FORUM

Performance Assessment and Translation of Physiologically Based Pharmacokinetic Models From acslX to Berkeley Madonna, MATLAB, and R Language: Oxytetracycline and Gold Nanoparticles As Case Examples

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ABSTRACT

Many physiologically based pharmacokinetic (PBPK) models for environmental chemicals, drugs, and nanomaterials have been developed to aid risk and safety assessments using acslX. However, acslX has been rendered sunset since November 2015. Alternative modeling tools and tutorials are needed for future PBPK applications. This forum article aimed to: (1) demonstrate the performance of 4 PBPK modeling software packages (acslX, Berkeley Madonna, MATLAB, and R language) tested using 2 existing models (oxytetracycline and gold nanoparticles); (2) provide a tutorial of PBPK model code conversion from acslX to Berkeley Madonna, MATLAB, and R language; (3) discuss the advantages and disadvantages of each software package in the implementation of PBPK models in toxicology, and (4) share our perspective about future direction in this field. Simulation results of plasma/tissue concentrations/amounts of oxytetracycline and gold from different models were compared visually and statistically with linear regression analyses. Simulation results from the original models were correlated well with results from the recoded models, with time-concentration/amount curves nearly superimposable and determination coefficients of 0.86–1.00. Step-by-step explanations of the recoding of the models in different software programs are provided in the Supplementary Data. In summary, this article presents a tutorial of PBPK model code conversion for a small molecule and a nanoparticle among 4 software packages, and a performance comparison of these software packages in PBPK model implementation. This tutorial helps beginners learn PBPK modeling,

provides suggestions for selecting a suitable tool for future projects, and may lead to the transition from acslX to alternative modeling tools.

Key words: acslX; Berkeley Madonna; MATLAB; PBPK modeling; R language.

Physiologically based pharmacokinetic (PBPK) modeling is a commonly used computational method in pharmacology and toxicology that describes the absorption, distribution, metabolism, and elimination of various chemicals in the body based on interrelationships among key physiological, biochemical, and physicochemical determinants (Bischoff, 1980; Ramsey and Andersen, 1984; Teorell, 1937; WHO, 2010; Yang et al., 1995). PBPK models have wide applications, including drug discovery and development (Jones et al., 2015; Rowland et al., 2011), the design of drug therapeutic regimens (Lin et al., 2015a; Moss et al., 2015), drug withdrawal interval estimation (Buur et al., 2006; Lin et al., 2016a; Riviere et al., 2016), and risk assessment of environmental toxicants and nanomaterials (Andersen et al., 1987; Andersen and Krishnan, 1994; Breckenridge et al., 2016; Campbell et al., 2016; Fisher et al., 1998; Li et al., 2010; Lin et al., 2015b; Mumtaz et al., 2012a; Reddy et al., 2005; Smith et al., 2014; Yang et al., 2015a).

Typically, a PBPK model for a particular compound is built using programming software, such as acslX (AEgis Technologies Group, Huntsville, Alabama, USA), Berkeley Madonna (University of California at Berkeley, California, USA), MATLAB (The MathWorks, Inc., Natick, Massachusetts, USA), and R language, among which acslX was the most commonly used PBPK modeling tool in the fields of toxicology, risk assessment (Lin et al., 2013; Martin et al., 2015; Reddy et al., 2005; Teeguarden et al., 2013; Yang et al., 2015b; Yoon et al., 2011), and veterinary pharmacology (Buur et al., 2006; Craigmill, 2003; Lin et al., 2016d; Yang et al., 2014a). These software systems are based on different computer languages (ie, semantics and syntax), thus the conversion of a PBPK model between different software is not straightforward. As a result, pharmacologists, toxicologists, and health risk assessors usually need to learn specific program attributes in order to know how to apply PBPK models built on an unfamiliar programming language. Therefore, the translation of PBPK model codes has become a critical issue limiting PBPK model application; this limitation has been emphasized in workshop reports by a panel of PBPK experts (Loizou et al., 2008; McLanahan et al., 2012) and also in a series of publications from the Agency for Toxic Substances and Disease Registry (el-Masri et al., 2002; Ruiz et al., 2010, 2011, 2014; Mumtaz et al., 2012a,b).

AEgis Technologies Group announced that acslX support and sales would be discontinued in November 2015. All published PBPK models using acslX will require recoding in a different software program and for future applications, new models need to be constructed using alternative tools. Given this situation, the Society of Toxicology (SOT) Biological Modeling Specialty Section (BMSS) has organized a series of webinars introducing various alternative modeling platforms. However, there are no articles detailing how to convert PBPK model codes between different language platforms. To our knowledge, there are no published performance assessments on these popular software packages.

To help address the PBPK model code translation issues and facilitate the transition of model coding from acslX to other software platforms for future toxicology and risk assessment applications, the objectives of this forum article were: (1) to demonstrate the performance of the 4 PBPK modeling software tested using 2 existing models from our laboratory, oxytetracycline and gold nanoparticles; (2) to provide a tutorial on how to convert PBPK model code from acslX to Berkeley Madonna, MATLAB, and R language; (3) to discuss the strengths and weaknesses of each software platform in the applications of PBPK models; and (4) to share the authors' perspectives about the future direction in this field. Recently published PBPK models for oxytetracycline and gold nanoparticles (Figure 1; representatives of small molecules and nanomaterials, respectively) using acslX were selected as examples because these models include multiple routes of exposure (ie, IV, IM, oral, and SC) and have been validated for route-, dose-, and species-extrapolation using independent data (Lin et al., 2015a, 2016b). This tutorial can also be a case study for beginners to learn PBPK modeling.

TRANSLATION OF MODEL CODES FROM ACSLX TO BERKELEY MADONNA, MATLAB, AND R LANGUAGE

In general, the PBPK modeling process includes several common modeling steps as listed in Figure 2 regardless of the software used. However, for each modeling step the specific-coding method can be different between software systems. Table 1 lists an overall comparison of the coding methods for several key modeling steps across the 4 program systems. Detailed descriptions of PBPK model code conversions from acslX to the other 3 software programs are provided in the Supplementary Data. Readers are also encouraged to watch the SOT BMSS webinars (Buyukozturk and Paxson, 2016; Hinderliter and Ruark, 2016; Loizou, 2016; McDougall, 2016; Phillips, 2016; Setzer, 2016) for additional information not covered in this article.

Note that there are many different approaches to recode PBPK models. We are showing one approach using 2 case examples. Recently, a series of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models (or termed biologically based dose response models) for the thyroid system and perchlorate in rats and humans of different ages that were originally coded in acslX (Lumen et al., 2013; McLanahan et al., 2008; Fisher et al., 2012, 2013) have been converted to R language (EPA, 2016). These works also provide examples regarding how to convert PBPK models from acslX to R language (EPA, 2016). Representative PBPK model codes for oxytetracycline and gold nanoparticles in acslX, Berkeley Madonna, MATLAB, and R language are provided in the Supplementary Data. Example acslX executable runtime files or MATLAB files are also provided in Supplementary Data. These example files are compiled together into a single Word document. Individual files in software-specific formats are available upon request.

