United States
Consumer Product Safety Commission

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CPSC Staff Statement on:

Guidance Document for use of Human Biomonitoring Data For Exposure Assessment

The U.S. Consumer Product Safety Commission (CPSC) contracted with the University of Cincinnati to develop a guidance document that describes how human biomonitoring (HBM) data can be used with toxicokinetic, anatomy, and physiology information of different kinds to calculate chronic doses of human exposure through reverse dosimetry. Staff plans to use the guide as a tool to inform how the data can promote consistency and transparency across exposure assessments.

This statement was prepared by the CPSC staff, and the University of Cincinnati produced the following report for CPSC staff. The statement and report have not been reviewed or approved by, and do not necessarily represent the views of, the Commission.

CPSC staff may assess a product's potential health effects to consumers under the Federal Hazardous Substances Act (FHSA). A "hazardous substance" under the FHSA includes products that are "toxic" under the FHSA or present other hazards enumerated in the statute. A substance that is "toxic" may be a "hazardous substance" under the FHSA if it has the potential to cause "substantial personal injury or substantial illness during or as a proximate result of any customary or reasonably foreseeable handling or use." Therefore, staff considers exposure and risk in addition to toxicity when assessing potential hazards of certain products under the FHSA.

The second part of the risk assessment process is exposure assessment, which consists of a review of the available exposure data for the chemical. Approaches for exposure assessment vary, and this document describes one of many potential approaches (reverse dosimetry). This guidance document provides a range of available approaches and does not commit CPSC staff to use any one or combination of these approaches when conducting future assessments.

CPSC staff considers human biomonitoring data to be a valuable resource when estimating aggregate exposure to chemical substances for individuals or population groups. CPSC staff acknowledge that these data should be compared with information on consumer products to determine possible source attribution from multiple product and non-product-related sources to aggregate exposure.

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Guidance Document for Use of Human Biomonitoring Data for Exposure Assessment

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List of Abbreviations

2,5-DCP = 2,5-dichlorophenol
A&P = Anatomy and Physiology
APHL = Association of Public Health Laboratories
ATSDR = Agency for Toxic Substances and Disease Registry
BEs = Biomonitoring Equivalents
BMI = Body Mass Index
BPA = Bisphenol A
BRAT = Biomarker Reliability Assessment Tool
CDC = Centers for Disease Control and Prevention
CEFIC = European Chemical Industry Council
CEM = Consumer Exposure Model
CHMS = Canadian Health Measures Survey
CPSC = Consumer Product Safety Commission
DAF = Dermal Absorption Factor
DBP = di-n-butyl phthalate
DEHP = di(2- ethylhexyl) phthalate
DiBP = diisobutyl phthalate
ECF = Exposure conversion factor
ECHA = European Chemicals Agency
EPA (or US EPA) = Environmental Protection Agency
FDAE = Forward Dosimetry Aggregate Exposure
F_{ue} = Urinary excretion fraction
F_{ub} = Fraction of parent compound in the plasma
GC-MS/MS = Gas Chromatography - Tandem Mass Spectrometry
GC-HRMS = Gas Chromatography-High Resolution Mass Spectrometry
GerES = German Environmental Survey
GFR = Glomerular Filtration Rate
GM = Geometric mean
GSD = Geometric standard deviation
HBM = Human biomonitoring
HBM4EU = Human Biomonitoring for the European Union
HEALS = Health and Environment-wide Associations based on Large population Surveys
HTTK = High-Throughput Toxicokinetics
HTPBTK = High Throughput Physiologically Based Toxicokinetic
ICE = Integrated Chemical Environment
ICC = Intraclass Correlation Coefficient
ICRP = International Commission on Radiological Protection
IPCS = International Programme on Chemical Safety
IRIS = Integrated Risk Information System
IVIVE = *In vitro* to *in vivo* extrapolation
LC-MS/MS = Liquid Chromatography - Tandem Mass Spectrometry
LOD = Limit of detection
NAM = New alternative methods

NAS = National Academies Press
NHANES = National Health and Nutritional Examination Survey
PBPK = Physiologically-based pharmacokinetic
PBS = Phosphate-Buffered Saline
PC = Polycarbonate
PCBs = Polychlorinated biphenyls
PFOA = Perfluorooctanoic acid
PFOS = Perfluorooctane sulfonic acid
QPPR = Quantitative Property-Property Relationship
QSAR = Quantitative Structure Activity Relationship
QSPR = Quantitative Structure Property Relationship
RED = Rapid Equilibrium Dialysis
RfD = Reference dose
RIVM = Rijksinstituut voor Volksgezondheid en Milieu (the Netherlands National Institute for Public Health and the Environment)
SEC = Source Emission Categories
SVOC = Semivolatile Organic Compound
tt-MA = trans-muconic acid
UFR = Urinary Flow Rate
UFRBW = Body Weight Adjusted Urinary Flow Rate
VOC = Volatile Organic Compound
WHO = World Health Organization

1.0 Introduction to Human Biomonitoring in CPSC Context

1.1 What is Biomonitoring and Why is it Useful?

Human biomonitoring is the measurement of chemicals in human bodily fluids or tissues. As described further in Section 2, the sampled biological matrix can include urine, blood, breast milk, saliva, lipids, hair or nails, although the quality of the quantification varies with the matrix. The chemical measured may be the parent chemical or metabolite(s). A key advantage of biomonitoring is that it integrates exposures across routes and pathways. That is, biomonitoring data reflect the total internal dose, regardless of the source(s) of exposure. Exposures can be observed with biomonitoring even if intake was not monitored or measured at the time of exposure. However, the relationship between exposure and the measurement in a biological matrix depends on the chemical half-life in the body and the frequency and timing of past and/or ongoing exposures, as discussed throughout this guidance. In addition, biomonitoring results may not be specific to the chemical of interest, since measured metabolite(s) may be shared with other common parent-chemical exposures, or the chemical of interest may itself be a metabolite of other chemical(s) to which the individual was exposed.

1.2 Framing based on CPSC Problem Formulation

CPSC staff may consider biomonitoring data when conducting exposure assessments of chemical substances found in consumer products. Occurrence of a chemical substance in a biological matrix provides evidence of internal exposure. During scoping, CPSC staff consider whether there is a plausible connection between occurrence of a chemical in biological matrices and consumer applications of chemical substances. Examples of scoping questions include:

- Is there information available on consumer application sources that may have contributed to exposure. Consumer applications include contact exposures through direct use of consumer products and mediated exposures (through indoor air, indoor dust, or other environmental media) from consumer products
- Are there multiple biomonitoring and consumer application data sources?
- Is there enough information to characterize temporal or spatial trends of biomonitoring data or consumer product uses?
- Is the range of biomonitoring occurrence levels narrow (e.g., mostly at or near detection levels) or wide (e.g., large distribution between minimum and maximum values)?

If there is a connection between biomonitoring data and consumer product applications established during scoping, CPSC staff may further consider use of biomonitoring data in developing risk assessments. There are multiple approaches to measure or estimate internal exposures. While biomonitoring provides a direct measurement of internal exposure, there is not a way to precisely estimate the contribution from various sources of exposure. CPSC staff may need to consider the contributions from various sources in their assessments.

Consumer exposure models estimate indoor environmental concentrations and doses associated with use of consumer products or presence of consumer articles. Consumer exposure models provide the flexibility to estimate exposures associated with a variety of exposure scenarios from multiple products. The exposure assessor needs to consider how a person would be exposed to one or more products and carefully construct modeling scenario(s) that describe that person's exposure.

Environmental monitoring data can be combined with age-specific exposure factors and activity patterns to estimate doses. Environmental monitoring data can be used in lieu of or to supplement consumer exposure modeled estimates. The environmental media considered will vary based on the chemical substance, as certain chemical substances are more likely to be present in certain media based on inherent physical-chemical properties and expected use patterns. Environmental monitoring data represent spatial and temporal conditions present at the time the sample was collected. Therefore, these data may or may not reflect scenarios associated with specific chemical substances used in consumer products. Nevertheless, environmental monitoring data can be used to characterize the typical range of occurrence of chemical substances in different environmental media and to ground-truth modeled estimates.

When exposure estimates from consumer exposure models or environmental monitoring data are combined with chemical-specific toxicokinetic data, typically the fraction of the chemical absorbed, estimate(s) of internal exposure or absorbed dose are derived.

Biomonitoring data can also be used to estimate the internal or absorbed dose of a chemical There are multiple approaches to derive internal doses based on biomonitoring data, and these approaches are the subject of this guide. The guide will characterize multiple sub-categories of biomonitoring data and the various approaches available for treating these different data types.

CPSC staff may use a variety of data sources to provide multiple lines of evidence when deriving exposure estimates. All of these approaches can either provide deterministic point estimates of exposure or ranges of exposure based on anticipated variability. It is beyond the scope of this guide to discuss ways to integrate data across multiple approaches. However, this guide will consider how to address variability present in biomonitoring data. Further, the guide will provide important context and associated uncertainties with interpreting different sub-categories of biomonitoring data for use in exposure assessment.

2.0 Evaluation of the Biomonitoring Study

Many of the considerations for evaluating biomonitoring data are most relevant in the context of designing and conducting a study. However, it is also important to consider the issues discussed in this section as part of data review. When evaluating biomonitoring studies for use in exposure assessment, CPSC staff considers:

- the relevance of the study population,
- the appropriateness of the sampling strategy,

- the adequacy of the analytical methods,
- the appropriateness of the biomarker, and
- the appropriateness of the choice of matrix.

This section concludes with a recommended study design for biomarker sampling for evaluating exposure from consumer products.

2.1 Study Population

CPSC staff plans to consider relevance of a study population with regard to fit-for-purpose project goals (see Section 1.2). This includes consideration of the demographics of the study population, the geographic representation of the population, and when samples were collected. For example, different projects may focus on exposures of a particular sub-populations (e.g., mothers and children in the study of Casas et al., 2018) or on wide-spread regional or national populations. Geographical coverage may be important, particularly if some aspects of exposure are expected to exhibit regional variability. CPSC staff plan to consider the degree to which the sampled population reflect the population of interest for the exposure assessment. Consideration of when samples were collected can be important, since some exposures change over time. Additional areas for consideration include age, sex, body mass index (BMI), race/ethnicity, and any other factor that would have an impact on the biomarker measurement or the relationship between the biomarker and internal dose.

CPSC staff plan to evaluate the sampling strategy as part of the consideration of the representativeness of the sample population. Key aspects of this evaluation include the sample size and the approach for acquiring the sample of individuals for the study (Namulanda et al., 2020). Random sampling methods are preferred over samples of convenience, such as the first 100 people at a clinic. If a sample of convenience is used, the study should explicitly report the strategy for recruitment and selection, in order to allow for an independent evaluation of any potential distortions or biases (NAS, 2006).

2.2 Sampling Strategy

The appropriate sampling strategy(s) depends on the biomarker being evaluated and the matrix being sampled. A single sampling time is usually sufficient for measurements in blood and lipid, both in light of the invasive nature of the sampling and because these matrices are typically used to evaluate chemicals with longer half-lives. Multiple blood samples over years, however, could be used to evaluate long-term trends.

Several approaches are commonly used for sampling urine. Spot sampling (collection of a single sample) or collection of the first morning void is commonly used, due to convenience. However, as discussed in greater detail in Section 3.8, these approaches can lead to systematic errors. In brief, population variability may be over-estimated (leading to an over-estimate of the 95th percentile, for example, and a corresponding over-estimate of exposure/risk) (Aylward et al., 2017). This is because the intra-individual variability in biomarker levels with time since time since exposure makes a large contribution to the total measured variability. Therefore, 24-hour composite samples, representing each individual's total exposure over a day, are preferred over spot sampling. In some cases, samples may be pooled over a longer period (e.g., a week), to better reflect each

individual's average exposure (Casas et al., 2018), although such an approach is more labor-intensive than pooling over a day.

Note that this use of composite or pooled samples is distinct from pooling samples from multiple individuals into one sample. Such pooling across individuals has the strong disadvantage of decreasing the estimate of population variability, or making it impossible to estimate variability, depending on how samples are pooled.

Hair is rarely used for biomonitoring (see Section 2.5), but evaluation of different segments of hair can provide a temporal record of exposure.

Additional considerations for evaluating the appropriateness of the choice of biomarker and biological matrix are addressed in Sections 2.4 and 2.5.

2.3 Analytical methods

Numerous factors must be considered in evaluating the validity of an analytical method. Vorkamp et al. (2021) and LaKind et al. (2014) have created comprehensive approaches to analyzing the validity of an analytical method. Important factors when analyzing analytical methods include:

1. Sample Preparation- Are sampling preparation procedures well established and applied routinely?
2. Standards- Are analytical standards for target biomarkers applied throughout the methodology or not, and are these standards commercially available?
3. Validation- Has this analytical method been validated multiple times? Are certified materials being used or are interlaboratory comparisons of the methodology results available? Is this method well-established in multiple laboratories?
4. Selectivity- Is there interference that indicates a measured concentration might not be related to the biomarker of interest?
5. Sensitivity- There are numerous factors to consider when thinking about the sensitivity.
    a. Have limits of detection (LOD) been determined for each biomarker of exposure within a biological matrix (how comprehensive are these analyses)?
    b. Are these LODs sufficiently below what a typical exposure would be?
    c. Do LODs remain similar from sample to sample over time? Or do they vary?
    d. Have similar LODs been obtained in other laboratories?
6. Accuracy- Has the accuracy of an analytical test been confirmed using external quality control measures (e.g., certified reference manuals, relevant interlaboratory comparisons)? Is the accuracy within limits given by the guidelines for validation of analytical methods? (e.g., ≤ 20% deviation from the concentration level of a biomarker)

7. Robustness- When there are slight changes in the analytical procedure, are there any variations in each sample? Would there be variations if the same procedure were repeated?
8. Recovery- Can between 80-120% of the biomarker of interest be recovered? If not, can internal laboratory standards compensate for these deviations?
9. Range/Linearity- Is this methodology precise and accurate for determining the concentration of biomarker in a specific biological matrix?
10. Method requirement- Does the instrumentation unambiguously identify and quantify a biomarker of interest (e.g., GC-MS/MS, LC-MS/MS, GC-HRMS)?
11. Feasibility- Is this analytical method both financially and technologically feasible for the biomarker of interest? Some factors for non-feasibility may include:
    a. Compound instability/volatility: Is the stability of a biomarker known? If a compound is exceedingly volatile, transportation and storage conditions may be adapted, but if it is not financially or technologically feasible to store it, this could be problematic.
    b. Matrix availability: If the matrix is too invasive or if it is too hard to preserve a given sample, it may not be feasible to sample from this matrix.

It is also important to note any limitations in the study that were identified by the study authors.

Quality control issues also apply to sampling and sample storage. Information may not always be available on the specifics of sampling and storage, but where such information is available, specifics of the sample collection and preparation should be evaluated. This includes factors such as the method of sample collection and storage, and the use of appropriate analytical methods (APHL, 2019; CDC, 2018; LaKind et al., 2014; Vorkamp et al., 2021).

Metabolites often undergo conjugation reactions (e.g., sulfation, glucuronidation) as part of Phase II metabolism. In such cases, it is important to ensure that both conjugated and unconjugated forms of the metabolite are measured. This is often done by including a hydrolysis step as part of the sample preparation, but it is important to ensure that the hydrolysis does not result in other changes to the metabolite. The documentation of the analytical approach should specify whether the analytical approach detects only free compound or free and conjugated.

2.4 Choice of Biomarker of Exposure

CPSC staff consider a number of factors in determining whether a potential biomarker is appropriate for estimating exposure (summarized by Aylward et al., 2017; Casas et al., 2018; LaKind et al., 2014; Vorkamp et al., 2021). At the most basic level, a biomarker of exposure should "vary consistently and quantitatively with the extent of exposure (especially at low doses)" (NAS, 2006). The limit of detection should be sufficiently sensitive, and the level of environmental exposure should be sufficiently high relative to endogenous generation of the biomarker for the biomarker levels to primarily reflect the exogenous exposure.

An exposure biomarker may be the parent chemical or a metabolite or a combination of multiple metabolites. The parent may be a more appropriate biomarker when metabolism is slow, while metabolites are often used when metabolism and elimination occur relatively rapidly (half-life less than about 8 hours). Even then, it is important to consider the impact of intra-individual variability (see Section 3.8). It may also be important to characterize inter-individual factors that may impact exposure and presence of biomarker. This can include age, education, genetic factors (e.g., metabolic polymorphisms), and smoking status (IPCS, 2001). Some of these factors may be known from other studies, while other relationships may be evaluated as part of the biomarker study. If there is variation in biomarker concentrations related to a confounding exposure, a different biomarker should be used. For example, US EPA (2020a) excluded a potential biomarker of 1-bromopropane in their analysis, because of questions regarding the specificity of the biomarker.

Choice of the biomarker requires an understanding of the chemical's toxicokinetics, including the chemical's metabolic pathway, key metabolites, whether those metabolites are shared with other chemicals, as well as whether the parent is itself a metabolite of other chemicals. In most cases, the biomarker should be specific to the exposure of interest. For example, benzene is metabolized to both phenol and trans, trans-muconic acid (tt-MA), but the latter is more appropriate as a measure of benzene exposure, since urinary phenol can also reflect exposure to phenol itself (ATSDR, 2007)[1]. Sometimes there are multiple metabolites that are potentially useful as biomarkers. In some cases, calculations are conducted based on the total concentration of the relevant metabolites (e.g., Smith et al., 2021). In other cases, a single metabolite is chosen for the quantitative calculation. In this case, the choice of biomarker may be based on not only confirmation of the uniqueness of the metabolite to that chemical, but also factors related to the ease or difficulty of analysis. The proportion of the parent exposure converted to that metabolite is reflected in the urinary excretion fraction (F_{ue}) (see Section 3.3.1). Conversely, a metabolite shared by several chemicals in a class may be used if the risk management needs are such that determining the exposures to the class of chemicals is sufficient, without identifying exposures to the specific parent chemicals.

2.5 Choice of Biological Matrix

Urine and blood are the most commonly-used matrices, with urine used predominantly for chemicals with shorter half-lives, while blood is used for chemicals with longer half-lives, which are often lipophilic. Breast milk, and other lipid-based matrices, are also sometimes used for lipophilic chemicals. Exhaled breath may be used for volatile chemicals. Some biomarker work has also been done with other matrices, including hair, nails, sweat, saliva and teeth, but these are more often used in a qualitative manner (e.g., determine whether past exposure has occurred with no need to quantify extent of exposure).

[1] As discussed by Hays et al. (2012), although tt-MA is a popular biomarker for occupational exposures to benzene, tt-MA confounding from sorbic acid is problematic at the much lower environmentally-relevant benzene exposure levels.

The choice of biological matrix is dependent on the characteristics of the biomarker as well as the issues noted in problem formulation. Choice of the matrix should consider the chemical/physical properties of the chemical of interest and metabolites (e.g., lipophilicity/water solubility, volatility), common routes of exposure, half-life of the parent chemical, excretion pathways, and invasiveness of potential matrices. The population being evaluated can also affect the choice of matrix. For example, urine can be collected from infants using specially designed diapers (NAS, 2006), but it can be difficult to obtain enough blood for analysis from an infant.

2.5.1 Urine

Urine is often used to estimate exposure to compounds that are water soluble and rapidly excreted from the body (Esteban and Castano, 2009; Aylward et al., 2017). As noted in Section 2.2, a common approach is to collect spot urine samples, but 24-hour or multi-day composite samples better reflect the average individual exposure, and thus the overall population variability (Aylward et al., 2017; Casas et al., 2018). If taking daily or multi-day composite samples is not feasible, techniques exist to determine the optimal number of spot samples (see Section 3.8).

Urine can also be used for evaluating chemicals that are slowly excreted from the body. Urinary levels of metals such as cadmium and lead have been strongly correlated with blood biomarker levels, but urine is much less invasive, urine samples are the "preferred non-invasive matrix in heavy metals biomonitoring" (Esteban and Castano, 2009). An advantage for slowly excreted chemicals is that they are more likely to be at steady state levels in the body, reducing variation in urine levels and the associated uncertainty. For slowly-excreted chemicals, urinary levels tend to reflect recent exposures, while blood levels are related to total body burden, and so reflect the exposure over a longer duration. A challenge with some of the slowly-excreted chemicals, such as metals, is that most of the excretion is in the feces, and F_{ue} can be very low. This results in higher variability, and thus higher uncertainty.

2.5.2 Blood

Blood is typically used for compounds that are not rapidly excreted from the body (HEALS, 2015). Depending on the biomarker, one may choose to analyze whole blood, serum, plasma, or cell types within the blood (Alves et al., 2014). Concentrations in blood are related to tissue concentrations, with blood considered the "universal link between all tissues of the organism," making it the preferred matrix for many contaminants (Alves et al., 2014). However, it is invasive to collect blood. Typically, no more than 20 mL can be collected at one time (Manno et al., 2014; Polkowska et al., 2004). The use of an invasive matrix may make it harder to acquire participants for a study, and ethical concerns for sampling children need to be considered (HEALS, 2015; Manno et al., 2014). The site of blood collection should also be considered. For example, venous blood sampling is preferred over a finger prick test for testing blood lead levels, because the latter test is prone to surface lead contamination if the hands are not properly cleaned (ATSDR, 2020).

