

# Introduction

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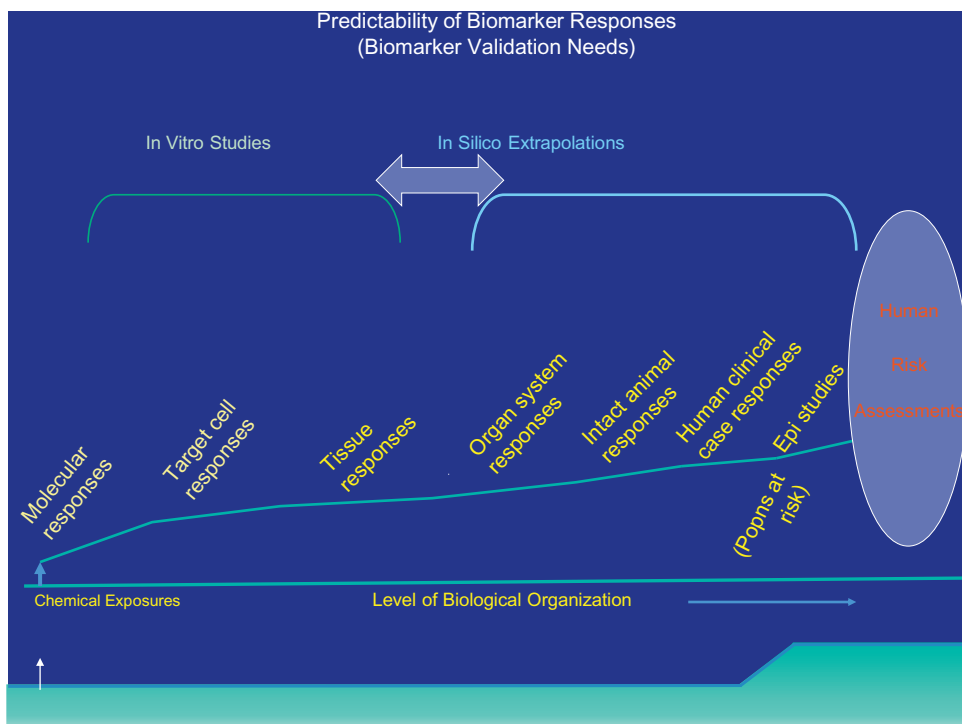
Bruce A. Fowler

ICF International, Fairfax, VA, USA

The evolving field of computational toxicology includes a number of related subdisciplines, some of which are reviewed in this book by international class authorities. The purpose of this chapter is to provide an overview of why computational modeling has become so important to the field of toxicology in general and to the practical needs of risk assessment in particular.

First, it should be noted that computational modeling is accepted as a powerful risk assessment tool for a number of important aspects of contemporary life. Weather forecasting models are an outstanding example; the results save lives every year by predicting the paths of hurricanes and other storms so that needed actions may be initiated *before* the arrival of the adverse weather condition, and loss of life and property may be prevented. In the pharmaceutical industry, potentially useful drugs are screened for dangerous side effects early in development based on chemical structure analyses. Computer modeling of adverse side effects has been used with good results for decades for helping to interpret the results of *in vitro* studies, guide more costly *in vivo* animal studies, and ultimately provide credible information on likely human health effects for risk assessment. This general approach is depicted in [Figure 1-1](#), which shows the forward extrapolation of basic scientific information through the basic levels of biological organization beginning with molecular reactions to cells to organs to whole organisms to human risk assessment. Aspects of this paradigm have been tried, tested, and vetted for a number of years; so there is circumspect reason to trust that information from these embedded components has proven useful in helping to protect the public's health. As these elements are better understood and their linkages more fully appreciated, results from computational toxicology will become more valuable. This book addresses merging information from different fields of science to provide a more coherent and richer view of mechanisms and risks.

More broadly, chemical modeling tools are being applied to the more than 80,000 chemicals in commercial use, plus 500–1,000 new ones each year. Both individual and mixtures' risks



**FIGURE 1-1** General diagram for utilizing computational toxicology methods to extrapolate basic molecular biomarker data from *in vitro* test systems to human health risk assessments.

are assessed to help prioritize regulatory or cleanup decisions. We live in a chemical-rich world, and there are quite simply not enough toxicologists or laboratory facilities capable of evaluating this large number of chemicals in a rapid and cost-effective manner. Yet society and societal decision makers must have guidance on chemical safety issues in a timely manner. Major chemical accidents, such as the Deepwater Horizon Gulf oil spill, are clear examples of this need and the effectiveness of computational modeling in providing needed answers with a short turnaround time so that important decisions could be undertaken.

The need for precision and expediency in risk assessment of chemicals exceeds the data available, and this is unlikely to change. To bridge this gap, creative computer modeling methods have been developed that consolidate and use all credible evidence. A strong underpinning of these approaches is the transparency and replication of each decision step. This book brings together some of the most promising methods that have been tested and successfully applied to real-world needs to meet the pressing challenge of assessing human risk from chemical exposures.

The dual overall goals of this book are to provide a summary of the state of the science of computational toxicology by presenting specific applications that have enhanced the response

to a defined risk assessment challenge and to suggest future research needs based on a synthesis of the extant knowledge. Additionally, important areas, such as high-throughput screening of large numbers of chemicals, not addressed in this book, are making great advances and hold promise for improving risk assessment when applied to specific risk assessment situations in the future.