Comparisons of simulation results (ie, model outputs) of oxytetracycline plasma concentrations following a single IV injection (5 mg/kg) and a single oral exposure (100 mg/kg) in dogs between the original acslX model and our recoded models are shown in Figures 3A and B, respectively. Simulation results from the acslX, Berkeley Madonna, MATLAB, and R language models were almost identical and visually superimposed. Determination coefficients (R^2) of linear regression analyses
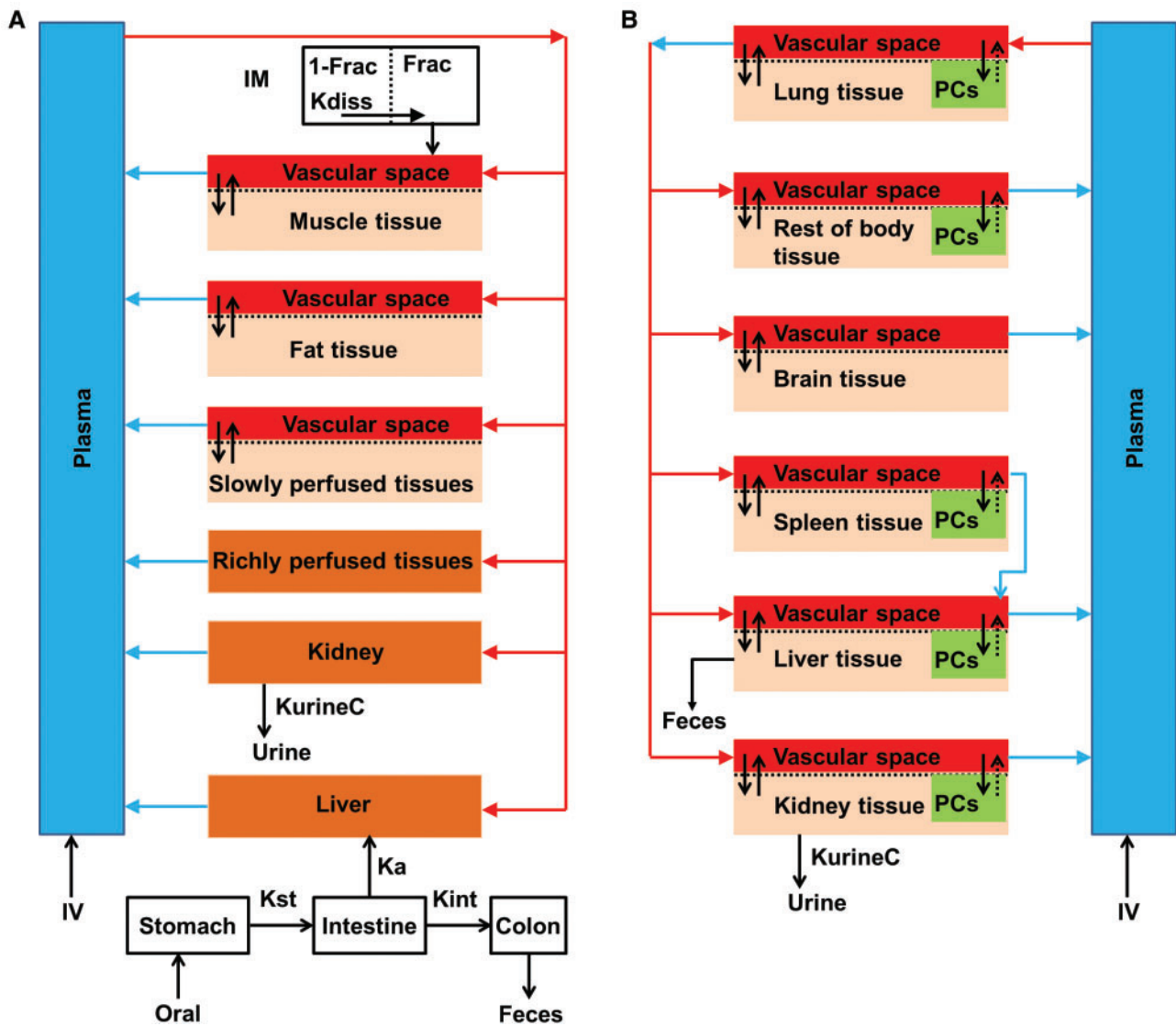
FIG. 1. A schematic of the PBPK model for oxytetracycline in canine (A) and for gold nanoparticles in swine (B). IM, IV, and oral represent intramuscular, intravenous, and oral exposures, respectively. Ka, Kst, Kint, KurineC, and Kdiss (unit: h^{-1}) represent intestinal absorption, stomach emptying, intestinal transition, urine elimination, and oxytetracycline dissolution rate constants, respectively. Frac, fraction of oxytetracyline that is immediately available for absorption following IM injection. PCs, phagocytic cells. These PBPK model structures were adapted from our earlier publications (Lin et al. 2015a; 2016b).
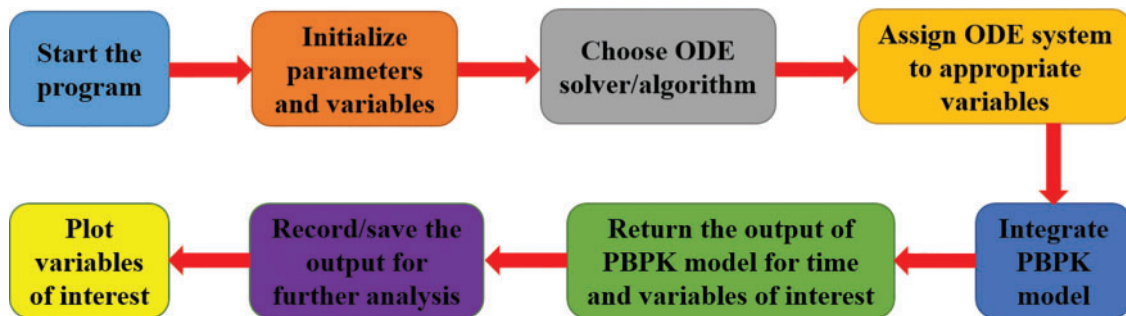

FIG. 2. A general flowchart of the common modeling steps among the 4 PBPK modeling systems. These 4 programming systems include acslX, Berkeley Madonna, MATLAB, and R language. The overall differences in the PBPK model coding among these 4 systems are listed in Table 1 and explained in detail in the text. PBPK, physiologically based pharmacokinetic.

Table 1. Comparison of the PBPK Model Converting Steps From acslX to the Other 3 Program Systems

Model Converting Steps	Initial Model	Recoded Models		
	acslX	Berkeley Madonna	MATLAB	R Language
Change the comment symbol (ie, the ! comments behind these symbols would be ignored by the software)		; or {}	%	#
Define the constants	use the "CONSTANT" command	delete the "CONSTANT" command	delete the "CONSTANT" command	delete the "CONSTANT" command
Define the sections	use section statements (eg, "INITIAL" or "DERIVATIVE")	all section statements can be deleted	delete the "CONSTANT" command all section statements can be deleted; the primary model file must commence with the command "function"	all section statements should be removed
State the integration algorithm	use algorithm IALG value	use the default algorithm or change the algorithm in the Parameter Window	call the ODE solver such as ode23 or ode45	use the ODE function, a built-in function of the R package "deSolve"
Differential equations	use the "INTEG" statement in the form of State=INTEG(deriv, ic)	use initializer and integrator equations in the form of d/dt(State) = … or State' = …	dY =…;	ODEPBPK <- function (Time, State, Pars)
Exposure/dosing equations	Exposure regimen-dependent, see the text for more details			
Simulation output interval	CINT = 0.1	DTOUT = 0.1	t_temp = ti:0.1:tf;	Time <- seq(from=0, to = 48, by = 0.1)
Plot mass balance equations	The value of Bal (mass balance equation) should be approximately 0.0 ± relative error tolerance of numerical integration throughout the simulation period regardless of the software used.			

Only the general differences among different program systems are listed in this Table. Please refer to the text for detailed explanation.

between acslX and Berkeley Madonna results, between acslX and MATLAB results, between acslX and R language results were 1.00, 0.87, and 1.00, respectively, for the IV exposure simulation (5 mg/kg) and were 1.00 for all for the oral exposure simulations (Table 2). Determination coefficients for other exposure scenarios were all close to 1.00, except the one between acslX and MATLAB results for the IV exposure (10 mg/kg), which was 0.86 (Table 2). Overall, these results from the recoded models are highly correlated with the original model simulations, indicating successful translation of the original oxytetracycline model from acslX to Berkeley Madonna, MATLAB, and R language.

Figures 3C and D show comparative simulations of plasma gold concentrations and liver gold amounts following a single IV injection (2 mg/kg) of gold nanoparticles in pigs between the original acslX model and our recoded models in Berkeley Madonna, MATLAB, and R language. Similar to oxytetracycline, the simulations of gold concentrations/amounts from different models were almost identical and the determination coefficients were close to 1.00 (ie, 0.96–1.00). These results suggest that we have also successfully translated the original gold nanoparticle model from acslX to Berkeley Madonna, MATLAB, and R language.

Besides determination coefficients from regression analyses, PBPK model performance can be assessed with mean absolute percentage error (MAPE) based on the following criteria: (1) acceptable prediction: MAPE < 50%; (2) good prediction: 10% < MAPE < 20%; and (3) excellent prediction: MAPE < 10% (Chen et al., 2015; Cheng et al., 2016; Mumtaz et al., 2012a; Rayer, 2007). As shown in Table 2, MAPE values for all comparisons were < 10%, except the one 18.67% for gold nanoparticles between acslX and Berkeley Madonna models. These results further confirm that both the oxytetracycline and gold nanoparticle models have been successfully translated from acslX to Berkeley Madonna, MATLAB, and R language, with the simulation outputs between each other correlated well.