Blood serum is lipid rich and so is a useful biomatrix for lipophilic substances such as halogenated flame retardants, and less invasive than sampling fatty tissue (Tay et al., 2019). Blood can also be used for evaluating rapidly excreted (typically more water-soluble) chemicals. In these cases, the issues of variability and the length of the half-life relative to the frequency of exposure need to be considered, as for less invasive urinary measurements. For very persistent chemicals, blood concentrations reflect the cumulative exposure, which can result in age-related trends in biomarker levels if the population exposure is decreasing with time. Serum lipid concentrations and the serum concentrations of lipophilic chemicals vary with recent meal consumption, but normalization to total serum lipid content stabilizes the estimated concentration of lipophilic substances (Phillips et al, 1989, as cited by Aylward et al., 2014). Aylward et al. (2014) also noted that lipid concentration in serum can affect the serum concentration of lipophilic chemicals even if they are not persistent, but that lipid adjustments have not been applied routinely to measurements of volatile organic compounds in blood. Similarly, binding to proteins in blood can affect the measured levels of the biomarker, but adjustment for protein binding is not done routinely.

2.5.3 Breast Milk and Lipids

The maternal body burden of fat-soluble compounds such as dioxins and brominated flame retardants can be evaluated with reverse dosimetry by sampling breast milk (Shen et al., 2007; Uehara et al., 2006; HEALS, 2015) to assess exposures to the mother. Breast milk data can also be used with a conventional exposure assessment approach to estimate early life exposure in babies (HEALS, 2015). Collecting breast milk is easy and non-invasive. Breast milk can vary in lipid concentration, which requires a lipid adjustment (mg biomarker/g lipid) to correct for variation in lipid concentration (Esteban and Castano, 2009).

Lipophilic compounds may rapidly diffuse from the blood to fatty tissues, which may warrant the use of a lipid as a biological matrix (NAS, 2006). Adipose tissues store lipophilic compounds in the body and may metabolize and release into the bloodstream at a slow rate. Sampling of adipose tissues represents long-term exposure to lipophilic compounds but is invasive and it is difficult to acquire a large sample size. The World Health Organization limits the use of adipose tissues to “ecological studies comparing fat from cadavers or surgical specimens to general population levels” (IPCS, 2000).

2.5.4 Exhaled Breath

Generally, volatile compounds with short half-lives are exhaled from the lungs, making exhaled breath a suitable biomatrix (Tang et al., 2015; Manno et al., 2014). Exhaled breath can be used to monitor both previous inhalation exposure to volatile chemicals and levels of volatile chemicals absorbed after oral or dermal exposure. After absorption, volatile compounds will travel to the lungs through the blood and will be expired (Tang et al., 2015). Exhaled breath is easy to collect, is inexpensive, and is non-invasive (Wilson and Monster, 1999). Methods are available for both direct breath analysis in real time and for indirect breath analysis (involving sample collection and concentration), but these involve specialized equipment (Tang et al., 2015).

2.5.5 Other Matrices (Hair, Nails, Teeth, Saliva, Sweat)

There are several matrices that can be used to detect the presence of biomarkers, but are currently not generally considered adequate or appropriate for quantitative estimates of exposure. An important exception is that hair concentrations of methylmercury have been used for exposure estimates, including as supporting data for the RfD developed by the US EPA (2001) in its IRIS assessment.

Hair can be used to characterize long term exposure to heavy metals such as methylmercury. If the hair is investigated in segments, it can provide a timeline of exposure (Esteban and Castano, 2009). Hair is a minimally invasive matrix to sample (HEALS, 2015). A limitation of this matrix is that washing is needed to remove chemicals that deposited *onto* the hair (as opposed to being excreted *into* the hair). However, washing can also alter the concentration of the chemicals within the hair, complicating the analysis (Esteban and Castano, 2009; Wilhelm and Idel, 1996; HEALS, 2015). The relatively large amount of hair that needs to be collected (50-200 mg of hair) can limit the use of this matrix (HEALS, 2015). Furthermore, it is difficult to associate levels of biomarker in hair to biomarker in blood and other tissues (Esteban and Castano, 2009).

Nails can provide information on the long-term exposure of inorganic chemicals such as heavy metals (Esteban and Castano, 2009; HEALS, 2015). It is advantageous to use nails as long-term exposures can be characterized in one small sample (HEALS, 2015). Nails can be prone to external contamination, but toenails are not as prone to contamination as fingernails (Esteban and Castano, 2009). For analyzing biomarker in nails, samples can be contaminated through use of nail polish, nail cutters, and medication.

Deciduous teeth can be used to detect exposures to metals such as lead and magnesium in children (HEALS, 2015). Teeth can provide information related to lifetime exposure in children (HEALS, 2015). Deciduous teeth are difficult to collect and are not easily available (Esteban and Castano, 2009).

Saliva may be a suitable matrix in cases where the biomarker has a low molecular weight (e.g., organic solvents, specific trace elements, selected pesticides), but does not bind strongly to proteins (HEALS, 2015; Esteban and Castano, 2009). Saliva is a non-invasive matrix that is easy and cheap to collect (Esteban and Castano, 2009), but may be less sensitive than other matrices (HEALS, 2015). Furthermore, there are other confounding factors that have made use of saliva in biomonitoring less widespread (Alves et al., 2014).

Sweat has been used in the past to detect exposure to certain compounds. In a study by Omokhodion and Crockford (1991), researchers were able to detect lead in the sweat of humans, but there was a poor correlation between levels of biomarker in sweat and biomarker in blood. Furthermore, it is a matrix that is not easily available in high quantities and is difficult to collect (Esteban and Castano, 2009). Although sweat is not yet appropriate for quantitative estimates of exposure, use of sweat for noninvasive real-time monitoring of exposures is an active area of research.

2.6 Study Design for Evaluating Exposures from Consumer Products

A key challenge for CPSC staff is that population-level biomonitoring data such as NHANES will provide general information on the population distribution of exposure, but unless questionnaire data or paired environmental monitoring data is also available, this exposure cannot be directly tied to any specific products. Targeted exposure studies are needed to allow for attribution of exposure to specific products or classes of products, and these targeted studies have specific needs for both sampling approaches and data analysis.

The study by Koch et al. (2014) provides a good example of a study design for assessing human exposure to personal care products. The study involved recruiting eight volunteers who provided detailed information about the use of personal care products, recorded and weighed product use in a detailed diary and collected every urine void over a 6-day collection period. Because every urine void was collected over 24 hours and over multiple days, complete urine elimination of analytes of interest could be determined on a daily basis (mass excreted in urine). This data could be matched with the exposures presumed from product labels, diary entries and weight of product used. This particular study also involved a 2-day washout period in which participants were provided replacement consumer products that did not contain the chemicals of interest. Declines in urine concentration of the analytes of interest were observed and quantified using this study design, thus confirming the source of the exposures.

3.0 Interpreting the Biomonitoring Data

3.1 Overview

Three types of data are needed to calculate exposures corresponding to measured biomonitoring data – (1) the biomonitoring data on a biomarker of interest, (2) chemical-specific toxicokinetic data, and (3) human anatomy and physiology data. Calculations can be done using either forward or reverse dosimetry. Forward dosimetry involves calculating the biomarker level corresponding to a given exposure in an environmental medium. CPSC staff consider exposure reconstruction or reverse dosimetry, calculating the exposure level(s), specifically the internal dose, corresponding to a specified biomarker level.

This section first addresses sources of biomonitoring data, followed by a discussion of concepts and equations used for reverse dosimetry. After the toxicokinetic concepts have been introduced in the context of the reverse dosimetry calculations, the text addresses sources of chemical-specific toxicokinetic data, as well as sources of anatomy and physiology data. Forward dosimetry is briefly addressed, followed by a brief discussion of guidance documents available for evaluating physiologically-based pharmacokinetic (PBPK) models. The section concludes with a discussion of methods for determining source contribution and considerations for addressing variability in biomonitoring data.

3.2 Sources of Biomonitoring Data

CPSC staff considers the source of biomonitoring data to determine whether it is relevant to the consumer population. Biomonitoring data can be derived from national data,

regional/large cohorts, small cohorts, or individual data (also called case study data). Pooled datasets combine samples from multiple individuals within a cohort. National biomonitoring data are derived from nationwide datasets. In the US, the largest national biomonitoring survey is the National Health and Nutrition Examination Survey (NHANES) and is conducted by the Centers for Disease Control and Prevention (CDC). Other national surveys include the Canadian Health Measures Survey (CHMS) and the German Environmental Survey (GerES). The HBM4EU (Human Biomonitoring for the European Union) project is a cooperative transnational effort. For the purpose of this guidance, a regional or large cohort is defined as 100 individuals or greater. Several states, including California and Minnesota, have their own biomonitoring programs. Small cohorts are defined as biomonitoring data for between 2 to 99 individuals.

3.3 Reverse Dosimetry Approaches

3.3.1 Introduction to Reverse Dosimetry and Exposure Reconstruction

As noted, reverse dosimetry is the process of back-calculating the exposure to a chemical (often via the oral route) that would be consistent with a measured biomonitoring level in humans. This process relies on measured biomonitoring data, information about the toxicokinetics of the chemical of interest, and human anatomy and physiology data. There are many methods of conducting reverse dosimetry, ranging from very simple methods (mass balance approach) to very complex (PBPK model estimations). This section presents the major approaches for reverse dosimetry, and is followed in Section 3.4 by a discussion of the sources of data to use in the calculations.

3.3.2 Urinary Mass Balance Approach

The mass balance approach is a simple and commonly-used method of estimating daily intakes from biomarkers in urine. It is called the mass balance approach because it relies on the balance between the mass or moles of exposure (intake) and mass or moles of analyte (parent compound or metabolite(s)) excreted (eliminated) in urine. The only toxicokinetic parameter required is urinary excretion fraction (F_{ue}). F_{ue} is a unitless term that accounts for the fraction of a dose that is consumed or applied and is absorbed, metabolized and ultimately eliminated in urine in the form of the biomarker of interest (F_{ue}=mass biomarker eliminated in urine/mass consumed of chemical of interest). The F_{ue} can be used when exposure is via the oral *or* dermal routes, and is a route-specific parameter, since it includes absorption via the relevant route. Note that this method cannot be used if there is substantial exposure via both the oral and dermal routes, because the oral and dermal F_{ue} values would be different and thus it is an indeterminant problem. The one exception is if some information is available about the relative relationship between exposures from the oral and dermal routes, or the relative absorption from the two routes. Inhalation exposure is not usually addressed using the mass balance approach. (See Section 3.3.3.)

The mass balance approach assumes that steady state has been reached. This means that this approach may be inappropriate for using spot samples to estimate exposures to compounds that are rapidly eliminated, due to the high intra-individual variability with

time, and the associated increase in uncertainty. (See Section 3.8 for a more detailed discussion of this issue.)

Oral Exposures

For compounds where the exposures are predominantly via oral ingestion (food, water, etc.), the solution to the mass balance equation is daily oral intake (amount consumed). Using the mass balance approach, daily oral intake may be calculated as follows (derived from Aylward et al., 2017):

$$DI = \frac{C*V}{BW*F_{ue}} \qquad \textit{Eq. 1}$$

Where: DI= Daily intake of the parent compound (mg/kg-day)
C= Biomarker concentration in urine (mg biomarker/L)
V= 24-hour urinary flow rate (L/day)
BW= Body weight (kg)
F_{ue}= Urinary excretion fraction (mg biomarker excreted/mg parent compound intake)

Urinary volume and flow vary from individual to individual due to differences in hydration status. There are several approaches to account for differences in hydration status, including adjustments based on creatinine excretion, osmolality (a measure of how concentrated the urine is), specific gravity and urine flow rate (L/hr). (See below for the equations and Section 3.8.1 for additional information on the implications of this adjustment for addressing variability.) Equation 2 is a mass balance equation that uses metabolite concentrations that are creatinine adjusted (derived from Koch et al., 2007):

$$DI = \frac{C_{cr}*Cr_e}{BW*F_{ue}} \qquad \textit{Eq. 2}$$

Where: DI= Daily intake of the parent compound (mg/kg-day)
C_{cr}= Creatinine adjusted concentration of analyte in urine (mg biomarker/g creatinine)
Cr_e= creatinine excretion rate (g creatinine/day)
BW= Body weight (kg)
F_{ue}= Urinary excretion fraction (mg biomarker excreted/mg parent compound intake)

Creatinine excretion varies with age, size, body weight, gender and race/ethnicity. Therefore, it is preferable to use a creatinine excretion rate that is directly relevant to the population of interest. For example, Koch et al. (2007) used body height and gender-based excretion data obtained from children of the same ethnicity as the children that provided urine for biomonitoring measurements, normalized to body weights of the individual subjects. They also conducted an alternative analysis based on age-specific data for daily excreted urine volume/kg body weight.

Either spot samples or 24-hour urine composites can be used with the mass balance approach, regardless of whether the creatinine correction is used. When taking a 24-hour urine composite, the daily urinary volume is known, decreasing uncertainty. When taking spot samples, one must make assumptions on how much urine an individual produces in a day (i.e., L/day). In addition, the total urine volume in the spot sample may be unknown, making calculations based on creatinine-adjusted concentration particularly useful for spot samples (Mage et al., 2008).

Dermal Exposures

For chemicals for which exposures occur predominantly via dermal exposures, the mass balance approach will calculate daily dermally applied dose (µg/kg-day). As for the mass balance equation for oral exposures, calculating a dermally applied dose requires knowing the urinary excretion fraction for an analyte (parent compound or metabolite) following a dermal dose. Thus, although this approach is not very common, dermal exposure may be quantified using the following equation:

$$DDD = \frac{C*V}{BW*F_{ue}} \qquad Eq.\ 3$$

Where: DDD= Daily dermal dose (µg/kg-day)
C= Biomarker concentration in urine (µg/L)
V= 24-hour urinary flow rate (L/day)
BW = body weight (kg)
F_{ue} = dermally derived urinary excretion fraction

The dermal F_{ue} is usually derived by comparing the oral and dermal bioavailability and then applying the relative bioavailability to the orally-derived F_{ue}.

Combined Oral and Dermal Exposure

As noted, the F_{ue} differs for the oral and dermal routes, since this parameter includes the absorption fraction via each route, respectively. This means that the mass balance approach cannot usually be applied if there are meaningful contributions from both routes. However, if there is information on the relative absorption via the two routes, or the relative internal dose from the two routes is known, then the amount of intake from the two respective routes can be calculated. Practically, this type of calculation requires information about relative exposures from the oral and dermal routes, such that a forward-based conventional exposure assessment can be conducted. In this situation, the biomonitoring data can be used to check the conventional exposure assessment.

Inhalation Exposures

There are no simple established approaches for back calculating air concentration from urinary biomonitoring data. In contrast to the oral and dermal routes, where the relationship between external exposure and internal dose is reflected in the absorption fraction (and incorporated into the F_{ue}), the inhalation absorption at steady state is determined by the blood:air partition coefficient (US EPA, 1994).

3.3.3 Exposure Conversion Factor Approach

Because the mass balance approach cannot be applied for the inhalation route, exposure conversion factors (ECFs) are commonly used for inhalation reverse dosimetry, in addition to being used for oral reverse dosimetry. ECFs may be calculated using simple linear regressions to evaluate the relationship between exposure concentrations and biomarker levels in urine or blood. This is commonly done for inhalation exposures, since exposure conversion factors can often be calculated from occupational cohort data where exposures are well documented (Hays et al., 2012). ECFs are also frequently used with classical pharmacokinetic or physiologically-based pharmacokinetic (PBPK) models to establish a relationship between the biomarker of interest and the external exposure (concentration in air) or oral intake (e.g., Brown et al., 2015; Lee et al., 2017). This method assumes steady state (Lee et al., 2017) and that the dose-biomarker relationship is linear (Brown et al., 2015). The ECF approach is needed when conducting reverse dosimetry calculations with a PBPK model because one cannot run a PBPK model "backwards." In other words, a PBPK model can be used for forward dosimetry calculations, to determine the concentration of a biomarker corresponding to a specified external air concentration, but a PBPK model cannot directly calculate the air concentration or oral dose corresponding to a specified biomarker concentration for a reverse dosimetry analysis. In order to address this issue, the model is run for a range of external air concentrations or oral/dermal doses to establish the linear range for the dose-biomarker relationship, and then the ratio between the air concentration or oral/dermal dose and the biomarker can be determined. This ratio is the ECF:

$$ECF = \frac{Air\ Concentration\ or\ Oral\ or\ Dermal\ Dose}{C_{biomarker}} \qquad Eq.\ 4$$

This equation may be rearranged to calculate an external exposure or intake dose level from a measured biomarker concentration.

$$Air\ concentration\ or\ Oral\ or\ Dermal\ Dose\ = ECF * C_{biomarker} \qquad Eq.\ 5$$

The same general principle applies whether the exposure part of the ratio is the air concentration or an oral or dermal dose, and whether the biomarker portion is concentration of chemical in blood or urine. When used to interpret data from PBPK modeling, ECFs are generally applied to biomarkers in blood, because the model is designed to calculate the concentration in blood. For example, the ECF approach was applied to steady-state solutions to chemical-specific PBPK or compartmental models for numerous chemicals (Aylward et al., 2010). More complex approaches combined ECFs with Monte Carlo sampling to evaluate uncertainty and variability in the calculation (Tan et al., 2006; Liao et al., 2007; Huizer et al., 2014).

3.3.4 Reverse Compartmental Model Approach

Converting Compartmental Model Equations for Reverse Dosimetry

Compartmental PK model equations may be rearranged to solve for daily absorbed dose. This approach can be used to estimate the external exposure and absorbed dose from internal samples such as blood, lipids, and tissues. This simple compartmental model

operates under a steady state assumption and lumps metabolism and excretion together into a half-life term. Any compartmental equation can be rearranged to solve for external dose. Egeghy and Lorber (2011) rearranged a one compartmental model to solve for external dose using serum[2] concentration.

$$\frac{d(C)}{dt} = \frac{D(t)}{V_d} - k * C(t) \qquad \textit{Eq. 6}$$

Where: D= Daily absorbed dose (mg/kg-day)
C= Serum concentration (mg/mL)
V_d= Volume of distribution (mL/kg)
k= First order elimination rate in the body (per day)

Application of this equation assumes steady state. Understanding that input will equal output, and that the serum concentration is constant with time, allows one to rearrange the equations as follows:

$$\frac{D}{V_d} = C * k \qquad \textit{Eq. 7}$$

One can then rearrange the equation to solve for D:

$$D = C * k * V_d \qquad \textit{Eq. 8}$$

The absorbed dose can be converted to a standard daily intake, external exposure, by dividing D by the absorption fraction.

$$DI = \frac{D}{AF} \qquad \textit{Eq. 9}$$

Where: DI= Daily intake (mg/kg-day)
D= Daily absorbed dose (mg/kg-day)
AF = Absorption fraction (unitless)

Eq. 9 can be re-arranged to calculated the daily absorbed dose by multiplying the daily intake, external exposure, by the absorption fraction. When the absorption fraction is close to one, the absorbed dose is similar to the daily intake.

$$D = DI * AF \qquad \textit{Eq. 10}$$

Where: DI= Daily intake (mg/kg-day)
D= Daily absorbed dose (mg/kg-day)
AF = Absorption fraction (unitless)

[2]The same approach would apply for blood, serum or plasma.

One can convert a variety of one compartmental model equations to reverse dosimetry equations, as shown in the following text.

Calculating Daily Dose from Blood or Serum

For some compounds, biomonitoring in blood is preferred because the compound is not readily excreted in urine (e.g., mostly excreted through bile and feces) and/or has a long half-life. This approach requires knowledge of the chemical's distribution in the body (volume of distribution). Note that the volume of distribution must be specific to the matrix in which the biomarker is measured (e.g., blood vs. serum), since protein binding can affect this parameter. This reverse dosimetry approach operates under a steady state assumption and that assumes that elimination is a first-order process (Egeghy and Lorber, 2011; Fromme et al., 2007):

$$D = C * k * V_d \qquad \textit{Eq. 11}$$

Where: D= Daily absorbed dose (mg/kg-day)
C= Serum concentration (mg/mL)
V_d= Volume of distribution (mL/kg)
k= First order elimination rate in the body (per day)

As above, the absorbed dose can be converted to a daily intake by dividing by the fractional bioavailability.