The applications of computational modeling presented cover a diverse range of exposures and needs for rapid risk assessment responses. They have been used to inform decision making in varying but challenging risk assessment situations confronting risk managers. These needs include risk of chemical mixtures encountered in the Deepwater Horizon Gulf oil spill; the identification of sensitive subpopulations as a function of age, gender, genetic inheritance, and diet; and the rapid development of preliminary health guidance values for emergency response situations. The computational methodologies have generated evidence-based, quantitative levels of risk. These methods include database mining; molecular pathway/network analyses; read-across matrices for REACH chemical registrations; alternatives to animal testing; and application of integrated QSAR, PBPK, and molecular docking approaches for predicting the toxicity of chemicals and their metabolites on an individual or mixture basis.

Optimism regarding the practical application of computational modeling stems not only from the examples discussed by experts in this book but also the 20+ years of successful and productive experience in the pharmaceutical industry in the design and evaluation of drugs. This clear track record of success will undoubtedly continue to expand. Computational toxicology is not a panacea that will resolve all current chemical risk assessment issues, but the judicious application of the available computational methods to specific problems **can** yield robust information to better inform wiser, cost-effective chemical risk decision making today. The examples provided should stimulate further advances in methods and importantly expand the number and types of risk assessment needs to which these methods may be credibly applied in a transparent manner.

A concerted effort has been made to provide international experts an opportunity to discuss applications in a readily understandable form so that persons with limited technical backgrounds can make optimal use of the information. Important practical advantages of computational toxicology for promoting chemical safety rest with initial screening of chemicals or drugs in order to focus limited laboratory resources on more precise and significantly important questions. A second important aspect from the perspective of risk assessment is the synthesis, analysis, and interpretation of data generated by laboratory studies. This bioinformatics aspect of computational methodologies is of ever-increasing importance, since modern molecular approaches to toxicology generate enormous quantities of complex and interrelated data sets. This voluminous amount of information must be analyzed, digested, and interpreted in order to be of practical use in risk assessment. A promising aspect for risk assessment in this area is the generation of molecular pathway analyses, which bring together several lines of information to gain insights into likely chemical modes of action. If the pathway analyses are sufficiently robust to predict cellular death or carcinogenic transformation, then the primary toxicity pathway or network of pathways may be designated as an adverse outcome pathway (AOP) and used for informing credible preliminary risk assessment decisions and further confirmatory laboratory research needs.

Specific chapters include PBPK, QSAR, and toxicity pathways for initial screening of chemicals; application of QSAR to chemical agents released into water environments; chemical mixtures; modeling of sensitive subpopulations for risk assessments; computational modeling of toxicogenomic data sets for risk assessment; and integrating systems biology approaches for predicting drug-induced liver toxicity. Other chapters focus on practical translation of computational methods for risk assessment; computational translation and integration of test data to meet risk assessment goals; computational translation of data from nonmammalian species to meet REACH informational strategies; development of *in silico* models for risk assessment; examples of simulations with a newly developed generic PBTK model for incorporating human biomonitoring data to meet REACH guidelines; use of public data sets for risk assessment, computational toxicology, and applications for risk assessment of pharmaceuticals; the decision forest—a novel pattern recognition method for *in silico* risk assessment; and translation of computational model results for risk assessment.

The value of these approaches has also been recognized by leading forward-thinking U.S. public health agencies, such as the NIEHS, FDA, EPA, and ATSDR, in fostering initiatives that utilize computational approaches alone or in combination with modern molecular toxicology biomarker tools. The excellent foreword to this book provides a prospective-looking overview of the interagency Tox21, and EPA ToxCast and NexGen programs, which are focused on moving the field of chemical risk assessment ahead by utilizing more modern 21st century tools and provides a rationale for why these approaches are important for risk assessment. It should be noted that the pharmaceutical and chemical industries have also been heavily committed to these approaches for many years and have made major contributions to thought leadership in this area.

It is hoped that the book will provide the reader with a good perspective of what is currently being done in computational toxicology and will stimulate insights that pinpoint where applications can better inform risk assessment decisions about chemicals and drugs.

Quantitative Structure-Activity  
Relationship (QSAR) Models,  
Physiologically Based Pharmacokinetic  
(PBPK) Models, Biologically  
Based Dose Response (BBDR)  
and Toxicity Pathways  
Computational Tools for Public Health

*Patricia Ruiz<sup>1</sup>, Xiaoxia Yang<sup>2</sup>, Annie Lumen<sup>2</sup>, and Jeff Fisher<sup>2</sup>*

<sup>1</sup>Agency of Toxic Substances & Disease Registry (ATSDR), U.S. Centers for Disease Control,  
Chamblee, GA, USA, <sup>2</sup>Food & Drug Administration, National Center for  
Toxicological Research, Jefferson, AR, USA

## INTRODUCTION

Human health risk assessment is “the process to estimate the nature and probability of adverse health effects in humans who may be exposed to chemicals in contaminated environmental media, now or in the future.”<sup>1</sup> Currently, most data required for human risk assessment are derived from toxicological studies conducted in laboratory animals. The “Toxicology in the 21st Century” initiative<sup>2</sup> expands the toxicity testing tools to include the development of alternative toxicity testing methods that examine pathways of toxicity (on a large scale) and the employment of dose-response and extrapolation modeling tools. While the latter methodology is in its infancy, several methodologies for dose-response

and extrapolation modeling are more mature. Over the last decade, physiologically based pharmacokinetic (PBPK) modeling has gained acceptance as a computational tool for use in public health assessments.<sup>3,4</sup> In this chapter, we present examples of quantitative structure-activity relationship (QSAR) models, physiologically based pharmacokinetic (PBPK) models, and biologically based dose response (BBDR) models that have been developed for use in public health assessments and advancing knowledge gained through *in silico* examinations of biological systems.