In the field of toxicology, currently there are no hard fast criteria for assessing the consistency of simulation results from a PBPK model coded in different programs. However, in the field of drug development, there are well-established approaches and criteria to compare whether the pharmacokinetics of 2 drugs (a reference drug and a test drug) is equivalent (FDA, 2003). Specifically, FDA assesses bioequivalence based on pharmacokinetic endpoints such as C_{max} (peak plasma concentration) and AUC (area under the plasma concentration time curve) that are reflective of rate and extent of absorption, respectively. In this study, we also determined the C_{max} and AUC ratios between the recoded models and the original model based on the simulation data from Figure 3. The purpose of our analyses was to assess the simulation performance of different models using the 2 key pharmacokinetic endpoints (C_{max} and AUC). We found that the calculated ratios were between 81% and 100% (Table 3).

We anticipate that performance assessment for PBPK models coded in different programs will become an issue in the future. Before the criteria of PBPK model performance assessment are established, we propose that one important check on performance of a PBPK model between different programming platforms can be carried out as follows: (1) compare the simulated concentration-time profile visually, (2) conduct regression analysis between initial model-predicted and recoded model-predicted results, (3) calculate the MAPE value between model outputs, (4) determine the C_{max} ratio between models, and (5) calculate the AUC ratio between models, as done in this study.
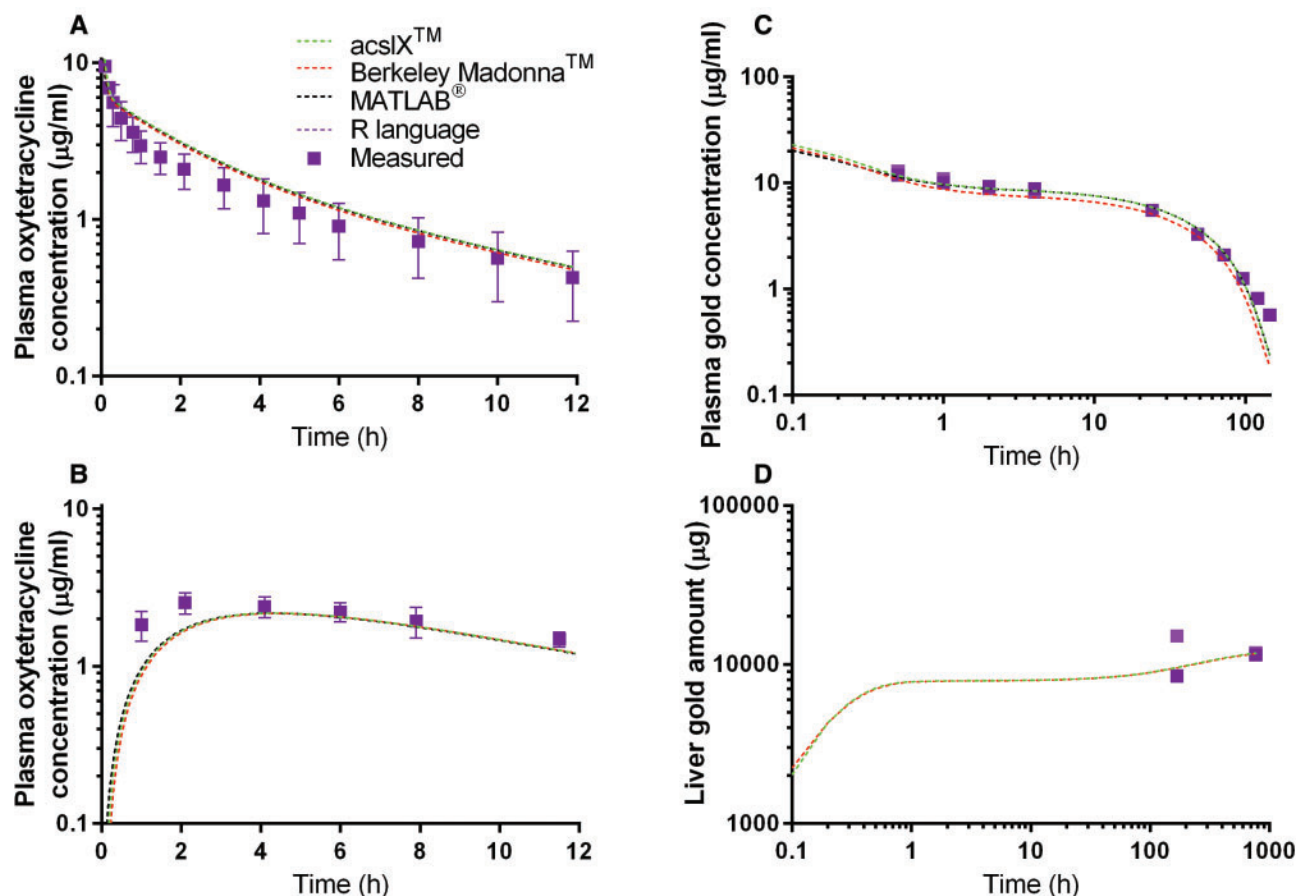
FIG. 3. Comparisons of simulation results between original and recoded models. Measured data represent plasma oxytetracycline concentrations in canine following a single 5 mg/kg IV (A) (Baggot et al., 1978) or 100 mg/kg oral exposure (B) (Immelman, 1977), or plasma gold concentrations (C) or liver gold amounts (D) in swine following a single 2 mg/kg IV exposure (Fent et al., 2009). Green, red, black, and purple dashed lines represent simulation results from acslX, Berkeley Madonna, MATLAB, and R language models, respectively. The simulated concentration/amount data from different models were so close that the kinetic curves were hardly distinguishable from each other (Online figure in color.).

THE NEED FOR A TUTORIAL

Due to the wide application of PBPK models in toxicology, there are numerous review articles and textbooks on this topic (Lin et al., 2016a; Peters, 2012; Reddy et al., 2005). However, the majority of existing works only provide theoretical descriptions of PBPK modeling methodology, without specific programming examples. As a result, it is difficult for beginners to learn programming techniques. This article represents useful examples and a structured solution to help overcome this limitation, thereby helping future students to acquire this technique.

The PBPK model codes can be adapted and expanded to create new PBPK models for other chemicals or nanomaterials. The PBPK models are applicable to small molecular weight drugs or nanomaterials in different species (ie, mice, rats, dogs, pigs, and humans). The models are coded to describe multiple routes of exposure or dosing (ie, oral, IV, IM, and SC administrations), both flow-limited and membrane-limited (or diffusion-limited) compartments, as well as both single and repeated exposures. Equations for other processes, such as protein binding and hepatic metabolism, are available from the literature (Lin et al., 2011; Loccisano et al., 2012; Yang et al., 2014b; Yoon et al., 2009) and can be easily incorporated into the present models.

ADVANTAGES AND DISADVANTAGES OF EACH SOFTWARE PACKAGE IN THE IMPLEMENTATION OF PBPK MODELS INTENDED FOR RISK ASSESSMENT

There are many guidance documents for PBPK-based risk assessment (Andersen and Krishnan, 1994; EPA, 2006; Loizou et al., 2008; Thompson et al., 2008; WHO, 2010; Yang et al., 1995). In general, the application of PBPK models in risk assessment involves 4 steps: (1) choice of critical studies; (2) selection and implementation of PBPK models; (3) evaluation of dose metrics; and (4) determination and extrapolation to human exposure levels. PBPK models should be selected to be consistent with the critical studies for deriving the point of departure in terms of the species, life stages, dose ranges, exposure routes and endpoints (dose metrics and target tissues), as well as the objectives of risk assessment. A checklist of key aspects that should be considered in selecting PBPK models for a given risk assessment has been provided in a PBPK modeling guidance document by the World Health Organization (WHO, 2010). This article focuses on discussing the advantages and disadvantages of each software package with respect to implementation of PBPK models in risk assessment, for which typically 3 aspects should be

Table 2. Determination Coefficients of Linear Regression Analyses and MAPE Values in the Comparisons Between Initial Model-Predicted and Recoded Model-Predicted Results

