, Derma, Fat, Intestine, Kidney, Liver, Rapidly Perfused Tissue, Slowly Perfused Tissue, Spleen, Static Lung, Stomach, and Venous Blood, as shown in Figure 3. ERDEM allows for multiple circulating compounds with multiple metabolites entering and leaving each compartment to afford pharmacokinetics and dosimetry simulations. Finally, the analysis and graphical representation of such data, in tabular, chart or animation form can be reported by ERDEM or other packages intended to handle physiologically annotated data, such as PAVA (43).

[Figure 3. ERDEM Structure]

In the case of individual purpose PBPK models, compartments are proposed and parameters are dedicated to the task. Hughes (CHAPTER) offers a "menu" of data needs to construct a dedicated PBPK model to follow the disposition of deltamethrin in male Long-Evans rats. Physiological parameters such as cardiac output and tissue/blood volumes were drawn from a previous PBPK model (10) with additional parameters, e.g., tissue to blood partition coefficients, adapted from Godin (33). Here "initial" *in silico* (QSAR) parameters or *in vitro* values (in units extrapolated to *in vivo* value equivalents) are required to initiate the modeling process. The output from the model (using these initial values) is then compared to *in vivo* experimental values (tissue concentrations, enzyme inhibition, urinary biomarkers, etc…). If the model output does not agree with in vivo experimental values, then the initial parameter values must be adjusted or new equations

(pathways, etc…) and parameters need to be considered and added to the model and the process repeated (fitting model output to *in vivo* experimental values).

In CHAPTER (X), Davis and colleagues examine the model fitting process through the use of Baysian statistics. They advocate establishing informative prior distributions (priors) of PBPK/PD parameter values from "initial" in silico (QSAR) predictions or *in vitro* assays. The model output from these *priors* would take the form of *posterior* distributions to be tested against newly acquired *in vivo* experimental values in an iterative process of renewal and augmentation.

The "depth" of this process is dependent on several factors: model intent (regulatory, exploratory or heuristic interests), available data, and exposure or toxicity end points/metrics. Ultimately, the model complexity is a product of how representative and complete the model needs to be to satisfy, for an example, a regulatory intent. The Journal of Biophysical Chemistry (jbpc@scrip.org) lists areas (parameter development) to be considered: pharmacology and physiology, structure-activity relationships, patch clamping, stochastic processes, computational chemistry, molecular docking, biomolecular modeling and structure. These study areas may be used to expand the modeling process by developing the parameters listed under physiological, biophysical, biochemical, pharmacodynamic as represented in Figure 2.

Physiology is captured in the model structures for laboratory animal species with intentions of extrapolation to human models. Generally, the animal (rodent) model is calibrated toward the "jump" to the human model and verified and validated against sets of animal data using sensitivity and uncertainty analyses. However, this process toward optimum animal models may be deviated from when more representative human data (mostly or entirely *in vitro* data) can be added. Several authors (Davis, Ellison, Furlong, Hodgson, Hughes) (CHAPTERS) have adopted this "style"/ approach which is most keenly identified with biochemical metabolism (Chambers, Kaneko, Ross)

(CHAPTERS). Greater depth of understanding about the efficiency and completeness of metabolism involving certain enzymatic processes (e.g., PON1) are essential for accounting for mass balance of active ingredient used in controlled dosing studies versus what is likely to occur through occupational or incidental human exposure. Extrapolations from High (laboratory animal study) to low dose human exposure conditions are as vexing as species to species extrapolations.

In this regard, we must bear in mind that rats and other laboratory species extensively metabolize OPs, carbamates and pyrethroids. For example, fewer metabolites of carbaryl are eliminated by humans than rats (7). Animal studies are intentionally set to test limits (high dose) to produce a wider variety of end products. Humans produce fewer metabolites. This may be due to variation in PON1 plasma protein content and activity among human individuals (action on the "oxon") as suggested by Furlong (CHAPTER). Experimental dosages in human volunteer studies are justifiably set at the low end of the dose-response curve. Therefore, we are unable to determine whether high dosages produce a wider variety of end products in humans. Models assist us in exploring the human situation in the absence of human in vivo volunteer study data. We expect these data gaps to be filled from well designed in vitro studies that consider enzyme activity and content in tissues with emphasis on genetics and gene expression among individuals (34, 35).