In each of the equations above, the two terms for rate of elimination and volume of distribution are always multiplied by each other ($kP*V_d$). This is also referred to as the clearance of a compound and is defined as the volume of the body (or blood or whichever body matrix/compartment that the compound is being measured in) that is cleared of a compound per unit of time (e.g., L/hr). Thus, when clearance is known for a compound (e.g., has been published in the literature), this can be used in place of the $k*V_d$ term, with appropriate adjustments for the units. Thus, in this case:

$$V_d\left(\frac{mL}{day}\right) = V_d\left(\frac{L}{hr}\right) * 1000\frac{mL}{L} * \frac{24}{1}\frac{hours}{day} \qquad \textit{Eq. 12}$$

Calculating Daily Intake from Body Lipids

To estimate daily intake of a biomarker with a half-life of days or weeks, it may be possible to sample body lipids. The following reverse dosimetry approach assumes steady state, that elimination is due to a first-order degradation process, that the substance distributes equally in body lipids, and if there are different biomarker isomers, that they exhibit no differences in toxicokinetic parameters (derived from Aylward and Hays, 2011; US EPA, 2020b):

$$DI = C_l * F_l * k \qquad \textit{Eq. 13}$$

Where: DI= chronic daily intake (mg/kg day)
C_l= lipid concentration (mg biomarker/kg lipid)
F_l= fraction of body weight that is lipid (assumed to be 25%)

k= elimination rate calculated from chemical half-life in lipids (ln(2)/half-life)

3.3.5 Multi-Compartmental Model Approach

New approaches for testing the toxicity of compounds using *in vitro* assays have created the need for developing approaches for extrapolating an assay concentration to some measure of external exposure so as to put those *in vitro* toxicity findings in an exposure context. One of the basic assumptions being made is that the cell culture (assay) nominal concentration is equivalent to blood or tissue concentration *in vivo*. A model is used to conduct reverse dosimetry to estimate an external dose that yields a "target" assay (blood) concentration in humans. The US EPA in collaboration with the NIEHS has developed methods for using toxicokinetic data to facilitate this *in vitro* to *in vivo* extrapolation (IVIVE). The same reverse dosimetry principles used for IVIVE apply to interpretation of data from biomonitoring in blood, since the *in vitro* chemical concentration is considered to be analogous to the concentration of the chemical in blood. However, as described later in this section, there are several caveats in using this approach to estimate human exposures, related to the assumptions in the model, and simplifications in the toxicokinetics. Importantly, the most commonly-described equations assume steady state, but urine is used much more frequently than blood for biomonitoring of chemicals. Perhaps because the focus has been on using such models for IVIVE of dose-response data and comparison with exposures estimated from urinary data in NHANES, no publications were located using the methods described here to estimate human exposures from concentrations in blood. Nonetheless, this approach does seem promising for future application for chemicals lacking appropriate human and animal toxicokinetic data.

Several publications have described the use of *in vitro* data, sometimes supplemented by *in silico* data, in combination with generic compartmental models (Wetmore et al., 2012; Wetmore, 2015; Wambaugh et al., 2018). Models described in these papers are used in the US EPA's HTTK package for IVIVE. The most commonly used generic multicompartmental model assumes zero-order uptake from the gut and 100% oral bioavailability. It includes three compartments – gut, liver, and the rest of the body. Removal from the body is based on renal clearance and hepatic metabolic clearance. The renal clearance is based on the glomerular filtration rate (GFR) and the unbound fraction of parent compound in the blood (F_{ub}). Hepatic clearance is based on the intrinsic metabolic clearance $CL_{int,h}$. It assumes first-order clearance using a "well-stirred" approximation (i.e., assuming that the concentration of the chemical is uniform across the liver). Thus, this model can express the steady state concentration in blood using only standard physiological parameters and chemical-specific parameters that can be determined relatively easily in human cells *in vitro.*

$$C_{SS} = \frac{k_{dose}}{(GFR*F_{ub})+\left(\frac{Q_l*F_{ub}*Cl_{int,h}}{Q_l+F_{ub}*Cl_{int,h}}\right)} \qquad \textit{Eq. 14}$$

Where: k_{dose}= Dose rate (mg/kg-hour)
C_{SS}= Blood Concentration in steady state (mg/L)

GFR= Glomerular Filtration Rate (L/hour-kg)
F_{ub}= Unbound fraction of parent compound in the blood (unitless)
Q_l= Liver blood flow (L/hour-kg)
$CL_{int,h}$= Whole-liver intrinsic clearance rate (L/hour-kg)

The equation for the steady state concentration can be rearranged to express daily intake (converting the dose rate from dose per hour to dose per day) as a function of the steady state blood concentration, standard physiological parameters, and chemical-specific parameters determined *in vitro.* Alternatively, Eq. 14 can be applied to determine the steady state concentration for a daily intake of 1 mg/kg-day (k_{dose} = 0.042 mg/kg-hr), and the result can be used as a conversion factor for the measured biomarker concentration (*Eq. 15*). This latter approach has the advantage of being mathematically more intuitive, and is the approach used by the U.S. EPA for IVIVE:

$$DI = \frac{C\ x\ 1\frac{mg}{kg}-day}{C_{ss}} \qquad Eq.\ 15$$

Where: DI = Daily intake (mg/kg-day)
C = Biomarker concentration in blood (mg biomarker/L)
C_{ss} = Steady state concentration of biomarker in blood at a dose of 1 mg/kg-day, calculated using Eq. 14.

Hepatic clearance per kg body weight may be derived from scaling human *in vitro* data (Ring et al., 2017):

$$CL_{int,h} = CL_{int} * hepatocellularity * M_{liver} * \frac{L}{10^6\ \mu L} * \frac{60\ min}{hr} \qquad Eq.\ 16$$

Where: $CL_{int,h}$ = Whole liver intrinsic clearance (L/hr)
CL_{int}= Intrinsic clearance rate measured from *in vitro* human hepatocytes (µL/min – million cells)
Hepatocellularity= millions of cells/kg of liver tissue
M_{liver}= Liver mass (kg)

The fraction unbound is measured by measuring the chemical concentration in phosphate-buffered saline (PBS) and dividing that value by the mean concentration in a matched plasma sample. The fraction in plasma can be used to calculate the fraction unbound in blood (Ring et al., 2017):

$$F_{ub} = \frac{F_{up}}{R_{b2p}} \qquad Eq.\ 17$$

Where: F_{up}= Fraction of chemical unbound in plasma (unitless)
F_{ub}= Fraction of chemical unbound in blood (unitless)
R_{b2p}= Constant ratio of blood to plasma concentration (see equation below)

R_{b2p} may be derived using Schmitt's Method (Schmitt, 2008 as cited in Ring et al., 2017):

$$R_{b2p} = 1 - Hct + Hct * K_{RBC2p} * F_{up} \qquad \textit{Eq. 18}$$

Where: Hct= Hematocrit (% red blood cells in blood)
K_{RBC2p}= Partition coefficient between red blood cells and plasma
F_{up}= Fraction of chemical unbound in plasma (unitless)

The httk model, which is written in R and available at https://cran.r-project.org/web/packages/httk/index.html implements the 3-compartment model discussed here, as well as 1-compartment, 2-compartment, and PBTK modeling. It allows for the use of built-in chemical toxicokinetic parameters for hundreds of ToxCast chemicals, or user-added kinetic data. It allows for both reverse dosimetry and forward dosimetry (calculating internal tissue concentrations from an oral or intravenous dosing regime). The more user-friendly interface that is part of NTP's Integrated Chemical Environmental (ICE) is not useful for reverse dosimetry of biomonitoring data. ICE includes preloaded toxicokinetic data for ToxCast chemicals, but the steady state concentrations are also preloaded and tied to specific assay responses.

There are a number of limitations to this use of a compartmental model with chemical-specific parameters measured *in vitro*, as described by Ring et al. (2017). At the most basic level, the high throughput *in vitro* assays often do not produce usable data. For example, Wetmore et al. (2012) were unable to detect any unbound chemical for 32% of the chemicals tested. Wetmore et al. (2012) also noted that their model did not include renal resorption, and that it assumed that hepatic clearance acts only on the fraction of chemical not bound to protein. Addressing either of these assumptions improved the predictivity of the model. Predictivity was also improved by incorporating an *in vitro* measure of bioavailability.

There are some differences among the specific parameters in the various papers published on using httk and reverse dosimetry for IVIVE. This variability reflects ongoing refinements and improvements to the methods. For example, f_{ub} has been re-measured for some highly bound chemicals, due to difficulty in calculating f_{ub} for highly bound chemicals in early work. More recent work has also measured the blood:plasma ratio, a parameter that was not evaluated initially. In light of the ongoing improvements to httk and the associated parameters, it is best to use the most current version of httk for calculations, rather than extracting parameters and doing calculations using EXCEL spreadsheets. The most recent data are reflected in the most current httk version, while other sources of parameters, such as EPA's CompTox Dashboard, may not have the most recent data (Ring and Wetmore, personal communication).

Because the model assumes steady state blood concentrations, uncertainty is higher when evaluating intermittent exposures to rapidly excreted compounds, since they may not be at steady state. Section 3.8 addresses issues related to intra-individual variability in blood concentrations and approaches for addressing this variability. Because the model considers

only first-order metabolism, it cannot account for enzyme induction or saturation of metabolic pathways (Wetmore et al., 2012). Wambaugh et al. (2015) tested the implications of the steady state assumption by replacing the assumption of a constant infusion dose with a dosing scenario of three daily doses, to better mimic exposure at meals or during consumer use. They found that, of 271 environmentally-relevant chemicals evaluated, 70% reached steady state within 28 days, and 90% reached steady state within 1000 days. The exceptions were a few highly bioaccumulative compounds, such as polychlorinated biphenyls (PCBs), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonic acid (PFOS). Wambaugh et al. (2015) also reported that for most chemicals, the 3-compartment model could predict C_{SS} with an accuracy similar to that of a more detailed perfusion-limited physiologically-based toxicokinetic model.

Ring et al. (2017) used a modification of httk to prioritize chemicals for human health risk research. They developed a population simulation based on demographic and anthropometric quantities from NHANES, and used a Monte Carlo sampling approach to estimate the oral equivalent doses associated with activity measures (e.g., the AC_{50}) from ToxCast assays. Exposure was estimated based on urine biomarkers in NHANES using a Bayesian approach, combined with far-field and near-field approaches for estimating exposure by product use class.

Wambaugh et al. (2015) compared the steady state concentrations based on *in vitro* data with those inferred for humans from *in vivo* studies for 11 environmental chemicals and 74 pharmaceuticals. The inferred *in vivo* results were calculated using a generic high throughput physiologically based toxicokinetic (HTPBTK) model that predicts non-steady state chemical time courses. They found a weak correlation between the predicted and observed results ($R^2 \sim 0.34$), with a tendency for the predictions from *in vitro* data to overestimate the concentrations estimated from *in vivo* data. The authors stated that[3] results were within about a factor of 3 for 65% of the chemicals, while 28% of the chemicals overestimated the steady state concentration by ~6x, and 8% of the chemicals overestimated it by 120x. The authors used the results to develop a triage framework providing information on the degree of confidence for the prediction for specific chemicals, and identifying chemical properties correlated with large deviations between observed and predicted behavior. The triage framework was then applied to 349 environmental chemicals and pharmaceuticals. They found that predictions were "on the order" for 140 chemicals (40%), over- or underestimated by >3.2x for 72 chemicals (20.6%), and over- or underestimated by >10x for 38 chemicals (10.9%). Overestimation exceeded underestimation by ~10x. In addition, 19 chemicals (5.4%) were classified as not reaching steady state (and so no steady state concentration could be calculated), and the plasma binding assay failed for 80 chemicals (23%). Note that these categories are based on predictions from the triage elements, not calculated or measured steady state concentrations *in vivo.*

[3] It is noted that the stated percentages add up to about 100%, but imply that all of the overestimates were either by 6x or 120x, an unlikely result. In addition, examination of Figure 4 of the paper suggests that there were several chemicals for which the literature value was under-estimated by large margins, particularly at higher steady state concentrations.

Wambaugh et al. (2018) conducted an evaluation of the use of *in vitro* toxicokinetic data and IVIVE methods by comparing the results of the IVIVE analysis with *in vivo* toxicokinetic data from 45 chemicals, including more than 26 non-pharmaceuticals, and one-compartment or two-compartment models. Some of their key conclusions were as follows:

- Bioavailability varied substantially for the non-pharmaceutical chemicals, and was often over-estimated by the *in vitro* methods.
- Total clearance was more underestimated for the nonpharmaceuticals than for pharmaceuticals.
- The steady-state, peak, and time-integrated plasma concentrations of nonpharmaceuticals was estimated with "reasonable accuracy."
- The predictions of plasma concentrations improved when experimental measurements of bioavailability were included.
- The combination of high throughput toxicokinetic methods and IVIVE was considered "sufficiently robust" to be use for prioritizing environmental chemicals based on health risk.
- For a large number of the chemicals, the actual error in the prediction of the steady state plasma concentration was >10x higher than the error predicted using the method of Wambaugh et al. (2015).

In considering the implications for using a similar approach to the IVIVE methods for using reverse dosimetry for interpreting human biomonitoring data, it is important to consider the problem formulations of the two approaches. First, Wambaugh et al. (2015) considered their approach adequate for *prioritizing* chemicals, in comparing potency estimates extrapolated from *in vitro* data with exposure estimates. This is a very different application from using biomonitoring data to estimate exposure. In particular, the two applications differ in the implications of conservative assumptions. Wetmore et al. (2012) stated that the simplifying assumptions associated with the multicompartmental model were all in the conservative direction. For example, overestimating bioavailability means that the estimated oral equivalent dose needed to reach a given steady state would be underestimated. If that dose estimate is used to establish an exposure limit or safe dose, underestimating the oral equivalent dose is health-protective. *However,* the same reasoning does not apply when using reverse dosimetry to estimate exposure. In that case, the overestimate of bioavailability would result in an underestimate of the dose resulting from a given scenario, which would not be a health-protective approach. In light of this conclusion and the overall uncertainty associated with the calculations, caution should be used in applying these methods. It is likely that the methods and available toxicokinetic parameters will continue to be refined. However, there is uncertainty associated with the use of *in vitro* toxicokinetic parameters and generic compartmental models for reverse dosimetry of biomonitoring data.

3.4 Sources of Chemical-Specific Toxicokinetic Data

3.4.1 Introduction

A key challenge for appropriately interpreting biomonitoring data is the availability of appropriate chemical-specific toxicokinetic parameters. Data availability can determine the

type of model used for reverse dosimetry. The mass balance approach for urine samples only requires knowledge of the Fractional urinary excretion (F_{ue}). A simple one-compartment pharmacokinetic model may require information on the absorption via the route of interest (oral, inhalation, dermal), the chemical's half-life, and the fraction of excretion to the biomatrix of interest (e.g., urine, feces, exhaled breath, etc.) (Aylward et al., 2012). A physiologically based pharmacokinetic model would require much more information but can be used to evaluate a variety of scenarios, including the time course of a biomarker under non-steady state conditions.

Appendix Table B2 shows how reverse dosimetry studies reviewed for this guide have sourced their toxicokinetic data. Sources of toxicokinetic data include from human data, animal data, *in vitro* data, and *in silico* or modelled data.

The following sections address sources of different types of chemical-specific toxicokinetic data, as well as strengths and weaknesses of data from different sources.

3.4.2 Human *In Vivo* Data

The ideal approach is to use human toxicokinetic parameters, since that minimizes the uncertainty associated with interspecies extrapolation. Human kinetic parameters can be obtained by searching the published literature or may be measured in the context of the biomonitoring study. Authoritative reviews, particularly Toxicological Profiles published by the Agency for Toxic Substances and Disease Registry (ATSDR), may provide relevant parameters, such as elimination half-life.

Compared to using other types of data, human data come with fewer uncertainties. Human toxicokinetic data are not common due to ethical concerns. As discussed for biomonitoring data, it is important to evaluate how representative kinetic parameters are of the human population. However, in the case of kinetic parameters, the key consideration is whether the parameter is appropriate for the population group used for the biomonitoring study, rather than whether it is representative of the entire population. In practice, average values are often used for toxicokinetic parameters, but it is important to consider the variability of that parameter across the population. For example, Aylward et al. (2017) noted that the F_{ue} for triclosan varies drastically among individuals (F_{ue} ranged from 20-80%). This means that using an average F_{ue} to calculate a daily intake will result in a narrower range of the population distribution of daily intake and an underestimate of high-end (upper-percentile) intakes. To alleviate this uncertainty, one could either derive a F_{ue} from the individual being analyzed or use simulated values with Monte Carlo sampling.

Another challenge is that the F_{ue} may not represent the entirety of a chemical's elimination, if urine samples were not collected for long enough in the study where the F_{ue} was determined. (Recall that the F_{ue} is chemical-specific, and so an F_{ue} determined in an intensive sampling study can be applied to variety of different biomonitoring studies conducted using different sampling strategies.) If urine voids were collected for less than five times the half-life of elimination, Eq. 19 can be used to extrapolate the urinary elimination to infinity (Poet et al., 2016).

$$Total\ mg\ excreted = \frac{mg_{urine}}{\left(1 - exp^{\left(-ln(2) \times \frac{t_C}{t_{1/2}}\right)}\right)} \qquad Eq.\ 19$$

Where: t_c = total time of urine collection (hr)

mg_{urine} = total mg of parent chemical excreted in the urine (as parent or metabolite)

The F_{ue} can then be calculated as the ratio of mg biomarker excreted/mg parent compound intake.

3.4.3 Animal *In Vivo* Data

Animal toxicokinetic data from controlled dosing studies have been used in reverse dosimetry studies if human toxicokinetic data are not available (Egeghy and Lorber, 2011; Fromme et al., 2007; Zhang et al., 2020). Although animal data can be incorporated into simple compartmental or mass balance analyses (Connolly et al., 2020; Zhang et al., 2020), it is more common for animal kinetic parameters to be incorporated into physiologically based pharmacokinetic (PBPK) models (Hubal et al., 2019). It is important when using animal data to ensure that this data is qualitatively and quantitatively relevant to humans.

Animal data has the advantage of being easier to acquire than human data. Certain animal toxicokinetic parameters, such as hepatic clearance, may be allometrically scaled to be more relevant to humans (Miura et al., 2019; 2020). However, such scaling assumes that all important species-related differences are due to size-related quantitative differences in metabolism. Qualitative differences, such as differences in metabolic pathways are not accounted for, and such differences would make use of the animal parameter problematic. It is also important to note that some parameters, such as bioavailability, do not correlate well with body weight, and therefore do not scale allometrically (Espie et al., 2009). Espie and colleagues also noted potential modifications to allometric scaling, including accounting for differences in bile flow and glomerular filtration rate, although in practice such adjustments appear to be rare.

The animal F_{ue} can be used directly for reverse dosimetry (Health Canada, 2020; Zhang et al., 2020) if no human F_{ue} or PBPK model is available. The animal F_{ue} would be obtained from a study specifically measuring that parameter. It may be compiled as part of a chemical-specific review, such as an ATSDR profile, but no general compilation of animal F_{ue} values was identified. As noted in the context of the human data, the F_{ue} is based on the total amount of biomarker excreted, relative to a specified intake/dose of the chemical of interest. Therefore, if the urine collection time in the animal study is less than five times the half-life of elimination, the F_{ue} should be extrapolated to infinity, using Eq. 19. However, caution is warranted when using an animal F_{ue}. The animal F_{ue} can differ substantially from the human F_{ue}, in part because of the different molecular weight cutoffs for glomerular filtration in rodents and humans. If an animal F_{ue} is used, it is important to recognize that the uncertainty is generally higher than if the F_{ue} were obtained from a human study, but the uncertainty is usually lower than when using human *in vitro* toxicokinetic data. However, no specific guidelines exist regarding use of animal F_{ue}s.

The "triple pack" approach has been used to extrapolate from *in vivo* rat dermal absorption data to humans. This is done by multiplying the rat *in vivo* dermal absorption factor (DAF) by the ratio of the human and rat *in vitro* DAFs. That is:

$$Triple\ pack\ DAF = rat\ in\ vivo\ DAF * (\frac{human\ in\ vitro\ DAF}{rat\ in\ vitro\ DAF}) \quad Eq.\ 20$$

However, Allen et al. (2021) recommended that the human *in vitro* DAF be used in preference to the triple-pack value. EFSA (2017) provided a tiered approach for estimating dermal absorption, starting with default data on closely-related products, then using *in vitro* human data, existing rat data, and the triple pack approach.

3.4.4 *In Vitro* Data

In vitro data from high throughput assays, sometimes supplemented by *in silico* data, have been used in reverse dosimetry applications (Wetmore et al., 2012; Wetmore, 2015; Wambaugh et al., 2018). As noted in Section 3.3.4, published applications have been primarily for IVIVE, but similar approaches could be used to interpret biomonitoring blood concentrations, recognizing the caveats discussed in 3.3.4.