Exposure Paradigms						R^2 (MAPE)			References
Route	Dose (mg/kg)	Number of Injections	Species	Matrix	Substance	Berkeley Madonna	MATLAB	R Language	
IV	5	1	Canine	Plasma	OTC	1.00 (4.13%)	0.87 (1.68%)	1.00 (0.22%)	(1)
Oral	100	1	Canine	Plasma	OTC	1.00 (2.07%)	1.00 (2.38%)	1.00 (0.15%)	(2)
IM	20	1	Canine	Plasma	OTC	1.00 (0.00%)	1.00 (0.70%)	1.00 (0.10%)	(3)
IV	10	1	Canine	Plasma	OTC	1.00 (4.14%)	0.86 (1.29%)	1.00 (0.16%)	(4)
IM	20	1	Canine	Plasma	OTC	1.00 (0.00%)	1.00 (0.82%)	1.00 (0.06%)	(5)
Oral	50	1	Canine	Plasma	OTC	1.00 (2.08%)	1.00 (2.47%)	1.00 (0.15%)	(2)
Oral	44	1	Canine	Plasma	OTC	1.00 (1.11%)	1.00 (1.70%)	1.00 (0.14%)	(6)
Oral	50	2	Canine	Plasma	OTC	1.00 (1.36%)	1.00 (1.59%)	1.00 (0.11%)	(2)
Oral	25	5	Canine	Plasma	OTC	1.00 (1.19%)	1.00 (1.22%)	1.00 (0.05%)	(7)
Oral	25	5	Canine	Liver	OTC	1.00 (1.24%)	1.00 (1.33%)	1.00 (0.07%)	(7)
Oral	25	5	Canine	Kidney	OTC	1.00 (1.13%)	1.00 (1.12%)	1.00 (0.06%)	(7)
Oral	25	5	Canine	Muscle	OTC	1.00 (1.68%)	1.00 (2.86%)	1.00 (1.68%)	(7)
IV	2	1	Swine	Plasma	AuNP	1.00 (18.67%)	0.96 (0.13%)	1.00 (0.01%)	(8)
IV	2	1	Swine	Liver	AuNP	1.00 (0.18%)	1.00 (0.00%)	1.00 (0.00%)	(8)

AuNP, gold nanoparticles; MAPE, mean absolute percentage error; IM, intramuscular; IV, intravenous; OTC, oxytetracycline; R^2, determination coefficient of a linear regression analysis; (1) Baggot et al. (1978); (2) Immelman (1977); (3) Immelman and Dreyer (1981); (4) von Wittenau and Yeary (1963); (5) Kikuvi et al. (2001); (6) Cooke et al. (1981); (7) von Wittenau and Delahunt (1966); (8) Fent et al. (2009). The ODE solvers used in different software platforms are Gear's stiff algorithm (ie, IALG = 2), Runge-Kutta 4, ode23 [Runge-Kutta (2,3)], and lsoda (ordinary differential equation solver for stiff or nonstiff system; the default method in the deSolve package) for acslX, Berkeley Madonna, MATLAB, and R language, respectively. R^2 and MAPE values were rounded to 2 decimal places.

Table 3. Comparison of the AUC and C_{max} Between Initial Model-Predicted and Recoded Model-Predicted Results

Dose Metrics	Route	Dose (mg/kg)	Number of Injections	Species	Matrix	Substance	Berkeley Madonna	MATLAB	R Language	References
AUC	IV	5	1	Canine	Plasma	OTC	95.81%	98.05%	99.95%	(1)
	Oral	100	1	Canine	Plasma	OTC	99.29%	100.10%	100.05%	(2)
	IV	2	1	Swine	Plasma	AuNP	84.99%	99.77%	100.28%	(3)
	IV	2	1	Swine	Liver	AuNP	99.36%	100.00%	100.00%	(3)
Cmax	IV	5	1	Canine	Plasma	OTC	95.71%	80.98%	100.11%	(1)
	Oral	100	1	Canine	Plasma	OTC	99.59%	99.82%	100.09%	(2)
	IV	2	1	Swine	Plasma	AuNP	93.06%	88.02%	100.00%	(3)
	IV	2	1	Swine	Liver	AuNP	99.07%	100.00%	100.00%	(3)

AUC, area under the concentration/amount curve from the first simulation time 0.1 h to the terminal simulation time that was dependent on the design of the selected studies (12 h for plasma OTC, 144 h for plasma AuNP, and 768 h for liver AuNP); AuNP, gold nanoparticles; C_{max}, maximum concentration/amount during the simulation time; IV, intravenous; OTC, oxytetracycline; (1) Baggot et al. (1978); (2) Immelman (1977); (3) Fent et al. (2009). Data are normalized as a percentage of the value predicted using the initial acslX model.

considered, including extrapolation across exposure paradigms, among species, and characterizing the population variability. A general comparison of these 4 software packages in the implementation of PBPK models in risk assessment is provided in Table 4 and is described in detail below.

Extrapolation across exposure paradigms
Extrapolation across exposure paradigms includes route-to-route, high- to low-dose, and short- to long-term (or vice versa) extrapolations. These extrapolations generally involve changing a few parameters describing the dosing scenario, such as the dose level, dosing interval, and exposure duration. This can be implemented in acslX, MATLAB, or R language by writing an executable file with the study-specific exposure parameter values to call the PBPK model to generate simulation results that are specific to a given study. In acslX, this executable file is called M script file or runtime file; in MATLAB it is a regular MATLAB file; and in R language it is just a simple R script file. In Berkeley Madonna, however, there is only 1 file, the Equation file, for the model; since extrapolation

across exposure paradigms only needs to change a few parameters, this can be done by assigning new values to those parameters in the Parameter Window. Alternatively, in the Equation file, by "Commenting in and out" (ie, using curved brackets, {}, to include or exclude a section of the parameters to be used or ignored by the software during a computer simulation) of a detailed section of parameter values for a specific species and/or experimental conditions, PBPK modeling may be achieved for the study of interest. An alternative approach is to create an individual model file for each study in Berkeley Madonna.

Interspecies extrapolation
Interspecies extrapolation requires changing more parameters, including body weight, cardiac output, tissue volumes, blood flows, and depending on the needs, some chemical-specific parameters. Similar to exposure paradigm extrapolation, we can write an executable file with new parameter values to call the PBPK model to run a new species-specific simulation in acslX, MATLAB, or R language. This approach is used in a recent

Table 4. A General Comparison of acslX, Berkeley Madonna, MATLAB, or R Language in the Implementation of PBPK Models in Risk Assessment

Application	acslX	Berkeley Madonna	MATLAB	R language
Extrapolation across exposure paradigms	By writing a M script file or runtime file with study-specific exposure parameter values to call the PBPK model to run a specific simulation	By changing a few relevant parameter values in the Parameter Window or creating a new model file	Similar to acslX	Similar to acslX
Interspecies extrapolation	By writing a M script file or runtime file with species- and study-specific parameter values to call the PBPK model to run a specific simulation	By creating a new model file for a specific simulation in a new species	Similar to acslX	Similar to acslX
Population uncertainty and variability analysis				
MC analysis	By selecting input parameters, distribution functions, setting parameters, and output parameters in a specifically-designed module for this purpose; easy to implement; does not require programming	Assign the distributions of model parameters by coding	Similar to Berkeley Madonna, but in a different language	Similar to Berkeley Madonna in a different language; can also use MCSim implemented in R via the deSolve package
MCMC analysis	MC Modeler and MCMC Sampler that are specifically designed for this purpose; example PBPK model using MCMC analysis is provided with instructions	Not available	Implement the MCMC approach by coding	Implement the MCMC approach by coding or use MCSim
Overall	Relatively easy to learn, has many examples and modules specifically designed for PBPK analyses, an excellent tool for implementation of PBPK models in risk assessment, requires medium-to-high level programming skills	Easy to learn, suitable for basic PBPK analyses except MCMC, requires medium level programming skills	Can perform all PBPK analyses, but requires medium-to high-level programming skills, the price of the software is relatively expensive	Similar to MATLAB, except it is a free software package

MC, Monte Carlo; MCMC, Markov chain Monte Carlo. MATLAB, R language, and Berkeley Madonna can be run in all the 3 common operating systems (ie, Macintosh, Windows, and Linux). MATLAB, R language, and acslX support parallel computing, but Berkeley Madonna does not.

PBPK model in acslX for risk assessment of the pesticide atrazine (Breckenridge *et al.*, 2016; Campbell *et al.*, 2016). However, due to the many changes in the parameter values, interspecies extrapolation can also be done by creating a new model for a specific species. This approach applies to all 4 software packages discussed in this article.

