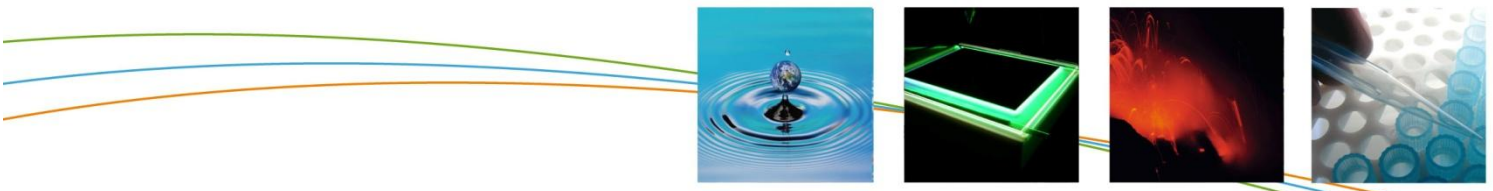
REPORT

Intarese Deliverable D86

Measuring and modeling: Combining PBPK modeling and human biomonitoring values

Roel Smolders & Greet Schoeters



Boeretang 200 – 2400 MOL – BELGIUM
Tel. + 32 14 33 55 11 – Fax + 32 14 33 55 99
vito@vito.be – www.vito.be

VAT BE-0244.195.916 RPR (Turnhout)
Bank 435-4508191-02 KBC (Brussel)
BE32 4354 5081 9102 (IBAN) KREDBEBB (BIC)

CHAPTER 1 ISSUE FRAMING: MEASURING

1.1 Introduction

Recently, it has become more and more obvious that there is a tremendous effect of adverse environmental conditions on the occurrence of chronic disease in the general population, ranging from e.g. diabetes due to bad nutritional habits to cardiovascular diseases due to environmental exposure to particulate matter. With the impact being substantial, it is an even bigger task to unravel the often very subtle and multi-causal interactions between the environment, life-style, individual genetic susceptibility and the occurrence of health effects in the general population. Willett (2002) estimates that for many frequent diseases such as stroke, coronary heart disease or type 2 diabetes, 70% to 90% of all attributable risks are due to life-style factors, which hence is potentially preventable.

Traditionally, environmental epidemiology studies the environment-related factors that directly affect the distribution of health and illness among populations, and aims at deriving causal relationships and insights among exposure and health data. Apart from a very limited number of cases (e.g. smoking and lung cancer), a "one cause – one effect" understanding of the relationship between environmental stressors and health effects is a simplistic misbelieve. Most outcomes of morbidity and mortality are caused by an intricate chain of events. Hence, epidemiology addresses the question whether an agent could cause a disease, not whether an agent did actually cause a specific adverse health effect (Bailey et al, undated).

To better understand the effects of exposure to environmental chemicals on public health, including internal dose as an intermediate step between environmental exposure data and health effect offers increased opportunities to further substantiate potentially relevant causal inference along an exposure-dose-response chain (EDR, Figure 1.1). Internal dose, i.e. *the amount of agent that enters a target after crossing an exposure surface*, refines exposure assessment by describing exposure to chemicals in terms of an internal metric rather than an external one. By this shift from external to internal, dose integrates a wide variety of processes from a very diverse nature, including biological, chemical and socio-economic ones (Table 1.1). The main aim of this framework is to move away from the simplistic "*distance from source*" approach, and to include "*a more refined and person-specific assessment*" of the link between environmental exposure and health effects ((National Research Council 2006; Lyons et al 2008, Smolders and Schoeters 2007).

Table 1.1 Dose as a metric of internal concentration allows integration of information from a disparity of sources

Information source	Added value along the EDR information chain
Time-activity patterns:	People frequent multiple micro-environments during the period of a day, a week, a lifetime... These different environments may all contribute in various ways to the total exposure profile of an individual and hence are reflected in the internal dose
Confounding factors:	Age, gender, ... may be confounding factors that have a profound effect on internal concentrations of pollutants
Socio-economic/lifestyle factors:	Housing conditions, smoking behavior, food consumption patterns,... may all be important factors that directly (as a source of contaminants) or indirectly (through alteration of general physical status) have an impact on internal dose
Pharmacokinetic information:	For a chemical to elicit a toxic response, contact needs to be made with the target tissue. Factors like absorption, distribution, metabolism and excretion (ADME) to a large extend drive this process
Individual susceptibility:	Differences in genetic composition also may influence sensitivity or susceptibility to pollutants

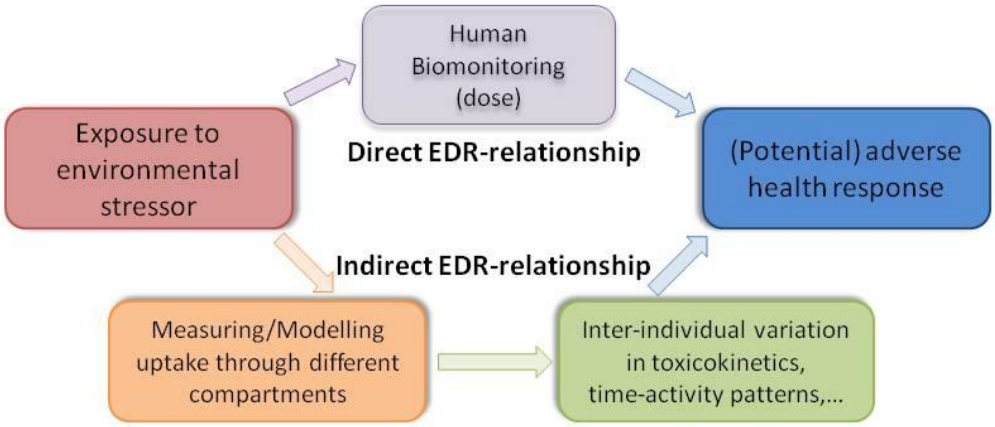
Apart from its advantage of being an integrative measure of exposure through multiple environmental compartments and time-scales (which offers important benefits for refining exposure assessment), including dose in the exposure-response relationship increases the potential of an environmental health impact assessment to make a shift from a population-based to a more individual environmental health impact assessment(Figure 1.1).

Including dose as an intermediate metric can be done in two different ways:

- Direct EDR-relationship: human biomonitoring (HBM) provides a direct means to quantify internal dose of a chemical by measuring the chemical, metabolite or reaction product in human tissues or specimens, such as blood, hair, or urine;
- Indirect EDR-relationship: separate pieces of information, describing (some of the) information sources outlined in Table 1.1, are combined, generally in some mathematical format, to derive a modeled estimation of internal concentration.

Rather than seeing these as two separate means to improve the relationship between exposure and effect along the EDR-chain, it are two different ways to describe the same relationship, and hence also should be used as mutually supporting lines of evidence. Human biomonitoring provides a direct observational measure of the internal dose of a chemical, but very often lacks the sensitivity and specificity to identify individual sources. Models provide a more mechanistic approach to relate dose to environmental exposure to specific exposure pathways, and/or to relate to dose to particular health effects, but require validation as they only represent an approximation of the hypothesized relationships between environmental exposure and associated health effects.

Figure 1.1 Outline of the exposure-dose-response (EDR) framework in Environmental health impact assessment



In the whole suite of modeled approximations, Physiologically-Based PharmacoKinetic (PBPK) or Physiologically-Based Toxicokinetic (PBTK) models play a key role in eliciting the relationship between external exposure and internal dose[1]. By mathematically representing the behavior of a compound following exposure, these models can be of great value for interpreting human biomonitoring values. They provide a mechanistic description of the phenomena involved in the complex ADME processes.

The main aim of this document is to examine the use of PBPK models to describe the relationship between exposure and dose along the EDR-chain, and clarify the mutual benefit of using both measured HBM data (i.e. direct EDR relationship) and modeled PBPK data (i.e. indirect EDR relationship) for improving environmental health impact assessment are discussed. In the end, both techniques to approximate internal dose can be mutually beneficial in improving the interpretation of human biomonitoring, and hence also the complex relationships between environmental exposure and health effects/

We will start by a very brief overview of the advantages of human biomonitoring techniques in addressing the EDR-relationship. However, a lot of information on this has already been aggregated, and is available elsewhere within the INTARESE toolbox.

1.2 HBM and the direct EDR-relationship

Human biomonitoring (HBM) data provides unique tools to directly assess the relationship between exposure to environmental contaminants and associated health effect. As a means to quantify internal dose, it is a valuable midway point between external concentrations and early health effects (Figure 1.2). A typical (research) study would illustrate that the exposure-dose-response continuum follows a logical progression of events from external to internal concentration, and further onto early detection of health effects (typically left-to-right progression in figure 1.1).

[1] In this document, the terms "pharmacokinetic/PBPK" and "toxicokinetic (PBTK)" are considered to have the same meaning

Human biomonitoring in such a research study uses a perceived relationship between exposure and health effect as the driving hypothesis, and biomonitoring data is used to further specify to which extend the particular chemical had entered the body. In this perspective, human biomonitoring data would not be an endpoint in itself, but a means to better describe internal processes and a more direct relationship with potential health effects. Generally, this type of research studies describes exposure, dose and response of (a limited number of) individuals in detail, and is a source for both hypothesis generation and testing.

In the last decade or so, another "type" of human biomonitoring data has emerged that has had a significant impact in how we nowadays see Environment & Health related issues. Broad population surveys, such as GerES in Germany, NHANES in the USA, or the Flemish Human Biomonitoring Program in Belgium have focused on quantifying background information on the exposure in the general population, without necessarily addressing immediate research questions (Table 1.2). Because often several thousands of individuals generally are included in this type of study, it would be both financially and logistically impossible to describe environmental exposure and health effects in the same detail is generally the case in the previously mentioned research studies. In large HBM survey projects, biomarker data are often gathered and reported without corresponding detailed external exposure data, leaving the relationship between internal and external exposure as one to be determined (Clewell et al 2008; Smolders et al 2009). The typical left-to-right progression from Figure 1.1 is not necessarily followed in a human biomonitoring survey project, and retracing source identification and identifying early warning signs of adverse health effects becomes a difficult task. Hence, for improved understanding of human biomonitoring data originating from survey projects, additional options for "source identification", "exposure reconstruction", or "population health relevance" are needed.