Wetmore et al. (2012) measured plasma protein binding using a high-throughput modification of the rapid equilibrium dialysis (RED) method. Similarly, they measured hepatic metabolism *in vitro* in primary human metabolites. Bioavailability was measured in a bidirectional permeability assay using Caco-2 cells, a cell line derived from a colon cancer. All of these assays were conducted using multiwell plates, facilitating high-throughput testing. Using these methods, a library of *in vitro* data for >900 pharmaceuticals and ToxCast chemicals has been developed and is part of the httk R package. In another approach to using *in vitro* data, Zhang et al. (2018) calculated F_{ue} values based on studies with human liver microsomes or S9 fractions. However, no studies were located quantitatively evaluating this approach relative to the use of *in vivo* data. In addition, it is not clear whether the F_{ue} used in this approach includes absorption, since the authors described the F_{ue} as "the fraction of OPs converted into their metabolites."

There are a number of advantages to using the *in vitro* data. The data are commonly derived from human cells, avoiding the need for interspecies extrapolation, and avoiding issues associated with animal testing. The data have also been collected in a standardized manner and are readily available in a library linked to a model for interpreting the data. However, as discussed in Section 3.3.4, the generic models for using *in vitro* data have a number of limitations and so should be used with caution. In contrast, allometric scaling of *in vivo* animal kinetic parameters such as the volume of distribution and clearance is a well-established practice (e.g., Clewell et al., 2008; Mahmood, 2020), although allometry may not apply for chemicals with elimination half-lives on the order of months to year. Overall, when *in vivo* animal data are available, such data are likely preferable to *in vitro* human data (Wood et al., 2017).

3.4.5 *In Silico* Data

In silico, or modeled data are often used to estimate toxicokinetic parameters for PBPK or compartmental modeling. In particular, the httk model discussed in Section 3.3.4 makes extensive use of tissue to plasma partition coefficients calculated using Quantitative Structure Property Relationships (QSPRs). These tissue to plasma partition coefficients can be calculated using Schmitt's method or other calculations (Pearce et al., 2017; Schmitt, 2008). The tissue to plasma partition coefficients can then be combined with species-specific tissue volumes to calculate the volume of distribution. Uncertainty in the *in-silico* estimates appears to be comparable to other risk assessment-related uncertainties. For example, Schmitt (2008) compared calculated and observed tissue to plasma partition coefficients for 14 tissues and 59 chemically-diverse drugs, and found that for 73% of the compounds analyzed, there was general agreement between the two values (less than a 3-fold difference between both values), but that the levels of agreement between calculated and observed partition coefficients depends on the tissue being analyzed. In a separate analysis of the kinetics of tetrabromobisphenol A, Miura et al. (2020) used *in silico* methods to estimate the octanol-water partition coefficient and unbound fraction in plasma, and then calculated tissue:blood partition coefficients based on these two parameters. In a broad evaluation of how well PBPK and Quantitative Structure-Activity Relationship (QSAR) models predict the volume of distribution (primarily of pharmaceuticals), Mathew et al. (2021) found that global QSAR models outperformed the PBPK methods, but recommended that the best approach involves "strategic integration of *in silico*, *in vitro*, and *in vivo* methods.

3.5 Sources of Anatomy and Physiology (A&P) Data

Human A&P data include parameters such as body weight, urinary volume, cardiac output, creatinine excretion, and others. The values of these parameters are not chemical-specific, but they may vary across populations. Therefore, it is preferable to use A&P data from the same study (or the same study population) as used for obtaining biomarker measurements. Use of study-specific data has the advantage of directly tying biomonitoring data to the A&P data, but this is possible only if the study authors used the data, or the primary data are provided in a format that connects the A&P data to the biomonitoring data. If such data are not available, A&P data may be obtained from a variety of sources. Some analyses obtained parameters from studies specifically designed to measure the parameter of interest ("dedicated studies" in Table 1, such as van Haarst et al., 2004). Some of these studies (e.g., Mage et al., 2008; Levey et al., 2009) developed regression equations describing how the parameter of interest varies across different populations. Results from dedicated studies are useful for deriving generalizations that can be applied in situations where the relevant study-specific data are not available, while regression equations can be used to account for important sources of variability when individual subject demographic data are available, but data on a specific parameter (e.g., creatinine excretion) are not available for that population. The other major source of A&P data is from compilations published as consensus values as guidance for use in kinetic or PBPK modeling (e.g., Brown et al., 1997; US EPA, 2011; ICRP, 2002; ECHA, 2008a, 2008b). Appendix Table B1 provides additional illustrative examples regarding where reverse dosimetry studies have sourced their A&P

data, particularly for cases where the parameter was obtained in the same study as the biomonitoring data.

Table 1. Sources of Anatomy and Physiology Data

Parameter	Source Type	Citation Example
Body weight	Same study	Apel et al., 2020
Body weight	Compilation	Institute of Medicine, 1998, as cited in Katsikantami et al., 2019
Urinary volume pregnant women, adult women, toddlers)	Compilation	Health Canada, 2020
Urinary volume (adults)	Dedicated study	Van Haarst et al., 2004, as cited in Aylward et al., 2017
Urinary volume (children)	Dedicated study	Miller and Stapleton, 1989, as cited in Fromme et al., 2014, 2016
Urinary volume (children and adults), tissue volumes, growth rates	Compilation	ICRP, 2002[4], as cited in Cok et al., 2020; Institute of Medicine, 1998, as cited in Katsikantami et al. 2019; US EPA, 2011, as cited in Cao et al., 2016
Creatinine excretion	Regression equation based on individual's gender, height, weight and age	Mage et al., 2008, as cited in Aylward et al., 2017 and related papers; Cockcroft and Gault, 1976, as cited by Mage et al., 2008
Creatinine excretion (adults)	Compilation (textbook)	Tietz et al., 2006, as cited in Qian et al., 2015
Creatinine excretion (children)	Dedicated study	Remer et al. 2002, as cited in Qian et al., 2015 and Koch et al., 2011
Glomerular filtration rate (GFR)	Dedicated study	Rule et al., 2004, as cited in Wetmore et al., 2012
Glomerular filtration rate (GFR)	Dedicated study, predicted from age, race, sex, and serum creatinine	Levey et al., 2009
Body lipid mass	Estimated from each individual's height and weight	Tay et al., 2019
Physiological parameters for PBPK models		

[4] Cited by name of the editor, Valentine

Parameter	Source Type	Citation Example
Organ volumes, fractional blood flows, cardiac output, alveolar ventilation	Compilation	ECHA 2008a, 2008b, as cited in Jongeneelen and Berge, 2011 Technical Guidance Documents for REACH
Body weight, fractional blood flows to tissues, tissue volumes, cardiac output and many other parameters	Compilation	Brown et al., 1997 (International Life Sciences Institute)
Blood flow to tissues, volume of tissues	Allometrically scaled using method of Clewell et al., 2014	Lin et al., 2020

3.5 Forward Dosimetry

Forward dosimetry analyses can be done by simply rearranging the equations presented in Section 3.3 to solve for the biomarker concentration. A common formal approach is to calculate Biomonitoring Equivalents (BEs) from existing toxicity reference values such as RfDs, and comparing the BE with the biomonitoring data. This approach has been used extensively by Health Canada to interpret biomonitoring data from the Canadian Health Measures Survey (CHMS) (Faure et al., 2020).

3.6 PBPK Modeling Approaches

As noted in prior Sections, PBPK modeling is frequently used when the available toxicokinetic data come from animal or *in vitro* studies. In brief, PBPK models describe the body as a series of compartments representing tissues or groups of tissues, with the flow rates, tissue volumes and metabolic parameters based on the physiology of the species of interest. Guidance is available for evaluating PBPK models (US EPA, 2006; IPCS, 2010; McLanahan et al., 2012). As noted by Caldwell et al. (2012), there are two types of uncertainty associated with PBPK models. Model uncertainty "refers to the lack of knowledge needed to determine whether the scientific theory on which a model was based is correct." Parameter uncertainty "refers to the lack of knowledge about the values of a model's parameters which leads to a distribution of values for each parameter" (Caldwell et al., 2012). Models may be inaccurate when they are not fit for purpose, or the logic behind the model does not correspond with the pharmacokinetics of a toxicant in the body.

3.7 Determining Source Contribution to External Exposure

As noted in the introduction, CPSC staff use biomonitoring data not simply to estimate exposure, but as part of a broader consideration of whether there is a plausible connection between the measured exposure and the occurrence of the chemical in specific product(s). Evaluation of this broader issue requires integrating the exposure estimate calculated from biomonitoring data using reverse dosimetry with exposure estimates from a combination of source-based models. As described in further detail in the rest of this section, this integration can be qualitative (based on an association of the biomonitoring-based

exposure estimate with the use of certain products), or quantitative (based on a comparison of the biomonitoring-based exposure estimate with estimates of exposure from specific sources, such as diet and household dust).

3.7.1 Qualitative Approaches

Qualitative approaches evaluate the association between the biomarker and exposures to specific product sources and/or other sources, such as consumption of certain foods and intake from the environment. In these cases, the biomarker measurement is quantitative, but the consideration of exposure from different sources (forward dosimetry) is evaluated on a yes/no basis. Questionnaires can be used to obtain individual-level data on the uses of products with specific materials or consumption of specific foods. For example, Smith et al. (2021) conducted a study of 100 adolescent girls to evaluate the potential for exposure to several phthalates and bisphenol A (BPA) based on family practices such as:

- the use of plastic containers to store food,
- microwaving in plastic,
- the presence of linoleum or vinyl floors at home,
- plastic or vinyl shower curtains, as well as
- recent consumption of certain foods, drinks, or any canned items, receipt contact, or consumption of fast foods.

Urinary levels of the phthalates and phthalate metabolites and of BPA were evaluated. Rather than using reverse dosimetry to estimate internal dose, the authors compared the biomarker measures with a nationally representative sample, after adjusting for age, BMI and time since last meal. These data were used to evaluate the association between the various exposures evaluated in the questionnaire and the chemicals of interest. A strength of this study is that urinary biomarker data were connected to data on product and food exposures on an individual level. However, given the short half-life of elimination of phthalates and BPA in urine and episodic exposure events associated with these types of products, the concentration of these analytes in urine are expected to be highly variable (Preau et al., 2010). For instance, for compounds such as BPA and di(2- ethylhexyl) phthalate (DEHP), Preau et al. (2010) found that the concentration of BPA and the DEHP metabolites in urine can vary up to 1000-fold within an individual within a single day. For these reasons, these types of studies would benefit from collecting more than a single spot urine sample. 24-hour voids or even longer would help identify potential associations between urinary concentrations of the analytes and exposure sources (Koch et al., 2014).

Oya et al. (2021) used a similar approach to evaluate the relationship between the urinary biomarkers of neonicotinoids in young children and behaviors related to various potential consumer product exposures, foods consumed, or method of food preparation. Urine was collected in an overnight diaper, and measurements were corrected for creatinine concentration and for absorption of the biomarker by the diaper material. This approach allowed for analysis of exposure of very young children in a non-invasive manner.

3.7.2 Quantitative Approaches

Several publications have used biomarker data in combination with forward dosimetry estimates of exposure to evaluate the potential contribution of different sources to the total exposure. Consideration of some of these examples in further detail illustrates some ways that CPSC staff can use biomarker data in combination with other exposure information, as well as some potential pitfalls in the analyses.

In an early application of the approach, von Goetz et al. (2010) estimated exposure to BPA for several different age groups from a variety of different food sources and products, including polycarbonate (PC) baby bottles and household dust. The total exposure estimated from these sources was compared with an aggregate exposure estimate based on national biomonitoring data (apparently using spot sampling) and reverse dosimetry, using an F_{ue} of 1. The authors found good agreement between the two approaches for adults, but that the total dose estimated for children and toddlers based on known sources was about half the estimate based on biomonitoring data. This relatively small difference may reflect uncertainty and variability in the biomonitoring data, or may indicate that an important source has not been accounted for. The authors did not provide information on the variability in the daily dose rates, making it impossible to fully consider the significance of the difference in estimated doses.

Cao et al. (2016) conducted a similar comparison between exposure estimated from environmental media and food, and that estimated from urinary concentrations, for di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DBP), and diisobutyl phthalate (DiBP). The exposure scenario-based approach was based on measurements in various regions of China. Monte Carlo simulation was conducted to estimate a distribution of total exposure from the various sources. The biomonitoring data came from a national survey of 203 (presumably spot) urine samples, and metabolite-specific F_{ue} values were applied. Although the biomonitoring sample size reflects a large cohort, the number is relatively small for a national survey. The authors found that the median intake from the two methods were roughly comparable for DEHP and DBP, but that the exposure estimates for DiBP from the scenario approach were about 1/3 that from the biomarker-based approach. Incomplete information was provided regarding the distributions for DiBP, making independent interpretation of the data challenging. The study authors suggested that the lower estimate in the scenario approach for DiBP may reflect other exposure pathways that were not part of the calculation, such as cosmetics, detergents, pharmaceuticals, or medical devices.

In a similar analysis, Fong et al. (2014) compared the daily intake of DEHP estimated based on urinary levels in a group of exposed workers with the daily intake estimated from personal air monitoring. They found that inhalation exposure contributed about 21% of total intake for workers in a high-exposure group, and about 5% in a low-exposure group.

Levasseur et al. (2021) evaluated exposure in 203 children from 190 families (a “large cohort”), using measurements from hand wipes, wristbands, spot urine samples, household dust, and household questionnaires. Three spot urine samples were collected over a 48-hour period, and a composite urine sample prepared from all three samples. The specific

gravity of this composite sample was measured, and the sample was evaluated for the biomarkers of interest. Analyses were conducted with the specific gravity corrected biomarker concentrations. This approach of multiple spot samples is an improvement over a single spot sample, but the authors did not provide sufficient information on the timing or spacing of the three samples to determine the degree to which the sampling approach approximates a 24-hour sample. Correlation analyses found many of the expected correlations between exposure sources and urinary biomarker levels. However, the authors also found that concentrations of triclosan in dust correlated significantly with urinary levels, as well as with exposures measured via wristband and handwipes. This result suggests that a source of triclosan other than personal care products may be important for children, since use of triclosan in soap would not be expected to be associated with triclosan in household dust.

At a more general level, Eichler et al. (2021) developed a modular mechanistic framework for predicting human exposure to semivolatile organic compounds (SVOCs). This framework is useful because, unlike volatile organic compounds (VOCs), SVOCs partition among multiple indoor compartments. Their approach provides a mechanistic framework for evaluating exposure scenarios for different source emission categories (SECs), with the goal of supporting high-throughput exposure estimates. The authors described mechanistic models, or process-based models, as ones that "rely on well-established physicochemical processes such as diffusion or sorption." The authors characterized SECs as solid, soft, frequent contact, applied, sprayed, or high temperature. Depending on the SEC, there is the potential for transport resulting in direct contact exposure, or emission to one of the environmental compartments (outdoor contributions, gas phase, airborne particles, dust, indoor surfaces, clothing). Mass transfer may occur between these compartments, ultimately resulting in "mediated exposure" via dermal uptake, inhalation, or ingestion. Equations and parameters were provided for estimating the transfer among compartments and estimating the resulting exposure. Exposures estimated with this sort of model could be used together with biomonitoring data to improve the estimates of the contribution of various sources to the overall exposure. Several other models are available to estimate exposure from consumer products. These include ConsExpo, developed by the RIVM (RIVM, 2017), the U.S. EPA's Consumer Exposure Model (CEM; U.S. EPA, 2019), as well as several other consumer models developed by the U.S. EPA and others.

Overall, these quantitative approaches can be used to determine important sources, and whether key exposure sources remain unaccounted for. As noted above, von Goetz et al. (2010) and Cao et al. (2016) drew conclusions based on differences in exposure estimates on the order of 2-3x.

When comparing exposure doses estimated using reverse dosimetry and aggregate exposures estimated from multiple sources using forward dosimetry, consideration of variability and uncertainty of the exposed group is important. CPSC staff recommends that a range of exposure estimates are generated for both approaches, if sufficient data are available. CPSC staff also recommends documentation of the approach and associated data sources. This information provides context for comparisons across multiple approaches

and associated use in risk assessment and risk management decisions. See Table 2 for potential outcomes.

Table 2. Examples of outcomes when comparing reverse dosimetry and forward-dosimetry aggregate exposure (FDAE) from multiple sources

Outcome	Potential Explanation
FDAE distribution is less than and does not overlap with Reverse Dosimetry Distribution	FDAE has likely not considered enough potential exposure source categories
Reverse Dosimetry Distribution is less than and does not overlap with FDAE Distribution	FDAE is likely aggregating exposure from too many sources; potential uncertainty in reverse dosimetry
Reverse Dosimetry and FDAE ranges overlap	Interpret both distributions and characterize associated variability and uncertainty; Characterize the magnitude of the difference of the central-tendency exposure estimate

3.8 Addressing Variability

3.8.1 Variation Related to Hydration Status

Differences in hydration status can have a substantial impact on the urinary flow rate, and thereby on the concentration of a biomarker in the urine. As noted in Section 3.3.2, a common approach for accounting for this variation is adjusting the concentration based on the creatinine concentration in the urine. This adjustment is based on the observation that the creatinine concentration varies less than the urinary flow rate, but creatinine excretion is not constant. For example, children excrete less creatinine relative to body weight than do adults. Creatinine excretion also varies systematically with dietary pattern, between lean and obese people, and across racial and ethnic groups. These limitations to creatinine adjustment have led to other adjustments to reflect hydration status, including adjustments based on osmolality (a measure of how concentrated the urine is), specific gravity and urine flow rate (L/hr).

Since 2009, NHANES has collected information on the volume of the complete urinary void, as well as the time since last void. This allows for the calculation of the urinary excretion rate of the analyte over the time period covered by the void, thus addressing the issue of hydration status without requiring the use of a surrogate such as creatinine concentration. The excretion rate is calculated using the following equation:

$$ER = \frac{C*V}{t} \qquad \textit{Eq. 21}$$

Where: ER = Excretion rate (ng/hr)
C = Concentration of biomarker in urine (ng/mL)
V = measured urinary composite void volume (mL)
t = time since last void (hours)

This relationship can also be expressed relative to body weight:

$$ER\ \left(\frac{ng}{kg} - hour\right) = \frac{C*V}{t*BW} \qquad \textit{Eq. 22}$$

Where: ER = Excretion rate (ng/kg-hr)
C = Concentration of biomarker in urine (ng/mL)
V = Measured urinary composite void volume (mL)
t = Time since last void (hours)
BW = Body weight (kg)

In these equations, the term V/t and V/(t x BW) describe the urinary flow rate (UFR, mL/hour) and body weight-adjusted urinary flow rate (UFRBW, mL/kg-hour), respectively.

Hays et al. (2015) used NHANES data to evaluate the relationship between UFR and UFRBW with age, sex, race/ethnicity, and body mass index (BMI). They found strong systematic variation in UFRBW with age, race/ethnicity and BMI, and in UFR with age, sex and race/ethnicity. In particular, they noted that urinary osmolality (a measure of urinary concentration) increases with BMI in all age categories, suggesting that urine is more concentrated on average in people with a higher BMI. These systematic variations had a substantial impact on case studies evaluating the relationship between chemical exposures and BMI. For example, the (unadjusted) urinary concentration of 2,5-dichlorophenol (2,5-DCP) increased with BMI. The trend remained when the concentrations were adjusted by osmolality to account for hydration status, but not when a creatinine adjustment was applied. The trend also remained for the mass excretion rate, but not for the body weight-adjusted mass excretion rate. The authors suggested that the systematic variation of UFR with BMI could result in "reverse causation," leading to misinterpretation of biomonitoring data. That is, rather than the exposure causing the health outcome, the health outcome of obesity (or, more broadly, obesity-associated disease) increases the *biomarker concentration* through the *reduced UFR*. Thus, there was an association between 2,5-DCP in the urine based on mass excretion rate, but not based on body-weight adjusted mass excretion rate.

Although the Hays et al. (2015) analysis focused on the relationship between biomarkers and effect measures, rather than reverse dosimetry, it also has implications for using urinary biomarkers to estimate exposure. The authors recommended that future analyses of health outcomes using urinary biomarker data should be conducted not only based on biomarker concentration, but also on the basis of mass excretion rate (ng/hr and ng/kg-hr). They also recommended that the evaluation include a clear hypothesis regarding "the relationship between exposure pathway, exposure metric and health outcome." For example, body weight would be expected to have a different impact when food is an important exposure source (since food intake may scale with body weight) than in situations where inhalation is a primary source (since inhalation intake does not scale directly with body weight). Similar considerations would apply in applying reverse dosimetry to interpret urinary biomarker data.

3.8.2. Intra-individual Variability, the Relationship to Half-Life and Population Distribution

As noted earlier in this guidance, it is important to distinguish between intra-individual variability (related to variability in timing of sample collection relative to exposure event) from inter-individual variability (related to differences in physiology or differences in actual exposures). This is particularly important when using data from spot urine samples (instead of 24-hour composite samples) to evaluate chemicals with short half-lives.