## APPLICATION OF STRUCTURE-ACTIVITY RELATIONSHIP (SAR) AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR)

Alternative methods, such as structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR), are invaluable tools for simulating necessary endpoints to correctly assess chemical hazards. SAR and QSAR models are mathematical relationships between the chemical's quantitative molecular descriptors and its toxicological endpoint.<sup>5-10</sup> SAR and QSAR are intended to complement, not replace, development and use of experimental, laboratory-based approaches. Some SAR and QSAR methods are based on a limited set of chemicals, whereas others are based on a vast or diverse database. Some are based on generic principles of toxicology; others use more specific mechanistic or mode of action information.<sup>6,8,10-15</sup>

A growing number of such methods and models are being developed for various specific toxicity endpoints, and are expected to play an increasingly important role in estimating and predicting toxicity for hazard and risk assessment. Many commercial and open source software packages, such as TOPKAT, CASE, and MultiCASE, have used QSAR models to predict human health effects and related toxicities.<sup>16-25</sup>

SAR/QSAR modeling plays an important and active role in the Agency of Toxic Substances and Disease Registry (ATSDR) programs in support of the agency mission to protect human populations from exposure to environmental contaminants.<sup>26</sup> It is used for cross-chemical extrapolation to complement the traditional toxicological approach when chemical-specific information is unavailable.<sup>6,10,26-28</sup> The key application of SAR/QSAR is filling chemical toxicity data gaps. QSAR methods are used to investigate adverse health effects and exposure levels, bioavailability, and pharmacokinetic properties of hazardous chemical compounds. QSAR analyses are incorporated into ATSDR documents (such as the Toxicological Profiles and chemical-specific health consultations) to support environmental health assessments and prioritization of environmental chemical hazards, and to improve study design.<sup>10</sup>

Two examples of the SAR/QSAR modeling were recently published.<sup>28,29</sup> In Ruiz et al.,<sup>28</sup> the Concise International Chemical Assessment Documents board requested quantitative structure toxicity relationship (QSTR) assessments of certain specific toxicity endpoints for ethylene glycol mono-*n*-alkyl ethers. These chemicals are a family of ethylene glycol ethers widely used as organic solvents and thinners for resins, paints, and dyes. QSAR models embedded in TOPKAT, a commercial toxicity prediction system, were applied to assess the toxicity potential of these compounds. Concordance between experimental and predicted mutagenicity data on many of these compounds confirmed the accuracy of QSAR model

predictions. Although mutagenicity and carcinogenicity were not indicated for the majority of these molecules, predictions indicated their potential developmental toxicity. Applying unique features of the QSAR model helps to identify substructures and structural features that may lead to increased developmental toxicity potential and thus gain insight into the substructures that may alter potential for developmental toxicity.

In another example reported by Pohl et al.,<sup>29</sup> QSAR analysis was used to identify chemicals that affect joint toxicity of chemicals. Chemicals such as xylene and toluene have been shown to interact with ethoxyethanols at the enzymatic level and influence their toxicity outcome, particularly testicular toxicity. A unique feature of TOPKAT called “QSAR similarity analysis” was used to identify chemicals that could cause developmental toxicity and are structurally similar to xylene and toluene. Unlike other similarity measures, QSAR similarity search, expressed as the Euclidian distance computed from the values of model descriptors, is property sensitive because it reflects the similarity of descriptor values between two molecules with respect to a specific property or endpoint.<sup>20</sup> When implemented, this type of search mines the associated database of a QSAR model to look for the most similar compounds, and it assigns surrogate chemicals and prediction confidence based on the similarity distance and concordance between the experimental and predicted values of these similar compounds.<sup>30,31</sup> These two articles demonstrate the importance of an *in silico* method as a tool that can supplement traditional approaches to risk assessment of chemical mixtures.

SAR/QSAR tools play a role in pharmaceutical chemicals, especially when the only characteristic known is the structure of a chemical. The pharmaceutical industry has successfully used two- and three-dimensional QSAR approaches to study drug receptor interactions. Because these methods allow screening of large libraries of molecules for potential activity, such techniques could also be used for evaluating environmental chemicals. Additionally, this approach could eliminate the need for resource-intensive laboratory work for an increasing number of chemicals.

Practical use of such models can help estimate toxicity of chemicals that lack experimental data. These tools can also help prioritize chemicals for screening and subsequent toxicity testing while saving cost and time, minimizing experimental animal testing, and optimizing overall use of resources. Recent laws, such as the European Union’s REACH regulation, are pushing acceptance of these methods by regulatory and public health communities in mitigating potential hazardous exposures. The versatility and utility of SAR/QSAR models could be further evaluated, demonstrated, and, if needed, refined through the *in vitro* data being generated through high-throughput and genomic studies. SAR/QSAR models are expected to play a critical role in toxicology and public health as the risk assessment process is modernized.

## PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING CASE STUDIES

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One major limitation to the PBPK models’ acceptance was that they were developed using different simulation languages (e.g., MatLab™, Simusolve, AcsIX™), thus making them difficult to use. Making the models more user-friendly will be an essential step to increase their use in the field by practitioners of risk and health assessments.