Figure 1.2 The role of biomonitoring in the "Exposure-Dose-Response" continuum (Redrawn from Clewell et al, 2008)

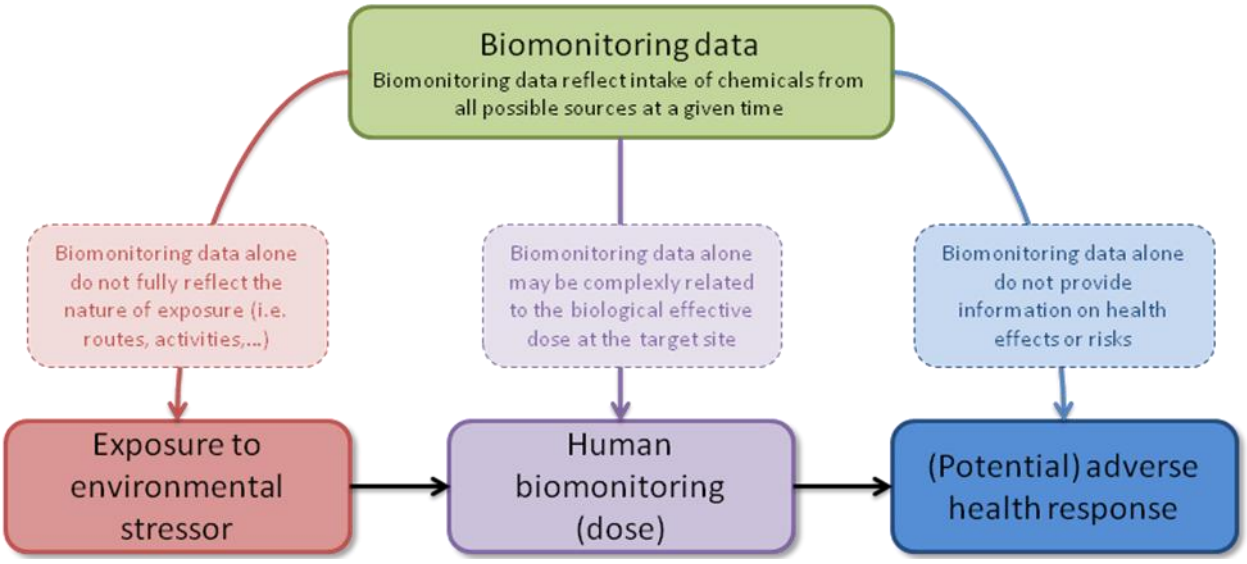


Table 1.2 A brief overview of some large scale HBM population surveys. URLs indicate where results for the different surveys can be obtained

Country	Survey name	URL for Results
Belgium	Flemish CEH	http://www.milieu-en-gezondheid.be/English/index.html
Canada	CHMS	http://www.hc-sc.gc.ca/ewh-semt/contaminants/human-humaine/index-eng.php
Czech Republic	Environmental Health Monitoring	http://www.szu.cz/topics/environmental-health/environmental-health-monitoring
Germany	GerES	http://www.umweltbundesamt.de/gesundheit-e/survey/index.htm
International	COPHES-project	http://www.eu-hbm.info/
International	ESBIO-inventory	http://www.hbm-inventory.org/scid/e-formv2/default.asp
International	WHO-human milk	http://www.who.int/foodsafety/chem/pops/en/index.html
USA	NHANES	http://www.cdc.gov/exposurereport/

Generally, these options need to take into account population variability in exposure and pharmacokinetic processes. One of the major themes in source identification for biomarkers of exposure, and the health-based interpretation that is associated with it, is the difference between source identification at the individual level and at the population level (Table 1.3). This discrepancy is also reflected in the opportunities for source identification for environmental stressors, and in the variability that is associated with this. Because detailed information on external exposure concentrations or time-activity patterns is often missing, source identification for large-scale survey projects mostly occurs at a population level. Although survey projects typically contain data on a large number of individuals, and hence reflect population variability in biomarker values, they tend to contain relatively limited information per individual regarding specific exposure scenarios or pathways. Most frequently, only one sample is taken per individual, hence omitting all information regarding the kinetic behavior of the biomarker studied (Mosquin et al 2009). This shift from individual to population requires taking into account the natural variability that is present in population exposure profiles, time-activity patterns or pharmacokinetic variability.

Table 1.3 Differential aspects of research and survey projects generating HBM data

	Research project	Survey project
# participants	Low	High
Exposure classification	High	Low
Aim	Hypothesis testing	Background values
Inter-individual variability	Low	High
Study control	High	Low
Source identification	Individual level	Population level

1.3 Improving the interpretation of HBM data through modeling

The aim of this document is to outline some (indirect) modeling approaches to improve the interpretation of survey-based HBM data. For these approaches to become successful however, they will need to take into account a number of requirements:

- **Inter-individual variability**: HBM data has the unique advantage that it includes person-specific exposure information like for example individual susceptibility, time-activity patterns, and life-style and consumer behavior in one measurement. Modeling approaches to interpret HBM data should as much as possible allow the opportunity to, as much as possible include person-specific data as well;
- **Dynamic exposure profiles**: Internal dose is not a static variable, but reflects the temporal and spatial variability in exposure. In this perspective, a HBM value is only a point estimate of a continuously fluctuating concentration of a contaminant in a human matrix. Modeling efforts should preferably enable scientists to include this variability;
- **Generic application**: The Fourth National Report on Human Exposure to Environmental Chemicals (CDC 2010) reports on the concentrations of over 200 different chemicals in human matrices of about 2400 participants across the United States. And it is foreseen that in the future, many more techniques to detect chemicals in human matrices will become available. Coming up with specifically suited models for each of these hundreds of different chemicals would be a daunting task, and would probably not be feasible. Therefore, generic models are needed that offer enough sensitivity to adequately describe indirect EDR-relationships, while at the same time offer sufficient flexibility to warrant routine application for a broad range of substances. Also, the models should offer an interface that can be understood by a broad suite of scientists, even without a specific background. Hence, easy computation and a user-friendly interface are essential;
- **Mechanistic information:** Obviously, a good model should as much as possible provide mechanistic information on the sources of contaminant exposure, and potentially also the relationship with health effects, and in an ideal situation predict how changes in exposure would result in changed internal dose. By adding this mechanistic information in outlining policy actions, the most important exposure routes may be identified that offer the best cost-benefit options for policy makers (Loizou et al 2008).

CHAPTER 2 PBPK MODELLING IN THE EXPOSURE-DOSE-RESPONSE CHAIN

2.1 The general purposes of data modeling

Human biomonitoring offers a direct description of the dose associated with aggregate exposure across all pathways, also including the physiological and pharmacological variability inherent to biological systems. At the same time however, there is a need to improve understanding about what the major exposure pathways are, which organs are targeted by a particular exposure pathway, and how different individuals with variable susceptibility and time-activity patterns are affected by variable exposure scenarios. It is clear that biomonitoring, as an integrative measure of exposure, cannot provide this information by itself, but would benefit from a more mechanistic approach to improve the understanding of EDR-relationships.

Recently Dahl et al (2009) dedicated a review on the conceptual framework of pharmacokinetic models. They argued that the main purposes of constructing and applying a pharmacokinetic model in environmental health impact assessment are to:

- Describe complex data;
- Test hypothesis;
- Make predictions.

The type of model to be developed is likely to differ depending of the intended use of the model, and the assessment of model quality include agreement with previous knowledge, agreement with observed data, and agreement between prospective model predictions and new experimental data. More specifically within the context of this document, a good model offers a representation of the mechanisms describing the physiological, pharmacological processes of relevance to xenobiotic exposure, and allows one:

1. To test whether assumed hypotheses are consistent with observe behavior,
2. To examine the sensitivity of a system to parameter variation,
3. To learn about processes not directly amenable to experimentation,
4. To utilize prior knowledge in the analysis of new data,
5. To naturally increase complexity of the model as information becomes richer, and
6. To predict system behavior under conditions not previously experienced.

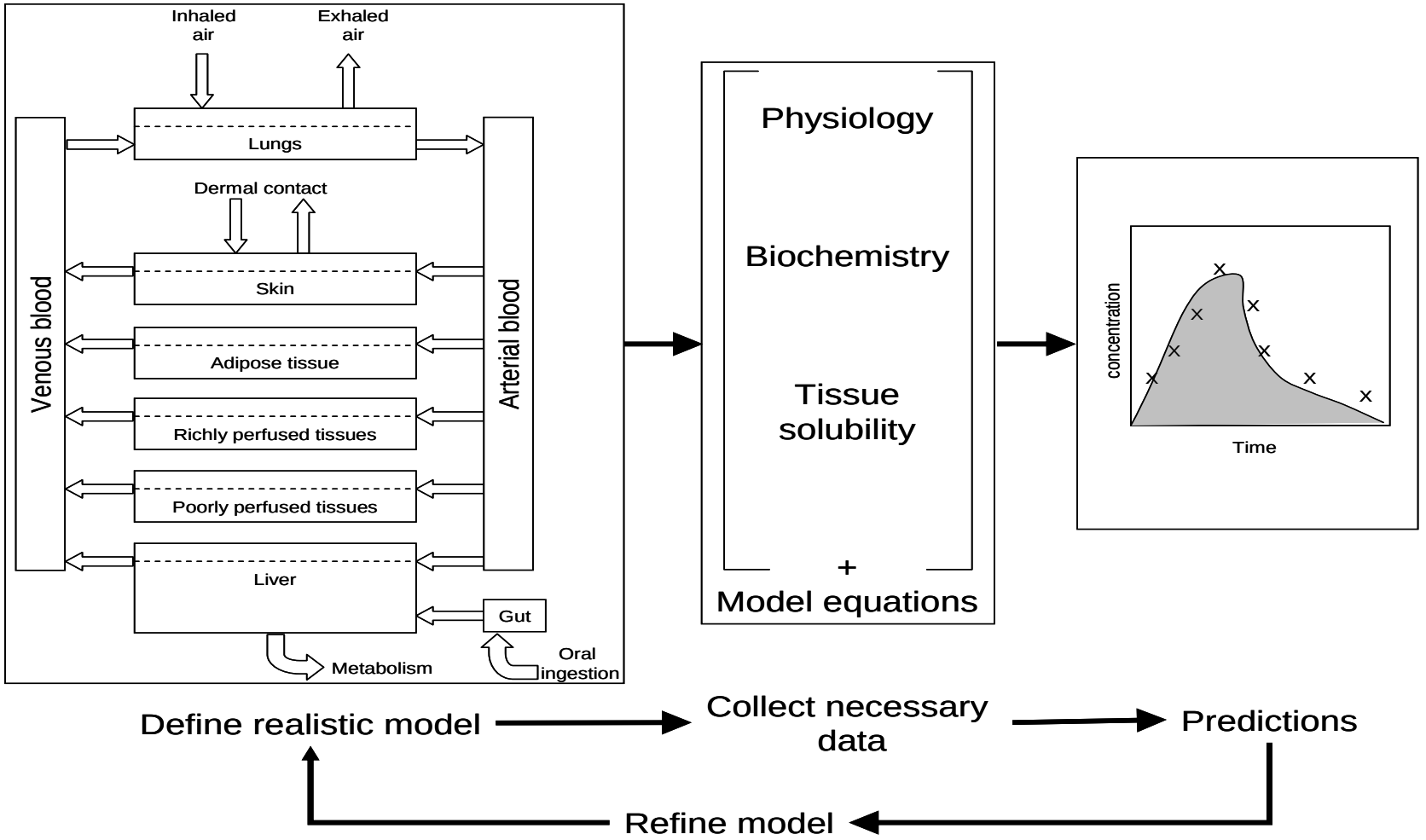
The main message of this document is that models can provide this type of mechanistic information, and hence provide an important added value for the interpretation of HBM data.