Aylward et al. (2012) conducted a detailed evaluation of the relationship among half-life, sampling time and measured variability. They found that the population distribution of biomarker concentrations is a function of the ratio of the elimination half-life and the exposure frequency (i.e., interval between repeated exposures). A larger ratio results in less variability, while a smaller ratio results in larger variability (Figure 1). This means that the intra-individual variability resulting from sampling at different times in the elimination curve can be a key driver of the apparent population variability. In the simulations conducted by Aylward and colleagues, they found that if the elimination half-life is less than ¼ - ½ the exposure interval, the P95:P50 ratio of the biomarker concentration was wider than the population variability in the dose rates used to calculate the biomarker concentrations. Conversely, as the half-life approaches the duration of the exposure interval, the predicted biomarker concentration either approaches the variability in the underlying dose distribution (for a constant dose rate scenario) or is less than the variability (for a varying dose rate scenario). The lower biomarker concentrations in the latter case reflects the averaging out of the biomarker concentrations over time when "elimination is slow enough (relative to exposure interval) to provide an integrated reflection of exposure events including those preceding the most recent event."

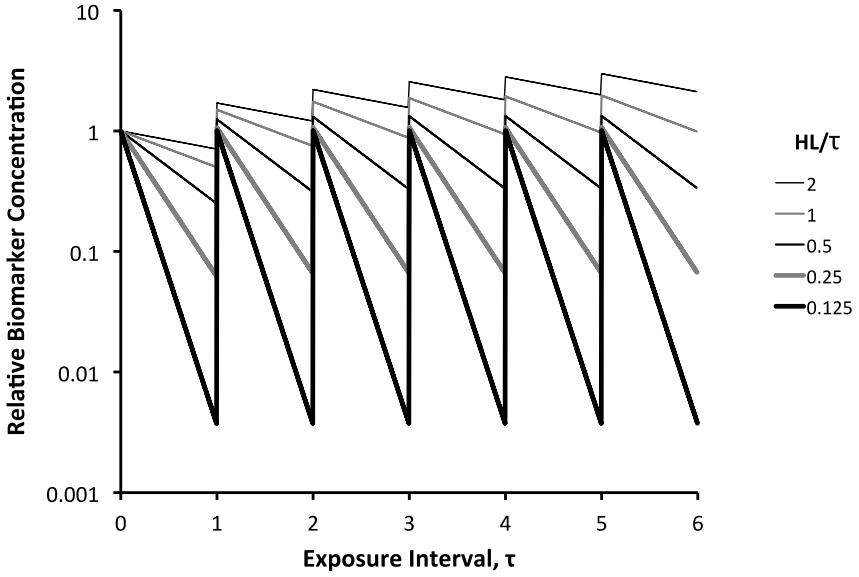

Figure 1: Simulated biomarker concentration vs. time curves for a repeated unit dose at a consistent interval assuming different values for half-life of elimination(HL) as a fraction of the exposure interval, τ.

Similarly, Bradman et al. (2012) found that urinary spot samples of organophosphate metabolites in children varied widely over the course of a week. If the concentration was elevated in a spot sample, it was likely that the child's exposure over the course of the week was elevated, but a single spot sample did not necessarily detect children with elevated exposures. These results are consistent with observation that most of a decay curve will be at concentrations below the time-weighted average.

Intraclass Correlation Coefficient (ICC)

The results of Aylward et al. (2012) mean that the variability in the population distribution may be over-estimated when the half-life is short relative to the exposure frequency. This over-estimation is of concern because exposure estimates often focus on the high end of the population distribution (e.g., the 95th percentile). The intraclass correlation coefficient (ICC) provides an approach for quantifying the relative contribution of intra-individual variability and inter-individual variability, and for calculating a better estimate of the overall population variability. The ICC is defined as the ratio of the logged variance between subjects and the total logged variance (Pleil and Sobus, 2013; Casas et al., 2018):

$$ICC = \frac{\sigma_\alpha^2}{\sigma_\alpha^2+\sigma_\varepsilon^2} \qquad \textit{Eq. 23}$$

Where: σ_α^2 = between subject logged variance
σ_ε^2 = within subject logged variance

An ICC can range between 0 and 1. An ICC of 0 means that 100% of the variability is due to *intra-individual* variability. In other words, biomarker measurements from spot sampling of any given individual may be any value across the entire distribution, and so a single spot sample is not a good estimate of the individual's mean exposure. Conversely, an ICC of 1 means that repeated measurements of an individual will stay the same, and so both the individual mean, as well as the population mean and distribution are well-characterized.

Pleil and Sobus (2013) described a method for estimating the distribution of long-term average exposures from a distribution of spot biomarker measurements using the ICC. They described three tiers of information for estimating the ICC. In Tier 1, no information is available on the ICC, and so the only option is to bracket the exposure estimates with the ICC bounds of 0 and 1. In Tier 2, the ICC can be inferred from other data, based on research experience or similarities to other chemicals. The choice of surrogate should be informed by similarities in half-life of the compound within humans and the exposure timing (e.g., similar with respect to presence in food, water, air, etc.). Pleil and Sobus (2013) recommended that Tier 2 estimates be treated with caution, and that in the absence of "articulated" repeated measurements, the most conservative assumption of m = 2 measurements/subject be used. Tier 3 requires an intensive sampling protocol that allows development of a robust ICC, as described by Aylward et al. (2017). (See Appendix A1).

As an example of Tier 1 bounding, consider a hypothetical distribution where there is one biomonitoring measurement per person from a group of 220 individuals, where the distribution of samples has a geometric mean GM = 1155 ng/L and geometric standard deviation GSD = 2.84 ng/L. If the ICC equals 1, there is no within-subject variability, and the measured GM and GSD are the GM and GSD of the distribution of long-term average exposures for the population.

At the other extreme, if the ICC equals 0, this means that all of the variability is due to intra-individual variability. In this case, the population GSD needs to be calculated based on the sample size:

$$SEM = \frac{GSD}{\sqrt{N}} = \frac{2.84}{\sqrt{220}} = 0.19 \qquad \textit{Eq. 24}$$

$$GSD = Exp(SEM) = 1.21 \qquad \textit{Eq. 25}$$

Table B3. Summary of Method Application and Quality Based on Type of Toxicokinetic Data

Type of Toxicokinetic Data	Types of TK Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature?	Reliable for chemical scenario? Short half-life.	Reliable for chemica scenario Long ha life.
Urinary excretion fraction (F_{ue})	Human	Urinary Mass Balance Approach	x	x	x	Yes	Yes, but F_{ue} can le to high variabilit and high uncertair
	Animal	Urinary Mass Balance Approach	x	x	x	Yes	Yes, but F_{ue} can le to high variabilit and high uncertair
	In Vitro	--	--	--	--	--	--
	Modeled	--	--	--	--	--	--
Half-life	Human	Compartmental model approach	x	x	x	Maybe	Yes
	Animal	Compartmental model approach	--	--	--	--	Yes
	In Vitro	--	--	--	--	--	--
	Modeled	--	--	--	--	--	--
Volume of Distribution	Human	--	--	--	--	--	Yes

<0.40	Poor
0.40-0.59	Fair
0.60-0.74	Good
≥0.75	Excellent

Note that a high ICC alone may not be sufficient for determining the adequacy of a sampling procedure. For example, the population distribution may not be fully characterized if there is seasonal or other longer-term variation in exposure (Aylward et al., 2017).

The ICC is positively correlated with the compound's half-life in the body (Aylward et al., 2017). For example, the authors found that triclosan (half-life of 10 hours) has a substantially higher ICC than BPA (half-life of 4-6 hours). The nature of the exposure (e.g., widespread vs. highly variable based on product use) also affects the ICC. (Aylward et al., 2017). The ICC may be affected by the nature of exposure as well. For instance, BPA has a low ICC because it is generally orally absorbed and its exposure is widespread across the population, decreasing the variability of exposure between individuals (Aylward et al., 2017).

Implications of Variability for Spot Sampling Design

The ICC can be increased by taking multiple samples from each individual, thereby increasing exposure estimation reliability. This raises the question of how many samples are needed to adequately assess exposure. Casas et al. (2018) used the Spearmen Brown equation to determine the number of samples needed to reach a specific ICC:

$$\rho_m = \frac{m*\rho}{1+(m-1)\rho} \qquad \textit{Eq. 28}$$

Where: ρ = ICC for one sample/pool
m = number of samples per individual needed
ρ_m = target ICC

Another approach is based on the coefficient of variation of repeated urinary concentrations (Li et al., 2020). These authors described the minimum number of spot or first morning void urine samples required to predict participant-specific mean concentration to be within 20% of the "true" values with a probability of 95% using the following equation:

$$k = \left(1.96 * \frac{CV}{20}\right)^2 \qquad \textit{Eq. 29}$$

Where: k = the minimum number of samples, as described in the text above
CV= the coefficient of variation of the log transformation (χ + 1) of measured concentrations

A challenge with approaches based on specific ICC values or the variation in a specific study is that the variability measure is tied to a specific study population, sampling scheme and method used to standardize urine concentrations. To address this issue, Verner et al. (2020) developed the Biomarker Reliability Assessment Tool (BRAT), freely available (currently as beta version) for download at https://www.magnoliasci.com/brat. User-specified inputs include biological half-life, intra-individual and inter-individual variability in exposure, exposure pattern, exposure period of interest, timing of sample collection, and standardization approach for urine dilution. The tool used a pharmacokinetic model, together with Monte Carlo sampling. Urine parameters (urine volume, time of urination, creatinine urinary concentration and urine specific gravity) were obtained from four men and four women, and the sampling approach randomly selects one of the eight individuals. The tool generates profiles of intake, internal dose, and urinary concentrations over the period of interest, and these profiles can be used to identify an appropriate sampling scheme.

3.8.3 Overall variability

The above discussion on variability has focused on the variability in individual parameters in the reverse dosimetry calculation. However, it is noted that overall population variability reflects the variability in these components, but is not a simple product of the component variability. A common approach for estimating the overall variability would be to conduct Monte Carlo sampling, based on the distributions for each parameter in the reverse dosimetry calculation, and use the sampling results to obtain an overall distribution of the estimated intake.

4.0 Case Studies

The case studies presented in Appendix A illustrate the principles presented in this guidance with specific chemical examples, and are accompanied by a spreadsheet that includes the actual equations. The case studies were chosen to illustrate the use of data from a diversity of sources and types of biomarkers. For each case study, the sources of the three data types (biomonitoring data, chemical-specific toxicokinetic data, and A&P data) are documented, followed by presentation of the equation(s) used for the reverse dosimetry calculation. The case studies illustrate (1) calculation of daily intake based on urinary spot sampling, (2) calculation of daily absorbed dose based on concentration in blood, and (3) use of in vitro toxicokinetic parameters to estimate daily intake from a concentration in blood. In addition, information is presented on the use of the ICC to refine the estimation of a population distribution from spot samples.

5.0 Conclusions and Recommendations

This section summarizes best practices across data types, based on a review of reverse dosimetry publications. (See Appendix B for summary tables.) The appropriate biomatrix for analysis depends strongly on the chemical half-life, and the analytical approach is often determined by the available data. The focus here is on simple compartmental models, due

to their ease of application. However, it is noted that in general, properly-validated PBPK models will improve the accuracy of the estimation. PBPK models are also the only way to address certain types of chemicals and metabolic issues. If toxicokinetic parameters are only available for an animal model, but other estimates of human metabolism (e.g., from *in vitro* studies) indicates substantial interspecies differences in metabolism, a PBPK model may be needed. In addition, simple compartmental models cannot address induction or saturation of metabolism; a PBPK model would be needed to address these situations, although induction or saturation is less likely to be a concern at environmentally-relevant exposure levels. With those caveats, several generalizations are still possible.

5.1 Urine as a Biomatrix

Urine is the preferred matrix for chemicals with a relatively short half-life. Sampling is non-invasive and methods for interpretation of urine data are well-established. Collection of 24-hour (or longer) composites helps to minimize intra-individual variability over the course of the day. If spot samples are used, the ICC should be included in the calculation of population variability. Hydration status should be considered by conducting the analysis based on both the biomarker concentration as well as based on the basis of mass excretion rate (ng/hr and ng/kg-hr). In considering the reverse dosimetry results as part of evaluating source contributions, it is important to have a clear hypothesis regarding the relationship between exposure pathway and exposure metric.

Reverse dosimetry can be conducted on urinary concentrations using a simple mass balance approach. This approach assumes that steady state has been reached. Steady state (or, more likely, periodicity, meaning that the concentration varies around a constant value) is a reasonable assumption in situations where there is a recurring pattern of exposure, particularly if 24-hour composite samples are used. For example, many dietary exposures and consumer items follow a similar daily pattern of exposure. However, steady state may not apply for products that are used very intermittently. In such situations, sampling from large population samples that would capture the intermittent nature of the exposure scenario would be important in accurately describing the population variability in exposure. In such cases, it would be important to ensure that any populations more likely to use the product of interest are adequately sampled.

Human toxicokinetic parameters are strongly preferred. Ideally these parameters would come from the same population(s) used as the basis of the anatomy and physiology data, but national-level anatomy and physiology data (particularly by age/sex grouping) are often used. Animal data can be used for the F_{ue}, with rats preferred over mice, but care is needed to ensure that animal and human toxicokinetics are sufficiently similar for the animal data to be a reasonable surrogate. There are currently no established methods for using *in vitro* or *in silico* toxicokinetic data to interpret urinary biomonitoring data.

The biomonitoring data may come from national-level data through small cohorts. Small cohorts have the advantage that it is possible to use an intense sampling design, as described in Section 2.5. Detailed activity questionnaires can be used for both small cohorts

and large cohorts up to about 100 to a few hundred individuals, helping to refine potential sources. National-level biomonitoring data are readily available without conducting additional studies, but do not include information about activities and potential sources of exposure. In addition, care is needed in evaluating spot sample data and distinguishing intra-individual from inter-individual variability.

5.2 Blood as a Biomatrix

Application of reverse dosimetry to blood concentrations generally requires knowledge of the chemical's volume of distribution and half-life (or the related parameters of clearance rate k or clearance=V_d*k) and fractional bioavailability. Blood is the preferred matrix for chemicals with long half-lives, and for lipophilic chemicals, which also generally have long half-lives. Although there is not a clear definition of long half-life in this context, a half-life of 36 hours or more is a reasonable cut-off, since the key issue is the elimination rate relative to the exposure frequency. This definition is fit for purpose, even though it is much shorter than common categorizations of short vs. long half-life.

Reverse dosimetry analyses with blood can be conducted using any size cohort. As for analyses with urine, the approaches presented here assume steady state. Achieving steady state is less of a concern for biomonitoring in blood than for biomonitoring in urine, mostly because the blood is the preferred matrix for chemicals with longer half-lives, and so the chemical is more likely to have reached steady state, and be less susceptible to short-term fluctuations in exposure. However, the internal dose for chemicals with extremely long half-lives (>1000 days) may still be in the accumulation phase, and so such chemicals may not have reached steady state, depending on the duration of exposure.

As for interpreting urine biomonitoring data, human toxicokinetic data are preferred, but animal data may be used, particularly if other (e.g., *in vitro*) data support the conclusion that humans are similar to animals. PBPK models are often used to interpret blood biomonitoring data, where human parameters can be scaled from the animal values and/or estimated as part of the parameter fitting process.

Although no case studies were found illustrating the use of *in vitro* toxicokinetic parameters for reverse dosimetry, first principles indicate that such data can be used for interpreting blood biomonitoring data. As described in Section 3.3.4, this can be done using a compartmental model and data on the intrinsic liver clearance and fraction unbound of the parent chemical. This approach has the advantage of using kinetic parameters obtained in human cells in a high-throughput approach, making it possible to evaluate data for many chemicals for which no *in vivo* kinetic data are available. Theoretically it would be possible to address contributions to population variability with such an approach. However, future methods development is needed to measure *in vitro* the variability in factors affecting estimated *in vivo* dose related to age, sex, or other population characteristics. As discussed in Section 3.3.4, there are also a number of caveats to this approach and the simplifying assumptions involved.

5.3 Lipids as a Biomatrix

Like blood, lipids can be used as a biomatrix for chemicals with long half-lives. However, due to the highly invasive nature of the sampling, lipids would be used as a biomatrix only in evaluating fat from cadavers or surgical specimens. The reverse dosimetry approach for lipids is similar to that used for evaluating biomarkers in blood. No case studies were located that applied reverse dosimetry to estimate exposure based on levels of biomarkers in human lipids.

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Appendix A – Case Studies

A1.0 Aylward et al., 2017 - Bisphenol A (BPA) and Triclosan; Urinary Spot Sampling of Rapidly Eliminated Chemicals

A1.1 Overview

As part of evaluation of the impact of intra-individual variability on spot sample results such as those collected in NHANES, Aylward et al. (2017) evaluated biomarker data and the implications for exposure assessment and reverse dosimetry using intensive data sets. The data sets included spot samples, 24-hour samples, and longer-term urine collection. Two case studies are presented here. The first is for BPA, a chemical for which the primary route of exposure is in food, and for which there is relatively little inter-individual variability. The second is for triclosan, for which the primary exposure occurs in personal care products. Because personal care products dominate exposure, there is both substantial inter-individual variability (depending on whether products containing triclosan are used) and potentially intra-individual variability in daily exposure (if the use of personal care products varies from day to day). Both BPA and triclosan have short half-lives, consistent with sampling of urine and the goal of the analysis of improving the interpretation of urinary biomarker data.

A1.2 Identify the necessary data

Biomonitoring Data

Two intensive sampling data sets were used for the BPA analysis, and one for the triclosan analysis. Each data set included information from eight subjects (four male and four female), from whom every urine void was collected for several days. Data for triclosan were from a study funded by the European Chemical Industry Council (CEFIC). Data for BPA were from the CEFIC study and one conducted by the US Centers for Disease Control and Prevention (CDC). The age ranges were 25-58 and 31-66 years for the CDC and CEFIC datasets, respectively. Urine samples were collected for 7 days for the CDC data set, and for 6 days for the CEFIC data. However, the subjects in the CEFIC study were instructed to abstain for 2 days (in the middle of the 6-day collection period) from certain personal care products that contain triclosan, and those days were excluded from the analysis, resulting in 4 days of spot samples for the analyses described by Aylward et al. (2017). For both chemicals, the biomarker of interest was the parent compound.

Chemical-Specific Toxicokinetic Data

Aylward et al. (2017) identified the following parameters for BPA and triclosan. The excretion half-life of BPA is 4-6 hours and the F_{UE} of BPA is 1 (Volkel et al., 2008). The half-life for triclosan is approximately 10 hours and the F_{UE} is 0.54 (Sandborgh-Englund et al., 2006).

Anatomy and Physiology Data

In order to estimate the daily intake in µg/day using the urinary mass balance approach (Section 3.3.2), the only additional parameter needed is the 24-hour urinary volume. The

authors used a volume of 1.7 liters/day (van Haarst et al., 2004). For analyses of the creatinine-adjusted concentration, the authors used a central tendency creatinine excretion rate for adults of 1.3 g/day (Mage et al., 2008). Aylward et al. (2017) also noted that the creatinine excretion rate can be calculated on an individual basis as a function of gender, height, weight and age (Mage et al., 2008).

A1.3 Conduct Reverse Dosimetry

Daily intake was estimated using Equation 1 (Section 3.3.2 of the main text), with a few modifications. Daily intake was estimated on a mass basis, rather than per unit body weight, and so there was no normalization by body weight. Because the purpose of the study was to inform the interpretation of distributions of spot sample data in large data sets, the concentration term was the 95th percentile urine concentration. Similarly, the creatinine-adjusted excretion rate was used with Equation 2 to estimate daily intake. These calculations are illustrated with the supplemental EXCEL® spreadsheet. The calculations shown are based on the geometric mean spot sample data from Table 4 of Aylward et al. (2017), and using the parameters used in that study. The spreadsheet shows the calculation of daily intake based on either mg/day or body weight adjusted (mg/kg-day). However, Aylward et al. (2017) did not present the intake corresponding to the measured urinary concentrations.

It is noted that a key focus of the Aylward et al. (2017) study was on the implications of variability in spot sample data. These results and their implications were discussed in the main text (Section 3.8).

A2.0 Egeghy and Lorber, 2011 – Perfluorooctane sulfonate (PFOS); Blood/Serum Sampling of Persistent, Lipid-Soluble Chemicals

A2.1 Overview

PFOS is a persistent, relatively non-volatile chemical that is found in many consumer products and the environment. People can be exposed to PFOS released from consumer products, including non-stick cookware, electronics, and oil and stain-protective coatings in carpets, food containers, and clothes. Humans are commonly exposed to PFOS through the environment. Industrial releases can lead to PFOS contamination of the air and water. People may also be exposed from the use of PFOS in fire-fighting foam (Egeghy and Lorber, 2011). Egeghy and Lorber (2011) used data from several biomonitoring studies of PFOS in blood to estimate intake, and compared the results with intake estimated based on the concentrations in several environmental media.