## A General Approach to Developing a Human PBPK Toolkit

A human physiologically based pharmacokinetic (PBPK) model toolkit is being developed to assist with site-specific health assessments. The kit, designed to better serve ATSDR's health assessors and state partners, will consist of a series of published human PBPK models coded in a common simulation language (Berkeley Madonna).<sup>4,32–36</sup> The ultimate goal is to develop an online PBPK database, where health assessors and other related health workers can access PBPK models quickly and easily. This collection of models will be called the "ATSDR toolkit."<sup>4,33</sup>

For model selection criteria, information such as number of data sets used to calibrate and evaluate the model, model maturity (number of predecessor models from which the model was derived), and author experience was used. At present, the toolkit includes models of environmental contaminants, including volatile organic compounds (VOCs) and metals.<sup>37–39</sup>

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### VOC MODELS

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A generic seven-compartment VOC model consisting of blood, fat, skin, kidney, and liver; rapidly and slowly perfused tissue compartments; and a gas exchange compartment was developed.<sup>39</sup> These compartments were included in the model based on their use in previously published PBPK models. The generic VOC PBPK model can be used for VOCs such as benzene (BEN), carbon tetrachloride (CCl<sub>4</sub>), dichloromethane (DCM), perchloroethylene (PCE), trichloroethylene (TCE), and vinyl chloride (VC).<sup>40–46</sup> All compartments were described as well mixed and flow-limited. Published literature provided chemical-specific and biochemical parameters for the models.<sup>47–54</sup> This model code allowed simulation of three routes of individual or simultaneous exposure: inhalation, oral, and dermal. However, lack of available published human data sets prevented comparison of the generic model predictions for the dermal route. In the current version of the model, it did not include original model simulations for metabolites and metabolite data; however, a critical improvement for post-screening use in the future will be incorporating metabolites information in this model, particularly when toxicity is mediated by metabolite(s).

The applicability of the model was first assessed by comparing it to the published human kinetic data for each VOC and the corresponding published model predictions. To ensure further the reliability of the model, the area under the concentration curve (AUC) for blood or exhaled breath for each VOC using both the generic and original published model was calculated as shown in Table 2-1. The mean of the sum of the squared differences (MSSDs) between model prediction and observation for each kinetic time course data set was also calculated; MSSD was computed by squaring the difference between a measured data point and the value of the simulation at the corresponding time. These squares were summed and then the sum was divided by the number of data points. The MSSD was thus determined for both the published model and for the generic VOCs model.<sup>39</sup> The VOC PBPK model was used to estimate the blood concentrations for the available minimal risk levels (MRLs) values<sup>55,56</sup> of each of the specific VOCs for which



**TABLE 2-1** Physiologically Based Pharmacokinetic (PBPK) Volatile Organic Compounds (VOCs) Model Comparison

VOCs	<i>AUC<sub>r</sub></i>		MSSD	
	Generic Model	Original Model	Generic Model	Original Model
BEN <sup>a</sup>	0.9	1.6	0.0008	0.0009
CCl <sub>4</sub> <sup>b</sup>	2.5	1.9	0.4515	0.2344
DCM <sup>c</sup>	1.1	1.1	3.8214	1.1722
PCE <sup>c</sup>	0.6	0.8	0.0805	0.0164
TCE <sup>c</sup>	0.8	0.8	0.0095	0.0089
VC <sup>b</sup>	1.2	1.1	0.1875	0.1831

BEN, benzene; CCl<sub>4</sub>, carbon tetrachloride; DCM, dichloromethane; PCE, perchloroethylene; TCE, trichloroethylene; VC, vinyl chloride.

<sup>a</sup>μM;

<sup>b</sup>ppm;

<sup>c</sup>mg/L.

**TABLE 2-2** Comparison of Minimal Risk Level (MRL) Simulated Blood Concentration of Each Solvent, Assuming Simultaneous Inhalation (24 h/day) and Oral Ingestion (4 Drinking Bouts per Day) to the Measured Blood Concentration of Solvent Reported by National Health and Nutrition Examination Survey (NHANES) 2003–2004. The Simulated Solvent Exposure Is Set to the MRL for Inhalation of the Solvent in Air and Ingestion of the Solvent in Water

	BEN <sup>+</sup>	CCl <sub>4</sub> <sup>+</sup>	DCM <sup>+</sup>		PCE <sup>+</sup>	TCE <sup>+</sup>	VC <sup>+</sup>
MRL <sup>*</sup>	0.003/0.0005	0.03/0.007	0.6/0.2	0.3/0.06	0.2/0.05	2/0.2	none
Exposure Duration	Chronic	Intermediate	Acute	Chronic	Acute	Acute	—
PBPK MODEL	Blood Concentration (ng/mL)						
Predicted Peak	0.04	0.40	18.12	6.70	10.76	111.65	—
NHANES <sup>**</sup>	Blood Concentration (ng/mL)						
	0.260 (0.210–0.320)	<LOD	< LOD		0.140 (0.091–0.300)	<LOD	ND <sup>**</sup>
Limit of Detection (LOD)	0.024	0.005	0.07		0.048	0.012	ND

BEN<sup>+</sup>, benzene; CCl<sub>4</sub><sup>+</sup>, carbon tetrachloride; DCM<sup>+</sup>, dichloromethane; PCE<sup>+</sup>, perchloroethylene; TCE<sup>+</sup>, trichloroethylene; VC<sup>+</sup>, vinyl chloride.