2.2 PBPK modeling and the indirect EDR-relationship

A central paradigm in toxicity states that "the presence of a chemical does not necessarily equal the presence of risk". Indeed, the expression of toxicity does not only dependent on (the route of) exposure, but also includes uptake-elimination processes, and eventually the contact between the biologically-active form of a chemical and the critical target receptor in the body to elicit a toxic response. These three elements, (1) exposure, (2) pharmacokinetics and (3) physiological dynamics form the basis of hazard and risk evaluation (Blaauboer 2003; Lipscomb et al 2004). The way chemicals are taken up, transported and excreted by individuals is an inherent source of interindividual variability, and are often not fully understood. Because of variability in absorption, distribution, metabolism and/or excretion (ADME) parameters, individual susceptibility to environmental contaminants can differ dramatically among individuals within a well-defined population (Dahl et al 2009, Bois et al 2010). In risk assessment, the internal exposure to a chemical at the target site for toxic effect is often referred to as a "target dose".

In recent years, physiologically based pharmacokinetic (PBPK) modeling has shown to be well-suited to calculate tissue doses of chemicals and their metabolites over a wide range of exposure conditions. Because these PBPK models are based on the human physiology and anatomy and summarize the behavior of chemicals in the body, they are often considered to be more realistic compared to empirical models (Beaudouin et al 2010; Jonsson 2001). In these PBPK models, the body is subdivided into a series of compartments that represent specific organs or lumped tissue and organ groups with appropriate volumes, blood flow rates, and pathways of metabolism (Figure 2.1). Routes of administration and exposure scenarios are included in their proper relationship to the overall physiological structure, and differences in exposure scenario are accounted for in the time sequence of the dose input terms. The transfer of chemicals between the different compartments is described by differential equations, with perfusion being the main limiting parameter for distribution of the chemical within a given compartment (Jonsson 2001).

Figure 2.1 An idealized approach for a PBPK model for simulation diffusion-limited tissue uptake and multi-route exposures. Dottet lines represent the separation of cellular matrix and tissue blood components (redrawn from Andersen et al (2003) and USEPA (2006)



2.3 Modeling individual exposure profiles

In its simplest form, two identical individuals or organisms (human or animal) should respond identical when in contact with the same stressor. In this ideal situation, interpretation along the EDR-chain would be very simple. A particular exposure would lead to a related internal dose, which could be determined by modeling or human biomonitoring, and this in its turn would cause an almost predefined health effect. In a real world however, this obviously is not the case. Mainly through (1) differences in exposure profiles among different individuals and populations and (2) inter-individual variability in pharmacokinetic parameters, different organisms will respond differently to their environment and in the way they process chemicals through ADME. This normal biological variability significantly complicates the interpretation of human biomonitoring data, as the relationship between the external concentration of a pollutant and the internal biomonitoring dose becomes blurred.

To gain better understanding of the factors responsible for the inter- and intra-individual variability of the target dose, using an indirect approach to better understand the EDR-chain offers great potential. By better understanding how variability of ADME processes among and within individuals influences the relationship between external concentrations and internal dose, interpretation of HBM data and source identification can be made much easier. PBPK models, if correctly built and parameterized, offer great potential in among others identifying the most relevant exposure routes, metabolites that may be of relevance, and relating target dose to health effects.

However, when developing PBPK models to understand population exposure profiles, the models need to take into account that not all individuals in a population share the same physiological or pharmacokinetic profile, nor does exposure in similar exposure scenarios lead to comparable biomarker values or health effects. A population-based estimate of exposure should account for the intrinsic heterogeneity in the population, both in the modeling of the disposition of the chemical in the body, and in the description of the exposure conditions (Bois 2001). Additionally, the biomonitoring data itself, considered as a whole, should reflect the variability in the population from which it arises (Lyons et al 2008). If source identification based on biomarker measurements is to be successful, factors such as appropriate exposure data availability, and pharmacokinetic variation need to be taken into account in the models used.

While PBPK models have become widely accepted tools for chemical risk assessment, the statistical calibration and validation of PBPK models has received comparatively little attention. This is particularly true for the exact values of the physiological and physicochemical parameters that need to fit into the differential equations, which are often not known with precision, especially not *in vivo* (Jonsson 2001). In order to properly account for the inter- and intra-individual variability inherent in toxicokinetic data, and entangle these variability (i.e. a biological reality) from uncertainty (i.e. a lack of appropriate data), proper means for model validation are required.

As PBPK models use biological information in order to predict the disposition of chemicals, genetic variability, differences in life stage, gender, ethnicity, or health status may affect the processes that control this disposition. Most PBPK models generally represent pharmacokinetic and –dynamic data for standard healthy adults. However, specifically susceptible subpopulations such as children, the elderly, and

health impaired individuals may not answer to this profile (Thompson et al 2009). Therefore, literature reviews have compiled physiological parameters for particular sub-populations, such as children (Price et al 2003a), adults (2003b), and the elderly (≥ 65 years of age) and health-impaired (Thompson et al 2009). Table 2.1 compares some pharmacokinetic parameter values for different subgroups of the population.

Table 2.1 Physiological parameters for children aged 6, 10, 14 years old and adults (data from Price et al (2003), and elderly aged >69 years (Data from Thompson et al 2009)

Parameters	**Children**			**Adult**	**Elderly***
	6 years	**10 years**	**14 years**		
Alveolar ventilation rate (L/h)	147	219	290	300	291,6
Cardiac output (L/h)	245	338	404	372	
Tissue blood rates (L/h)					
Liver	19,5	39,9	54,9	96,7	65,9
Brain	59,1	53,7	46,9	42,4	
Adipose tissue	12,9	16,7	17,7	19,3	26,3
Slowly perfused tissues	7,5	15,1	27,6	61,1	
Rest of body	146,3	212,8	256,7	152,4	
Tissue volumes (L)					
Liver	0,62	0,87	1,26	1,8	1,26**
Brain	1,31	1,36	1,39	1,4	1,22**
Adipose tissue	3,68	6,25	11,49	14,9	
Slowly perfused tissues	5,71	10,17	18,41	35,9	
Rest of body	8,37	11,26	11,64	8,17	

* Average data

** assumes a transformation of tissue weight of 1000g per liter

These differences in physiological variables among different subpopulations may be important, as the contribution of different environmental compartments in the ADME of chemicals may vary substantially with age. For example, liver blood flow increased five-fold from 6-year olds to adults, and DeWoskin and Thompson (2008) described how differences in renal clearance parameters at different life stages may significantly alter the disposition of environmental toxicants.

Generally, PBPK models are developed to model internal doses of a specific chemical based on external exposure concentrations. As input into these models, concentrations of a chemical in different environmental compartments (air, water, soil), food, or specific lifestyle sources (e.g. smoking) are used to predict internal concentrations. Recently, the more sophisticated models have also included population variability in pharmacokinetic parameters, mainly using a Bayesian approach (see further). Hence, the current state-of-the-art allows researchers to develop good approximations of the

distribution of critical chemicals in the general population, based on both variability in exposure profile and pharmacokinetic variability.

The approach described above is generally referred to as "forward dosimetry", as it uses input data from environmental compartments to calculate the expected biomarker values (the left-to-right approach already mentioned in Figure 1). This forward dosimetry approach has a lot of potential to offer for source identification. It provides an objective way to evaluate the impact of different exposure routes by comparing calculated biomarker values (originating from PBPK modeling) with measured biomarker values (coming from population human biomonitoring).

2.4 Towards screening-level generic PBPK models

One of the major problems associated with the inclusion of PBPK models in the EDR-chain is the need to ascertain that models and their associated input data adequately describe the processes they are designed to estimate. The increasing use of tissue dosimetry in environmental health impact assessment also necessitates the need to develop internationally recognized good modeling practices (Loizou et al, 2008). The availability of suitable toxicokinetic data may be a bottleneck for routine application of PBPK modeling in interpreting HBM data since the availability and generic applicability of validated models remains relatively sparse. However, it has also been advocated that it may not always be necessary to have full PBPK models as long as sufficient toxicokinetic information is available to relate HBM data with hazard data (Hays and Aylward, 2008). For substances where toxicokinetic data is lacking, generalized toxicokinetic models are being developed which allow for a rapid screening-level approach to PBPK modeling. Below, a number of examples of (open access) screening-level generic PBPK model approaches are presented:

IndusChemFate

IndusChemFate is a generic PBTK model developed by Cefic LRI in collaboration with IndusTox Consult as part of the project "*Development of a computer programme with a multi-level modeling tool for the estimation of biomonitoring equivalent guidance values for chemical agents related to health based exposure rates for inhalation, oral intake and/or skin exposure*."

IndusChemFate contains algorithms as QSPRs (=Quantitative Structure-Property Relationships) for blood:air and tissue:blood partitioning, which allows it to provide useful information even when experimental partition characteristics of a compound are lacking. IndusChemFate is a generic PBTK-model for the derivation of human biomonitoring equivalent guidance values (BEGV) for multiple (data-poor) chemicals. It is a *first tier* or screening tool that requires a minimum of input data. It makes it possible to estimate biological monitoring guidance values as equal to airborne limit values. The model IndusChemFate is programmed in Visual Basic and runs in MS Excel. The data input proceeds via input in two worksheets of the Excel-file. Output is presented as numerical listing in time and in graphs and is presented in the same Excel-file. The model is provided as freeware with an open source code. The

IndusChemFate model and a user manual are publicly available from the CEFIC-LRI website (http://www.cefic-lri.org/lri-toolbox/induschemfate).

MEGen

MEGen (Model Equation Generator) software is a 'proof of concept' intuitive user interface for the rapid generation and analysis of PBPK models which was successfully developed by CEFIC LRI in collaboration with the UK's Health and Safety Laboratory. MEGen enables a user to describe physiology, biology and toxicology in order to output a set of mathematical equations that emulate the information supplied by the user and constitute a PBPK model. During this process, the software interrogates a built-in database, supplying pertinent data for use within the model. The resulting mathematics can be translated and imported into a number of commercial modeling packages where it may be visualized and exercised.

Another feature of the MEGen model building process is the provision of a transparent and auditable trail. MEGen provides a schematic diagram of a built model along with a corresponding table listing the value, units, source, origin and reference for each parameter specified. The diagrams and tables can be exported directly into documents prepared in standard word processors. MEGen is freely available under the General Public License regulation at http://xnet.hsl.gov.uk/megen/.

PKQuest

This is a slightly older program to construct PBPK models, which includes a " Standard human" and "Standard rat" data set. It has a relatively simple user interface and graphical output, and has recently been revised to a Java application (PKQuest_Java) (Levitt 2002, 2009). PKQuest_Java is designed for the non-specialist who would like to attempt some PBPK modeling without acquiring detailed training or expensive software. PKQuest, along with different detailed examples, is freely available through www.pkquest.com.