A2.2 Identify the necessary data

<u>Biomonitoring Data</u>

Blood biomonitoring data were extracted from several different studies (Calafat et al., 2007; Olsen et al., 2003a, b; Olsen et al., 2005; Hansen et al., 2001; Calafat et al., 2006; Olsen et al., 2004; Olsen et al., 2007b; Kannan et al., 2004; Apelberg et al., 2007). Table A2-1 summarizes key characteristics of the respective studies. As shown, the studies ranged

from national studies to regional cohorts to small cohorts. Most evaluated serum, but two evaluated plasma for part of the cohort, and one evaluated cord blood. One study evaluated pooled serum, and two evaluated both plasma and serum.

Table A2-1. Summary of Studies Measuring General Population Blood Concentrations of PFOS

Citation	Total *n* Evaluated	Blood Fraction Evaluated	Other comments
Calafat et al., 2007	2094	Serum	NHANES 2003/2004
Calafat et al., 2007	1562	Serum	NHANES 1999/2000
Olsen et al., 2003a, b	645	Serum	Red Cross
Olsen et al., 2005	356	Serum 1974; Plasma 1989	178 each from 1974 and 1989
Hansen et al., 2001	24	Serum	--
Calafat et al., 2006	23	Pooled serum	--
Olsen et al., 2004	238	Serum	Elderly people from Seattle, WA
Olsen et al., 2007b	40, 100	Plasma for 2005 Serum for 2000	--
Kannan et al., 2004	75, 30, 70	Serum	3 locations in the US
Apelberg et al., 2007	299	Cord blood	Baltimore, MD
Calafat et al., 2007	2094	Serum	NHANES 2003/2004

Chemical-Specific Toxicokinetic Data

Egeghy and Lorber (2011) estimated the first-order elimination rate kP as 0.00039/day, based on the median half-life of about 4.8 years reported by Olsen et al. (2007a) from occupational data. Due to the lack of human data, the volume of distribution (V_d) was estimated using data from monkey studies. Egeghy and Lorber (2011) bounded the estimates of volume of distribution with a low estimate of 200 mL/kg (Andersen et al., 2006) and a high estimate of 3000 mL/kg (extrapolated from Griffith and Long, 1980; Noker and Gorman 2003).

Anatomy and Physiology Data

No anatomy and physiology data are necessary to calculate the daily intake dose using this method.

A2.3 Conduct Reverse Dosimetry

Daily intake was estimated using Equation 7 (Section 3.3.4 of the main text), and assuming steady state. The authors noted that it can be reasonable to assume that steady state has been reached for adults who have been exposed at background levels for a "reasonably long" period of time.

The supplemental EXCEL® spreadsheet illustrates the calculation of the average daily intake of PFOS, based on the geometric mean of PFOS in serum in the NHANES 2003-2004 data of 20.7 ng/mL. The calculation is shown for the bounding estimates of the volume of

distribution, and agrees with the range of intakes estimated by the authors. The intake calculated based on body burden biomarker levels was compared with the intake estimated by measuring the concentration of PFOS and precursors in various environmental media, and calculating intake using standard equations.

A3.0 Wetmore et al., 2015 – Chlorpyrifos (Case Study 3A)/Acetaminophen (Case Study 3B); Use of *In Vitro* Toxicokinetic Parameters to Estimate Daily Intake

A3.1 Overview

As part of an effort to expand the use of *in vitro* toxicology data, including data developed in high throughput cell culture assays such as ToxCast, high throughput toxicokinetic (httk) data and methods have been developed to aid in *in vitro* to *in vivo* extrapolation (IVIVE) (Wambaugh et al., 2015; Wetmore 2015; Wetmore et al., 2012, 2013, 2015; Ring et al., 2017). The reverse dosimetry approaches used for IVIVE can also be used to interpret data from biomonitoring in blood, since the *in vitro* chemical concentration is considered to be analogous to the concentration of the chemical in blood. Most of the available publications on these methods focus on presenting the overall calculation methods and high-level summaries across multiple chemicals. However, Wetmore et al. (2013, 2015) presented calculations for several individual chemicals and provided the underlying *in vitro* toxicokinetic data. The case studies presented here were based on the data on chlorpyrifos and acetaminophen presented in Wetmore et al. (2015). Unfortunately, that paper did not provide results for chemicals that are more relevant to consumer exposure. It is also noted that Pearce et al., (2017) have developed the httk R package to support calculations (including population distributions) using the httk data. (See also Breen et al., 2021.) Instead of using EXCEL® to try to replicate previous calculations, it is highly recommended to do the calculations in R, using the calc_analytic_css function in the httk package. This approach minimizes the potential for error and ensures that the most recent available toxicokinetic parameters are used. Those parameters (current as of the completion of this guide) are provided in the chemphysicaldata tab of the supplemental EXCEL® file with this guide. Note that previous versions of httk included a calc_oral_equiv function, but this function is no longer included. The function calc_mc_oral_equiv uses a Monte Carlo simulation and is also not recommended for this application. Instead, using calc_analytic_css allows the calculation of a C_{ss} which can then be used to calculate an oral equivalent dose, as described in A3.3. Since use of the R code is recommended, the supplemental EXCEL® file does not include a tab for the case studies here. However, this text does describe the steps in the process, for illustrative purposes.

A3.2 Identify the necessary data

<u>Biomonitoring Data</u>

As noted, the Wetmore et al. (2015) study is not a classic biomonitoring study. Instead, it describes the calculation of an internal dose corresponding to an *in vitro* concentration. The context of the analysis is interpreting ToxCast data using *in vitro* to *in vivo* extrapolation (IVIVE), but the same methods can be applied to calculate the daily intake

that corresponds to a specified concentration of a chemical in the blood. Because this case study is illustrative, with a focus on the equations used, no actual biomonitoring data were used in the case study. Instead, a blood concentration of 10 μM can be used for convenience. Based on the chlorpyrifos molecular weight of 350.6 g/mol, 10 μM corresponds to a blood concentration of 3.51 mg/L. For acetaminophen, the molecular weight of 151.2 g/mol means that 10 μM corresponds to a blood concentration of 1.51mg/L.

Chemical-Specific Data

The blood concentration is used to estimate daily intake using the multi-compartmental model approach described in Section 3.3.5, and assuming steady state. In this approach, two chemical-specific parameters are needed and are measured using high-throughput techniques. These parameters are the fraction of chemical unbound in plasma and the intrinsic clearance rate of the chemical of interest in human hepatocyte culture. Values from httk can be obtained from the chemphysicaldata tab of the supplemental EXCEL® file with this guide. The fraction unbound in plasma is shown in column AI (Human.Funbound.plasma), and the *in vitro* intrinsic clearance is shown in column AC (Human.Clint).[6] Where multiple values are provided for a given parameter, these values reflect the median, and upper and lower credible bound, in that order.

Several manipulations are required to convert the two high-throughput toxicokinetic parameters into toxicokinetic data useable in the reverse dosimetry calculation. The toxicokinetic data required for the reverse dosimetry approach are the whole-liver intrinsic clearance rate and the unbound fraction of parent compound in the blood. This latter parameter reflects the free (unbound) compound that can be metabolized. A third chemical-specific parameter (the blood to plasma concentration ratio) is calculated from the fraction unbound in plasma.

The fraction unbound in plasma is determined by measuring the chemical concentration in phosphate-buffered saline (PBS) and dividing that value by the mean concentration in a matched plasma sample. The fraction in plasma can be used to calculate the fraction unbound in blood (Ring et al., 2017):

$$F_{ub} = \frac{F_{up}}{R_{b2p}} \qquad \textit{Eq. A30}$$

Where: F_{up}= Fraction of chemical unbound in plasma (unitless)

[6] Note that values obtained from the httk package differ in some cases from those obtained from Wetmore et al. (2015) supplemental table 3. The fraction unbound in plasma can be found in supplemental table 3A of Wetmore et al. (2015), column E (Mean % Unbound), and converted from a % to a fraction. Intrinsic clearance rate in human hepatocyte culture can be found in supplemental table 3B of Wetmore et al. (2015), column AK (Adjusted Clearance). These differences reflect the ongoing improvements in the IVIVE program and insights on appropriate scaling methods (personal communication with EPA). As noted in Section 3.3.5, methods for estimating the *in vitro* metabolic parameters have improved with time, resulting in some modifications to the parameters, particularly for highly-bound chemicals; the most recent data are reflected in the httk package.

F_{ub}= Fraction of chemical unbound in blood (unitless)
R_{b2p}= Constant ratio of blood to plasma concentration (see equation below)

R_{b2p} may be derived using Schmitt's Method (Schmitt, 2008 as cited in Ring et al., 2017):

$$R_{b2p} = 1 - Hct + Hct * K_{RBC2p} * F_{up} \qquad Eq.\ A31$$

Where: Hct= Hematocrit (% red blood cells in blood)
K_{RBC2p}= Partition coefficient between red blood cells and plasma

Calculation of the partition coefficient between red blood cells and plasma (K_{RBC2p}) involves several different parameters and partition coefficients in addition the F_{up}. For the case studies shown here, K_{RBC2p} was obtained from the httk R package, as noted in the accompanying Case Study spreadsheet.

Additional calculations are needed to calculate the whole liver intrinsic clearance rate from the *in vitro* data. The whole liver intrinsic clearance rate is based on the intrinsic clearance rate measured from human hepatocytes *in vitro,* the hepatocellularity in millions of cells, and liver mass:

$$CL_{int,h} = CL_{int} * hepatocellularity * M_{liver} * \frac{L}{10^6\ \mu L} * \frac{60\ min}{hr} \qquad Eq.\ A32$$

Where: $CL_{int,h}$ = Whole liver intrinsic clearance (L/hr)
CL_{int}= Intrinsic clearance rate measured from *in vitro* human hepatocytes (µL/min – million cells)
Hepatocellularity= millions of cells/kg of liver tissue
M_{liver}= Liver mass (kg)

Two different data sets have been used for hypocellularity and liver mass[7]. The parameters provided by Wetmore et al. (2015) are used in the primary calculations on the accompanying case study spreadsheet.

Anatomy and Physiology Data

The only anatomy and physiology data required for this approach are the liver blood flow, the glomerular filtration rate (GFR), and hematocrit. The mean glomerular filtration rate of 6.7 L/hour is based on the data of Rule et al. (2004). The mean liver blood flow is 90 L/hour, based on the results of Davies and Morris (1993). The httk package uses a default hematocrit value of 0.44[8].

[7] Both Wetmore (2015) and Wetmore et al. (2015) obtained the hepatocellularity from Barter et al. (2007), but they reported different values. Wetmore (2015) used a value of 137 x 10^6 hepatocytes/g liver, while Wetmore et al. (2015) used 110 x 10^6 hepatocytles/g liver. The reason for this difference is not clear. Similarly, Wetmore (2015) and Wetmore et al. (2015) reported different values for the liver mass or volume, based on Johnson et al. (2005), with no explanation for the difference. Wetmore (2015) used 1820 g, while Wetmore et al. (2015) used 1596 g. Here, the specific gravity of the liver is assumed to be 1, as is common in pharmacokinetic modeling. The product of hepatocellularity and liver weight (i.e., the number of hepatocytes/liver) differs by about 40% for the two approaches.

[8] More recent analyses, such as Breen et al. (2021), use physiological parameters scaled to body weight.

A3.3 Conduct Reverse Dosimetry

The reverse dosimetry calculations were conducted as described in Section 3.3.5, using Equations 13 and 14 in the main text. Equation 13 presents the steady state concentration of the chemical in blood as a function of the dose rate, GFR, fraction of the parent compound unbound in the blood, liver blood flow rate, and whole liver intrinsic clearance. Note that, although the parameter of interest is the dose rate, Equation 13 describes the steady state concentration in blood, because this equation follows a logical progression. The equation can be rearranged to express daily intake (converting the dose rate from dose per hour to dose per day) as a function of the steady state blood concentration, standard physiological parameters, and chemical-specific parameters determined *in vitro*.
Due to the complexity of the calculations and number of parameters, the relevant equations are repeated here from the main text.

$$C_{SS} = \frac{k_{dose}}{(GFR*F_{ub})+(\frac{Q_l*F_{ub}*Cl_{int,h}}{Q_l+F_{ub}*Cl_{int,h}})} \qquad Eq.\ A33$$

Where: k_{dose}= Dose rate (mg/kg-hour)
C_{SS}= Blood Concentration in steady state (mg/L)
GFR= Glomerular Filtration Rate (L/hour-kg)
F_{ub}= Unbound fraction of parent compound in the blood (unitless)
Q_l= Liver blood flow (L/hour-kg)
$CL_{int,h}$= Whole-liver intrinsic clearance rate (L/hour-kg)

Whole liver intrinsic clearance and fraction unbound in blood are calculated as described in Appendix Section 3.2.

<u>Estimating daily intake</u>

As discussed in Section 3.3.5 of the main text, Eq. 13 (here Eq. A4) can be applied to determine the steady state concentration for a daily intake of 1 mg/kg-day (k_{dose} = 0.042 mg/kg-hr). The result can then be used as a conversion factor for the measured biomarker concentration (Eq. 14 in the main text, here Eq. A5). This approach has the advantage of being mathematically more intuitive than rearranging Eq. A4 to solve for dose, and is the approach used by the U.S. EPA for IVIVE:

$$DI = \frac{C\ x\ 1\frac{mg}{kg}-day}{C_{ss}} \qquad Eq.\ A34$$

Where: DI = Daily intake (mg/kg-day)
C = Measured biomarker concentration in blood (mg biomarker/L)
C_{ss} = Steady state concentration of biomarker in blood at a dose of 1 mg/kg-day, calculated using Eq. 13 (mg/L)

A4.0 Pleil and Sobus (2013), Use of the ICC

This is not a full case study, but rather explains how the ICC can be used in interpreting biomonitoring data. The ICC is a measure of how much of the total variability in a sampling of population-based biomonitoring data is due to intra-individual variability (discussed in Section 3.8.2 of the main text). The application is shown in the Case Study 4 tabs of the accompanying spreadsheet.

In this example, the user enters the spot sample geometric mean (GM) and geometric standard deviation (GSD) for the biomonitoring data of interest, such as data from NHANES. The spreadsheet automatically calculates the distribution of long-term average exposures for the population for the bounds of ICC = 0 (all the variability is due to intra-individual variability) and ICC = 1 (all variability is due to inter-individual variability). Additional user-specified ICC values and number of samples per individual (m) can be entered, based on ICC data obtained in smaller, dedicated studies (rows 13 and 14). Based on the ICC, the spreadsheet calculates a modified GSD for the population (row 8 of the spreadsheet). This modified GSD reflects the actual population distribution. Thus, when ICC = 1, and all variability is due to inter-individual variability, the modified GSD is the same as the original GSD. When ICC = 0, all variability is due to intra-individual variability, and the modified GSD is smaller than the original GSD. Other values of ICC < 1 result in intermediate values of the modified GSD. The modified GSD can then be used together with the GM to calculate the 95th percentile.

The new GSD can now be used to estimate the 95th percentile of the population exposure:

$$95^{th}\ percentile\ =\ GM\ +\ 1.64GSD \qquad \textit{Eq. 35}$$

The 95th percentile concentrations calculated for the three combinations of ICC and m in the case study are shown in Row 9 of the spreadsheet.

The spreadsheet was initially designed for determining the percentage of people who would potentially exceed the BE for a given distribution. Because the focus of this guide is on reverse dosimetry, the BE line has been removed. However, the box for entering the user-specified BE has not been removed, because removing the range would remove all of the curves.

Appendix B – Supplemental Tables

This Appendix provides two types of data summaries. Table B1 provides additional information on sources of A&P data, and illustrative reverse dosimetry analyses that used various types of A&P data from various sources. Table B1 is intended to supplement the information provided in Table 1 of the main document.

Table B1 is organized according to the four main sources of A&P data discussed in the main document. The data may have been obtained from the same study in which the biomonitoring was conducted ("same study"). The data may come from a study specifically designed to characterize the parameter in the population ("dedicated study"). A regression equation may have been developed to describe how the parameter varies with other population characteristics, either as part of a dedicated study, or in a publication reviewing multiple data sets ("regression equation"). Finally, the parameter may be based on compilations or default values that are published as guidance documents ("compilation"). The values in the compilation may themselves have been obtained from dedicated studies or regression equations, but for ease of reference, are noted here simply as the compilations. All of the approaches noted here are legitimate and scientifically valid approaches for obtaining A&P data.

Tables B2 and B3 summarize the types of biomonitoring data and toxicokinetic data, the reverse dosimetry approach used with different data types, and types of situations where the data types can be used appropriately. Of necessity, only brief conclusions can be presented in this format, and the reader is encouraged to review these tables in conjunction with the more nuanced conclusions and recommendations in Section 5.0 of the main document.

Table B1. Sources of Anatomy and Physiology Data by Data Type – Illustrative Studies

Type of A&P Data	Same Study	Compilation	Dedicated Study	Regression Equation
Body Weight	Apel et al., 2020; Cok et al., 2020; Koch et al., 2011; Lakind et al., 2011; Lu et al., 2014; Wittassek et al., 2007	Institute of Medicine, 1998, as cited in Katsikantami et al. 2019	--	--
Body Lipid Mass	Tay et al., 2019[9]	US EPA (2020b) (assumed proportions without reference)	--	Tay et al., 2019
Creatinine Excretion	--	Tietz et al., 2006, as cited in Qian et al., 2015	Remer et al. 2002, as cited in Qian et al., 2015, Koch et al., 2007, 2011	Cockcroft and Gault, 1976, as cited in Mage et al., 2008; Mage et al., 2008, as cited in Aylward et al., 2017, Reyes and Price, 2018, and others; Kawasaki et al, 1991, as cited in Fong et al., 2014
Glomerular Filtration Rate (GFR)			Rule et al., 2004, as cited in Wetmore et al., 2012	Levey et al., 2009
Urinary Volume	Apel et al., 2020; von Goetz et al., 2010; Moos et al., 2017; Wittassek et al., 2007	Institute of Medicine, 1998, as cited in Katsikantami et al. 2019; US EPA, 2011, as cited in Cao et al., 2016; ICRP, 2002, as cited in Cok et al., 2020, Connolly et al., 2020,	Van Haarst et al., 2004, as cited in Aylward et al., 2017; Miller and Stapleton, 1989, as cited in Fromme et al., 2014, 2016	--

[9] Calculated based on individual BMI, age and sex, based on a regression equation

Type of A&P Data	Same Study	Compilation	Dedicated Study	Regression Equation
		Lakind et al., 2011; Lu et al., 2014 (default); Health Canada, 2020 (pregnant women, adult women, toddlers)		

Table B2. Summary of Method Application and Quality Based on Type of Biomonitoring Data

Type of Biomonitoring Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature? (at least once)	Reliable for Chemical Scenario? (Short Half-Life)	Reliable for Chemical Scenario? (Long Half-Life)	Example of Best Practices Publication/Comments
Urine Spot Samples (with or without creatinine adjustment)	Urinary Mass Balance Approach	x	x	x	If elimination half-time is short relative to exposure frequency, bounding should be done using ICC to address variability.	Yes, but low F_{ue} can lead to high variability and high uncertainty	Koch et al., 2007; Aylward et al., 2012. A short elimination half-life relative to exposure interval can lead to over-estimation of the variability in the population distribution; correction for the relative contribution of intra- and inter-individual variability via the ICC is needed. Assumes steady state or periodicity, which is reasonable if product has a consistent daily pattern of use.
24-hour Urine Composites	Urinary Mass Balance Approach	x	x	x	Yes	Yes, but low F_{ue} can lead to high variability and high uncertainty	Wittassek et al., 2007. This approach is preferred over spot samples, because it captures the entire daily excretion, and so intraindividual variability has less impact for short half-life chemicals. Assumes steady state. May need to extrapolate to longer

Type of Biomonitoring Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature? (at least once)	Reliable for Chemical Scenario? (Short Half-Life)	Reliable for Chemical Scenario? (Long Half-Life)	Example of Best Practices Publication/Comments
							durations to capture entire excretion (Poet et al., 2016)
Whole Blood, serum or plasma	Reverse Compartmental Model	x	x	--	If elimination half-time is short relative to exposure frequency, bounding should be done using ICC to address variability.	Yes	Fromme et al., 2007; Egeghy and Lorber, 2011; Tay et al., 2019. Assumes steady state; long half-life chemicals have less variability across time. Calculation for serum includes consideration of body lipid mass.
	Multi-Compartmental Model	x	--	--	If elimination half-time is short relative to exposure frequency, bounding should be done using ICC to address variability.	High uncertainty common	Theoretically possible application of IVIVE methods, but no published examples located. High uncertainty raises issues – see main text for details.

Type of Biomonitoring Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature? (at least once)	Reliable for Chemical Scenario? (Short Half-Life)	Reliable for Chemical Scenario? (Long Half-Life)	Example of Best Practices Publication/Comments
	Exposure Conversion Factors	x	x	x	If elimination half-time is short relative to exposure frequency, bounding should be done using ICC to address variability.	Yes	Clewell et al., 2008. Used in combination with PBPK modeling.
Breast Milk Samples	Reverse Compartmental Model Approach (Lipids)	x	x	x	No	Yes	US EPA, 2020b (HBCD), based on Aylward and Hays, 2011. Intake expressed as function of mass HBCD/mass lipid in the body[10].
Adipose Tissue	Reverse Compartmental Model Approach (Lipids)	x	x	x	No	Yes	US EPA, 2020b (HBCD), based on Aylward and Hays, 2011. Intake expressed as function of mass HBCD/mass lipid in the body.

