Biomonitoring: Uses and Considerations for Assessing Non-Occupational Human Exposure to Pesticides

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3.1 INTRODUCTION

Biomonitoring is an important tool that can be used to evaluate human exposure to pesticides by measuring the levels of pesticides, pesticide metabolites, or altered biological structures or functions in biological specimens or tissues (Barr *et al.*, 2005b; Needham *et al.*, 2007; Needham *et al.*, 2005). These measurements in biological media, referred to as "biomarkers", reflect human exposure to pesticides through all relevant routes, and can therefore be used to monitor aggregate and cumulative exposures. Aggregate pesticide exposure is defined as exposure to a single pesticide from all sources, across all routes and pathways (USEPA, 2001a). Cumulative pesticide exposure is defined as exposure to multiple pesticides that can cause the same toxic effect via a common biochemical mechanism (USEPA, 2001a). The complexity of aggregate and cumulative pesticide exposures often obscures the linkages between exposure measurements and potential human health effects. Therefore, biomonitoring offers a means to clarify these critical relationships. However, careful interpretation of biomonitoring data is necessary to accurately assess human exposure to pesticides and the associated human health risks.

The purpose of this chapter is to provide an overview of the state-of-the-science for pesticide biomonitoring research. We first present the fundamental concepts and primary uses of biomonitoring, and then highlight the major criteria required for the selection and use of biomarkers in population-based exposure studies. Next we focus on factors that affect the use and interpretation of biomarkers of exposure for current-use pesticides. We conclude by identifying critical data gaps and research needs in the field of biomonitoring; the consideration of these factors in future studies will better inform assessments of exposure, dose, and risk.

3.2 LINKING PESTICIDE EXPOSURE TO HEALTH EFFECTS

The relationships between human exposure to pesticides and possible health outcomes can be described using an exposure-effect continuum (Angerer *et al.*, 2006; Needham *et al.*, 2007; Needham *et al.*, 2005; NRC, 1987). As shown in Figure 1, major components of this continuum include exposure, internal dose, biologically effective dose, early biological effects, and ultimate health effects. Pesticide *exposure* refers to human contact with pesticides in environmental media (Zartarian *et al.*, 2005). Sources of pesticide exposure include such media as dust, soil, air, water, and food, and routes of pesticide exposure include inhalation, ingestion, and dermal contact. (The course that a pesticide takes from exposure source to exposure route is the exposure pathway (Zartarian *et al.*, 2005)). The amount of pesticide that enters the human body after crossing an exposure surface (e.g., skin, lung tissue, gastrointestinal tract) is referred to as the *internal dose* (ID) (USEPA, 2001b), and the amount of absorbed pesticide that reaches the target sites where biochemical alterations or adverse effects occur is the *biologically effective dose* (BED) (USEPA, 2001b). The BED leads to *early biological effects* (EBE), which are the structural and functional impairments within the body (resulting from pesticide exposures) that correlate with, and possibly predict, the ultimate health effects (NRC, 2006).

It is often difficult to directly relate exposure measurements to observed human health effects, considering the many sources and routes of environmental pesticide exposure. The exposure-effect continuum for pesticides can presumably be clarified with an understanding of the underlying biological functions and processes. In Figure 1, ID, BED, and EBE are highlighted as the key links between exposure and effects, and are therefore the focus of biomonitoring studies. Unfortunately, these key links (especially BED and EBE) often occur in inaccessible human tissues (e.g., liver, lung, and brain) and are therefore difficult to measure directly. However, surrogate biological measurements of ID, BED, and EBE, and factors governing these measures, can be made in readily available human fluids and tissues including blood, urine, saliva, semen, skin, breast milk, and expired air. These surrogate biological measurements are referred to as biomarkers of exposure, effect, and susceptibility (NRC, 1987).

Biomarkers of exposure include measurements of pesticides, pesticide metabolites, and modified molecules or cells (e.g., DNA and protein adducts) in biological tissues/fluids (e.g., blood) or excreta (e.g., urine). These biological measurements are directly related to the dose of a pesticide (ID and BED in Figure 1) and are a function of pesticide exposure.