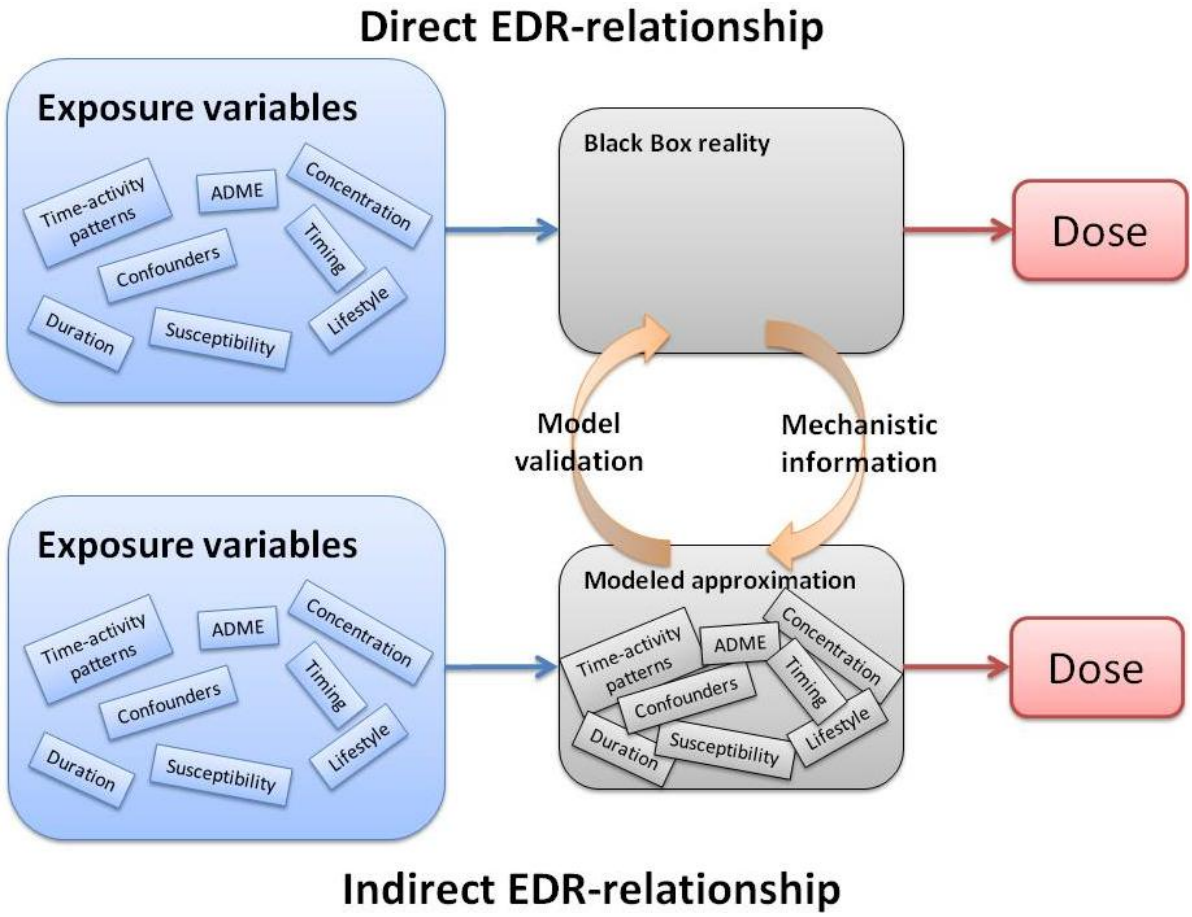
2.5 Measuring & Modeling along the EDR-chain

Recently, there has been significant interest, but also worry, about interpretation of human biomonitoring in a policy making context. The NRC-report on human biomonitoring clearly stated that "*The ability to generate new biomonitoring data often exceeds the ability to evaluate whether and how a chemical measured in an individual or population may cause a health risk or to evaluate its sources and pathways of exposure*" (National Research Council, 2006). By integrating both a direct and indirect description of the EDR-relationship however, improved interpretation of HBM data may be achieved.

In this interaction between direct and indirect EDR-relationships, HBM data offer a means to quantify the "black box reality" of aggregate and cumulative exposure (Figure 2.2). However, identifying which uptake routes, lifestyle or confounding factors, or time-activity patterns are responsible for the measured dose, remains unknown. Hence,

for interpreting HBM data, mechanistic information needs to be found through a modeled approximation.

Figure 2.2 Direct (HBM) and indirect (modeled) EDR-relationships provide mutual benefit to understand EDR-relationships



At the same time however, the modeled approximation only is what it is, a model that provides an 'informed guess' on how exposure is related to the target dose. Hence, the indirect EDR-relationship should be validated by measurement data, for which obviously HBM data would be the prime candidate. To make better use of body burden/biomarker data in the process of public health tracking, two essential scientific research tools, models and measurements, must be better integrated. Models provide the means to integrate and interpret measurements, design hypothesis driven experiments, and predict the effectiveness of risk management strategies. Measurements, in turn, provide tests of the models and ''ground truth'' (Sohn et al 2004).

To conclude this chapter, it should be clear that HBM and PBPK modeling are interrelated, and both offer important advantages to improve the knowledge on the relationship between environmental exposure and associated health effects. Both approaches have their merits and draw-backs, and can only achieve their greatest potential when used as a tandem.

In the following chapter, we will give some examples how combining HBM and PBPK modeling has offered increased insight into otherwise poorly understood relationship between environmental exposure and health effects.

CHAPTER 3 COMBINING MEASURING AND MODELING: SOME APPLICATIONS

3.1 Introduction

In practice, PBPK models and human biomonitoring share the same goal: gaining more insight in the relationship between the presence of contaminants in the environment and potential health effects. Both approaches have significant merits, but also suffer disadvantages. Also, Sohn et al (2004) argued that in spite of the consensus on how to build PBPK models, there is much less consensus on how to use these models to find environmental determinants of chronic disease from biomonitoring. The new challenge in PBPK modeling is to develop a framework for the application of PBPK models to large and often poorly characterized human populations that have highly variable exposures, activities, physiology, and pharmacokinetics (Bois 2001, Sohn et al 2004). The key question is whether and how well the variation in source-to-dose relationships can be quantified against the noise contributed by these other variables and uncertain factors.

In the following, some applications of combined measuring of biomarkers and pharmaco- and/or toxicokinetic modeling are given. The main aim of the examples is to illustrate how both approaches interact, and are mutually beneficial.

3.2 Exposure reconstruction

One of the main options of combining PBPK models and HBM data is to make inferences about environmental exposure scenarios for biomarker data collected in population-based studies (Mosquin et al 2009). Biomarker data from large-scale HBM surveys often adequately reflects population variability, yet frequently lack the necessary activity information to take into account biomarker kinetics, or to develop aggregate exposure profiles. Exposure scenarios can be variable, with multiple possible routes and time variability of exposure. While these uncertainties generally remain unaddressed in HBM surveys, the additional use of PBPK models may offer further insight in exposure reconstruction.

Therefore, a modeling approach may be particularly useful to provide further insight in the most important parameters that determine observed differences in biomarker values among and/or within individuals. For example, Gosselin et al (2006) used the biologically based toxicokinetic model developed by Carrier et al (2001) to estimate the dynamic profile of MeHg in both blood and hair. While many PBPK models used a simple one compartmental model to describe the fate of MeHg in the human body (assuming a steady-state in MeHg uptake and excretion), the Carrier-model accounts for fluctuating MeHg levels in fish, but also for fluctuating fish consumption patterns. Through the combined use of measuring and modeling, Gosselin et al (2006) was able to describe the bilateral links between the MeHg daily intake and the mercury concentration in

blood and hair samples collected at any given time following the onset of any MeHg ingestion period. It was clearly illustrated that the steady-state is not valid for MeHg toxicokinetic modeling, and the probable time duration of MeHg exposure is essential when back-calculating MeHg intakes. Particularly, it was concluded that biomarker studies would be better off focusing more on gathering data on the period during which the individual might have consumed contaminated food, rather than the need to quantify the daily MeHg intake.

Also Sohn et al (2004) provided a good example of the synergy that is created when biomonitoring data and PBPK models are integrated for reconstructing population exposure. Combining venous blood concentrations of TCE (trichloroethylene) and PBPK modeling showed that population-scale variability of pharmacokinetics dominate the uncertainty of the predicted TCE concentration in air, and suggested that one should not spent excessive resources obtaining exposure onset and duration data if the primary objective is reducing uncertainty in the predicted concentration of TCE in air. The authors concluded that in order to achieve optimal synergy among biomarker measurements and PBPK modeling, the persistence of the biomarker should be long relative to exposure duration for estimating long-term, or population scale, exposure effects.

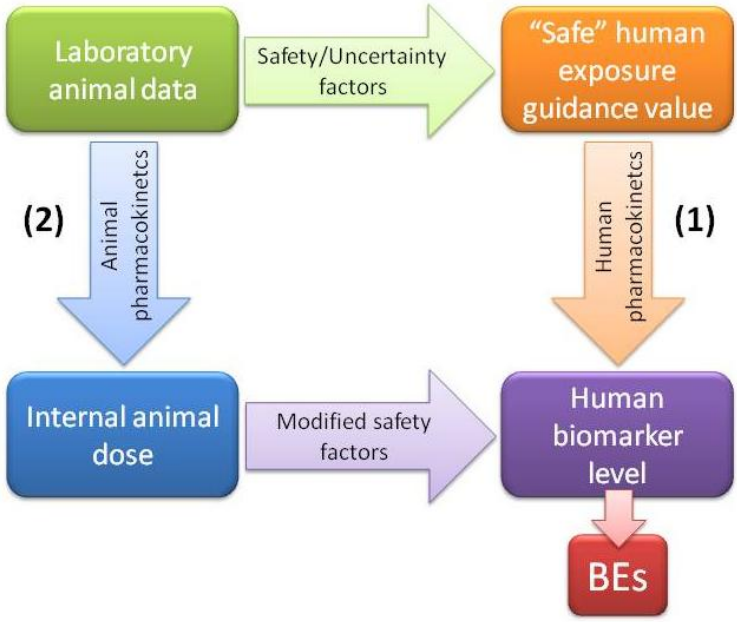
As a final example, also Ruiz et al (2010) used PBPK modeling to gain further insight in urinary cadmium biomarker data from the most recent NHANES biomonitoring survey. From these data, the authors predicted urinary Cd concentrations by age at various intake doses from 10 to 100 µg Cd/day, and particularly demonstrated the marked elevated uptake of Cd in 6-11 year old children. The model also predicted a 1.4- to 1.6-fold higher urinary Cd excretion in females compared to males in all age groups. The study demonstrated that computational techniques such as PBPK models can be useful predictors for delineating population subgroups at special risk as a function of age and gender.

3.3 Biomonitoring equivalents (BEs)

In an effort to improve the framework for the interpretation of biomonitoring data from HBM surveys, Hays and co-workers have introduced the concept of the Biomonitoring Equivalent (BE). BEs are defined as the concentration of a chemical (or metabolite) in a biological medium consistent with defined exposure guidance values or toxicity criteria including reference doses and reference concentrations, minimal risk levels or tolerable daily intakes (Hays et al 2007, 2008). These exposure guidance values are estimates of the daily exposure to a chemical that are believed to be without appreciable health risks, and are used regulatory agencies as guidelines for making risk management decisions.