[10] The same approach was used for adipose tissue, blood, serum, and breast milk, after normalizing to concentration in terms of ng/g lipid, assuming lipid content in whole blood and serum is 25%, lipid content in hair is 6%, and density of serum is 1.024 g/mL. Use of feces and fetal tissue is also noted, without explanation of how lipid normalization was done.

Type of Biomonitoring Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature? (at least once)	Reliable for Chemical Scenario? (Short Half-Life)	Reliable for Chemical Scenario? (Long Half-Life)	Example of Best Practices Publication/Comments
Hair	Reverse Compartmental Model Approach (Lipids)	x	x	X	No	Not broadly validated	US EPA, 2020b (HBCD)- Used a lipid model to estimate daily intake in hair. Assumes that lipid content in hair is 6%

Table B3. Summary of Method Application and Quality Based on Type of Toxicokinetic Data

Type of Toxicokinetic Data	Types of TK Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature?	Reliable for chemical scenario? Short half-life.	Reliable for chemical scenario? Long half-life.	Example of Best Practices Publication/Comments
Urinary excretion fraction (F_{ue})	Human	Urinary Mass Balance Approach	x	x	x	Yes	Yes, but low F_{ue} can lead to high variability and high uncertainty	Koch et al, 2007. This is the most common approach for reverse dosimetry based on urine data.
	Animal	Urinary Mass Balance Approach	x	x	x	Yes	Yes, but low F_{ue} can lead to high variability and high uncertainty	Health Canada, 2020; Zhang et al., 2020. The animal Fue can be used if human data are not available.
	In Vitro	--	--	--	--	--	--	--
	Modeled	--	--	--	--	--	--	--
Half-life	Human	Compartmental model approach	x	x	x	Maybe	Yes	Fromme et al., 2007, US EPA, 2020b
	Animal	Compartmental model approach	--	--	--	--	Yes	Tay et al., 2019. Greater uncertainty than use of human half-life
	In Vitro	--	--	--	--	--	--	--
	Modeled	--	--	--	--	--	--	--
Volume of Distribution	Human	--	--	--	--	--	Yes	Theoretically valid if appropriate data available, but no examples located.

Type of Toxicokinetic Data	Types of TK Data	General Approach Used	Valid, sound equation?	Used in peer reviewed literature? (at least once)	Used in Grey Literature?	Reliable for chemical scenario? Short half-life.	Reliable for chemical scenario? Long half-life.	Example of Best Practices Publication/Comments
	Animal	Compartmental model approach	x	x	--	Not applicable	Yes	Fromme et al., 2007; Egeghy and Lorber, 2011. Not used for short half-life chemicals because blood monitoring and compartmental model not used for these chemicals.
	In Vitro	--	--	--	--	--	--	--
	Modeled	--	--	--	--	--	--	--
In vitro – intrinsic clearance and fraction unbound in plasma	Human	--	--	--	--	--	--	--
	Animal	--	--	--	--	--	--	--
	In Vitro	--	--	--	--	--	--	--
	Modeled	Multi-Compartmental Model Approach	x	--	--	No	High uncertainty common	Theoretically possible application of IVIVE methods, but no published examples located. High uncertainty raises issues – see main text for details.

Appendix C – Search Strategy

A multipronged search strategy was used to both identify relevant information on relevant methods and to identify examples of the use of reverse dosimetry for case studies. This multipronged approach included direct searching of authoritative websites, "tree searching" (also known as forward and backward searching) from a limited set of key references, and review of references identified in interviews with experts. Each of these approaches is discussed in more detail below. Note that an iterative process was used. That is, articles identified from searching bibliographic databases such as PubMed or from other searches were themselves used as the basis for forward and backward searches.

Authoritative Website Search

To find relevant articles and information for the guidance document, an authoritative website search was conducted between October and December 2020. To do this, multiple agency websites were searched for information on the use of biomonitoring data in exposure assessments. These agency sites include:

- Association of Public Health Laboratories (https://www.aphl.org/programs/environmental_health/nbn/Pages/default.aspx)
- California Environmental Contaminant Biomonitoring Program (https://biomonitoring.ca.gov/)
- CDC (https://www.cdc.gov/biomonitoring/index.html)
- European Chemicals Agency (https://echa.europa.eu/)
- European Environmental Agency (https://www.eea.europa.eu/)
- German Environmental Survey (https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/german-environmental-survey-geres)
- German Federal Institute for Risk Assessment (https://www.bfr.bund.de/en/home.html)
- HBM4EU (https://www.hbm4eu.eu/), including the video from the "International Hybrid Conference on Human Biomonitoring for Science and Chemical Policy."
- Health Canada (https://www.canada.ca/en/health-canada.html)
- International Society of Exposure Science (http://ises2020ca.org/program/)
- Japan Environment and Children Study (http://www.env.go.jp/chemi/ceh/en/)
- Minnesota Biomonitoring Program (https://www.health.state.mn.us/communities/environment/biomonitoring/index.html)
- NHANES (https://www.cdc.gov/nchs/nhanes/index.htm)
- USEPA, including ExpoBox https://www.epa.gov/expobox/exposure-assessment-tools-approaches-exposure-reconstruction-biomonitoring-and-reverse and

America's Children and the Environment (US EPA) (https://www.epa.gov/americaschildrenenvironment/ace-biomonitoring)
- WHO (https://www.who.int/)

These sites were searched for potential case studies:

- German Environmental Survey (https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/german-environmental-survey-geres)
- Health Canada (https://www.canada.ca/en/health-canada.html)
- Japan Environment and Children Study (http://www.env.go.jp/chemi/ceh/en/)
- USEPA, including ExpoBox https://www.epa.gov/expobox/exposure-assessment-tools-approaches-exposure-reconstruction-biomonitoring-and-reverse and America's Children and the Environment (US EPA) (https://www.epa.gov/americaschildrenenvironment/ace-biomonitoring)

For each website, the search function within that site was used to search for guidance documents and articles. The following search terms were used:

- Biomonitoring

Relevant references were extracted from this search. For websites where the term "biomonitoring" received more than 100 hits, the search was further narrowed using all the following search terms in succession.

- Biomonitoring AND exposure assessment
- Biomonitoring AND pharmacokinetic
- Biomonitoring AND toxicokinetic
- Biomonitoring AND methods
- Biomonitoring AND reverse dosimetry
- Biomonitoring AND external exposure

In each search, the information on the search page(s) was screened for relevance. If a research paper appeared on a search page, the abstract was screened to determine relevance. This was done by continuously searching page by search page until none of the hits on a given page were relevant.

Additional targeted searches were conducted for a few websites that were of particular interest, either because of prior knowledge of the existence of guidance documents from the respective organizations, or because of an expectation that the website would have relevant information. The search terms used for each of these supplemental searches were as follows:

- HBM4EU: Exposure reconstruction AND hbm data
- ATSDR (searched toxicological profiles on benzene, phenol and lead for data on toxicokinetic parameters useful for reverse dosimetry assessment, as illustrative cases).

- CDC site: “human samples” AND collection
- WHO: human exposure assessment
    - The original citation from the WHO site was in Korean, but the citation was used to find the original IPCS Environmental Health Criteria (EHC) document on Human Exposure Assessment.

Tree Search (October-December 2020)

Three types of “seed materials” were used for the tree search. The goal of this searching was to identify articles that would either provide useful general information for the guidance document or would be useful as case studies. The first approach used PowerPoint files of presentations that that were either initially provided by CPSC staff or were identified from searches of the authoritative websites. These presentations were used only for backward searching of cited references:

Aylward, L., Hays, S. (2013). Biomonitoring equivalents and interpretation: Current activities [PowerPoint slides]. Summit Toxicology, LLP. Bozeman, MN. Retrieved from https://www.umweltbundesamt.de/sites/default/files/medien/355/dokumente/aylward_and_hays_2013_biomonitoring_equivalents_and_interpretation.pdf

Aylward, L., Hays, S. (2015). Biomonitoring equivalents – Current activities and use of toxicokinetic modeling [PowerPoint slides]. Summit Toxicology, LLP. Bozeman, MN. Retrieved from https://www.umweltbundesamt.de/sites/default/files/medien/355/dokumente/aylward_and_hays_2015_biomonitoring_equivalents_current_activities_and_use_of_toxicokinetic_modeling_0.pdf

Calafat, A. (2011). Biomonitoring for exposure assessment: Challenges and future directions [PowerPoint slides]. Centers for Disease Control. Sacramento, CA. Retrieved from https://biomonitoring.ca.gov/sites/default/files/downloads/CalafatSGPNov2011.pdf

Fillol, C., & Vandentorren, S. (n.d.). National human biomonitoring programme in France: Selection of substances and prioritization of biomarkers [PowerPoint slides]. Institut de Veille Sanitaire. Retrieved from https://www.umweltbundesamt.de/sites/default/files/medien/378/dokumente/clemence_fillol_national_human_biomonitoring_programme_in_france_selection_of_substances_and_prioritization_of_biomarkers.pdf

Nong, A. (2016). Pharmacokinetic modeling of health and exposure measures to support health risk interpretations. Health Canada. Retrieved from https://www.umweltbundesamt.de/sites/default/files/medien/378/dokumente/andy_nong_pharmacokinetic_modeling_of_health_and_exposure_measures_to_support_health_risk_interpretations.pdf

Wambaugh, J. (2017). Fun with high throughput toxicokinetics. Environmental Protection Agency. Retrieved from https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NCCT&dirEntryId=337839

Zidek, A. (2016). Use of biomonitoring data under Canada’s chemicals management plan. Existing Substances Risk Assessment Bureau. Retrieved from

https://www.umweltbundesamt.de/sites/default/files/medien/378/dokumente/angelika_zidek_use_of_biomonitoring_data_under_canadas_chemicals_management_plan.pdf

The second approach to tree searching used conference proceedings that were identified in the review of the authoritative websites. Backwards searching was conducted with the following conference proceedings to identify relevant articles:

State of California Environmental Contaminant Biomonitoring Program Scientific Guidance Panel. https://dev.biomonitoring.ca.gov/sites/default/files/downloads/SGPTrans1204008.pdf

International Hybrid Conference HBMC2020- Human Biomonitoring for Science and Chemical Policy. https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/human-biomonitoring-for-science-chemical-policy

The third approach involved backward and forward searching from a limited number of key articles. These key articles were identified based on articles identified by the CPSC staff, relevant guidance documents found during the authoritative web search, and literature review articles that discussed an important overarching topic. Searching was done as an iterative process. Backward searching was conducted by reviewing the text and reference list of these publications. Forward searching was conducted using the Web of Science "cited by" feature. The following references were used as seed articles. Additional information on the screening criteria is provided below, after the list of articles screened.

Apel, P., & Ougier, E. (2017). 1st substance-group specific derivation of EU-wide health-based guidance values. HBM4EU: Deliverable report WP 5- Translation of results into policy. Retrieved from https://www.hbm4eu.eu/wp-content/uploads/cmdm/3390/1524411798_HBM4EU_D5.2_1st-substance-group-specific-derivation-of-EU-wide-health-based-guidance-values.pdf

Aylward, L. L., Hays, S. M., & Zidek, A. (2017). Variation in urinary spot sample, 24 h samples, and longer-term average urinary concentrations of short-lived environmental chemicals: implications for exposure assessment and reverse dosimetry. Journal of exposure science & environmental epidemiology, 27(6), 582-590.

Aylward, L. L., Hays, S. M., Smolders, R., Koch, H. M., Cocker, J., Jones, K., ... & Bevan, R. (2014). Sources of variability in biomarker concentrations. Journal of Toxicology and Environmental Health, Part B, 17(1), 45-61.

Berman, T., Goldsmith, R., Levine, H., & Grotto, I. (2017). Human biomonitoring in Israel: Recent results and lessons learned. International journal of hygiene and environmental health, 220(2), 6-12.

Calafat, A. M., Ye, X., Wong, L. Y., Bishop, A. M., & Needham, L. L. (2010). Urinary concentrations of four parabens in the US population: NHANES 2005–2006. Environmental health perspectives, 118(5), 679-685.

Carlson, K. R., & Garland, S. E. (2015). Estimated phthalate exposure and risk to pregnant women and women of reproductive age as assessed using four NHANES biomonitoring data sets (2005/2006, 2007/2008, 2009/2010, 2011/2012).

Consumer Product Safety Commission Directorate for Hazard Identification and Reduction. Retrieved from https://www.cpsc.gov/s3fs-public/Estimated%20Phthalate%20Exposure%20and%20Risk%20to%20Women%20of%20Reproductive%20Age%20as%20Assessed%20Using%202013%202014%20NHANES%20Biomonitoring%20Data.pdf
Clark, K. E., David, R. M., Guinn, R., Kramarz, K. W., Lampi, M. A., & Staples, C. A. (2011). Modeling human exposure to phthalate esters: a comparison of indirect and biomonitoring estimation methods. Human and Ecological Risk Assessment: An International Journal, 17(4), 923-965.
Egeghy, P. P., & Lorber, M. (2011). An assessment of the exposure of Americans to perfluorooctane sulfonate: a comparison of estimated intake with values inferred from NHANES data. Journal of exposure science & environmental epidemiology, 21(2), 150-168.
Esteban, M., & Castaño, A. (2009). Non-invasive matrices in human biomonitoring: a review. Environment international, 35(2), 438-449.
Faure, S., Noisel, N., Werry, K., Karthikeyan, S., Aylward, L. L., & St-Amand, A. (2020). Evaluation of human biomonitoring data in a health risk based context: An updated analysis of population level data from the Canadian Health Measures Survey. International journal of hygiene and environmental health, 223(1), 267-280.
Hartmann, C., Uhl, M., Weiss, S., Koch, H. M., Scharf, S., & König, J. (2015). Human biomonitoring of phthalate exposure in Austrian children and adults and cumulative risk assessment. International journal of hygiene and environmental health, 218(5), 489-499.
Hays, S. M., Aylward, L. L., & Blount, B. C. (2015). Variation in urinary flow rates according to demographic characteristics and body mass index in NHANES: potential confounding of associations between health outcomes and urinary biomarker concentrations. Environmental health perspectives, 123(4), 293-300.
HEALS (2015). Guidelines for appropriate “biomarker of exposure” selection for EWAS studies. Deliverable 4.2, WP4 Human Biomonitoring. Retrieved from http://www.heals-eu.eu/wp-content/uploads/2013/08/HEALS_D4.2.pdf
Horvat, M., Sarigiannis, D., Handakas, E., Karakitsios, S., & Gotti, A. (2017). Report on the optimal methodology for exposure reconstruction from HBM data. HBM4EU: Deliverable Report WP 12-from HBM to exposure. Retrieved from https://www.hbm4eu.eu/wp-content/uploads/2018/09/Deliverable-12.2-Report-on-the-optimal-methodology-for-exposure-reconstruction-from-HBM-data.pdf
Joas, R., Casteleyn, L., Biot, P., Kolossa-Gehring, M., Castano, A., Angerer, J., ... & Horvat, M. (2012). Harmonised human biomonitoring in Europe: activities towards an EU HBM framework. International journal of hygiene and environmental health, 215(2), 172-175.
Jongeneelen, F. J., & Berge, W. F. T. (2011). A generic, cross-chemical predictive PBTK model with multiple entry routes running as application in MS Excel; design of the model and comparison of predictions with experimental results. Annals of occupational hygiene, 55(8), 841-864.
Manno, M., Sito, F., & Licciardi, L. (2014). Ethics in biomonitoring for occupational health. Toxicology letters, 231(2), 111-121.

National Research Council (2006). Human Biomonitoring for Environmental Chemicals. Washington, DC: The National Academies Press. Retrieved from https://doi.org/10.17226/11700
Paustenbach, D., & Galbraith, D. (2006). Biomonitoring: Is body burden relevant to public health?. Regulatory Toxicology and Pharmacology, 44(3), 249-261.
Pearce, R. G., Setzer, R. W., Strope, C. L., Wambaugh, J. F., & Sipes, N. S. (2017). Httk: R package for high-throughput toxicokinetics. Journal of statistical software, 79(4), 1.
Pleil, J. D., & Sobus, J. R. (2013). Estimating lifetime risk from spot biomarker data and intraclass correlation coefficients (ICC). Journal of toxicology and environmental health, part A, 76(12), 747-766.
Pleil, J. D., & Sobus, J. R. (2016). Estimating central tendency from a single spot measure: A closed-form solution for lognormally distributed biomarker data for risk assessment at the individual level. Journal of Toxicology and Environmental Health, Part A, 79(18), 837-847.
Polkowska, Ż., Kozłowska, K., Namieśnik, J., & Przyjazny, A. (2004). Biological fluids as a source of information on the exposure of man to environmental chemical agents. Critical reviews in analytical chemistry, 34(2), 105-119.
Schulte, P.A., and G. Talaska. 1995. Validity criteria for the use of biological markers of exposure to chemical agents in environmental epidemiology. Toxicology 101(1-2):73-88
Shen, H., Main, K. M., Virtanen, H. E., Damggard, I. N., Haavisto, A. M., Kaleva, M., ... & Schramm, K. W. (2007). From mother to child: investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere, 67(9), S256-S262.
Sobus, J. R., DeWoskin, R. S., Tan, Y. M., Pleil, J. D., Phillips, M. B., George, B. J., ... & Edwards, S. W. (2015). Uses of NHANES biomarker data for chemical risk assessment: trends, challenges, and opportunities. Environmental health perspectives, 123(10), 919-927.
Steckling, N., Gotti, A., Bose-O'Reilly, S., Chapizanis, D., Costopoulou, D., De Vocht, F., ... & Jagodic, M. (2018). Biomarkers of exposure in environment-wide association studies–Opportunities to decode the exposome using human biomonitoring data. Environmental research, 164, 597-624.
Tan, C., Dary, C., Chang, D., Ulrich, E., Van Emon, J., Xue, J., ... & Zartarian, V. G. (2013). Biomonitoring—An Exposure Science Tool for Exposure and Risk Assessment. US Environmental Protection Agency, EPA/600/R-12/039. Retrieved from https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NERL&dirEntryId=243731
Tay, J. H., Sellström, U., Papadopoulou, E., Padilla-Sánchez, J. A., Haug, L. S., & de Wit, C. A. (2019). Serum concentrations of legacy and emerging halogenated flame retardants in a Norwegian cohort: Relationship to external exposure. Environmental research, 178, 108731.
US EPA (2013). Interpreting biomonitoring data and using pharmacokinetic modeling in exposure assessment. Risk Assessment Training and Experience Program (RATE), EXA 408. Retrieved from https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=514937

US EPA (2020b). Risk Evaluation for Cyclic Aliphatic Bromide Cluster (HBCD). Office of Chemical Safety and Pollution Prevention, EPA Document #740-R1-8006. Retrieved from https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluation-cyclic-aliphatic-bromide-cluster-hbcd
Vincente, J., Buekers, J., Bessems, J., David, M. (2019). Case-study report on HBM-indicators. HBM4EU: Deliverable Report WP 12-from HBM to exposure. Retrieved from https://www.hbm4eu.eu/work-packages/additional-deliverable-5-3-case-study-report-on-hbm-indicators/
Vogel, N., Conrad, A., Apel, P., Rucic, E., & Kolossa-Gehring, M. (2019). Human biomonitoring reference values: Differences and similarities between approaches for identifying unusually high exposure of pollutants in humans. International Journal of Hygiene and Environmental Health, 222(1), 30-33.
World Health Organization, International Labour Organization, & Inter-Organization Programme for the Sound Management of Chemicals. (2008). Uncertainty and data quality in exposure assessment (Vol. 6). World Health Organization. Retrieved from https://apps.who.int/iris/handle/10665/44017
Wilson, H. K., & Monster, A. C. (1999). New technologies in the use of exhaled breath analysis for biological monitoring. Occupational and environmental medicine, 56(11), 753-757.

Each article was screened for relevance for use in the guidance document. Studies were screened to identify ones that included any of the following information:

- Exposure calculations based on biomarker data, with documentation of biomarker and toxicokinetic data used
- A description of choices for selecting an appropriate biomarker or biological matrix for a chemical substance
- A review of the literature on the use of human biomonitoring data
- A description of interindividual variability in a sample population or providing general population age or sex-specific default values
- A description of the use of human, animal, *in vitro*, modeling, or read-across toxicokinetic data and their use in interpreting human biomonitoring studies
- A description of methods for differentiating inter- and intra-individual variability in using biomonitoring data

The initial project plan envisioned screening literature to identify two types of relevant references: (1) methods documents and (2) examples of use of HBM data for estimating exposure. We envisioned using the former group of references to identify criteria for evaluating potential case studies based on:

a. Whether the HBM data and/or toxicokinetic data used in the examples are reliable and relevant *on their own merits*, and

b. Whether the reported HBM data and/or toxicokinetic data used in the examples are reliable and relevant *for use in estimating human exposure*.