\*Inhalation concentration (ppm)/Oral ingestion rate (mg/kg-day);

\*\*NHANES 2003–2004. 95th percentiles of blood concentration (in ng/mL) for U.S. population, ND = Not Done.

biomonitoring data on human blood levels were available from the National Health and Nutrition Examination Survey (NHANES).<sup>57</sup> Steady-state VOC concentrations in venous blood were then compared with NHANES data using these simplified assumptions about exposure frequency and duration (Table 2-2).<sup>39</sup> If the measured NHANES blood levels were below those estimated from the simulations, the exposures were regarded as “safe.”

## METALS MODELS

Published human metals PBPK models for arsenic, mercury, and cadmium were reviewed, using Berkeley Madonna to select and recode the best model available based on performance, accuracy, and reproducibility.<sup>38</sup> Human physiological and chemical-specific parameters describing the absorption, distribution, and blood and tissue partitioning of arsenic (As), mercury (Hg), and cadmium (Cd) were obtained from the literature.<sup>1,58–62</sup> The PBPK models allow individual and simultaneous simulation of different routes of exposure.

A published Cd toxicokinetic model describes aggregated lung, liver, kidney, blood, and other tissues.<sup>60–61</sup> Intake by oral and inhalation routes is transferred to an uptake pool that distributes to three blood compartments. The model predicted the urinary concentrations of Cd that are considered a surrogate for body burden in assessing health risk from exposure, including the sex- and age-stratified geometric urinary mean. This model was used to predict the creatinine-corrected urinary Cd concentrations among women and men from the *Fourth National Report on Human Exposure to Environmental Chemicals*,<sup>37</sup> as shown in Table 2-3.

The recoded human PBPK model for arsenic consists of interconnected submodels for inorganic arsenic and its metabolites, monomethyl arsenic (MMA), and dimethyl arsenic (DMA).<sup>58</sup> The model includes compartments for lung, liver, gastrointestinal (GI) tract, kidney, muscle, brain, skin, and heart. Simulated arsenic exposures are controlled by a series of parameters that allow single or continuous dietary exposures to inorganic arsenic in the +3 or +5 valence state or exposures via drinking water. The recoded model

TABLE 2-3 Dietary Cadmium Intake, Model Predictions, and Geometric Mean Urinary Cadmium Concentrations in Nonsmoking Male U.S. Population (National Health and Nutrition Examination Survey: NHANES 2003–2004)

Age Group (Years)	Men			Women		
	Urinary Cd (µg/g creatinine)*		Cd Intake GM (µg/day)	Urinary Cd (µg/g creatinine)*		Cd Intake GM (µg/day)
	Measured	Predicted		Measured	Predicted	
6–11	0.088 (0.071 – 0.11)	0.101 (0.071 – 0.11)	15.0	0.088 (0.072 – 0.108)	0.172 (0.152 – 0.188)	13.5
12–19	0.074 (0.066 – 0.083)	0.087 (0.078 – 0.095)	19.7	0.103 (0.089 – 0.118)	0.163 (0.136 – 0.190)	15.1
20–39	0.125 (0.114 – 0.137)	0.137 (0.082 – 0.190)	22.4	0.179 (0.159 – 0.202)	0.285 (0.182 – 0.386)	16.2
40–59	0.208 (0.184 – 0.234)	0.214 (0.188 – 0.241)	22.1	0.342 (0.305 – 0.383)	0.427 (0.377 – 0.477)	16.5
≥ 60	0.366 (0.324 – 0.414)	0.226 (0.221 – 0.232)	17.6	0.507 (0.460 – 0.558)	0.453 (0.447 – 0.459)	14.4

\*From [60], 200 GM = geometric mean.

adequately simulated experimental human data found in the published literature. A visual comparison showed that the model performance corresponded well with that of the original model.<sup>38</sup> Performance was also evaluated by calculating values for percent median absolute performance error (MAPE%), median performance error (MPE%), and root median square performance error (RMSPE%) based on estimates of performance error (PE).<sup>38</sup>

The recoded human toxicokinetic model for methylmercury is based on the model described by the Carrier et al. model,<sup>59</sup> which consists of a total body compartment. Methylmercury enters this compartment from the GI tract by a first-order process. The amount of methylmercury in blood is proportional to that in the total body compartment.<sup>1,59</sup> The recoded model reproduced all the simulations of the original model.<sup>38</sup> A visual comparison showed that the model performance corresponded well with that of the original model. The model performance was evaluated by calculating a value for MAPE%, MPE%, and RMSPE% based on PE. The model could simulate and accurately predict the available total body burden of mercury experimental data, and its predictions were similar to those observed experimentally and found in published literature.<sup>38</sup> Overall, the current model could integrate various experimental data that are critical determinants of methylmercury kinetics. It also duplicates the time courses of various tissue burdens for different dose regimens and exposure scenarios.

In this chapter, a review of the ATSDR's progress toward making PBPK models available to the scientific community and health assessors, by bridging the gap between development and use, was represented. This human PBPK toolkit was done by recoding the best available published multiple simulation languages' PBPK models into a single simple simulation language (Berkeley Madonna). When completed, the models will be packaged into a human PBPK toolkit. Currently, the recoded models include three high-ranking metals and some commonly encountered VOCs from ATSDR's priority list of environmental contaminants. It has demonstrated that the toolkit can be used in the assessment of biomonitoring results as a screening tool. One major advantage of the human PBPK toolkit being developed at ATSDR is that it can be applied in the field by practitioners of risk and health assessments.