The concept of BEs is an approach that uses available pharmacokinetic data and forward dosimetry to calculate levels of biomarkers associated with these exposure guidance values (Figure 3.1).

Figure 3.1: Schematic diagram showing the concept for calculating BEs and possible routes for deriving a Biomonitoring Equivalent. Numbers (1) and (2) refer to specific sections in the following text (redrawn from Hays et al 2007)



The BE concept preferentially relies on human pharmacokinetic data to relate external dose to biomarker concentrations (pathway (1) in Figure 3.1). However, also when only animal-based pharmacokinetic information is available (pathway (2)), this may be applied within the concept, taking into account the appropriate uncertainty or safety factors.

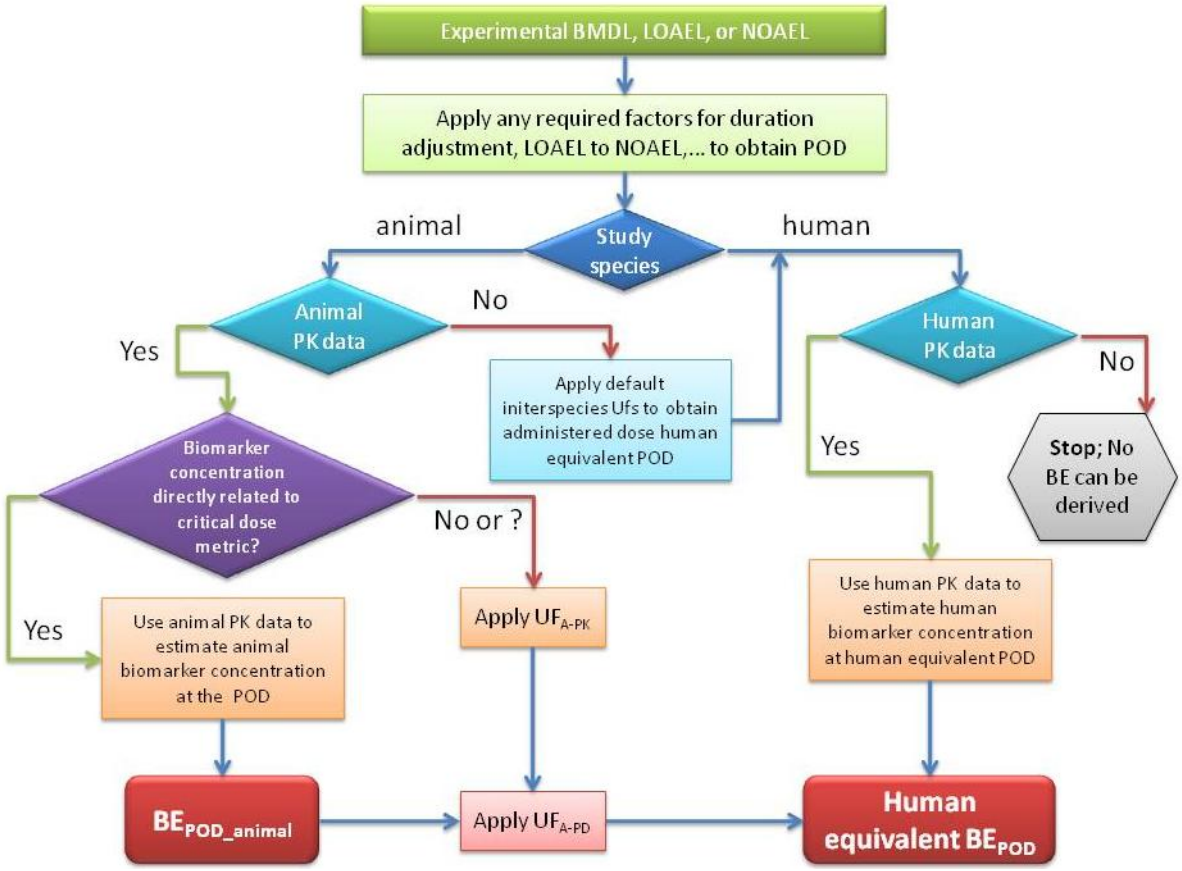
There are two basic elements of the derivation process illustrated in Figure 3.2:

- Identification of the biomarker concentration at the human equivalent Point of Departure (BE_{POD});
- Identification of a target margin of exposure (MOE) to be applied to the BE_{POD} to derive the BE value commensurate with the exposure guidance value.

Practically, a chemical-specific BE can be derived for many chemicals using existing pharmacokinetic information. The BE derivation process includes:

- Compiling existing tolerable exposure reference values and the approaches used to calculate them;
- Compiling and reviewing existing pharmacokinetic information available for translating exposure/intake (mg/kg/day or ppm in air) to internal dose metrics (blood or urine concentrations);
- Reviewing information on the mode of action (MOA) for each endpoint for which an exposure guidance value was derived;
- Determining the best available biomarker and assessing whether existing biomarkers used in biomonitoring studies are interpretable;
- Deriving the appropriate BE values (BE_{POD}, BE_{RfD});
- Independent peer-review of the BE.

Figure 3.2: Flowchart of the process for derivation of BE_{POD} values under combinations of animal and human toxicity data and either animal or human pharmacokinetic data or models. Key steps in the derivation include the evaluation of the application of default uncertainty factor components based on understanding regarding the relationship between the biomarker and the critical or relevant dose metric.



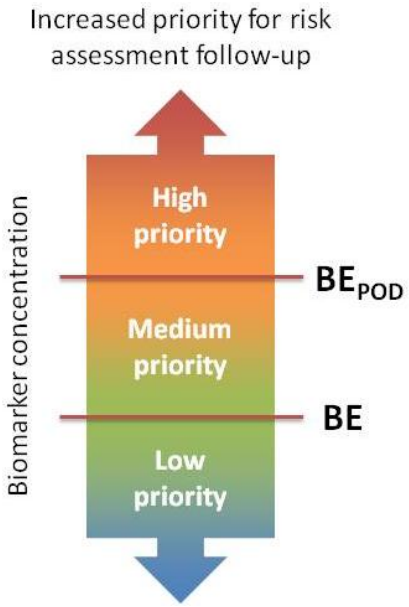
BMDL, lower bound on the benchmark dose; LOAEL, lowest observed adverse effect level; NOAEL, no observed adverse effect level; POD, point of departure; UFA-PK, pharmacokinetic component of the default inter-species uncertainty factor; UFA-PD, pharmacodynamic component of the default inter-species uncertainty factor.

It needs to be stressed that BE values are screening values. They can be used to provide a screening level assessment of measured blood or urine levels of a chemical in population- or cohort-based studies. Comparison of measured biomonitoring levels to BE values can provide an initial evaluation of whether the measured values in a given study are of low, medium, or high priority for risk assessment follow-up and inform whether there is a need for additional studies on exposure pathways, potential health effects, other aspects affecting exposure or risk, or other risk management activities.

BE values are not diagnostic criteria or "bright lines" between safe and unsafe levels. They cannot be used to evaluate the likelihood of an adverse health effect in an individual or even among a population. Exposure guidance values are set at levels that are designed to be health-protective for daily exposure for a full lifetime of exposure, while, depending on the chemical, biomonitoring data may be informative only about recent exposure levels. An exceedance of the BE value in a single sample of blood or urine may or may not reflect continuing elevated exposure and does not imply that

adverse health effects are likely to occur, but can serve as an indicator of relative priority for further risk assessment follow-up (Figure 3.3).

Figure 3.3: Interpretation of population biomonitoring data in exceedance of BEs (redrawn from Hays et al 2008)



Hays and coworkers have applied this BE-concept, which explicitly uses pharmacokinetic information to relate exposure guidance values to biomonitoring data, for a number of chemicals. Tables 3.1 and 3.2 provide an overview of chemicals for which BEs have been developed in respectively plasma and urine.

Table 3.1 Overview of available chemical-specific BEs in blood

Chemical	BE_{RfD}	BE_{POD_human}	BE_{POD_animal}	Reference
2,4-D (2,4-dichlorophenoxyacetic acid, µg/L)	5	170	540	Aylward LL, Hays SM. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S37-S48*
Cadmium (µg/L)	1.4-1.7	4.4-5.3	-	Hays SM, Nordberg M, Yager JW, Aylward LL. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S49-S56*
Polychlorinated dibenzo-p-dioxins and dibenzofurans (ng TEQ/kg)	31-74	31-74	-	Aylward LL, LaKind JS, Hays SM. 2008 *Journal of Toxicology and Environmental Health Part A*, 71: 1499-1508
Toluene	3-50	10-170	90-830	Aylward LL, Barton HA, Hays SM. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S27-S36*
Trihalomethanes (pg/ml): Chloroform Dibromochloromethane Bromodichloromethane Bromoform	 230 80 20 130	 750 270 190 420	 4.400 2.200 670 2.900	Aylward LL, LaKind JS, Hays SM. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S68-S77*

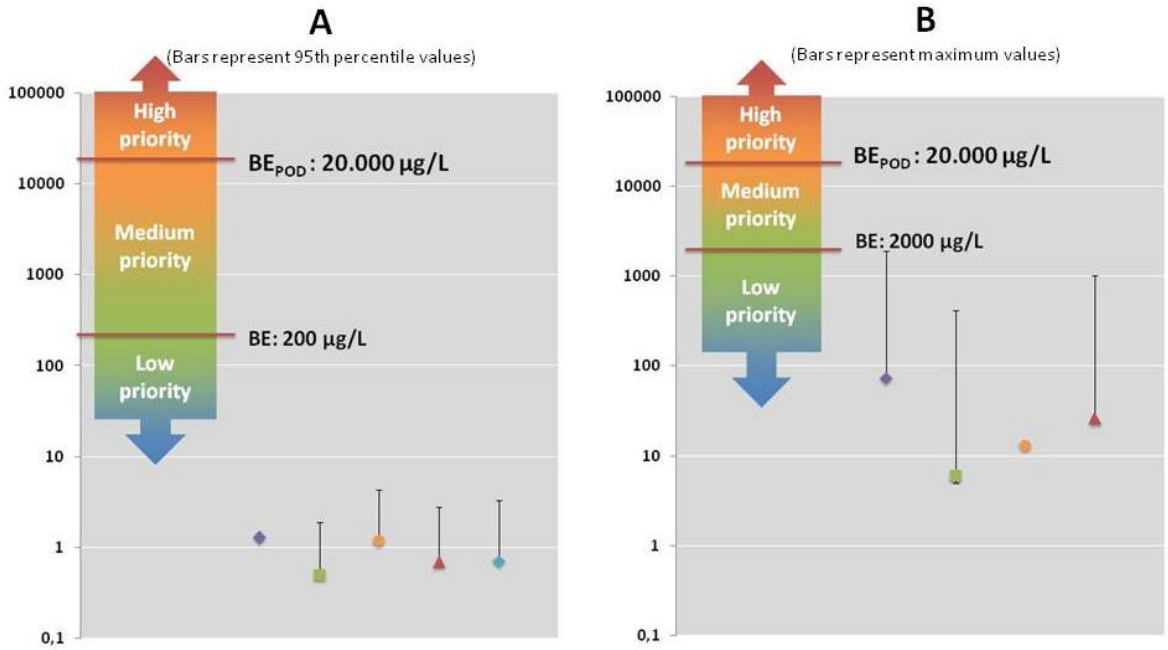
Table 3.2 Overview of available chemical-specific BEs in urine