Biomarkers of effect include measurements of biochemical, physiological, or behavioral alterations that result as a consequence of pesticide exposure. Some examples of biomarkers of effect include biological measurements of endogenous and inflammatory responses, measurements of DNA, protein, cell, tissue, and organ damage/modification, and observations of tumors or cancer cell clusters. These biological measurements reflect EBE (Figure 1), but are often difficult to ascribe to a specific pesticide exposure event.

Biomarkers of susceptibility include measurements of an individual's inherent ability to respond to pesticide exposures. These measurements include observations of molecular properties and functions, such as genetic polymorphisms and enzyme activities, which can affect the rates of pesticide absorption, distribution, metabolism, and elimination (ADME), along with an individual's biochemical disposition towards disease progression or repair. Biomarkers of

susceptibility are affected by a suite of exogenous and endogenous sources, and therefore, may be difficult to link to a specific pesticide exposure event.

Although not apparent in Figure 1, complex biochemical relationships, physiology (e.g., blood-flow rates), and toxicokinetic parameters (e.g., tissue:blood partition coefficients) link ID to BED and EBE. We illustrate some of these intricate relationships using examples of biomarkers of exposure, effect, and susceptibility in Figure 2. In this figure, squares represent biomarkers of exposure (which can be measured inside or outside the body), ovals represent biomarkers of effect, and a triangle represents biomarkers of susceptibility. The directional arrows Figure 2 represent the potential interactions between biological measures and processes. Since the biomarkers of effect and susceptibility shown in this figure are often difficult to attribute to a specific pesticide exposure event, the illustrated biomarkers of exposure, which include parent pesticides, pesticide metabolites, and pesticide adducts, remain the focus of pesticide biomonitoring research. For the remainder of this chapter we will focus specifically on biomarkers of exposure to assess human exposure to pesticides.

3.3 THE USES OF BIOMONITORING

Biomarkers of exposure from samples of human tissue, fluids, and excreta offer qualitative or quantitative evidence of pesticide exposure. These measurements are particularly useful in exposure research because they can highlight population-based exposure trends and improve estimates of pesticide exposure and dose.

3.3.1 Assessing Population-Based Exposure Trends

Biomonitoring is commonly used as a surveillance tool to identify baseline exposure levels in a population, trends in exposure levels over time, and unique sub-populations with higher exposure levels. Multiple biomonitoring studies have been conducted in the United States (U.S.) and abroad to evaluate human exposure to pesticides; examples of these studies are listed in Table 1. (A more comprehensive list of biomonitoring studies can be found elsewhere (Bouvier *et al.*, 2005)). Several of these studies have focused on exposure to specific pesticides within particular sub-populations, including pregnant women (e.g., the Center for the Health Assessment of Mothers and Children of Salinas [CHAMACOS] study (Castorina *et al.*, 2003)) and children (e.g., Children's Total Exposure to Persistent Pesticides and Other Persistent Organic Pollutants [CTEPP] study (Morgan *et al.*, 2007; Morgan *et al.*, 2005; Morgan *et al.*, 2008; Wilson *et al.*, 2004), the Minnesota Children's Pesticide Exposure Study [MNCPES] (Quackenboss *et al.*, 2000), and the German Environmental Survey for Children [GerES IV] (Becker *et al.*, 2008)). However, as clearly shown in Table 1, the Centers for Disease Control and Prevention's (CDC) ongoing National Health and Nutrition Examination Survey (NHANES) is the most comprehensive source of pesticide biomonitoring data, providing thousands of yearly measurements of individual biomarkers, stratified by age, sex, and race/ethnicity (CDC, 2003a). The *National Report on Human Exposure to Environmental Chemicals* (NER), a publication of the NHANES data, allows scientists and health officials to evaluate the specific pesticides to which the U.S. population is commonly exposed, to track trends in exposure levels over time,

and to set priorities on human exposure and human health research efforts (CDC, 2002; CDC, 2003b; CDC, 2005).