In the future, model developers and model users should work together to encourage use and acceptance of computational tools such as the human PBPK toolkit in the decision-making process. Such information exchange and shared expertise will

- Lead to the tools being more often used in the field;
- Increase awareness of their advantages and limitations; and
- Promote integration of the toolkit into the options available for decision makers.

It has been shown that models available in multiple simulation languages can be recoded into one simulation language. Thus, the end user has to learn only one simple language, rather than a multitude of computer languages, to derive the predictions needed for risk assessments. Such efforts will facilitate the models' integration into risk assessment processes.

The growing field of computational toxicology will produce innovative, increasingly available tools for chemical risk assessment. High-throughput screening and *in vitro* testing could change toxicity testing strategies. Computational tools are a necessary component for the development of new toxicity testing methods.

## Physiological Model for Bisphenol A

Bisphenol A (BPA), a high production volume chemical commonly used to harden polycarbonate plastics and epoxy resins, is present in a wide variety of consumer products, such as hard plastic products and the lining of metal food and beverage cans.<sup>63</sup> The Food and Drug Administration (FDA) estimates of average dietary exposure to BPA are 0.2–0.4  $\mu\text{g}/\text{kg}$  body weight (bw)/day for infants and 0.1–0.2  $\mu\text{g}/\text{kg}$  bw/day for children and adults.<sup>64</sup> As currently stated on the FDA's website, "scientific evidence at this time does not suggest that the very low levels of human exposure to BPA through the diet are unsafe"; however, the FDA also realizes that uncertainties exist with regards to the overall interpretation of the experimental data. Thus, the FDA is still pursuing more investigations to clarify uncertainties about the potential risk of BPA on human health.<sup>65</sup>

One area of BPA research at the National Center for Toxicological Research (NCTR) is pharmacokinetic studies on BPA, in which several species (rats, mice, monkeys) have been dosed with deuterated BPA, including at different reproductive states and ages.<sup>66–71</sup> Phase II conjugation (metabolism) of BPA is a detoxification pathway resulting in production of BPA-monoglucuronide (BPA-glu) conjugates and much smaller amounts of BPA-sulfate conjugates.<sup>72</sup> BPA is subject to substantial presystemic metabolism in the liver and small intestine, resulting in low oral bioavailability. Since oral ingestion of BPA is the primary route of exposure, a quantitative understanding of first pass metabolism is important for estimating the potential health risks from exposure to BPA. In monkeys and adult humans, BPA conjugates (BPA-c) are eliminated mainly by renal excretion. In rodents, BPA conjugates, mainly excreted into the feces (as the aglycone BPA) via the bile, are subject to enterohepatic circulation.<sup>73</sup> Small amounts of BPA-c are excreted in urine of rodents.

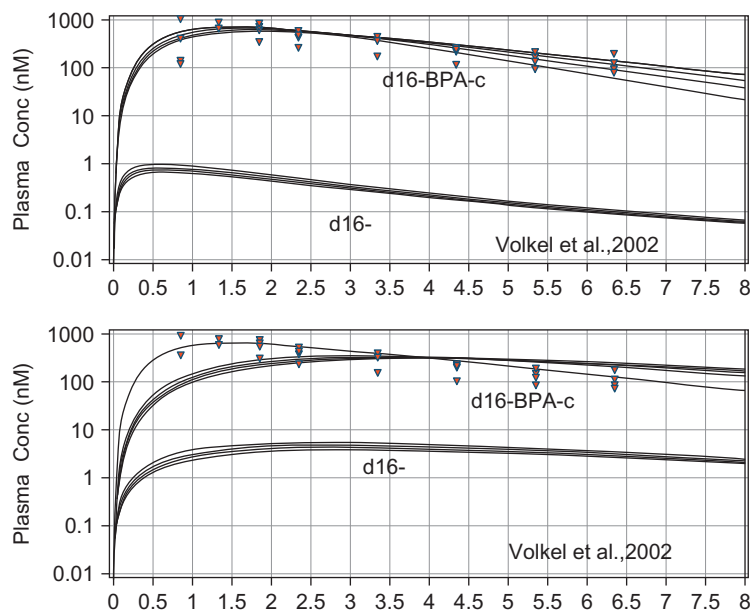
At NCTR, the PBPK models for BPA are under development for rodents, while an adult and infant monkey model for BPA was recently constructed and used to predict internal doses of BPA in human adults and infants.<sup>74</sup> These PBPK models, calibrated with deuterated BPA, will assist in dose-response analyses as new toxicological data become available and provide a foundation to help interpret reports of unusual concentrations of native BPA in rodents or humans. Contamination of biological samples with native BPA is an issue for any laboratory involved in measuring low levels of native aglycone BPA in blood or urine.<sup>75</sup> Several approaches have been used to minimize possible background contamination, e.g., use of stable isotope labeled BPA, and/or use of glass and polypropylene plastic products during the sampling and analytical process.<sup>75,76</sup>

The model development for BPA was based on previous BPA modeling work by Teeguarden et al.; the rodent and human PBPK model for BPA consists of four compartments for BPA and a volume of distribution for BPA-glu.<sup>77</sup> They investigated plasma protein binding of BPA by ultrafiltration of rat plasma for BPA concentrations ranging from 5833 to 877017 nM. Equations were incorporated in the PBPK model to account for nonspecific plasma protein-bound BPA and unconjugated BPA. BPA doses simulated by Teeguarden et al.<sup>77</sup> included oral administration of 100 mg/kg of  $^{14}\text{C}$ -BPA to male and female Fischer 344 rats<sup>72</sup> and intravenous (IV) dosing of 10 mg/kg of BPA in female DA/Han rats.<sup>78</sup> In addition, the model was used to extrapolate from rats administered large doses of BPA to low doses in humans. The human BPA model,<sup>77</sup> as well as the human BPA model reported by Fisher et al.,<sup>74</sup> relied on pharmacokinetic data reported by Volkel et al.<sup>79</sup> In this human study,

5 mg/individual of d<sub>16</sub>-BPA was given in hard gelatin capsules (54–90 µg/kg bw).<sup>79</sup> The conjugated aglycone BPA was below the limit of detection in the human plasma (10 nM) and urine samples (6 nM).