Chemical	BE_{RFD}	BE_{POD_human}	Reference
2,4-D (2,4-dichlorophenoxyacetic acid)	300 µg/g creatinin	30.000 µg/g creatinin	Aylward LL, Hays SM. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S37-S48*
Acrylamide	8 pmoles/g globin	25 pmoles/g globin	Hays SM, Aylward LL. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S57-S67*
Cadmium	1.7-2.0 µg/g creatinin	2.5-6.3 µg/g creatinin	Hays SM, Nordberg M, Yager JW, Aylward LL. 2008. *Regulatory Toxicology and Pharmacology, 51 (Supplement 1): S49-S56*
Cyfluthrin	260-310 µg/g creatinin	2600-3100 µg/g creatinin	Aylward LL, Hays SM, Gagné M, Krishnan K. *2009. Regulatory Toxicology and Pharmacology,55: 268-295*
Di(2-ethylhexyl)phthalate	Depending on number of metabolites taken into consideration		Aylward LL, Hays SM, Gagné M, Krishnan K. *2009. Regulatory Toxicology and Pharmacology,55: 249-258*
Phthalate esters: Diethyl phthalate Di-n-butyl phthalate Benzylbutyl phthalate	 18 mg/L 0.2-2.7 mg/L 3.8-31 mg/L	 180 mg/L 2-27 mg/L 38-310 mg/L	Aylward LL, Hays SM, Gagné M, Krishnan K. *2009. Regulatory Toxicology and Pharmacology,55: 259-267*

As an example on the use of BEs in the interpretation of biomarker data, Aylward et al (2010) recently used data from several biomonitoring surveys in the USA and Canada to illustrate the value of the BE approach in the context of risk assessment. From the available pharmacokinetic data, it was found that 2,4-D is eliminated in urine either as the unchanged parent compound (80-95%) or as a conjugate, with urinary half-lives on the order of 1 day. Based on these data, is was assumed that continuous exposure for more than one week results in a steady state, in which the amount excreted daily in urine would be approximately equivalent to the amount absorbed each day.

Because 2,4-D is excreted as the parent compound in urine, urinary biomarker data were used as well. It was recognized however that from a toxicological point of view, plasma concentrations probably would have been more informative for predicting target tissue concentrations and responses. Following the BE-framework outlined in Figures 3.1 and 3.2, a comparison between urinary 2,4-D concentrations in both the general population (Figure 3.4a) and occupationally exposed pesticide applicators (Figure 3.4b) could be compared with the appropriate BEs.

Figure 3.4: Comparison of urinary 2,4-D levels in (A) the general population, and (B) occupationally exposed applicators in the context of the BE value corresponding to respectively (A) the USEPA RfD for general population chronic exposures and (B) the USEAP occupational risk assessment levels.



For additional information, see Aylward et al (2010).

From this assessment, it was concluded that current use patterns and risk management efforts by industry and government are likely keeping average exposure to 2,4-D for the general population and in occupationally exposed groups well below current noncancer reference values.

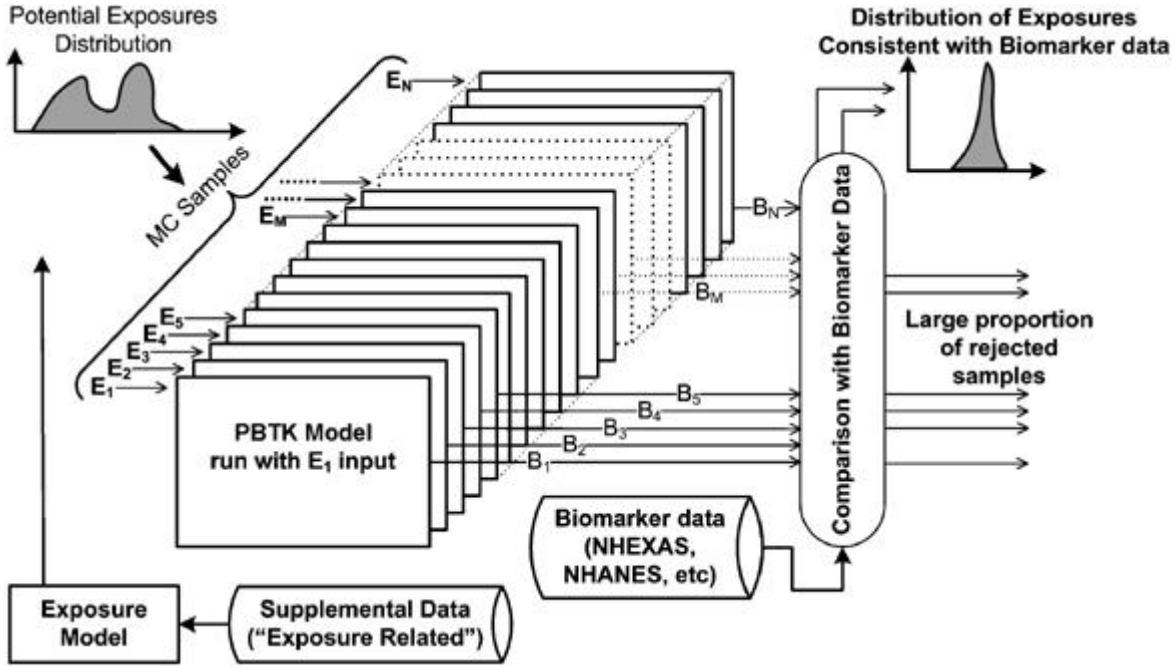
3.4 Reverse dosimetry

Recently, a novel approach to the use of PBPK models in source identification for the interpretation of biomarker values has been introduced, which explicitly aims at source identification. Establishing the relationship between biomarker data and environmental sources involves the reconstruction of past external exposure and has been termed "exposure reconstruction" or "reverse dosimetry" (Lyons et al 2008). By using advanced statistical methods such as Markov Chain Monte Carlo (MCMC) simulations, this reverse dosimetry approach has already been able to show its added value for source identification. The main issue however in this reverse dosimetry is that it needs to take into account the inherent variability of the population from which the HBM data arises. In the following, we dig a little deeper into the possibilities to include reverse dosimetry for improved source identification.

As was already mentioned earlier, biomarker data are often gathered and reported without concomitantly collecting corresponding detailed external exposure data in the relevant environmental compartments. Hence, the PBPK model should in this setup be reversed, and human biomonitoring data can be transformed to equivalent exposure concentrations (Tan et al 2006). This approach is generally referred to as reverse dosimetry, and basically is defined as "the estimation of the environmental exposures that would be consistent with the measured biomonitoring data" (Clewell et al 2008). Reverse dosimetry uses pharmacokinetic data in combination with information regarding the nature of the potential exposures, to infer the exposures that are likely to have resulted in the measured biomonitoring results. The fundamental problem underlying reverse dosimetry is to relate a measured internal dose, or tissue concentration to an unmeasured external exposure or dose. Typically, the relationship between exposure and dose however is such that an inverse does not exist, is unstable, or is not unique. The reverse dosimetry problem hence is a problem of statistical inference: we wish to determine an estimate of exposure for the general population based on biomonitoring data collected from a representative sample of that population. This statistical aspect of the problem can be addressed by combining a Bayesian analysis with a population model (Lyons et al, 2008).

Figure 3.5 provides a simplified schematic of a computational framework for exposure reconstruction showing major components and processes. Available supporting or complementary exposure-related data can provide "prior estimates" of exposures for individuals and populations. These estimates in turn can then be improved by using PBPK modelling and inversion techniques along with corresponding biomarker data (Georgopoulos et al 2009). Although this methodology is still under full development, and should currently fit more under "horizon scanning" than under actual application, Georgopoulos et al (2009) envisions that it should be possible to develop a comprehensive exposure reconstruction framework that allows source identification/exposure reconstruction following aggregate (i.e. from multiple exposure rouses) and cumulative (i.e. for multiple chemicals) exposures, and provide user-friendly computational tools for use by the exposure/risk assessment and management communities. In addition, the modelling framework outlined in Figure 3.5 could also be used for estimating distributions of physiological and biochemical PBPK model parameters for individuals and populations that are consistent with available biomarker data.

Figure 3.5 A simplified schematic of a computational framework for exposure reconstruction showing major components and processes. Available exposure-related data can provide "prior estimates" of exposures, which in turn can be used in conjunction with biomarkers and PBTK modeling to obtain improved estimates of exposures and doses (Taken from Georgopoulos et al (2008))

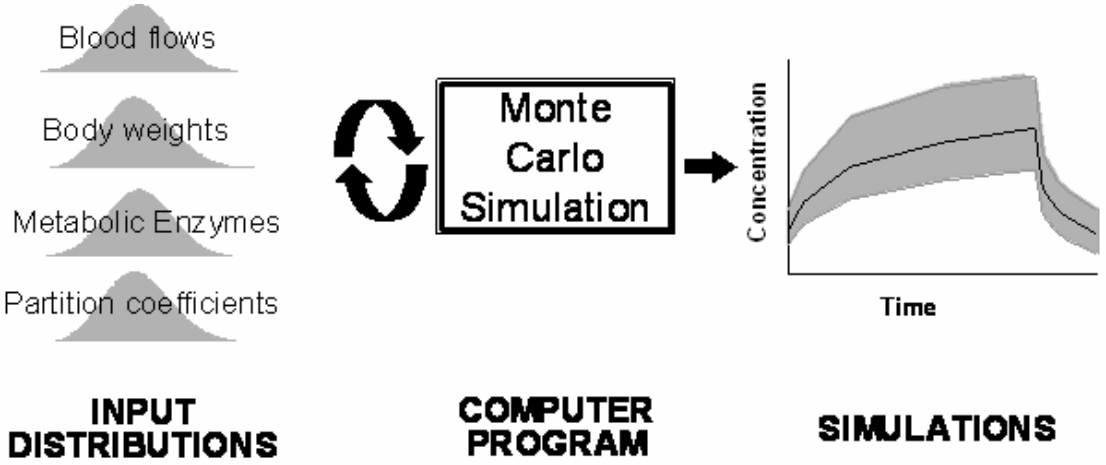


Using a Bayesian approach

An issue associated with both the forward and reverse dosimetry approach described earlier is that from a mathematical point of view, these models are highly parameterized and must be fitted or calibrated to experimental data in order to make accurate predictions of internal dose or reverse exposure scenarios. While other methods such as maximum likelihood estimation or least squared error approaches have been proposed, a Bayesian approach called Markov Chain Monte Carlo (MCMC) analysis has frequently been used to calibrate these complex models (Bois 2001; Hack 2006; Allen et al 2007).