Numerous biomarkers are measured in the ongoing NHANES study to assess human exposure to organochlorine (OC) insecticides, organophosphate (OP) insecticides, carbamate insecticides, pyrethroid insecticides, and a variety of herbicides. Many OC insecticides for which biomarkers are measured (e.g., hexachlorobenzene and dichlorodiphenyltrichloroethane [DDT]) are no longer in use, or have restricted use in the U.S. However, because of their relatively high persistence in the environment and in the body, these biomarkers can still be measured in human specimens such as blood. Unlike the persistent OC insecticides, many current-use OP insecticides (e.g., chlorpyrifos and malathion), carbamate insecticides (e.g., carbofuran and propoxur), pyrethroid insecticides (e.g., permethrin and deltamethrin), and herbicides (e.g., 2,4-dichlorophenoxyacetic acid [2,4-D] and atrazine) are environmentally and biologically non-persistent (although some of their degradates may remain in the environment for a longer period of time). Therefore, biomarkers of these pesticides reflect more recent environmental exposures (i.e., hours or a few days). Since many of these non-persistent pesticides are still in use, biomonitoring can be used to identify the current exposure trends, to aid in the design of mitigation strategies to reduce broad-scale exposures, and to assess the effectiveness of exposure-mitigation efforts (CDC, 2005).

3.3.2 Improving Estimates of Exposure and Dose

Biomonitoring data can improve estimates of dose derived from exposure and kinetic models, since biomarker measurements consider all routes of pesticide exposure, and all physical, behavioral, and physiological sources of variability. Additionally, biomonitoring data can improve and validate existing exposure and kinetic models that are needed in population exposure studies where biomonitoring data are not available.

In the absence of biomonitoring data, dose (i.e., ID and BED) can be approximated using deterministic or probabilistic exposure models coupled with classical toxicokinetic (TK) or physiologically-based toxicokinetic (PBTK) models (a description of PBTK models is included in Chapter 4 of this section). Exposure models estimate route-specific exposures using measurements of contact duration and pesticide concentrations in environmental and personal samples (e.g., air, soil, dust, water, food, and skin samples). Since sample concentrations can vary over time and space (e.g., outside vs. inside), repeated measures are often necessary to highlight exposure variability and to improve exposure estimates. Additionally, since pesticide exposures can vary according to subject-specific traits and activities, exposure models must consider observations of behavioral (e.g., hand-to-mouth activities for non-dietary ingestions and food intake for dietary ingestion) and physical (e.g., exposed skin surface area for dermal exposure and ventilation rates for inhalation exposure) sources of variability.

Once route-specific exposure estimates are produced from the exposure models, they are used as the input terms for TK or PBTK models to generate a dose estimate. Numerical constants used in the kinetic models (describing the ADME processes) are frequently derived from *in vitro* measurements and from *in vivo* rodent studies. Therefore, uncertainty may be associated with these kinetic parameter estimates.

Considering the many sources of variability and uncertainty associated with dose estimates from exposure and kinetic models, biomonitoring is now recognized as a valuable

quantitative tool that can be used in concert with exposure and kinetic models to improve dose estimates. Moreover, biomonitoring can help explain the relationships between exposure and biomarker measurements using a forward dosimetry approach, and can be used to work backwards from biomarker measurements to exposure estimates using a reverse dosimetry approach. This information can be used to improve the human health risk assessment of pesticides.

3.3.2.1 Forward Dosimetry

Forward dosimetry is an approach that can be used to understand the quantitative relationships between pesticide exposures and observed biomarker concentrations. In forward dosimetry, estimated or measured pesticide concentrations from environmental and personal (non-biological) samples are used as inputs into probabilistic or deterministic exposure models to estimate pesticide dose. The dose estimate (based on aggregate intake) is then compared to a measured biomarker concentration; the results from this comparison provide necessary information regarding the important sources and routes of human exposure to pesticides and can be used to identify missing sources and routes of exposure. This information is valuable for the interpretation of existing biomarker data, and for the design and execution of future population-based exposure studies. Forward dosimetry can also be used to estimate biomarker levels resulting from pesticide exposures at regulatory/guidance levels (e.g., reference doses or concentrations [RfDs and RfCs]) that are considered to be acceptable or safe (Hays *et al.*, 2007). Comparing these estimated values to observed levels from population-based biomonitoring studies is particularly useful for human health risk assessment. The methods and applications of forward dosimetry are more thoroughly discussed in Chapter 4 of this section. To date, few exposure and biomonitoring studies have been designed to use this forward dosimetry approach (Morgan *et al.*, 2007; Morgan *et al.*, 2005; Wilson *et al.*, 2007).