Population uncertainty and variability analysis
A key aspect that must be considered in using PBPK models for risk assessment is the uncertainty and variability of pharmacokinetics and pharmacodynamics within species and between species. Population variability is a result of interindividual variations in physiology, biochemistry, and molecular biology due to genetic and environmental factors. If the distribution of each model parameter is known, population variability analysis can be conducted using Monte Carlo simulation. Monte Carlo simulation can be conveniently implemented in acslX, in which there is a module specifically for this purpose. PBPK model users simply need to select input parameters, distribution function, output parameters, and setting parameters (eg, mean, standard deviation, lower bound, and/or higher bound), thus minimal programming is required. In Berkeley Madonna, MATLAB, and R language, there is no ready-to-use module, but the model code can be revised to incorporate the distribution of each parameter, thereby generating population simulation results. Another tool that is specifically designed to conduct Monte Carlo stochastic simulations in PBPK models is GNU MCSim (Bois, 2009). This tool has been extensively used in PBPK model population variability analysis (Bois *et al.*, 1996; Chiu *et al.*, 2014; Covington *et al.*, 2007; Lyons *et al.*, 2008; Stamyr *et al.*, 2015). MCSim is written in C and, like other GNU software, can be run in a Linux operating system. A recent version of MCSim (MCSim under R) can be implemented in the R program for use on a Windows computer as part of the deSolve package (Soetaert *et al.*, 2010). We used the deSolve package in our models (Supplementary Data). In addition, Dr Weihsueh A. Chiu from Texas A&M University has created an alternative (using minGW) program that allows MCSim to run in a Windows system.

Although population variability analysis can be conducted using Monte Carlo simulation by assigning the distributions of model parameters, in most cases the distributions of many parameters are unknown or uncertain and assumptions have to be made in order to generate population simulation results, which are of relatively high uncertainty. In PBPK models, when new data become available the prior distributions of model parameters can be updated to generate posterior distributions that more accurately represent the population characteristics of model parameters using a hierarchical Bayesian population approach with Markov chain Monte Carlo (MCMC) simulation. This approach involves the following aspects: (1) specification of the hierarchical population statistical model, (2) specification of prior distributions of model parameters; (3) estimation of the posterior distributions of model parameters using MCMC technique by incorporating newly available data; and (4) evaluation of convergence, the consistency of estimated parameters, and model fit (Chiu *et al.*, 2014).

In the field of toxicology and risk assessment, the most commonly used tool for MCMC analysis in PBPK models is MCSim. Detailed instructions about the software installation, implementation, and application in risk assessment are available in the User's Manual and in several recent publications (Bois, 2009; Bois *et al.*, 1996; Chiu *et al.*, 2014; Covington *et al.*, 2007; Lyons *et al.*, 2008; Stamyr *et al.*, 2015). Previously, MCSim could only be implemented in a Linux operating system. With the recent

availability of MCSim in a Windows system and in light of the importance of MCMC analysis in the application of PBPK models in risk assessment, it is expected that the use of MCSim to conduct population variability analysis to support risk assessment will increase. MATLAB and R language can also be used to conduct MCMC analysis in PBPK models (Krauss *et al.*, 2015; Thompson, 2012). However, these are less commonly used compared with MCSim. In acslX, there are MC Modeler, MCMC Sampler, and example PBPK models using MCMC analysis that were specifically designed to help users to conduct MCMC analyses in PBPK models, making it an excellent software package for risk assessment. On the other hand, to the authors' knowledge, Berkeley Madonna has not been used or may not be feasible to conduct MCMC analysis.

User-friendliness and simulation time
Other factors that should be considered in the development and application of PBPK models in risk assessment are the ease to learn, the user-friendliness, and the simulation time. AcslX has multiple user-friendly plugin modules that allow the user to conduct several common PBPK analyses, including sensitivity analysis, parameter estimation, and Monte Carlo analysis. Many additional reference codes are also available in the literature (Lin *et al.*, 2016b; Yang *et al.*, 2014b; Yoon *et al.*, 2011). It also has a window in its user interface showing all project files linked together that allows easy organization, navigation, and implementation of project files. Therefore, acslX was an easy-to-learn PBPK modeling tool and suitable for implementation of PBPK models in risk assessment. However, the price for an academic version of acslX was much higher than that of Berkeley Madonna. The latter is also easy-to-use and has similar plugin analysis modules (ie, curve fitting, parameter estimation, and sensitivity analysis). Published PBPK model codes based on Berkeley Madonna are also available in the literature (Liang *et al.*, 2016; Mumtaz *et al.*, 2012b; Weijs *et al.*, 2014).

When compared with acslX and Berkeley Madonna, MATLAB is a more powerful high-level programming tool that is widely used by engineers and scientists across disciplines, including life science, electronics, automotive, and finance. Its prices vary depending on the version and the number of toolboxes that are included. It can be used to develop PBPK models and conduct all necessary PBPK analyses, but medium-to-high level programming is required, so it is relatively difficult to use. R language is also a powerful high-level programming platform that is used in a number of areas, especially in statistical analyses. The advantage of R language is that it is freely distributed, but R language is not commonly used to develop PBPK models at this stage.

In terms of simulation time, Berkeley Madonna is relatively faster, ie, with 1 click ("run the model"), the simulation curves for all parameters are ready to view within 1–2 s depending on the computer used (the computer used to run these simulations has an Intel Core i7-6700 Processor [Quad-Core, 8M Cache, up to 4.00 GHz] and 32 GB of RAM). MATLAB is a relatively larger program and takes a little longer time to run, but it has many different toolboxes, algorithms or solvers that can be used to optimize the code to increase the simulation speed. For example, the gold nanoparticle PBPK model in MATLAB was optimized using the conventional Euler approximation discretization method. This technique helps run the program for a fixed time step defined by $dT = 0.0002$ (h^{-1}). In this way, the optimized model ran about several times faster than the original model (the exact time of each simulation depends on the computer, MATLAB version, ODE solver, and the time step

[*dT*]). Both the initial and the optimized gold nanoparticle MATLAB models are provided in the Supplementary Data.

Overall, to the authors' experience, Berkeley Madonna is relatively easy for beginners to get started and is sufficient to develop basic PBPK models to conduct regular PBPK analyses (eg, parameter estimation, route-to-route extrapolation, and Monte Carlo analysis). Besides the present recoding tutorial, another article specifically introducing PBPK model development using Berkeley Madonna is also available (Yang and Lu, 2007). For experienced PBPK modelers, MATLAB and R language are more challenging, but more versatile and may be more suitable for complex projects, especially for projects involving MCMC analysis. If population variability analysis is the major goal, MCSim is recommended to be used as the procedure of using MCSim to conduct MCMC analysis in PBPK models is well documented.

Besides acslX, Berkeley Madonna, MATLAB, and R language, another general-purpose programming language that may be used to develop PBPK models is Python (Python Software Foundation, Delaware, USA). Also, while the above-described model code conversion methods should be of value for PBPK modelers, they may be less informative for risk assessors or toxicologists who only want to use PBPK models and have no or very limited programming experience. To facilitate model application by nonmodelers, one way is to convert a code-based PBPK model to a graphical user interface (GUI). In this regard, MATLAB, Shiny (a web application framework for R), and Python all have GUI development capabilities. A discussion of the detailed method of GUI development is beyond the scope of this article, but a general procedure based on MATLAB is provided in the Supplementary Data. In addition to the general-purpose programming tools, PBPK models can also be developed using custom software such as Simcyp Simulator (Certara, Princeton, New Jersey, USA) and GastroPlus (Simulations Plus, Inc., Lancaster, California, USA). These commercial proprietary software systems are commonly used to develop PBPK models for use in drug discovery and development. They can also be used to conduct *in vitro* to *in vivo* extrapolation and develop PBPK models for environmental chemicals and drugs to aid toxicity assessment (Adeleye *et al.*, 2015; Bois *et al.*, 2010; Wetmore *et al.*, 2012).

FUTURE PERSPECTIVE

The issues and challenges in the development and application of PBPK models for risk assessment have been discussed in another forum article (McLanahan *et al.*, 2012). This article focuses on the impact of the unavailability of acslX in the implementation of PBPK models in risk assessment, which obviously requires learning a new software package to recode published models or to develop a new model for future applications. In this regard, the present tutorial can help minimize this impact. In addition, from a broader view in terms of future direction in this field, some thoughts to consider include.

Training
Besides this tutorial, another direct approach to address the impact of unavailability of acslX is to offer hands-on training on other PBPK modeling software packages to individuals who previously used acslX and have not yet used other tools. In this regard, the PBPK workshops offered by Dr Raymond S.H. Yang at Colorado State University and by a group of PBPK modelers at ScitoVation offer excellent opportunities to receive relevant training using Berkeley Madonna. However, these workshops are typically short-term (ie, approximately 1 week), thus the participants can learn the concepts and have some basic hands-on practice, but there is not enough time to complete a whole project. The long-term goal of training should be to include a PBPK modeling course in university curricula, similar to what Dr Jeffrey W. Fisher did at The University of Georgia. In this aspect, a semester-based PBPK modeling course has been approved and will be offered through Kansas State University Global Campus. The objective of this course is to provide longer-term systematic PBPK modeling training and it is expected that each student should complete a whole PBPK modeling project by the end of the semester.