Monte Carlo analysis is generally used to evaluate the propagation of variability through a model, and results in an estimate of the variance in model output (Figure 3.6). This estimation is achieved by randomly sampling model parameters from defined distributions and running the model for a large number of iterations. The Bayesian approach requires having these defined 'prior' distributions that reflect the belief or knowledge on the distribution of PBPK variables. A Monte Carlo implementation of a PBPK model can be viewed as conducting *in silico* studies in a large number of humans with diverse physiology (Tan et al 2006, Bois et al (in press)).

Figure 3.6 A graphical overview of Monte Carlo simulations, where the distribution of internal concentrations is simulated by repeatedly sampling input values based on the distribution of individual parameters in a population(taken from USEPA 2006)



The analysis of the reverse dosimetry problem consists of the following steps:

- specification of the probability model: specification of the joint probability distribution and the specification of prior parameter distributions
- Bayesian inference: Calculation of the posterior distribution conditioned on the observed biomonitoring data using MCMC simulation and calculation of expected values for exposure
- Evaluation of the results: comparison of prior and posterior distributions of exposure using MC simulation to general model predictions for the observed biomonitoring data, evaluation of parameter independence, and comparison with previously obtained results (Lyons et al, 2008)

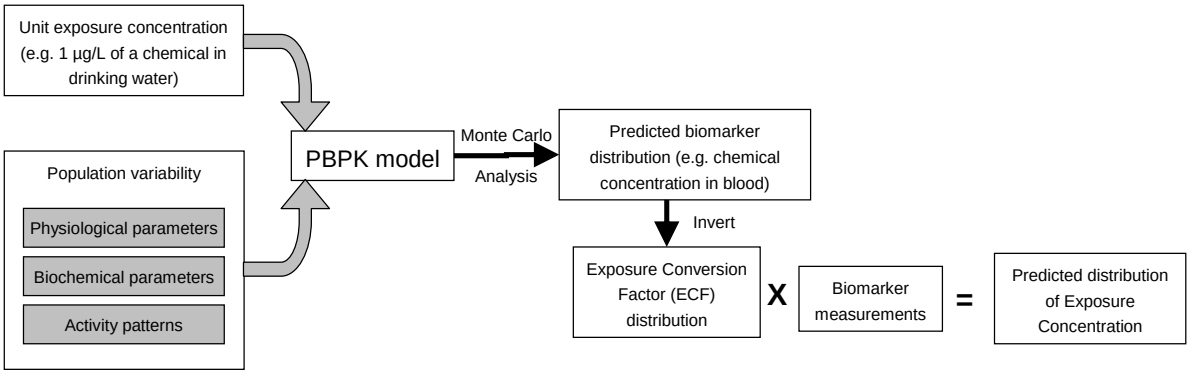
Several examples how MCMC analysis was used in forward PBPK modeling occur. Among others, the method was used to estimate methylmercury exposure of US women of childbearing age (Allen et al 2007), benzene (Yokley et al 2005) or perchloroethylene (Covington et al 2006), while Yang et al (2009) used it to describe multiroute chloroform exposure. In order to take into account the higher mentioned (sub) population variability in pharmacokinetic parameters, Price et al (2003) reported the development of the Physiological Parameters for PBPK Modeling (P^3M) computer program, a source of data for human physiological parameters. From this database, records can be randomly retrieved with specification of constraints on age, gender, and ethnicity. These output sets can be used as inputs to Monte Carlo-based PBPK models of interindividual variation in dose.

3.5 Using exposure conversion factors (ECF)

In their case study describing source identification for chloroform, Tan and co-authors (2007) described a relatively simple approach to reverse dosimetry using an "exposure conversion factor" (ECF). First, a Monte Carlo analysis was performed with varying the timing of sampling and different exposure scenarios. A reference chloroform concentration in water (= 1 µg/l) was then used to predict the distribution of chloroform concentrations in blood in pg/ml). The resulting output distributions were

then inverted to obtain a distribution of an ECF in (µg/l in water)/ (pg/ml in blood). The distribution of the ECF can be multiplied by any observed chloroform concentration in blood to estimate a distribution of chloroform concentrations in water to which the individual might have been exposed. This approach is graphically outlined in figure 3.7.

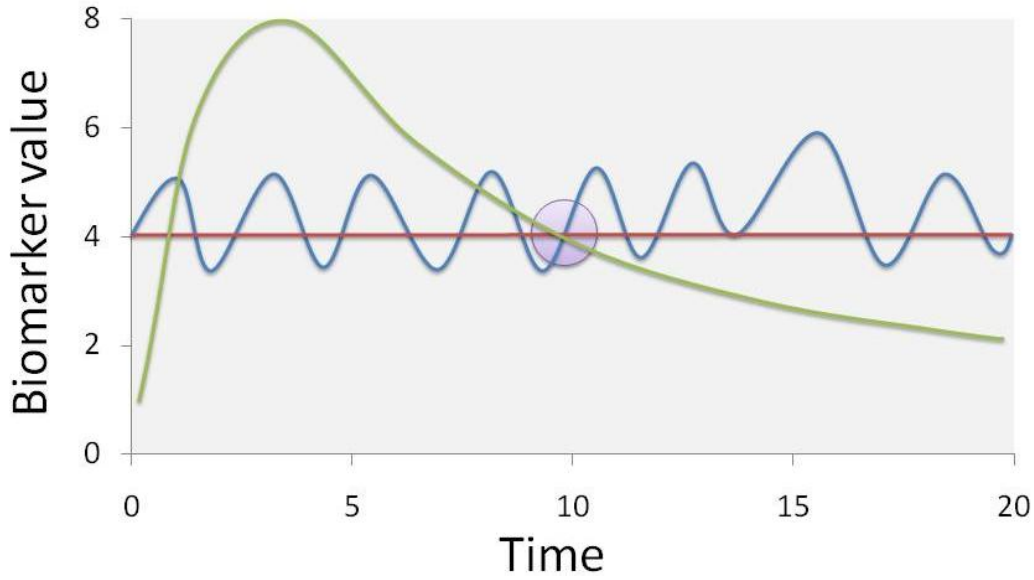
Figure 3.7 Schematic outline of the ECF approach for reverse dosimetry (Tan et al 2007; Clewell et al 2008)



3.6 Interpretation of repeated HBM measurements

Typically, large-scale human biomonitoring surveys gather biomarker measurements from single time points. These data are then used to make inferences about longer periods of toxicant intake, assuming that biomarker values are representative of steady-state conditions. However, steady-state conditions require stable biokinetics, a constant rate of exposure, and a dynamic equilibrium among different body tissues. Figure 3.8 gives an indication of how a single sampling time may not be representative of steady state biomarker concentrations. At the specific sampling time (t = 12), the biomarker value can be the result of different exposure scenarios. The dashed line implies one high peak exposure episode, the full line a continuous fluctuation around a steady-state situation, and the dotted line a completely steady-state situation. Obviously, information on the biomarker pharmacokinetics is issue has a significant effect on both source identification and potential associations with health effects.

Figure 3.8 A generic example indicating how a single biomarker value may not be representative for exposure assessment



An example on how this assumption of steady-state may be important for source identification can be found in historical blood-Pb levels. Fifty years ago, blood-Pb levels were very well correlated with the emission of Pb from car emissions, as tetraethyl lead as an antiknocking agent was the main source of Pb emission in the environment. It can be assumed that blood-Pb levels were more or less in a steady state as air concentrations were not changing rapidly. Recently, this has been validated for different European Blood-Pb datasets (Smolders et al, 2010). Since the outphasing of tetraethyl lead in gasoline, the contribution of this continuous exposure has declined rapidly, and currently childhood lead exposure in the US is thought to occur primarily through paint, dust, and soil ingestion (Bartell et al, 2004). These activities are episodic in nature and seem to occur sporadically and at varying rates (Wong et al, 2000).

Hence, assuming that biomarker values are representative for a steady-state concentration in the measured matrix may not be a justified assumption, and may require additional investigation. By repeated sampling of individuals with particularly high and particularly low biomarker values (e.g. > P_{95} and < P5), more insight in the pharmacokinetic behavior of the biomarker can be obtained. In combination, having an additional detailed questionnaire aimed at recording specific exposure scenarios during the time period between samplings, more insight into source identification can be obtained. The time-lag between sampling obviously is dependent on the half-life of chemicals, and the matrix that is used for biomarker determination.

Chen and co-authors (2009) outlined a stochastic approach using Markov Chain Monte Carlo (MCMC) simulations and a PBPK model to estimate inhalation exposure to TCE, based on repeated measurements in venous blood. Their procedure illustrated that estimating environmental exposure from repeated biomarker measurements could be achieved with very high precision given known exposure duration.

An additional approach, proposed by Bartell et al (2004) could be the inclusion of measuring the same chemical, but in different matrices, thus reflecting different pharmacokinetic properties of the biomarker. For example, measuring cadmium in both

blood and urine could give an indication of the long-term steady state exposure (urine) but also of the more recent, dynamic exposure (blood). Combining these both data may provide increased resolution for source identification. Already earlier, Henderson (1995) proposed the use of multiple biomarkers of varying half-lives to distinguish among different possible exposure scenarios.

Finally, one promising approach to improve the understanding of the kinetics of biomarkers is to use multiple biomarkers to describe the presence of a chemical in various matrices (i.e. biomarker batteries). By combining a PBPK model (which describes the expected distribution of a chemical among different matrices in the body) with biomarker battery data (quantifying the parent compound or metabolites in different matrices), greater sampling flexibility may be achieved. Mosquin et al (2009) argued that when a compound is simultaneously measured in for example blood and urine, they can be considered different biomarkers. The availability of multiple biomarkers then leads to a trade-off in the design of studies: is it more efficient to sample one biomarker over multiple time points or more biomarkers at a single time point? From a practical point of view, the latter may be preferred, as it is generally much easier and cost efficient to collect multiple biomarkers at a single sampling event in general population studies

In illustrating this observation by using a well-validated PBPK model for chlorpyrifos, Mosquin et al (2009) concluded that collecting biomarker samples at additional time points tends to be more effective than measuring multiple biomarkers at one time point, based on reducing the mean absolute percentage error (MAPE).

CHAPTER 4 CONCLUSIONS

Environmental health impact assessment requires public health tracking, and public health tracking requires a process for linking body burdens to a distribution of population doses. In order to achieve this requires sufficient and reliable information about population exposure and dose to those pollutants that have the most significant contribution to the observed health effect (Sohn et al 20044). By combining measuring (in the form of human biomonitoring) and modeling (in the form of PBPK models), a synergy is obtained. Models provide the means to integrate and interpret measurements, design hypothesis-driven experiments, and predict the effectiveness of risk management strategies. Biomonitoring in turn provides tests of the model and "ground truth" (Sohn et al, 2004).

CHAPTER 5 REFERENCES

Allen BC, Hack CE, Clewell HJ. Use of Markov Chain Monte Carlo analysis with a physiologically-based pharmacokinetic model of methylmercury to estimate exposures in U.S. women of childbearing age. Risk Analysis 27: 947-959.