3.3.2.2 Reverse Dosimetry

Reverse dosimetry (exposure reconstruction) is an approach that can be used to work backwards from biomarker measurements to estimates of human exposure to environmental pesticides. Reverse dosimetry, like forward dosimetry, requires the use of exposure models and kinetic models to address heterogeneity in environmental exposure measurements, and in the rates of ADME. This method, utilizing modeling results and measurements of biomarkers from observational studies, has the potential to yield exposure estimates that can be compared to regulatory/guidance levels (Hays *et al.*, 2007).

Unfortunately, reverse dosimetry requires the use of numerical model inversion techniques, and does not yield a unique solution, but rather, a range of potential exposure scenarios (Clewell *et al.*, 2008). Thus, there is uncertainty associated with exposure reconstruction estimates. Reducing uncertainty in exposure estimates will rely on an improved understanding of likely exposure scenarios and the factors affecting variability in toxicokinetic properties. Specific inversion techniques used to reconstruct environmental exposures are discussed in Chapter 4 of this section, and a more extensive review of up to date methods for exposure reconstruction, as well as the mathematical fundamentals of a computational

framework, are presented in Georgopoulos, *et al.* (Georgopoulos *et al.*, 2009). To our knowledge, there are currently no observational studies of environmental pesticide exposure that have been designed to use this approach.

3.4 BIOMARKER SELECTION AND USE

Biomarkers of exposure should be, at a minimum, sensitive, specific, valid, biologically relevant, and easy to collect (i.e., practical) in order to be useful as a surveillance tool and for improving quantitative estimates of exposure and dose (Metcalf and Orloff, 2004). Here we examine against these criteria the most commonly used biomarkers of pesticide exposure. Table 2 lists the 45 individual biomarkers of pesticide exposure, grouped by pesticide class, that were measured in NHANES during 1999-2002 (CDC, 2005); these data represent the most comprehensive set of published pesticide biomarker data to date. Listed for each biomarker in this table is the sample matrix used for analysis (addressing the issue of practicality), the overall geometric mean (GM) published in CDC's latest NER (addressing the issue of sensitivity), and the minimum number of parent compounds (addressing the issue of specificity). We use this information here to evaluate individual analytes as useful biomarkers of pesticide exposure.

3.4.1 Sensitivity

Undetectable levels of pesticide biomarkers can be an indication of infrequent and/or low-level pesticide exposures, or an indication of insufficiently-sensitive analytical methods. It has been suggested that biomarkers should be measureable even at very low doses, and should vary consistently and quantitatively with respect to exposure (Bernard, 1995; NRC, 1987). Despite advances in analytical techniques that have allowed the measurement of pesticides at ultra-trace levels, sensitivity issues still impair the quantitation of individual analytes in biological samples. In CDC's most recent NER (CDC, 2005), GM values for individual biomarkers were not calculated when the proportion of results below the analytical limit of detection (LOD) was greater than 40%. As shown in Table 2, out of a total of 45 individual pesticide biomarkers measured in NHANES during 1999-2002, a total GM value was calculated for only 6 biomarkers based on the most recent survey data (CDC, 2005). (Total GM values were reported for an additional 7 biomarkers based on the 1999-2000 survey data; the apparent drop in sensitivity from the 1999-2000 data to the 2001-2002 data may indicate a decrease in exposure to individual pesticides over time). Considering these data, there is insufficient sensitivity to measure the majority of these analytes as biomarkers of environmental pesticide exposure. We note that several computational methods can be used to impute values for measurements that fall below the analytical LOD (e.g., LOD divided by 2 or LOD divided by the square root of 2 (Hornung and Reed, 1990)). Discussions of the most appropriate ways to treat values below the analytical LOD have been published (Helsel, 2005; Pleil and Lorber, 2007); however, this is still a topic of much debate.

3.4.2 Specificity

A useful pesticide biomarker should be specific for a parent compound of interest (Metcalf and Orloff, 2004). Specific pesticide biomarkers can be used to assess aggregate exposure, since the biomarker measurement reflects exposure to one parent compound from all exposure sources and through all exposure routes. Table 2 shows that a little over half (28 out of 45) of the biomarkers of pesticide exposure measured in NHANES during 1999-2002 were specific for a single parent compound (CDC, 2005), and therefore, are suitable for the assessment of aggregate exposure. In many cases, measured pesticide *metabolites* are not specific biomarkers because they are common to multiple parent compounds. This situation is clearly demonstrated with the six dialkyl phosphate metabolites of OP insecticides, which include dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). These six metabolites can be produced from the metabolism of several different OP insecticides (e.g., chlorpyrifos, diazinon, malathion, and parathion). Therefore, when using these biomarkers to assess exposure, the relative contribution from each OP insecticide must be known to accurately quantify the contribution from a single parent compound.