As viewed by Hodgson et al (CHAPTER), circulating concentrations of parent compound are rapidly distributed to tissues and organs with portions of the absorbed mass being metabolized (activated) to more toxic structures or degraded to less toxic structures with both activated and degraded species being redistributed to sites of action or to be further metabolized and eliminated. Toxicity is generally seen as the end-point of the ADMET process although once a mass of pesticide active ingredient is absorbed, "random walk" of toxicant to the site of action (36, 37) is altered by competing processes of distribution and metabolism (degradation and activation). This "mass-balance"

relationship is best exemplified by the disposition of the organophosphorus (OP) insecticides, as described comparatively with the n-methyl carbamates by Moser (CHAPTER) and also by Timchalk and others (CHAPTER) in the context of a PBPK model.

The complexity of the ADMET process and thus the PBPK/PD model is largely dependent on chemical structure. Most PBPK/PD models are initially developed to address two-dimensional (2-D) structures. Quantitative structure activity relationships (QSAR) can provide "initial" in silico PBPK/PD parameter predictions that can be used to test PK and PD mechanisms. Ruark and colleagues (CHAPTER) examine (2-D) QSAR model development of enzyme (trypsin, chymotrypsin and acytylcholinesterase) inhibition bi-molecular rate constants for OP insecticides with the aim of filling pharmacodynamic data gaps in PBPK/PD models. Chang et al. (CHAPTER) have developed a mechanistic 3-D QSAR model to predict hydrolysis rates of pyrethroids via rat serum carboxylesterase by looking at specific stereoselective molecular descriptors based on a ligand-based pharmacophore query. Okamoto (CHAPTER) looked to chiral (3-D) chemistry to address toxicity of pyrethroid insecticides. As explained by Suderlund (CHAPTER), neurotoxicity of pyrethroids is highly stereospecific. Current PBPK models (10, 33) do not address the dynamic neurophysiology of pyrethroid mode of action (MOA). The toxicity metric is confined to predictions of mass in brain. Suderlund (CHAPTER) argues that quantitative descriptors of pyrethroid effects on sodium channel currents are not useful indices of toxicity to be used in PBPK/PD models which returns us to the issue of "breadth" and "depth" of understanding in exposure-dose modeling.

We view this "breadth" and "depth" issue as a balance between the amount of effort and cost to develop shallow but effective/useful exposure-dose (PBPK/PD) models versus development of models that explore the single elements, e.g., percutaneous absorption, MOA, genomics, metabolomics and proteomics. Most PBPK/PD modelers agree that the model must be representative of the test system. The next question is how

complete the model has to be to address the issue of intent, e.g., regulatory.

Several stochastically applied exposure-dose models (38, 39) consider the dose metric to end in the vascular compartment with the aim of satisfying urinary biomarker concentrations. These models abridge the ADMET process by "leaping" from the exposure-absorption cusp to elimination. The circulating concentration of parent active ingredient becomes the dose (toxicity) metric without regard to drug distribution/metabolism or "random-walk" to target tissues. This situation is particularly vexing when the parent active ingredient is "activated" to a more toxic structure as is the case for most thiophosphate and di-thiophosphate (OP) active metabolism to toxic oxons (4, 34) (moser(CHAPTER); Ruark(CHAPTER); Ellison(CHAPTER); Hodgson(CHAPTER)(40); chambers(CHAPTER)).