Aylward LL, Morgan MK, Arbuckle TE, Barr DB, Burns CJ, Alexander BH, Hays SM. 2010. Biomonitoring data for 2,4-Dichlorophenoxyacetic Acid in the United States and Canada: Interpretation in a public health risk assessment context using biomonitoring equivalents. Environmental Health Perspecties 118: 177-181.

Bartell SM, Griffith WC, Faustman EM. 2004. Temporal error in biomarker-based mean exposure estimates for individuals. Journal of Exposure Analysis and Environmental Epidemiology 14: 173-179.

Bailey LA, Gordis L, Green M, Rothstein P. Reference guide on epidemiology. In: Reference Manual on Scientific Evidence: 2nd Edition. 60p. Available online at: http://www.fjc.gov/public/pdf.nsf/lookup/6.epide.pdf/$File/6.epide.pdf.

Beaudouin R, Micallef S, Brochot C. 2010. A stochastic whole-body prysiologically based pharmacokinetic model to assess the impact of inter-individual variability on tissue dosimetry over the human lifespan. Regulatory Toxicology and Pharmacology 57: 103-116.

Blaauboer BJ. 2003. The integration of data on physico-chemical properties, in vitro-derived toxicity data and physiologically based kinetic and dynamic as modeling tool in hazard and risk assessment. A commentary. Toxicology Letters 138: 161-171.

Bois FY. 2001. Applications of population approaches in toxicology. Toxicology Letters 120: 385-394.

Bois FY, Jamei M, Clewell HJ. 2010. PBPK modeling of inter-individual variability in the pharmacokinetics of environmental chemicals. Toxicology (in press)

Carrier G, Bouchard M, Brunet RC, Caza M. 2001. A toxicokinetic model for predicting the disuse distribution and elimination of organic and inorganic mercury following exposure to methyl mercury in animals and humans. II. Application and validation of the model in humans. Toxicology and Applied Pharmacology 171: 50-60

Chen C-C, Shih M-C, Wu K-Y. 2009. Exposure estimation using repeated blood concentration measurements. Stochastic Environmental Research and Risk Assessment 24: 445-454

Clewell HJ, Tan YM, Campbell JL, Andersen ME. 2008. Quantitative interpretation of human biomonitoring data. Toxicology and Applied Pharmacology 231: 122-133.

Covington TR, Gentry PR, Van Landingham CB, Andersen ME, Kester JE, and Clewell HJ. 2006. The use of Markov chain Monte Carlo uncertainty analysis to support a Public Health Goal for perchloroethylene. Regulatory Toxicology and Pharmacology 47:1-18.

Dahl SG, Aarons L, Gundert-Remy U, Karlsson MO, Schneider Y-J, Steimer J-L, Troconiz IF. 2009. Incorporating physiological and biochemical mechanisms into pharmacokinetic-pharmacodynamic models: A conceptual framework. Basic & Clinical Pharmacology & Toxicology 106: 2-12.

Elinder CG, Lind B, Kjellstorm T, Linnman L, Friberg L. 1976. Cadmium in kidney cortex, liver and pancreas from Swedish autopsies: estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. Archives of Environmental Health 31: 292-301.

Feil R. 2006. Environmental and nutritional effects on the epigenetic regulation of genes. Mutation Research – Fundamental and Molecular Mechanisms of Mutagenesis 600: 46-57.

Georgopoulos PG, Sasso AF, Isukapalli SS, Lioy PJ, Vallero DA, Okino M, Reiter L. 2009. Reconstructing population exposures to environmental chemicals from biomarkers: Challenges and opportunities. Journal of Exposure Science and Environmental Epidemiology 19: 149-171.

Gosselin NH, Brunet RC, Carrier G, Bouchard M, Feeley M. 2006. Reconstruction of methylmercury intakes in indigenous populations from biomarker data. Journal of Exposure Science and Environmental Epidemiology 16: 19-29.

Hack CE. Bayesian analysis of physiologically based toxicokinetic and toxicodynamic models. Toxicology 221: 241-248.

Haque FN, Gottesmann II, Wong AHC. 2009. Not really identical: Epigenetic differences in monozygotic twins and implications for twin studies in psychiatry. American Journal of Medical Genetics Part C – Seminars in Medical Genetics 151C: 136-141.

Hays SM, Aylward LL, LaKind JS, et al. 2008. Guidelines for the derivation of Biomonitoring Equivalents: Report from the Biomonitoring Equivalents Expert Workshop. Regulatory Toxicology and Pharmacology 51: S4-S15.

Hays SM, Becker RA, Leung HW, Aylward LL, Pyatt DW. 2007. Biomonitoring equivalents: A screening approach for interpreting biomonitoring results from a public health risk perspective. Regulatory Toxicology and Pharmacology 47: 96-109.

Henderson RF. 1995. Strategies for the use of biomarkers of exposure. Toxicology Letters 82/83: 379-383.

Hubal EAC, Sheldon LS, Burke JM, McCurdy TR, Barry MR, Rigas ML, Zartarian VG, Freeman NCG. 2000. Children's exposure assessment: A review of factors influencing children's exposure, and the data available to characterize and assess that exposure. Environmental Health Perspectives 108: 475-486.

Jonsson F. 2001. Physiologically based pharmacokinetic modeling in risk assessment: Development of Bayesian population methods. Uppsala University, Division of Pharmacokinetics and Drug Therapy. 46p.

Levitt DG. 2002. PKQuest: A general physiologically based pharmacokinetic model. Introduction and application to propranolol. BMC Clinical Pharmacology 2, 21p.

Lipscomb JC, Meek ME, Krishnan K, Kedderis GL, Clewell H, Haber L. 2004. Incorporation of pharmacokinetic and pharmacodynamic data into risk assessments. Toxicology Mechanisms and Methods 14: 145-158.

Loizou G, Spendiff M, Barton HA, Bessems J, Bois FY, Bouvier d'Yvoire M, Buist H, Clewell HJ, Meek B, Gundert-Remy U, Goerlitz G, Schmitt W. 2008. Development of good modeling practice for physiologically based pharmacokinetic models for use in risk assessment: The first steps. Regulatory Toxicology and Pharmacology 50: 400-411.

Lyons MA, Yang RSH, Mayeno AN, Reisfeld B. 2008. Computational toxicology of chloroform: Reverse dosimentry using Bayesian inference, Markov Chain Monte Carlo simulation, and human biomonitoring data. Environmental Health Perspectives 116: 1040-1046.

Mathers JC. 2008. Epigenomics: A basis for understanding individual differences? Proceedings of the Nutritional Society 67: 390-394.

Mosquin PL, Licata AC, Liu B, Sumner SCJ, Okino MS. 2009. Reconstructing exposure from small samples using physiologically based pharmacokinetic models and multiple biomarkers. Journal of Exposure Science and Environmental Epidemiology 19: 284-297.

National Research Council. 2006. Human biomonitoring for environmental chemicals. Committee on Human Biomonitoring for Environmental Toxicants. The National Academies Press, Washington DC. USA. 292p.

Price K, Haddad S, Krishnan K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. Journal of Toxicology and Environmental Health A, 66: 417-433.

Price PS, Conolly RB, Chaisson CF, Gross EA, Young JS, Mathis ET, Tedder DR. 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. Critical Reviews in Toxicology 33: 469-503.

Ruiz P, Mumtaz M, Osterloh J, Fisher J, Fowler BA. 2010. Interpreting NHANES biomonitoring data, cadmium. Toxicology Letters (in press).

Satarug S, Baker JR, Urbenjapol S, Haswell-Elkins M, Reilly PEB, Williams DJ, Moore MR. 2003. A global perspective on cadmium pollution and toxicity in non-occupationally exposed populations. Toxicology Letters 137: 65-83.

Slob W, Krajnc EI. 1994. Interindividual variability in modeling exposure and toxicokinetics: A case study on Cadmium. Environmental Health Perspectives 102: 78-81.

Smolders R, Schoeters G. 2007. Identifying challenges and opportunities to establish an exposure-dose-response triad for Europe. ESBIO Deliverable 3.1; online available at: http://www.eu-humanbiomonitoring.org/doc/esbio_del_wp3.pdf

Smolders R, Schramm K-W, Stenius U, Grellier J, Kahn A, Trnovec T, Sram R, Schoeters G. 2009. A review on the practical application of human biomonitoring in integrated risk assessment. Journal of Toxicology and Environmental Health Part B – Critical Reviews 12: 107-123

Smolders R et al. Availability and comparability of human biomonitoring data across Europe: a case-study for blood-Pb levels. Science of the Total Environment (In press).

Sohn MD, McKone TE, Blancato JN. 2004. Reconstructing population exposure from dose biomarkers: inhalation of trichloroethylene (TCE) as a case study. Journal of Exposure analysis and Environmental Epidemiology 14: 204-213.

Stapleton HM, Kelly SM, Allen JG, Mcclean MD, Webster TF. 2008. Measurement of polybrominated diphenyl ethers on hand wipes: Estimating exposure from hand-to-mouth contact. Environmental Science & Technology 42: 3329-3334.

Tan Y-M, Liao KH, Clewell HJ. 2007. Reverse dosimetry: interpreting trihalomethanes biomonitoring data using physiologically based pharmacokinetic modeling. Journal of Exposure Science and Environmental Epidemiology 17: 591-603.

Tan Y-M, Liao KH, Conolly RB, Blount BC, Mason AM, Clewell HJ. 2006. Use of a physiologically based pharmacokinetic model to identify exposures consistent with human biomonitoring data for chloroform. Journal of Toxicology and Environmental Health, Part A: 69: 1727-1756.

Thompson CM, Johns DO, Sonawane B, Barton HA, Hattis D, Tardif R, Krishnan K. 2009. Database for physiologically based pharmacokinetic (PBPK) modeling: Physiological data for healthy and health-impaired elderly. Journal of Toxicology and Environmental Health, Part B, 12: 1-24.

Thompson CM, Sonawane B, Barton HA, DeWoskin RS, Lipscomb JC, Schlosser P, Chiu WA, Krishnan K. 2008. Approaches for applications of physiologically based pharmacokinetic models in risk assessment. Journal of Toxicology and Environmental Health, Part B. 11: 519-547

USEPA. 2006. Approaches for the application of physiologically based pharmacokinetic (PBPK) models and supporting data in risk assessment. National Center for Environmental Assessment, Washington, DC. EPA/600/R-05/043F. 123p.

Willett WC. 2002. Balancing life-style and genomics research for disease prevention. Science 296: 695-698.

Wong EY, Shirai JH, Garlock TJ, Kissel JC. 2000. Adult proxy responses to a survey of children's dermal soil contact activities. Journal of Exposure Analysis and Environmental Epidemiology 10: 509-517.

Xue J, Zartarian V, Moya J, Freeman N, Beamer P, Black K, Tulve N, Shalat S. 2007. A meta-analysis of children's hand-to-mouth frequency data for estimating nondietary ingestion exposure. Risk Analysis 27: 411-420.

Yang Y, Xu X, Georgopoulos PG. 2009. A Bayesian population PBPK model for multiroute chloroform exposure. Journal of Exposure Science and Environmental Epidemiology (in press)