While non-specific pesticide biomarkers are not ideal for assessing aggregate exposure, they can be useful for assessing cumulative exposure, which involves exposure to multiple parent compounds involving a common mechanism of toxicity. In a case study of the CHAMACOS cohort (Castorina *et al.*, 2003), the six non-specific dialkyl metabolites of OP insecticides (i.e., DMP, DMTP, DMDTP, DEP, DETP, and DEDTP) were measured in the urine of 446 pregnant women to assess cumulative OP insecticide exposures. Here, OP insecticide cumulative dose equivalents (calculated using the relative potency factor [RPF = the ratio of the toxic potency of a given chemical to that of an index chemical] of each relevant OP insecticide in the cumulative assessment group) were calculated using non-specific biomarker measurements to assess exposure risks for the pregnant women (Castorina *et al.*, 2003). This application demonstrates the utility of non-specific biomarkers for the assessment of cumulative exposure and dose.

3.4.3 Validity

A selected biomarker of pesticide exposure should be a valid indicator of an underlying exposure event. That is, a biomarker measurement should accurately reflect the magnitude of exposure to a specific pesticide. Unfortunately, many pesticides breakdown in the environment to produce degradates that are chemically equivalent to biological metabolites. Therefore, biomarker levels can reflect exposure to the parent pesticides and to their environmental degradates. For example, the OP insecticide chlorpyrifos can degrade in the environment to 3,5,6-trichloro-2-pyridinol (TCPy), which is commonly measured as a urinary biomarker of chlorpyrifos exposure. In residential settings, exposures to chlorpyrifos and TCPy can occur from several sources such as soil, dust, air, and food, and through several routes including inhalation, ingestion, and dermal contact (Morgan *et al.*, 2005). This information, combined with the fact that toxicological research has shown that rats orally exposed to TCPy excreted all of it unchanged in their urine (Timchalk *et al.*, 2007), indicates that humans likely excrete in their urine substantial amounts of unchanged TCPy as a function of environmental TCPy exposure. This scenario, particularly in residential settings, can lead to an overestimation of

exposure to the parent compound when relying on biological *metabolites* as urinary biomarkers of exposure (Duggan *et al.*, 2003). This issue is widely applicable in pesticide biomonitoring research, because any pesticide that is hydrolytically metabolized in the body is likely to be metabolized in the environment.

3.4.4 Biological Relevance

A biomarker of exposure ideally should be relevant to the exposure-effect continuum shown in Figure 1 (Metcalf and Orloff, 2004; NRC, 2006). In other words, the most useful biomarkers not only reflect pesticide exposures, but increase our knowledge of the underlying biological events that lead to potential health effects (Schulte and Talaska, 1995). We discussed in the previous section that TCPy is a commonly measured urinary metabolite of chlorpyrifos. Although not specific to chlorpyrifos, DEP and DETP can also be measured in the urine to assess environmental chlorpyrifos exposure. However, each of these urinary metabolites is the result of detoxifying biochemical reactions (Timchalk *et al.*, 2007). Since the *in vivo* toxicity of chlorpyrifos is a result of bioactivation into chlorpyrifos-oxon (CPO), the measurement of CPO in a biological matrix is presumably more biologically relevant than that of a detoxified compound (Timchalk *et al.*, 2007). Thus, provided that adequate analytical techniques exist, and the stability of the chemical in the matrix is sufficient, the measurement of a biologically relevant compound is preferable compared to the measurement of detoxification products. (Currently CPO is difficult to measure in samples of human blood (Timchalk *et al.*, 2002)). However, by measuring products of both activation and detoxification, the underlying biological processes of the exposure-effect continuum may be better explained. Additionally, the measurement of multiple compounds can be useful in identifying factors that may confound biomarker measurements (e.g., environmental metabolite residues and metabolic variations) (Timchalk *et al.*, 2007).