As illustrated by Hodgson (CHAPTER), the stoichiometry of OP distribution and metabolism impacts body burden, toxicity, and recovery/quantitation of biomarkers. When biomarkers are considered to be the "gold-standard" for accounting for exposure and calculating risk (41), the stoichiometry of distribution and metabolism of OPs (Moser(CHAPTER); Timchalk(CHAPTER); Ruark(CHAPTER); Ellison(CHAPTER); Furlong(34); Hodgson(CHAPTER); chambers(CHAPTER); Ross(CHAPTER)), carbamates (33) Moser) and pyrethroids (Hughes (CHAPTER); Kaneko(CHAPTER) ; Ross(CHAPTER); Davis(CHAPTER); Okamoto(CHAPTER)), or most any pesticide, must be considered to account for mass balance. Reliance on a single, readily/reliable (easily detectable) biomarker to address risk characterization in the absence of a clear view and understanding of stoichiometry is hazardous and may be misleading.

Metabolic pathways (42) afford the best initial view of stoichiometric accountability. In the absence of historic metabolic information, "initial" *in silico* QSAR PBPK/PD parameter predictions (Chang, CHAPTER) may serve as rational "priors" to populate the start-up model (Davis, CHAPTER). Referring back to the idea of model development being a detective story (26), we

conclude with a scheme/plan of discovery from "initial" *in silico* QSAR to PBPK/PD development, as depicted in Figure 4.

[Figure 4. In-silico Discovery Scheme]

This cyclical process sequentially informs both computational chemistry (QSAR) and PBPK/PD modeling (43). It is a "two-way" street. Physicochemical systems impact biological systems in a tortuous manner (44) and the nature of this interaction can be gleaned from iterative testing of the PBPK/PD model in a recursive or variational/perturbational "pulley" construct but working toward the same goal, a unified Physicochemical Structure Activity Pharmacokinetic/Pharmacodynamic model (PSA/PPM). We expect *in silico* ADME visualization tool (43) to substantially assist this process. We see ADMET parameters in layers under the exposure-dose paradigm based on phase distribution of a congeneric series of chemicals between the ectobiophase/ parabiophase/ endobiophase (37, 44). We recognize that this vision requires QSAR databases and corresponding biological response data to support PSA/PPM modeling. We anticipate that this volume will indeed stimulate interest in this union.

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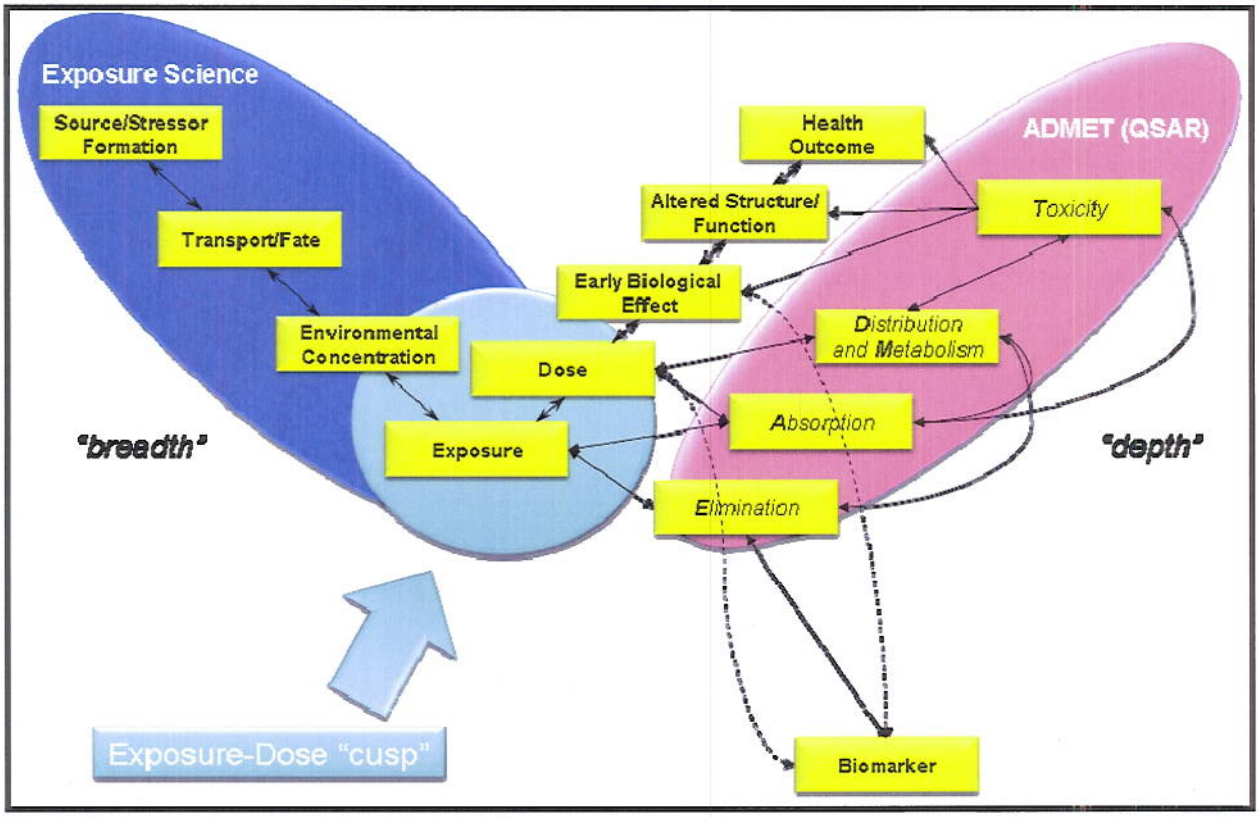