However, the number of potential case studies identified where reverse dosimetry was used was sufficiently small that this distinction did not end up being useful. Instead, the

references were reviewed to identify the type and source of (1) biomonitoring data, (2) toxicokinetic data, and (3) anatomy and physiology data. Based on this information, consideration of study quality and data quality based on the two criteria in the previous paragraph, as well as consideration of the half-life category for each chemical (in order to identify a range of half-lives), a mix of case studies was identified that reflects a variety of combinations of data types.

Studies Identified by Experts

Multiple interviews of experts in the biomonitoring field were conducted February and March 2021. Interviewers recommended articles that pertained to using reverse dosimetry to estimate external exposures.

The following experts were interviewed:

- Scott Hancock (Health Canada), Kristin Macey (Health Canada), Devika Poddalgoda (Health Canada), Andy Nong (Health Canada) – February 11, 2021
- Chris Brinkerhoff (US EPA), Erin Hines (US EPA), Peter Egeghy (US EPA), Andrea Pfahles-Hutchens (US EPA) – February 23, 2021
- Jon Sobus (US EPA) and Joachim Pleil (former US EPA) – March 11, 2021

Numerous articles were recommended by the interviewees. These articles were scanned for useful information and were evaluated as potential case studies. The following articles were recommended by interviewees, many of which had previously been identified in the other phases of the literature searching. These articles were then screened for utility of the information and for their potential utility as case studies:

Angerer, J., Bird, M. G., Burke, T. A., Doerrer, N. G., Needham, L., Robison, S. H., ... & Zenick, H. (2006). Strategic biomonitoring initiatives: moving the science forward. Toxicological Sciences, 93(1), 3-10.

Bastiaensen, M., Gys, C., Malarvannan, G., Fotache, M., Bombeke, J., Bamai, Y. A., ... & Covaci, A. (2021). Short-term temporal variability of urinary biomarkers of organophosphate flame retardants and plasticizers. Environment International, 146, 106147.

Caudill, S. P. (2015). Confidence interval estimation for pooled-sample biomonitoring from a complex survey design. Environment international, 85, 40-45.

Egeghy, P. P., & Lorber, M. (2011). An assessment of the exposure of Americans to perfluorooctane sulfonate: a comparison of estimated intake with values inferred from NHANES data. Journal of exposure science & environmental epidemiology, 21(2), 150-168.

Egeghy, P. P., Cohen Hubal, E. A., Tulve, N. S., Melnyk, L. J., Morgan, M. K., Fortmann, R. C., & Sheldon, L. S. (2011). Review of pesticide urinary biomarker measurements from selected US EPA children's observational exposure studies. International journal of environmental research and public health, 8(5), 1727-1754.

Kim, D., Andersen, M. E., Chao, Y. C. E., Egeghy, P. P., Rappaport, S. M., & Nylander-French, L. A. (2007). PBTK modeling demonstrates contribution of dermal and inhalation exposure components to end-exhaled breath concentrations of naphthalene. Environmental health perspectives, 115(6), 894-901.

Lee, S., Tan, Y. M., Phillips, M. B., Sobus, J. R., & Kim, S. (2017). Estimating methylmercury intake for the general population of South Korea using physiologically based pharmacokinetic modeling. Toxicological Sciences, 159(1), 6-15.

Lorber, M., & Egeghy, P. P. (2011). Simple intake and pharmacokinetic modeling to characterize exposure of Americans to perfluoroctanoic acid, PFOA. Environmental science & technology, 45(19), 8006-8014.

Morgan, M. K., Sobus, J. R., Barr, D. B., Croghan, C. W., Chen, F. L., Walker, R., ... & Clifton, M. S. (2016). Temporal variability of pyrethroid metabolite levels in bedtime, morning, and 24-h urine samples for 50 adults in North Carolina. Environmental research, 144, 81-91.

Morgan, M. K., Nash, M., Barr, D. B., Starr, J. M., Clifton, M. S., & Sobus, J. R. (2018). Distribution, variability, and predictors of urinary bisphenol A levels in 50 North Carolina adults over a six-week monitoring period. Environment international, 112, 85-99.

Phillips, M. B., Sobus, J. R., George, B. J., Isaacs, K., Conolly, R., & Tan, Y. M. (2014). A new method for generating distributions of biomonitoring equivalents to support exposure assessment and prioritization. Regulatory Toxicology and Pharmacology, 69(3), 434-442.

Pleil, J. D., & Sobus, J. R. (2013). Estimating lifetime risk from spot biomarker data and intraclass correlation coefficients (ICC). Journal of toxicology and environmental health. Part A, 76(12), 747–766. https://doi.org/10.1080/15287394.2013.821394

Pleil, J. D., & Sobus, J. R. (2016). Estimating central tendency from a single spot measure: A closed-form solution for lognormally distributed biomarker data for risk assessment at the individual level. Journal of toxicology and environmental health. Part A, 79(18), 837–847. https://doi.org/10.1080/15287394.2016.1193108

Pleil, J. D., Stiegel, M. A., & Risby, T. H. (2013). Clinical breath analysis: discriminating between human endogenous compounds and exogenous (environmental) chemical confounders. Journal of breath research, 7(1), 017107. https://doi.org/10.1088/1752-7155/7/1/017107

Sarigiannis, D. A., Karakitsios, S. P., Handakas, E., Simou, K., Solomou, E., & Gotti, A. (2016). Integrated exposure and risk characterization of bisphenol-A in Europe. Food and Chemical Toxicology, 98, 134-147.

Sobus, J. R., Tan, Y. M., Pleil, J. D., & Sheldon, L. S. (2011). A biomonitoring framework to support exposure and risk assessments. Science of the total environment, 409(22), 4875-4884.

Sobus, J. R., DeWoskin, R. S., Tan, Y. M., Pleil, J. D., Phillips, M. B., George, B. J., Christensen, K., Schreinemachers, D. M., Williams, M. A., Hubal, E. A., & Edwards, S. W. (2015). Uses of NHANES Biomarker Data for Chemical Risk Assessment: Trends, Challenges, and Opportunities. *Environmental health perspectives*, *123*(10), 919–927. https://doi.org/10.1289/ehp.1409177

Tan, Y. M., Sobus, J., Chang, D., Tornero-Velez, R., Goldsmith, M., Pleil, J., & Dary, C. (2012). Reconstructing human exposures using biomarkers and other "clues". Journal of toxicology and environmental health. Part B, Critical reviews, 15(1), 22–38. https://doi.org/10.1080/10937404.2012.632360

US EPA (2020a). Risk Evaluation for 1-Bromopropane (n-Propyl Bromide). Office of Chemical Safety and Pollution Prevention. EPA Document #740-R1-8013. https://www.epa.gov/sites/production/files/2020-08/documents/risk_evaluation_for_1-bromopropane_n-propyl_bromide.pdf

US EPA (2020b). Risk Evaluation for Cyclic Aliphatic Bromide Cluster (HBCD). Office of Chemical Safety and Pollution Prevention, EPA Document #740-R1-8006. Retrieved from https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluation-cyclic-aliphatic-bromide-cluster-hbcd

Bibliographic database search

A formal literature search was done between January and February 2021, to find potential case studies and other articles that would be useful in developing the guidance for using reverse dosimetry data to estimate exposure. The searches were conducted using Google Scholar, UC library, and Web of Science. The following search terms were used, with each set of search terms being used for each of the bibliographic databases.

- “simple pharmacokinetic model” AND “QSAR” AND “exposure”
- “simple pharmacokinetic model” AND “IVIVE” AND “exposure”
- “simple pharmacokinetic model” AND animal AND “exposure”
- Compartmental PK model AND “exposure” AND “QSAR”
- Compartmental PK model AND “exposure” AND “IVIVE”
- Compartmental PK model AND “exposure” AND animal
- “compartmental model” AND “reverse dosimetry”
- “reverse dosimetry” AND “exposure” AND “QSAR”
- “reverse dosimetry” AND “exposure” AND “IVIVE”
- “reverse dosimetry” AND “exposure” AND animal
- Biological monitoring AND fatty tissue
- Occupation* AND biomonitor* AND “daily intake”

- Toxicokinetic* AND animal AND biomonitor*

Specific search terms were also used to find case studies specific to consumer products. A total of 40 useful references were found using these search terms. The following search terms were used in Google Scholar, UC library, and PubMed:

- Consumer AND biomonitor* AND "daily intake"
- Consumer AND biomonitor* AND "reverse dosimetry"
- Consumer AND biomonitor* AND "exposure reconstruction"

Screening search results to identify potential case studies

Each article found from literature searching was scanned to see if it could qualify as a potential case study. To qualify as a potential case study, the following must apply:

- The study must utilize reverse dosimetry to estimate human exposure based on biomonitoring data
- The study must identify the parameters used and the source of each parameter (or how it was derived)

Case studies were eliminated from consideration if:

- The study utilized PBPK modelling approaches that are not the exposure conversion factor approach
- The study did not adequately address limitations (professional judgment was used to determine this). For example, Zhang et al. (2020) did not adequately address how using a pig F_{ue} may impact the results of the reverse dosimetry section of their study. Therefore, it was excluded from consideration.

The broader search was supplemented by targeted searching to identify studies relevant to the use of *in vitro* toxicokinetic data, particularly in the context of *in vitro* to *in vivo* extrapolation. This searching was based on key authors in the field (Wambaugh, Wetmore, Ring), as well as the terms httk and IVIVE.

Using these general selection criteria, 56 potential case studies were identified in the literature search. A total of 367 potential references were found that provided information relevant to the guidance document, of which 110 were cited in this document.

Appendix D –Expert Interviews

Several interviews of governmental scientists were conducted in the course of developing this guidance. The purpose of the interviews was to better understand how biomonitoring data and reverse dosimetry are being used in exposure assessments by the respective organizations, to identify issues associated with the application, and to obtain additional specific examples illustrating approaches used.

Interviews were conducted with the following groups:

- Health Canada - February, 2021: Scott Hancock, Kristin Macey, Devika Poddalgoda, Andy Nong

- US Environmental Protection Agency, February, 2021: Chris Brinkerhoff, Erin Hines, Peter Egeghy, Andrea Pfahles-Hutchens

- US Environmental Protection Agency, March, 2021: Joachim Pleil (former US EPA), Jon Sobus

The following questions were used as starting points for the interviews. Additional follow-up questions depended on the specific responses received.

--Can you provide example(s) of how you or your organization has estimated human exposure from human biomonitoring data and toxicokinetic data? (We have been doing a lot of digging into the literature, as I'll explain more on the call. Areas where we are particularly lacking in examples are for inhalation exposure and methods when we don't have human toxicokinetic data, particularly using in vitro and read-across methods, although other examples are also welcome.)
--In these examples, what key issues did you consider in interpreting the human biomonitoring data and interpreting the available toxicokinetic data?
--What were some of the challenges encountered and how did you address these challenges?
--What lessons did you learn for the chemical or class?
--What warnings do you have on pitfalls to avoid?

The results of the interviews were used to identify relevant publications and assessments to review for the methods discussed in the guidance. Information gained in the interviews was used to inform the content of this guide. We are grateful to the scientists who we interviewed for sharing their time and knowledge.

Appendix E – Definitions related to this Guide

The intended audience for this guide is practitioners with a working knowledge of biomonitoring data, toxicokinetic data, and exposure assessment. In several places throughout the guide, terms are used to characterize or group different kinds of chemicals, data, or models related to the use of Biomonitoring. Rather than define each term as it comes up throughout the guide, all of these terms were collected in this Appendix. For some terms, specific and citable definitions are available. In these cases, the definition is followed by a number that refers to the source list at the end of this glossary. For other terms, there is a more generic guideline or rule-of-thumb associated with these terms.

Chemical Specific Terms:

Endogenous - Naturally occurring chemicals that occur within the human body

Exogenous - Chemicals that have a non-human or external source.

Lipid soluble, also lipophilic - Chemicals with a logKoW > 4 (23)

Metabolite – Chemical compound that results from a chemical reaction in the body. A substance produced directly by a biotransformation of a chemical. Often, a chemical that enters the body is rapidly metabolized or otherwise difficult to measure or distinguish from external contamination. A metabolite may be more stable and also may be eliminated in urine, making it more accessible and easier to measure. (22)

Moderately-excreted compounds- Chemicals with an elimination half-life in the human body of 8-36 hours.

Non-volatile compound - Chemicals with a boiling point > 400 degrees Celsius (17; exact cutoff varies by organization)

Parent chemical - Chemicals prior to any metabolism; some parent chemicals may retain their chemical structure and integrity as they travel through the human body.

Rapidly-excreted, also short-lived compounds - Chemicals with an elimination half-life in the human body less than about 8 hours.

Semi-volatile organic compound (SVOC) - Chemicals with a boiling point between 250 and 400 degrees Celsius (17; exact cutoff varies by organization)

Slowly-excreted, also long-lived compounds - Chemicals with an elimination half-life in the human body of > 36 hours. This is a shorter half-life than often used to define this category, but is a pragmatic definition relative to typical biomonitoring sampling times.

Volatile organic compound (VOC) - Organic compounds that evaporate readily into the air (5). Chemicals with a boiling point < 250 degrees Celsius (17; exact cutoff varies by organization)

Water Soluble Compound - Chemicals with a logKoW < 1 or water solubility >10 mg/L

Exposure Assessment Terms:

Average daily dose, also intake, also applied dose - Dose rate averaged over a pathway-specific period of exposure expressed as a daily dose on a per-unit-body-weight basis. The ADD is usually expressed in terms of mg/kg-day or other mass-time units (2)

Biomonitoring - Systematic standardized measurement of concentration of a substance or its metabolites in human tissues (such as blood, urine, milk) (4); a method used to assess human exposure to chemicals by measuring a chemical, its metabolite, or a reaction product in human tissues or specimens, such as blood and urine (19).

Environmental monitoring - The process of sampling and analyzing the occurrence of chemicals in external media, such as air, water, food, or dust
Exposure pathway - The course an agent takes from the source to the target. (3) The route a substance takes from its source (where it began) to its end point (where it ends), and how people can come into contact with (or get exposed to) it. An exposure pathway has five parts: a source of contamination (such as an abandoned business); an environmental media and transport mechanism (such as movement through groundwater); a point of exposure (such as a private well); a route of exposure (eating, drinking, breathing, or touching), and a receptor population (people potentially or actually exposed). When all five parts are present, the exposure pathway is termed a completed exposure pathway. (5)
Exposed population or subpopulation - Also called receptor, a group of individuals who come into contact with the chemical of interest
Exposure source - The origin or starting point of a chemical; an object that releases the chemical into the environment; the origin of an agent for the purposes of an exposure assessment. (20)
Exposure scenario - A combination or description of an exposure source, exposure pathway, and exposed population (at a minimum) that define the conditions or situations where exposures occur. (3)
Exposure factors, and activity patterns - Human physiological and behavioral conditions that are used to calculate an average daily dose from an external media concentration. Examples of exposure factors include body weight, ingestion rates, inhalation rates, and skin-surface-area. Examples of activity patterns include time spent awake, time spent indoors, time spent at background vs. locations with elevated exposures.
External media, also medium - The material that the source chemical substance is contained within, such as water, soil, dust, food, consumer products. Some external media can also be characterized as exposure sources while others are better characterized as exposure pathways.
Internal dose, also uptake - The amount of a chemical substance that has been absorbed across absorption barriers per bodyweight per day. (1)
Personal monitoring - The process of sampling exposure in the immediate vicinity of an absorption barrier, such as measuring the concentration of air in an individual's breathing zone.
Source contribution - The relative contribution of one exposure source compared to another or compared to total exposure. This can be displayed in rank-order or as percent of the whole if multiple sources in an aggregate exposure assessment are quantified.

<u>Terms specific to Reverse Dosimetry and Biomonitoring:</u>
24-hour sample - Samples collected in 24-hour intervals. (10)
Absorption - The process of taking in. For a person or an animal, absorption is the process of a substance getting into the body through the eyes, skin, stomach, intestines, or lungs. (5)
Allometric scaling - Scaling of physiological rates or quantities to relative growth and size (mass or volume) of one animal species relative to another animal species. The relationship is generally written as $A = a(B)^k$, where A is the physiological process, B is a measure of the size of the organism (e.g., body weight) and a and k are constants (7)
Biological matrix – A discrete material of biological origin that can be sampled and processed in a reproducible manner. Examples are blood, serum, plasma, urine, feces, saliva, sputum, and various discrete tissues. (21)

Biomarker - (Short for biological marker) an objective measure that captures what is happening in a cell or an organism at a given moment. May measure exposure, response, or susceptibility. (8)
Clearance – The process of removal of a chemical from the body. This includes renal clearance - the volume of blood or plasma that could be freed of a specified constituent in a specified time (usually one minute) by excretion of the constituent into the urine through the kidneys (16) and metabolic clearance – the conversion of the parent chemical into one or more of its metabolites.
Compartmental model – A mathematical description of the flow of a chemical through the body. May contain a central compartment that represents the whole body (or plasma) where distribution occurs nearly instantaneously (one-compartment model) or an additional compartment (two-compartment model) where the distribution is affected by additional processes such as metabolism or sequestration into fat. PBPK models are an example of more complex compartmental models. Compartment models help characterize a chemical's kinetic behavior, and they are useful in deriving values for a chemical or drug's distribution in the body or clearance from the blood (i.e., half-life) (18)
Creatinine - A white crystalline strongly basic compound $C_4H_7N_3O$ formed from creatine and found especially in muscle, blood, and urine (15). Creatinine excretion is often used to normalize chemical concentrations in urine, because it is roughly constant on a daily basis and not affected by how concentrated the urine is, although creatinine excretion does vary with age, size, body weight, gender and race/ethnicity.
Distribution - Movement of a substance from the site of entry to other parts of the body. (9)
Elimination - The toxicokinetic process responsible for the removal or expulsion of a substance from the body. (9)
Forward dosimetry – (Mathematical calculations by which) an external exposure associated with a critical health effect or exposure guidance values (for example, presented in mg/kg bw/day) is converted to an internal dose. (6)
Half-life - The time it takes for half the original amount of a substance to disappear. In the context of biomonitoring, it is the time required for half of the original dose to be removed from the body, either as the parent compound or metabolite, i.e., the elimination half-life.
Intraclass correlation coefficient (ICC) - Calculated as the ratio of the between-person variance to the total variance (within + between). The values vary between 0 and 1. Higher values (greater than 0.5) indicate that variance between individuals is greater than variance within individuals and low ICC values indicate within-person variation is large compared to between-person variation. (10)
Lipid - A large and diverse group of organic compounds that contain primarily carbon and hydrogen atoms with a lesser amount of oxygen. Most lipids are insoluble in water but will readily dissolve in other lipids and in organic solvents. (9)
Lipid weight – Measurements that are based on the lipid fraction of the weight of the sample (1)
Mass balance - Method for evaluating kinetics of a chemical by accounting for all (major) inputs and outputs (intake and elimination) of a chemical. Ensuring mass balance allows input (dose) to be calculated based on measured output.
Metabolism - The conversion or breakdown of a substance from one form to another by a living organism. (5)
Physiologically-based pharmacokinetic (PBPK) model - A model that estimates the dose to a target tissue or organ by taking into account the rate of absorption into the body, distribution

among target organs and tissues, metabolism, and excretion on the basis of interplay among critical physiological, physicochemical, and biochemical determinants (18)
Plasma - The non-cellular, fluid portion of whole blood. (9)
Reverse dosimetry – The process of back-calculating the exposure to a chemical that would be consistent with a measured biomonitoring level in humans.
Serum - The clear yellowish fluid that remains from blood plasma after fibrinogen, prothrombin, and other clotting factors have been removed by clot formation (14)
Simple pharmacokinetic model, also one-compartment model – A model that estimates the change in concentration in one compartment over time given a specified exposure regime. It takes what comes in, or dose; subtracts what goes out via an elimination rate constant, k; and calculates the change in concentration of a chemical over time. (11)
Spot sample - Samples that are collected at a single point in time
Toxicodynamics - The determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response to an environmental agent (sometimes referred to as pharmacodynamics). (2)
Toxicokinetics - The determination and quantification of the time course of absorption, distribution, biotransformation, and excretion of chemicals (sometimes referred to as pharmacokinetics) (2)
Urine - Waste material that is secreted by the kidney, is rich in end products (as urea, uric acid, and creatinine) of protein metabolism together with salts and pigments, and forms a clear amber and usually slightly acid fluid. (13)
Volume of distribution - The volume of body fluid in which a compound is apparently distributed. It may consist of plasma, interstitial fluid, and intercellular fluid. (9)
Wet weight – Measurements that are based on the wet (or whole) weight of the sample. This in contrast to dry weight. (1)
Whole-blood - Blood with all its components (as white and red blood cells, platelets, and plasma) intact that has been withdrawn from a donor into an anticoagulant solution. (12)

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