3.4.5 Practicality

To be useful in large-scale studies, biomarkers should be easy to obtain, store, and analyze (Metcalf and Orloff, 2004; NRC, 2006). As previously mentioned, biomarkers of exposure can be measured in samples of human tissues or fluids, and in samples of human excreta. In large pesticide biomonitoring studies (see Table 1), blood and urine are the most commonly used human tissues/fluids and excreta, respectively, because they are, relative to other matrices (e.g., breast milk, adipose tissue, cord blood, feces), abundant in supply, collected using relatively non-invasive techniques (particularly urine), and can be analyzed using well-established methods. In fact, all of the NHANES biomarker measurements listed in Table 2 were derived from samples of human blood and urine (CDC, 2005). Still, appropriate quality assurance/quality control measures must be in place when using blood and urine for pesticide biomonitoring research. Biomarkers, whether parent compounds, metabolites, or adducts, may not be stable in a biological matrix (or in a sample preparation matrix [e.g., solvent]) if archived prior to analysis. Additionally, samples that are inappropriately collected or stored can be subject to chemical contamination. Any changes for which biomarker levels are not adjusted

(i.e., loss due to instability or increase due to contamination) will influence chemical measurements and ultimately lead to inaccurate estimates of exposure and dose.

Another major issue of practicality in biomonitoring studies is the collection of biological samples from sensitive subpopulations, such as children. Children may be more highly exposed to pesticides than adults, due to obvious differences in diet, environment, and daily activities. Increased exposure can have a particularly large impact on children considering their smaller body masses, immature physiological systems, and rapid physical development (Needham and Sexton, 2000; O'Rourke *et al.*, 2000). Therefore, it is important to better understand children's exposure to pesticides using biomonitoring. One of the main difficulties in using biomonitoring to assess pesticide exposures in children are the logistics of sample collection. For children that are able to provide blood or urine samples, it may be difficult to acquire the volume necessary for chemical analysis. In addition, urine collection for very young children may require alternative approaches such as using urine bonnets (collection devices placed under toilet seats), disposable diapers, or diapers with removable inserts (Hu *et al.*, 2004).

3.5 FACTORS AFFECTING THE USE AND INTERPRETATION OF BIOMARKERS OF EXPOSURE

3.5.1 Kinetics

Toxicokinetic properties affect the concentrations of pesticides and pesticide metabolites in the body, the biological matrices best suited for biomarker analysis, and the variability in biomarker levels over time (Clewell *et al.*, 2008; Hays *et al.*, 2007). Thus, the selection, use, and interpretation of biomarkers should consider rate constants that describe ADME processes. Here we demonstrate, using simplified theoretical models, the impact of varying kinetic rate constants on predicted biomarker levels. For these examples, we use simple one-compartment toxicokinetic models, and assume that kinetic processes are first-order (i.e., dependent on the concentration of one reactant). Concentrations of pesticide metabolites in the blood are shown in response to a fictitious week-long random exposure profile (exposure during five days of the week but not during the weekend), repeated over nine weeks. While these models do not consider nonlinear processes such as metabolic induction or saturation, they are suitable to demonstrate the importance of kinetic functions in biomonitoring studies.

Elimination from the body is often discussed in terms of a pesticide's biological persistence; this is typically measured as a biological half-life, which is the time required for the biomarker level to decrease by one-half in the absence of further input (i.e., exposure). Biologically persistent pesticides, including many OC insecticides, are eliminated from the body over the course of months or years, whereas biologically non-persistent pesticides, including many current-use OP, pyrethroid, and carbamate insecticides, and herbicides, are eliminated from the body over the course of hours or days (CDC, 2005).

In Figure 3, we highlight the effect of elimination rate on pesticide levels in the body. Here we show the blood metabolite levels over time of three different pesticides, each with the same uptake rate, but with different elimination rates; biological half lives in this example are postulated as 1 day, 1 week, or 4 weeks. The blood concentration curves in this figure demonstrate that the elimination rate linearly affects the eventual stable level of each biomarker.