Education
Besides the short-term or longer-term training, the establishment of graduate education programs focusing on PBPK and other related pharmacokinetic modeling training in academic institutions is highly encouraged and is in great need. PBPK modeling and application are specialized techniques and becoming more and more widely used in various fields that would require more and more competent PBPK modelers.

Sharing
As pointed out by other PBPK modelers, it is critical and necessary to publish PBPK model codes for subsequent model applications. More and more PBPK models have published their codes along with the articles (Liang *et al.*, 2016; Lin *et al.*, 2016c; Weijs *et al.*, 2014; Yang *et al.*, 2014b; Yoon *et al.*, 2011), but there are many others that do not. The sharing of model codes, especially codes in different software packages, is important for PBPK modelers to learn different programming languages, and this will definitely advance this field.

Collaboration
With the ever increasingly competitive funding situations, nowadays there are few academic laboratories that solely focus on PBPK model development and application because simply proposing to develop a PBPK model does not justify a grant support and without grants academic laboratories cannot maintain. Collaboration between industrial, governmental, and academic institutions on projects involving PBPK modeling is highly recommended to maintain support for research, training, and application of PBPK models in academia.

The essence of systems biology
Despite the fact that PBPK modeling is a relatively mature technology, new applications and research opportunities continue to appear on the horizon. If one traces the development of systems biology back to the teachings of Wiener (1948), one would perceive that PBPK modeling possesses the essence of systems biology in the integration of biomedical sciences with mathematics, physics, engineering, and "computing machines." In that regard and as a case in point for the application of PBPK modeling, the recent advances in environmental carcinogenesis in the "Halifax Project" where a major effort by some 200 cancer biologists and toxicologists, working on the basis of the 11 hallmarks of cancer, concluded that low-dose exposures of mixtures of environmental pollutants, which work in concert, may induce environmental carcinogenesis (Goodson *et al.*, 2015). PBPK, with the incorporation of pharmacodynamic modeling, would be an ideal platform for conducting these types of complicated experiments on computers. Pushing the envelope further, artificial intelligence may very well play an active role in such endeavors.

In summary, the present PBPK model recoding methods fill a gap in the pharmacological and toxicological literature. This article provides a tutorial for beginners to learn PBPK modeling techniques, assesses the simulation performance for PBPK models coded in different software programs, presents a general comparison of each software package in the implementation of PBPK models in risk assessment, offers suggestions for PBPK modelers to select a suitable alternative modeling tool for future projects, and may help facilitate the transition from acslX to alternative modeling tools (eg, Berkeley Madonna, MATLAB, and R language) in the PBPK modeling community.

SUPPLEMENTARY DATA

Supplementary data are available at *Toxicological Sciences* online.

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REFERENCES

Adeleye, Y., Andersen, M., Clewell, R., Davies, M., Dent, M., Edwards, S., Fowler, P., Malcomber, S., Nicol, B., Scott, A., *et al.* (2015). Implementing toxicity testing in the 21st century (TT21C): Making safety decisions using toxicity pathways, and progress in a prototype risk assessment. *Toxicology* **332**, 102–111.

Andersen, M. E., Clewell, H. J., 3rd, Gargas, M. L., Smith, F. A., and Reitz, R. H. (1987). Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* **87**, 185–205.

Andersen, M. E., and Krishnan, K. (1994). Physiologically based pharmacokinetics and cancer risk assessment. *Environ. Health Perspect.* **102(Suppl 1)**, 103–108.

Baggot, J. D., Powers, T. E., Powers, J. D., Kowalski, J. J., and Kerr, K. M. (1978). Pharmacokinetics and dosage of oxytetracycline in dogs. *Res. Vet. Sci.* **24**, 77–81.

Bischoff, K. B. (1980). Current applications of physiological pharmacokinetics. *Fed. Proc.* **39**, 2456–2459.

Bois, F. Y. (2009). GNU MCSim: Bayesian statistical inference for SBML-coded systems biology models. *Bioinformatics* **25**, 1453–1454.

Bois, F. Y., Jackson, E. T., Pekari, K., and Smith, M. T. (1996). Population toxicokinetics of benzene. *Environ. Health Perspect.* **104(Suppl 6)**, 1405–1411.

Bois, F. Y., Jamei, M., and Clewell, H. J. (2010). PBPK modelling of inter-individual variability in the pharmacokinetics of environmental chemicals. *Toxicology* **278**, 256–267.

Breckenridge, C. B., Campbell, J. L., Clewell, H. J., Andersen, M. E., Valdez-Flores, C., and Sielken, R. L., Jr. (2016). PBPK-based probabilistic risk assessment for total chlorotriazines in drinking water. *Toxicol. Sci.* **150**, 269–282.

Buur, J., Baynes, R., Smith, G., and Riviere, J. (2006). Use of probabilistic modeling within a physiologically based pharmacokinetic model to predict sulfamethazine residue withdrawal times in edible tissues in swine. *Antimicrob. Agents Chemother.* **50**, 2344–2351.

Buyukozturk, F., and Paxson, R. (2016). SimBiology as a platform for PBPK modeling. *Society of Toxicology Biological Modeling Webinar Series*. Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

Campbell, J. L., Jr., Andersen, M. E., Hinderliter, P. M., Yi, K. D., Pastoor, T. P., Breckenridge, C. B., and Clewell, H. J. 3rd. (2016). PBPK model for atrazine and its chlorotriazine metabolites in rat and human. *Toxicol. Sci.* **150**, 441–453.

Chen, W. Y., Cheng, Y. H., Hsieh, N. H., Wu, B. C., Chou, W. C., Ho, C. C., Chen, J. K., Liao, C. M., and Lin, P. (2015). Physiologically based pharmacokinetic modeling of zinc oxide nanoparticles and zinc nitrate in mice. *Int. J. Nanomed.* **10**, 6277–6292.

Cheng, Y. H., Lin, Y. J., You, S. H., Yang, Y. F., How, C. M., Tseng, Y. T., Chen, W. Y., and Liao, C. M. (2016). Assessing exposure risks for freshwater tilapia species posed by mercury and methylmercury. *Ecotoxicology* **25**, 1181–1193.

Chiu, W. A., Campbell, J. L., Jr., Clewell, H. J., 3rd, Zhou, Y. H., Wright, F. A., Guyton, K. Z., and Rusyn, I. (2014). Physiologically based pharmacokinetic (PBPK) modeling of interstrain variability in trichloroethylene metabolism in the mouse. *Environ. Health Perspect.* **122**, 456–463.

Cooke, R. G., Knifton, A., Murdoch, D. B., and Yacoub, I. S. (1981). Bioavailability of oxytetracycline dihydrate tablets in dogs. *J. Vet. Pharmacol. Therap.* **4**, 11–13.

Covington, T. R., Robinan Gentry, P., Van Landingham, C. B., Andersen, M. E., Kester, J. E., and Clewell, H. J. (2007). The use of Markov chain Monte Carlo uncertainty analysis to support a Public Health Goal for perchloroethylene. *Regul. Toxicol. Pharmacol.* **47**, 1–18.

Craigmill, A. L. (2003). A physiologically based pharmacokinetic model for oxytetracycline residues in sheep. *J. Vet. Pharmacol. Ther.* **26**, 55–63.

el-Masri, H. A., Mumtaz, M. M., Choudhary, G., Cibulas, W., and De Rosa, C. T. (2002). Applications of computational toxicology methods at the Agency for Toxic Substances and Disease Registry. *Int. J. Hyg. Environ. Health* **205**, 63–69.

EPA. (2006). *Approaches for the Application of Physiologically Based Pharmacokinetic (PBPK) Models and Supporting Data in Risk Assessment*. National Center for Environmental Assessment, Office of Research and Development, US Environmental Protection Agency, Washington, DC. Available at: https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryID=157668.

EPA. (2016). Peer Review of EPA's Biologically Based Dose-Response (BBDR) Model for Perchlorate in Drinking Water-Final List of Peer Reviewers, Notice of the Public Peer Review Meeting and Final Peer Review Charge Questions, pp. 87553–5. Available at: https://www.federalregister.gov/documents/2016/12/05/2016-29108/peer-review-of-epas-biologically-

based-dose-response-bbdr-model-for-perchlorate-in-drinking.

FDA. (2003). Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products - General Considerations. (Revision 1). U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER) Available at: http://www.fda.gov/ohrms/dockets/ac/03/briefing/3995B1_07_GFI-BioAvail-BioEquiv.pdf.