Figure 1: The "source-to-outcome continuum" diagram that represents the variety of macroscopic (breadth) and microscopic (depth) variables, parameters, and modeling considerations of systems complexity inherent in decision support systems required for risk assessment of environmental chemicals.

Table 1: Examples of factors and process/system considerations, both qualitative and quantitative in nature, that showcase determinants of percutaneous disposition.

Factors/steps involved in Percutaneous Disposition	
Transfer	skin, clothing + inanimate surface**
Substantivity to skin**	
Volatility**	
Release from vehicle**	Varies with solubility in vehicle, concentration, and pH, et al.
Wash effects*	Wash resistance; Wash enhancement
Rub effects**	Rub resistance; Rub enhancement
Kinetics of skin penetration**	Influenced by anatomical site, degree of occlusion, intrinsic skin condition, animal age, concentration of dosing solution, surface area dosed, frequency of dosing, post absorption, etc.
•Tissue disposition	
•Binding – all layers**	
•Anatomic pathways	
•Lateral spread**	
•Vascular perfusion**	
•Cutaneous metabolism**	
•Excretion kinetics**	

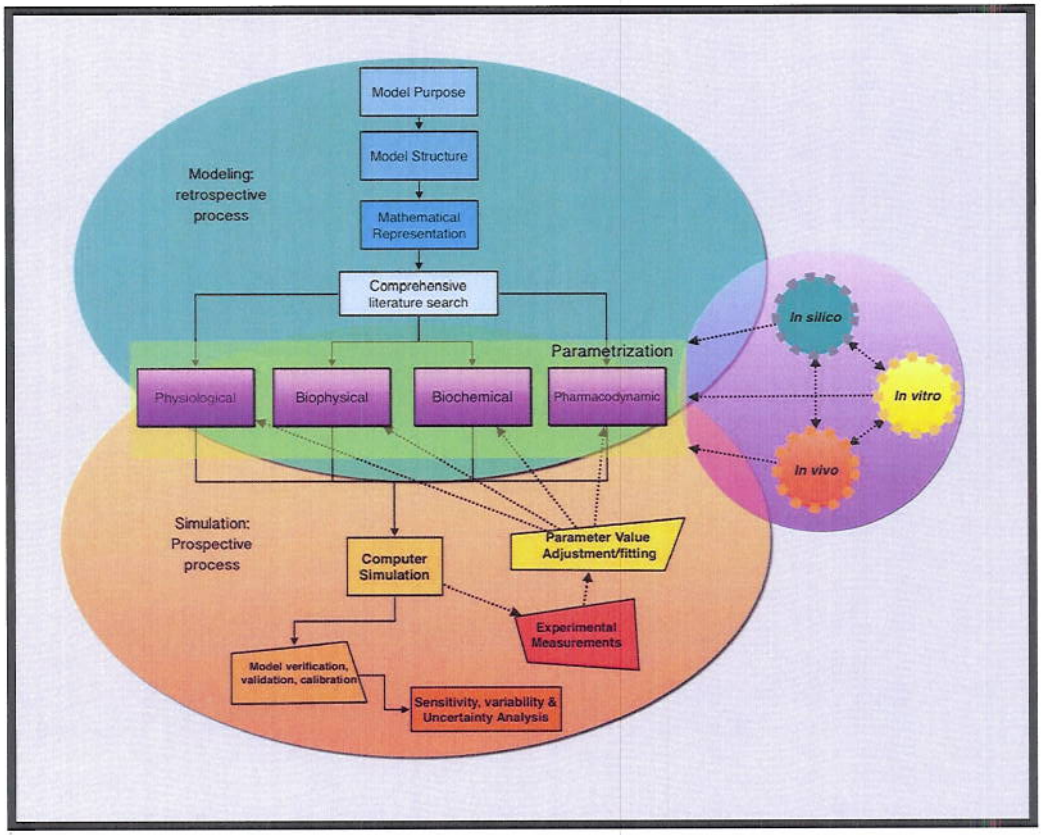

Figure 2: A grand-scheme workflow that delineates the iterative ADME/PK modeling (retrospective) and simulation (prospective) components, from model purpose, structure, Representation and needs to the tightly coupled parametrization process and parameter "fruits" that result of in silico, in vitro, in vivo inquiry, tightly coupled to calibration, validation and uncertainty/variability bounding.