Later, Fisher et al.<sup>74</sup> constructed a PBPK model for BPA in adult and infant rhesus monkeys, administering IV or oral bolus doses of 100 µg/kg of d<sub>6</sub>-BPA.<sup>70</sup> A sensitive and selective LC-MS/MS method was employed to determine the concentrations of both unconjugated aglycone and total BPA after enzymatic digestion with β-glucuronidase and sulfatase.<sup>75</sup> D<sub>6</sub>-BPA was used to avoid confounding the samples by native BPA contamination. In this PBPK model, phase II metabolism of BPA in the small intestine enterocytes was proposed to simulate the reduced serum aglycone BPA concentrations following oral administration. Also, systemic uptake of BPA metabolites (BPA-c) from the intestinal enterocytes was proposed to describe the rapid appearance of high serum levels of BPA-c after oral administration of BPA. In contrast to rats, urinary excretion of BPA-c is the major pathway for the elimination of BPA in non-human primates. Using the adult monkey model parameters for BPA and BPA-c, simulations of human ingestion of d<sub>16</sub>-BPA<sup>79</sup> were accomplished to predict the unconjugated BPA concentrations in serum (Figure 2-1).

Currently, a maturing and adult rat PBPK model for BPA and BPA-c is in development to describe the age-dependent dosimetry of unconjugated BPA in serum.<sup>80</sup> While other PBPK models for rodents have been developed for BPA,<sup>77,81–83</sup> none of the PBPK models describe BPA deposition in young rats. Additionally, presystemic metabolism of BPA in the GI tract has not been adequately characterized in the previous models, or enterohepatic recirculation of BPA-c excreted in bile. Collectively, this suite of BPA PBPK models developed at NCTR for rodents, monkeys, and humans will provide a quantitative tool to



**FIGURE 2-1** Simulations depicting plasma levels of the aglycone BPA and a metabolite, BPA-glucuronide (BPA-c),<sup>74</sup> from 4 adults who ingested deuterated BPA.<sup>79</sup> Only measurements of BPA-c in plasma were reported. The two panels represent model predictions in the 4 human volunteers based on *in vivo* nonhuman primate PBPK kinetics studies in which the monkeys were either dosed with deuterated BPA in an aqueous form (top panel) or with food (bottom panel).<sup>74</sup>

compare the dosimetry of BPA across species, life stages, and routes of administration and to conduct dose-response assessments.

## BBDR HPT Axis Modeling

Pharmacokinetic analyses of the hypothalamic-pituitary-thyroid (HPT) axis several decades ago have helped us to understand complex relationships between thyroid hormone production, transport, distribution, and metabolism and the biological actions of thyroid hormones.<sup>84,85</sup> Recently, biologically based dose response (BBDR) models for the HPT axis were developed for adult rats<sup>86–88</sup> to better understand selected aspects of the HPT axis from a quantitative perspective. These models predicted changes in serum thyroid hormones in response to iodide deficiency or chemical insult<sup>87</sup> but did not describe the intracellular nature of thyroid hormone action (e.g., binding and transcription). One reason these BBDR models of the HPT axis were developed was to gain insights into the relationship between serum thyroid hormones and the status of the HPT axis, as measured by serum thyroid stimulating hormone concentrations, serum iodide concentrations, and thyroidal iodide stores (thyroidal stores of thyroid hormones). Linking changes in serum thyroid hormone levels with adverse outcomes mediated by the HPT status is difficult, particularly for the lower end of the dose-response spectrum. The endocrinology literature is replete with “high dose, sledge hammer” responses of the HPT axis to thyroid active compounds, and a dearth of carefully designed studies exists for examining the HPT axis at moderate or low doses of thyroid active chemicals.

The recent BBDR HPT axis models<sup>86–88</sup> for adult rats include submodels for dietary iodide, thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3). These submodels are dependent on each other with interactions described using linear and nonlinear equations. Key biological processes described in the BBDR HPT axis models are the primary HPT axis negative feedback loop, TSH regulation of thyroidal gland by controlling the thyroid sodium iodide symporter (NIS) protein, the formation of thyroid hormones and the secretion of T4 and T3, and recycling of iodide liberated from deiodinase enzymes acting on thyroid hormones. TSH serum concentrations, under the control of serum levels of T4 (negative feedback loop), controlled the thyroid gland. Nested Michaelis–Menten equations were used to describe the control of TSH on thyroidal uptake of iodide by the NIS and the rate of formation of pools of bound precursor thyroid hormones in the thyroid gland, from which thyroid hormones were produced and secreted into systemic circulation. The rationale was to develop one set of model parameters that would describe, within reason, the data obtained from several laboratories. The BBDR HPT axis models evaluated iodide deficiency in albino rats using data published in the literature<sup>86</sup> in Long Evans rats using data collected as part of a larger research program,<sup>89</sup> and in euthyroid adult rats dosed with perchlorate, a drug and a food and environmental contaminant that blocks uptake of thyroidal iodide.<sup>87</sup> Significant strain differences in HPT axis responses to iodide deficiency were observed. Serum thyroid hormones and TSH changed less in the Long Evans rats compared to the Sprague Dawley rats on a comparable iodide deficient diet. The reason for this is unknown. Another discovery was that perchlorate may act on the thyroid gland by another mechanism, in