Fent, G. M., Casteel, S. W., Kim, D. Y., Kannan, R., Katti, K., Chanda, N., and Katti, K. (2009). Biodistribution of maltose and gum arabic hybrid gold nanoparticles after intravenous injection in juvenile swine. *Nanomedicine* **5**, 128–135.

Fisher, J., Lumen, A., Latendresse, J., and Mattie, D. (2012). Extrapolation of hypothalamic-pituitary-thyroid axis perturbations and associated toxicity in rodents to humans: Case study with perchlorate. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* **30**, 81–105.

Fisher, J. W., Li, S., Crofton, K., Zoeller, R. T., McLanahan, E. D., Lumen, A., and Gilbert, M. E. (2013). Evaluation of iodide deficiency in the lactating rat and pup using a biologically based dose-response model. *Toxicol. Sci.* **132**, 75–86.

Fisher, J. W., Mahle, D., and Abbas, R. (1998). A human physiologically based pharmacokinetic model for trichloroethylene and its metabolites, trichloroacetic acid and free trichloroethanol. *Toxicol. Appl. Pharmacol.* **152**, 339–359.

Goodson, W. H., 3rd, Lowe, L., Carpenter, D. O., Gilbertson, M., Manaf Ali, A., Lopez de Cerain Salsamendi, A., Lasfar, A., Carnero, A., Azqueta, A., *et al.* (2015). Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead. *Carcinogenesis* **36(Suppl 1)**, S254–S296.

Hinderliter, P. M., and Ruark, C. D. (2016). PBPK modeling in the R project for statistical computing. *Society of Toxicology Biological Modeling Webinar Series.* Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

Immelman, A. (1977). Blood levels of oxytetracycline in dogs after oral administration. *J. South Afr. Vet. Assoc.* **48**, 183–186.

Immelman, A., and Dreyer, G. (1981). Oxytetracycline plasma levels in dogs after intramuscular administration of two formulations. *J. South Afr Vet. Assoc.* **52**, 191–193.

Jones, H. M., Chen, Y., Gibson, C., Heimbach, T., Parrott, N., Peters, S. A., Snoeys, J., Upreti, V. V., Zheng, M., and Hall, S. D. (2015). Physiologically based pharmacokinetic modeling in drug discovery and development: A pharmaceutical industry perspective. *Clin. Pharmacol. Therap.* **97**, 247–262.

Kikuvi, G. M., Mitema, E. S., and Buoro, I. B. (2001). The pharmacokinetics of a long-acting oxytetracycline formulation in healthy dogs and in dogs infected with Ehrlichia canis. *Vet. Res. Commun.* **25**, 391–400.

Krauss, M., Tappe, K., Schuppert, A., Kuepfer, L., and Goerlitz, L. (2015). Bayesian population physiologically-based pharmacokinetic (PBPK) approach for a physiologically realistic characterization of interindividual variability in clinically relevant populations. *PLoS One* **10**, e0139423.

Li, M., Al-Jamal, K. T., Kostarelos, K., and Reineke, J. (2010). Physiologically based pharmacokinetic modeling of nanoparticles. *ACS Nano* **4**, 6303–6317.

Liang, X., Wang, H., Grice, J. E., Li, L., Liu, X., Xu, Z. P., and Roberts, M. S. (2016). Physiologically based pharmacokinetic model for long-circulating inorganic nanoparticles. *Nano Lett.* **16**, 939–945.

Lin, Z., Fisher, J. W., Ross, M. K., and Filipov, N. M. (2011). A physiologically based pharmacokinetic model for atrazine and its main metabolites in the adult male C57BL/6 mouse. *Toxicol. Appl. Pharmacol.* **251**, 16–31.

Lin, Z., Fisher, J. W., Wang, R., Ross, M. K., and Filipov, N. M. (2013). Estimation of placental and lactational transfer and tissue distribution of atrazine and its main metabolites in rodent dams, fetuses, and neonates with physiologically based pharmacokinetic modeling. *Toxicol. Appl. Pharmacol.* **273**, 140–158.

Lin, Z., Gehring, R., Mochel, J. P., Lave, T., and Riviere, J. E. (2016a). Mathematical modeling and simulation in animal health - Part II: Principles, methods, applications, and value of physiologically based pharmacokinetic modeling in veterinary medicine and food safety assessment. *J. Vet. Pharmacol. Ther.* **39**, 421–438.

Lin, Z., Li, M., Gehring, R., and Riviere, J. E. (2015a). Development and application of a multiroute physiologically based pharmacokinetic model for oxytetracycline in dogs and humans. *J. Pharm. Sci.* **104**, 233–243.

Lin, Z., Monteiro-Riviere, N. A., Kannan, R., and Riviere, J. E. (2016b). A computational framework for interspecies pharmacokinetics, exposure and toxicity assessment of gold nanoparticles. *Nanomedicine (Lond)* **11**, 107–119.

Lin, Z., Monteiro-Riviere, N. A., and Riviere, J. E. (2015b). Pharmacokinetics of metallic nanoparticles. *Wiley Interdiscip Rev Nanomed Nanobiotechnol.* **7**, 189–217.

Lin, Z., Monteiro-Riviere, N. A., and Riviere, J. E. (2016c). A physiologically based pharmacokinetic model for polyethylene glycol-coated gold nanoparticles of different sizes in adult mice. *Nanotoxicology* **10**, 162–172.

Lin, Z., Vahl, C. I., and Riviere, J. E. (2016d). Human food safety implications of variation in food animal drug metabolism. *Sci. Rep.* **6**, 27907.

Loccisano, A. E., Campbell, J. L., Jr., Butenhoff, J. L., Andersen, M. E. and Clewell, H. J. 3rd. (2012). Evaluation of placental and lactational pharmacokinetics of PFOA and PFOS in the pregnant, lactating, fetal and neonatal rat using a physiologically based pharmacokinetic model. *Reprod. Toxicol.* **33**, 468–490.

Loizou, G. (2016). A free to use PBPK modeling platform. *Society of Toxicology Biological Modeling Webinar Series.* Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

Loizou, G., Spendiff, M., Barton, H. A., Bessems, J., Bois, F. Y., d'Yvoire, M. B., Buist, H., Clewell, H. J., 3rd, Meek, B., *et al.* (2008). Development of good modelling practice for physiologically based pharmacokinetic models for use in risk assessment: the first steps. *Regul. Toxicol. Pharmacol.* **50**, 400–411.

Lumen, A., Mattie, D. R., and Fisher, J. W. (2013). Evaluation of perturbations in serum thyroid hormones during human pregnancy due to dietary iodide and perchlorate exposure using a biologically based dose-response model. *Toxicol. Sci.* **133**, 320–341.

Lyons, M. A., Yang, R. S., Mayeno, A. N., and Reisfeld, B. (2008). Computational toxicology of chloroform: Reverse dosimetry using Bayesian inference, Markov chain Monte Carlo simulation, and human biomonitoring data. *Environ. Health Perspect.* **116**, 1040–1046.

Martin, S. A., McLanahan, E. D., Bushnell, P. J., Hunter, E. S., 3rd., and El-Masri, H. (2015). Species extrapolation of life-stage physiologically-based pharmacokinetic (PBPK) models to investigate the developmental toxicology of ethanol using in vitro to in vivo (IVIVE) methods. *Toxicol. Sci.* **143**, 512–535.

McDougall, R. (2016). Demonstration of a methylene chloride PBPK model in MATLAB. *Society of Toxicology Biological*

Modeling Webinar Series. Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

McLanahan, E. D., Andersen, M. E., and Fisher, J. W. (2008). A biologically based dose-response model for dietary iodide and the hypothalamic-pituitary-thyroid axis in the adult rat: Evaluation of iodide deficiency. *Toxicol. Sci.* **102**, 241–253.

McLanahan, E. D., El-Masri, H. A., Sweeney, L. M., Kopylev, L. Y., Clewell, H. J., Wambaugh, J. F., and Schlosser, P. M. (2012). Physiologically based pharmacokinetic model use in risk assessment–Why being published is not enough. *Toxicol. Sci.* **126**, 5–15.

Moss, D. M., Marzolini, C., Rajoli, R. K., and Siccardi, M. (2015). Applications of physiologically based pharmacokinetic modeling for the optimization of anti-infective therapies. *Exp. Opin. Drug Metab. Toxicol.* **11**, 1203–1217.

Mumtaz, M., Fisher, J., Blount, B., and Ruiz, P. (2012a). Application of physiologically based pharmacokinetic models in chemical risk assessment. *J. Toxicol.* **2012**, 904603.