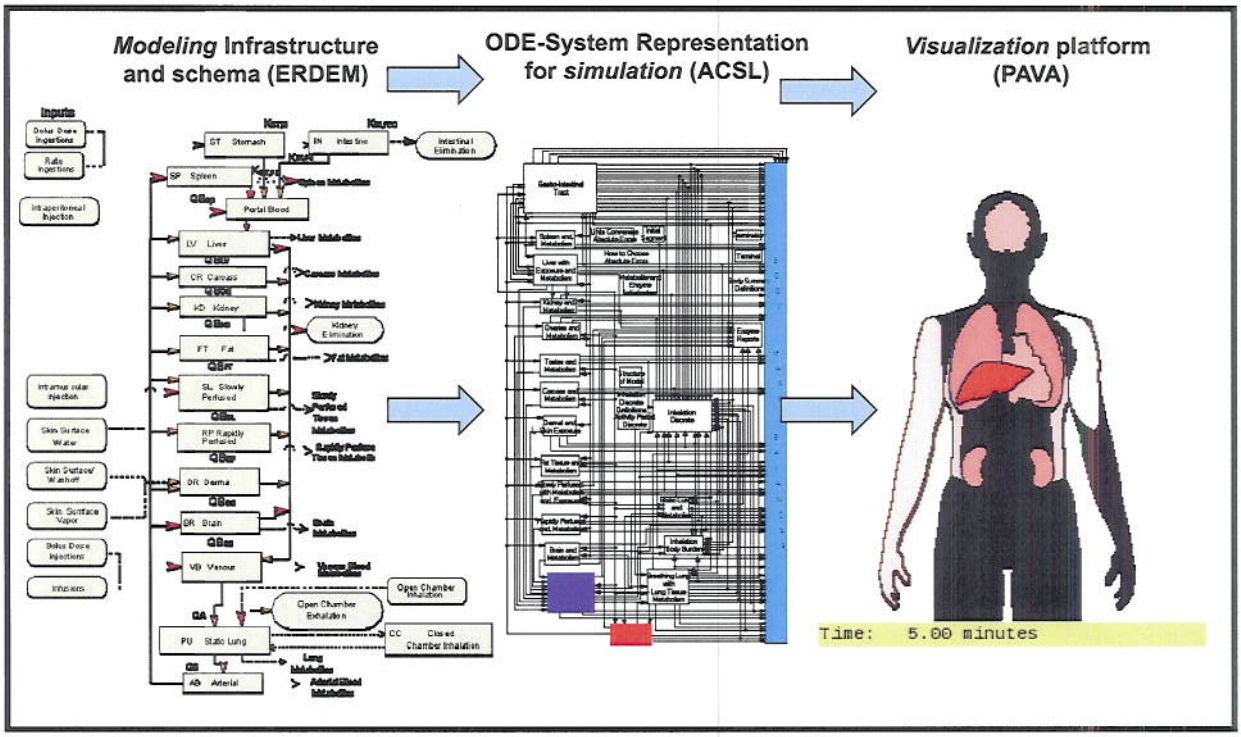

Figure 3: Generalized model constructs, simulation platforms and analysis / visualization platforms comprising the suite of needed components for quantitative ADME modeling *in silico*. For demonstration purposes, we have provided an overview structure of ERDEM, ACSL graphical ODE representation of ERDEM as coded, and a simulation output that contains physiologically annotated data rendered using PAVA as examples.