That is, doubling the biological half-life also doubles the eventual blood concentration. For the biomarker with a 4-week half life (representing moderate biological persistence), levels in the blood vary in the short term in response to exposure, but also continue to increase over the entire 9-week period. In fact, the magnitude of the short-term variability in relation to total variability is only marginal. Therefore, measurements of biomarkers of persistent pesticides that are made close in time are likely to be more similar than distant measurements. Considering this observation, biomarkers of persistent pesticides likely reflect exposures that have occurred over the previous months and even years, and can therefore be used to monitor seasonal effects and age-related effects in a population. For the pesticide with a 1-day half life, levels in the blood also vary in the short term, but do not accumulate with time. Therefore, non-persistent biomarkers reflect only the variability in recent exposures having occurred over the previous hours or days. These biomarkers are useful in evaluating recent exposure events, but require information regarding the time of sampling in relation to the time of exposure.

While Figure 3 demonstrates that the elimination rate can affect the eventual stable biomarker levels, Figure 4 demonstrates that the uptake rate (including absorption, distribution, and metabolism) can also affect biomarker levels, albeit in a somewhat dissimilar fashion. In this example, the elimination rate is held constant with half life of 1 week, and the uptake rate is varied; the uptake half times in this example are postulated as 1 hour, 4 hours, or 24 hours. Here we see the inverse effect of varying the elimination rate; that is, the faster the pesticide is absorbed, distributed, and metabolized, the higher the eventual steady state concentration. Additionally, the longer the uptake half time, the smoother the short-term fluctuation in biomarker levels. This scenario is applicable when we consider the different routes of pesticide exposure. For example, exposure to a pesticide through inhalation, ingestion, and dermal contact can all contribute to the internal pesticide dose, and we can assume that the pesticide will be eliminated at the same rate regardless of the exposure route. However, uptake of the pesticide through the skin may occur more slowly than through the gut, and uptake through gut may occur more slowly than through the lungs. Therefore, as shown in Figure 4, the magnitude and variability of the biomarker level will depend on route of exposure.

These examples of the influence of rate constants on biomarker levels highlight the importance of using kinetic models when designing biomonitoring studies and evaluating existing biomarker data. Moreover, these examples highlight the importance of not only understanding the most recent environmental exposure, but also, the exposure history for any given subject (i.e., between-subject differences in biomarker levels can occur because of true differences in environmental exposure levels or because of differences in recent exposure history). Finally, these examples highlight the importance of understanding the prominent routes of exposure when deciphering observed biomarker data. All of these factors are particularly important when using biomarker levels to quantify exposure and dose for the purpose of human health risk assessment.

3.5.2 Urinary Excretion Rate

In the previous section, we highlighted the effects of kinetic rates on pesticide biomarker levels. We noted that, while biomarkers of persistent pesticides typically reflect long-term exposure trends, those of non-persistent biomarkers reflect recent exposure events. Moreover, we pointed out that the relative variability in closely-spaced biomarker measurements of

persistent pesticides is smaller than that of non-persistent pesticides. Thus, biomarkers of non-persistent pesticides are only useful in evaluating recent exposure events when information is known about the time of sampling in relation to the time of exposure. Further complicating the assessment of biomarkers of exposure to non-persistent pesticides is the fact that most of these biomarkers are measured in the urine (see Table 2), and are therefore subject to variations in urinary excretion rates. Here we discuss the major considerations and current approaches for analyzing urinary biomarker measurements of current-use non-persistent pesticides.

Since pesticide exposures can vary over short periods of time, simple "snapshot" measurements of environmental and personal samples may not accurately assign the exposure profile. Likewise, since the concentrations of urinary biomarkers of non-persistent pesticides track closely with recent exposures, snapshot measurements of urine concentration may also misclassify an exposure profile. Nevertheless, biomarker concentration measurements from "spot" urine samples are very common in observational studies (see Table 1) to minimize the cost of sample analysis and burden on study participants.