Mumtaz, M. M., Ray, M., Crowell, S. R., Keys, D., Fisher, J., and Ruiz, P. (2012b). Translational research to develop a human PBPK models tool kit-volatile organic compounds (VOCs). *J. Toxicol. Environ. Health A* **75**, 6–24.

Peters, S. A. (2012). *Physiologically-Based Pharmacokinetic (PBPK) Modeling and Simulations*, pp. 1–430. John Wiley & Sons, Inc., Hoboken, New Jersey.

Phillips, M. B. (2016). How to translate an acslX PBPK model into the MATLAB language. *Society of Toxicology Biological Modeling Webinar Series*. Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

Ramsey, J. C., and Andersen, M. E. (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.* **73**, 159–175.

Rayer, S. (2007). Population forecast accuracy: does the choice of summary measure of error matter. *Popul. Res. Policy Rev.* **26**, 163–184.

Reddy, M. B., Yang, R. S. H., Clewell, H. J., and Andersen, M. E. (2005). *Physiologically Based Pharmacokinetic Modeling - Science and Applications*, pp. 1–420. John Wiley & Sons, Inc., Hoboken, New Jersey.

Riviere, J. E., Gabrielsson, J., Fink, M., and Mochel, J. (2016). Mathematical modeling and simulation in animal health. Part I: Moving beyond pharmacokinetics. *J. Vet. Pharmacol. Therap.* **39**, 213–223.

Rowland, M., Peck, C., and Tucker, G. (2011). Physiologically-based pharmacokinetics in drug development and regulatory science. *Annu. Rev. Pharmacol. Toxicol.* **51**, 45–73.

Ruiz, P., Aylward, L. L., and Mumtaz, M. (2014). Application of pharmacokinetic modelling for 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure assessment. *SAR QSAR Environ. Res.* **25**, 873–890.

Ruiz, P., Fowler, B. A., Osterloh, J. D., Fisher, J., and Mumtaz, M. (2010). Physiologically based pharmacokinetic (PBPK) tool kit for environmental pollutants–metals. *SAR QSAR Environ. Res.* **21**, 603–618.

Ruiz, P., Ray, M., Fisher, J., and Mumtaz, M. (2011). Development of a human Physiologically Based Pharmacokinetic (PBPK) Toolkit for environmental pollutants. *Int. J. Mol. Sci.* **12**, 7469–7480.

Setzer, R. W. (2016). Dynamic modeling using MCSim and R. *Society of Toxicology Biological Modeling Webinar Series*. Available at: https://www.toxicology.org/groups/ss/BMSS/index.asp. Accessed February 28, 2017.

Smith, J. N., Hinderliter, P. M., Timchalk, C., Bartels, M. J., and Poet, T. S. (2014). A human life-stage physiologically based pharmacokinetic and pharmacodynamic model for chlorpyrifos: development and validation. *Regul. Toxicol. Pharmacol.* **69**, 580–597.

Soetaert, K., Petzoldt, T., and Setzer, R. W. (2010). Solving differential equations in R: Package deSolve. *J. Stat. Softw.* **33**, 1–25.

Stamyr, K., Mork, A. K., and Johanson, G. (2015). Physiologically based pharmacokinetic modeling of hydrogen cyanide levels in human breath. *Arch. Toxicol.* **89**, 1287–1296.

Teeguarden, J. G., Housand, C. J., Smith, J. N., Hinderliter, P. M., Gunawan, R., and Timchalk, C. A. (2013). A multi-route model of nicotine-cotinine pharmacokinetics, pharmacodynamics and brain nicotinic acetylcholine receptor binding in humans. *Regul. Toxicol. Pharmacol.* **65**, 12–28.

Teorell, T. (1937). Kinetics of distribution of substances administered to the body. I. The extravascular modes of administration. *Arch. Int. Pharmacodyn. Ther.* **57**, 205–225.

Thompson, C. M., Sonawane, B., Barton, H. A., DeWoskin, R. S., Lipscomb, J. C., Schlosser, P., Chiu, W. A., and Krishnan, K. (2008). Approaches for applications of physiologically based pharmacokinetic models in risk assessment. *J. Toxicol. Environ. Health B Crit. Rev.* **11**, 519–547.

Thompson, Z. (2012). Statistical Estimation of Physiologically-based Pharmacokinetic Models: Identifiability, Variation, and Uncertainty with an Illustration of Chronic Exposure to Dioxin and Dioxin-like-compounds. Available at http://search.proquest.com/docview/1013441804/fulltextPDF/3983B185C37B4FE5PQ/1?accountid=11789. Department of Epidemiology and Biostatistics, College of Public Health, University of South Florida.

von Wittenau, M., and Yeary, R. (1963). The excretion and distribution in body fluids of tetracyclines after intravenous administration to dogs. *J. Pharmacol. Exp. Therap.* **140**, 258–266.

von Wittenau, M. S., and Delahunt, C. S. (1966). The distribution of tetracyclines in tissues of dogs after repeated oral administration. *J. Pharmacol. Exp. Therap.* **152**, 164–169.

Weijs, L., Roach, A. C., Yang, R. S., McDougall, R., Lyons, M., Housand, C., Tibax, D., Manning, T., Chapman, J., Edge, K., *et al.* (2014). Lifetime PCB 153 bioaccumulation and pharmacokinetics in pilot whales: Bayesian population PBPK modeling and Markov chain Monte Carlo simulations. *Chemosphere* **94**, 91–96.

Wetmore, B. A., Wambaugh, J. F., Ferguson, S. S., Sochaski, M. A., Rotroff, D. M., Freeman, K., Clewell, H. J., 3rd, Dix, D. J., Andersen, M. E., *et al.* (2012). Integration of dosimetry, exposure, and high-throughput screening data in chemical toxicity assessment. *Toxicol. Sci.* **125**, 157–174.

WHO. (2010). *Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment*, pp. 1–97. World Health Organization (WHO), International Programme on Chemical Safety (IPCS), Geneva, Switzerland.

Wiener, N. (1948). *Cybernetics: Or Control and Communication in the Animal and the Machine*. Paris, (Hermann & Cie) and Cambridge, MA, (MIT Press) ISBN 978-0-262-73009-9.

Yang, F., Yang, Y. R., Wang, L., Huang, X. H., Qiao, G., and Zeng, Z. L. (2014a). Estimating marbofloxacin withdrawal time in broiler chickens using a population physiologically based pharmacokinetics model. *J. Vet. Pharmacol. Ther.* **37**, 579–588.

Yang, R. S. H., el-Masri, H. A., Thomas, R. S., Constan, A. A., and Tessari, J. D. (1995). The application of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling for

exploring risk assessment approaches of chemical mixtures. *Toxicol. Lett.* **79**, 193–200.

Yang, R. S. H., and Lu, Y. (2007). The application of physiologically based pharmacokinetic (PBPK) modeling to risk assessment. In *Risk Assessment for Environmental Health* (M. Robson and W. Toscano, Eds.), pp. 85–120. San Francisco, CA: John Wiley & Sons, Inc.

Yang, R. S. H., Weijs, L., McDougall, R., and Housand, C. (2015a). The application of PBPK modeling, the Bayesian approach, and the utilization of Markov Chain Monte Carlo simulation in risk assessment. In *Toxicology and Risk Assessment* (A. M. Fan, E. M. Khan and G. V. Alexeeff, Eds.), pp. 265–299. Singapore: Pan Stanford Publishing Pte. Ltd.

Yang, X., Doerge, D. R., Teeguarden, J. G., and Fisher, J. W. (2015b). Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A. *Toxicol. Appl. Pharmacol.* **289**, 442–456.

Yang, X., Morris, S. M., Gearhart, J. M., Ruark, C. D., Paule, M. G., Slikker, W., Jr., Mattison, D. R., Vitiello, B., Twaddle, N. C., Doerge, D. R., *et al.* (2014b). Development of a physiologically based model to describe the pharmacokinetics of methylphenidate in juvenile and adult humans and nonhuman primates. *PLoS One* **9**, e106101.

Yoon, M., Nong, A., Clewell, H. J., 3rd, Taylor, M. D., Dorman, D. C., and Andersen, M. E. (2009). Evaluating placental transfer and tissue concentrations of manganese in the pregnant rat and fetuses after inhalation exposures with a PBPK model. *Toxicol. Sci.* **112**, 44–58.

Yoon, M., Schroeter, J. D., Nong, A., Taylor, M. D., Dorman, D. C., Andersen, M. E. and Clewell, H. J. 3rd. (2011). Physiologically based pharmacokinetic modeling of fetal and neonatal manganese exposure in humans: describing manganese homeostasis during development. *Toxicol. Sci.* **122**, 297–316.