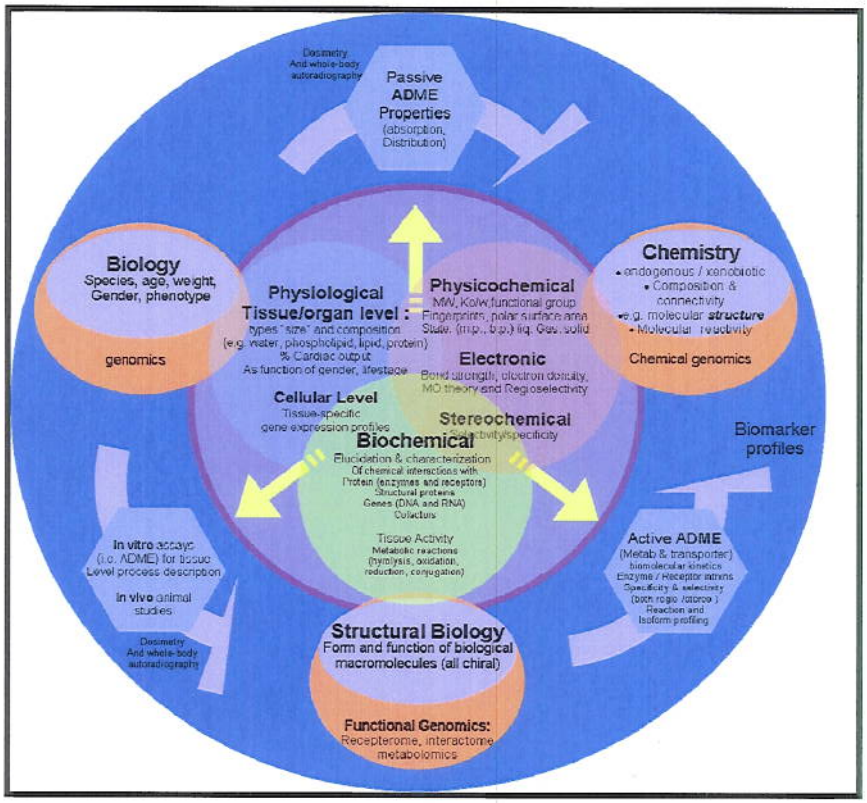

Figure 4: Circular workflow of required parameter components and needs (Orange "genomic" pots) means of acquiring information assets (hexagons) that satisfy the knowledge gaps between needed components (*in silico / in vitro / in vivo* inquiry and modeling) and inherent chemical properties (inner circle) that give rise to some of the underlying microscopic processes and extrapolations for determining and resolving parameters.