addition to blocking uptake of iodide in the adult Sprague Dawley rats. The BBDR HPT axis model could not predict the rapid changes in serum TSH and serum thyroid hormones observed after perchlorate administration, based solely on depletion of thyroidal iodide caused by blocking its uptake.

BBDR HPT axis models for the fetal and young<sup>88,90,91</sup> rats and humans are in development. The goal is to predict changes in the HPT axis during the maturation phase *in utero* and in the young as a consequence of iodide deficiency and exposure to perchlorate. The fetus and young are thought to be sensitive to alternations in thyroid hormones, particularly the brain, during critical windows of development. Extending the BBDR HPT axis models to address these sensitive subpopulations is important to help protect the fetus and young from irreversible neurotoxicity by predicting perturbations in the serum thyroid hormone profiles that may be associated with adverse neurotoxicity.

In the lactating rat and postnatal day (PND) 14 nursing pup, an HPT axis model was recently calibrated for each dietary iodide intake, ranging from 39–6.4  $\mu\text{g}/\text{day}$  for euthyroid lactating rats to 1.2–0.31  $\mu\text{g}/\text{day}$  for iodide-deficient lactating rats.<sup>92</sup> In this BBDR HPT axis model, calibrations were carried out for each iodide diet. A single set of key model parameters described the euthyroid conditions, while key model parameters were changed in a dose-dependent manner to describe iodide deficient conditions. Serum T4 dosimetrics in the PND14 pup were calculated for the iodide-deficient conditions and compared to neurodevelopmental outcomes in adult offspring.<sup>89,92</sup>

Another effort ongoing in parallel with the lactating rat BBDR HPT axis model is the pregnant woman and fetus BBDR HPT axis model for moderate iodide deficiency and exposure to perchlorate.<sup>91</sup> Iodide deficiency in pregnant women, leading to hypothyroxinemia and hypothyroidism, has been shown to cause neurodevelopmental toxicity to the fetus.<sup>93–95</sup> A BBDR model for the HPT axis in the pregnant woman and the fetus was developed for near-term pregnancy (40 weeks) to quantitatively evaluate the effects of a range of iodide intake rates on serum-free thyroxine (fT4) levels, and the interactions of co-exposure to iodide and perchlorate on serum fT4 levels. The average intake of iodide for women of reproductive age (25–45 years) was estimated to be 145–197  $\mu\text{g}/\text{day}$  by the FDA Total Diet Study (2003–2004), and the mean intake dose of perchlorate from food and water for females in age group 15–44 years was calculated to be 0.083  $\mu\text{g}/\text{kg bw}/\text{day}$ .<sup>96,97</sup>

The BBDR HPT axis model is composed of four maternal and fetal submodels (iodide, perchlorate, T4, and T3). PBPK submodels for iodide and perchlorate were integrated based on the primary mode of action of perchlorate to inhibit competitively the sodium iodide symporter mediated uptake of iodide into the thyroid gland, and serum thyroid hormones were described using single compartments. Model parameterization for euthyroid conditions was carried out in a “piecewise” manner conforming to several quantitative thyroidal system constraints as determined from various literature sources.<sup>98–104</sup> The model was then extended to predict serum T4 and T3 levels in the mother and fetus for lower iodide intake conditions and for co-exposure to perchlorate. Maternal hypothyroxinemia is a condition in which the circulating free thyroxine (fT4) levels are in the lower regions of the normal euthyroid reference ranges with no expected change in the TSH levels beyond its normal reference range.<sup>105</sup> In marginal iodide deficiency conditions, evidence exists for thyroidal autoregulation prior to the engagement of the hypothalamus (TSH stimulation) to maintain thyroid hormone homeostasis.<sup>106</sup> Such a phenomenon was



accounted for in the model by the adjustment of maternal parameters, like the renal clearance rate for iodide and the peripheral degradation rate for T3, as a function of iodide intake. The simulated maternal and fetal thyroid hormone levels, for perchlorate exposure, were in good agreement with the observed levels in the few epidemiological data available in the literature.<sup>107</sup> The dose-response model was thus successfully calibrated for euthyroid, mild to moderate iodide deficiency, and environmental and dietary perchlorate exposure conditions.<sup>91</sup>

This BBDR model for the HPT axis offers a valuable quantitative analysis, providing a better understanding for the conditions that may put the fetus at risk for perchlorate exposure and potential adverse neurodevelopmental outcomes as well as an assessment of the current exposure conditions for perchlorate. Expanding the deterministic BBDR HPT axis model for the pregnant mother and fetus to include distributions for model parameters (using Monte Carlo methods) will help provide model simulations that better represent a population of pregnant women. The significance of such computational tools lies in their ability to offer a fundamental framework for the biological systems and chemicals of interest by integrating the various conceptual, quantitative, and mechanistic segments of information available to better characterize the dose-response relationships and aid human health risk assessment measures to make informed regulatory decisions.

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