First morning voids are often selected as representative spot urine samples because they integrate over the longest period of time; samples collected at some other random time during the day may also be employed primarily for convenience to the subject. Despite their widespread use, biomarker concentration measurements from spot urine samples can not provide a value for total excreted material which is necessary to estimate previously absorbed dose. This would require some additional knowledge of total urine volume, elapsed time, and some measures of uptake and elimination kinetics. In Figure 5 we show respective levels of urinary biomarker concentration (ng/L) and total excreted biomarker mass (ng) over a two day period (here we assume a constant rate of urine production and allow reasonable variations in the time between excretions). Calculated levels of concentration and excreted mass vary in daytime and evening measurements as a result of randomly assigned episodic exposures of different durations and magnitudes. Of particular note in Figure 5 are the first morning void estimates where levels of excreted mass are considerably elevated reflecting the longer period of accumulation in the bladder. This figure demonstrates that without adjusting for volume of the urine void and the time since last void, biomarker concentration levels can erroneously assign a pesticide dose.

Our simple example in Figure 5 does not address additional real-world variability in rate of urine production due to interindividual differences in age, sex, ethnicity, etc., and intraindividual differences affected by hydration, work load, environmental conditions, and other factors. As such, a measurement of urinary concentration alone can not be expected to provide anything more than a qualitative view into the current exposure state of the subject. Because urine output is not constant either within measurements from an individual or among individuals, creatinine adjustment of urinary metabolite concentrations has typically been performed to correct for urine dilution among spot samples (Barber and Wallis, 1986). The thought process behind this procedure is if creatinine excretion is reasonably constant, then the presence of higher concentration of creatinine in the urine would indicate that the urine is concentrated (i.e., the person has a low hydration state prior to sample collection) and the presence of lower concentrations would indicate that the sample was dilute (i.e., the person had a high hydration state prior to sample collection); the variations in hydration state are adjusted by dividing the urinary pesticide concentration by the creatinine concentration. Research has indicated, however, that for a given individual, metabolic variations including diurnal metabolism, disease status, vitamin supplementation, diet, and other factors can alter the rate of creatinine excretion (Boeniger *et al.*, 1993). Furthermore, interperson variation in creatinine excretion is large,

especially when the population is heterogenous (i.e., multiple ages, sexes, race/ethnicities) (Barr *et al.*, 2005c). Thus, creatinine adjustment of urinary data in population studies will often introduce, rather than reduce, variability (Barr *et al.*, 2005c). An alternative to creatinine adjustment is specific gravity adjustment (Hauser *et al.*, 2004; Meeker *et al.*, 2005). However, since specific gravity is largely dependent upon the dissolved solids in urine which are primarily derived from creatinine, specific gravity and urinary creatinine are highly correlated.

Urine samples can be collected over a period of time (e.g., 2-hr, 8-hr, 12-hr, and 24-hr urine voids) to better understand variations in urine volume and how they impact estimates of dose. Twenty-four hour urine voids that are *individually* collected and assessed may provide the information necessary to calculate total excreted biomarker mass and to estimate dose. However, 24-hour urine voids are often difficult to accurately collect, as they are burdensome to observational study participants. Additionally, 24-hour urine voids require large amounts of storage space and many collection and analysis materials.

3.6 SUMMARY AND SUGGESTIONS FOR FUTURE RESEARCH

The field of biomonitoring has greatly expanded over the past several decades (Needham *et al.*, 2007). A wealth of biomarker data has been generated to better understand population-based pesticide exposures in the U.S. and abroad (see Table 1). Pesticide biomarker measurements allow the assessment of trends and variability in population-based exposures, the improvement of pesticide dose calculations, and the validation of existing exposure and dosimetric models. However, without additional information, such as exposure measurement and activity pattern data and modeling techniques, it is often difficult to determine the most important routes of exposure, the magnitude and duration of exposures, and the impact of these exposures on human health risk. While this additional information is sometimes lacking in large-scale biomonitoring studies (see Table 1), it is vital for improving our understanding of the exposure-effect continuum (Figure 1).

Forward dosimetry is an approach that can be used to relate exposure and biomonitoring data to better understand important sources and routes of pesticide exposure. Reverse dosimetry is an approach that can be used to infer pesticide exposures from biomonitoring data when exposure data is limited or unavailable. This application of biomonitoring is critically important for protecting human health because our laws, regulations, and guidelines are based on measurements of pesticides in environmental media. It is now possible, using these available approaches, to enhance our capability to interpret biomarker measurements as a function of aggregate and cumulative exposure. The proper application of these forward and reverse dosimetry approaches can improve our understanding of the relationships between exposure, dose, health effects, and risk.

Biomarkers should be sensitive, specific, valid, biologically relevant, and practical to be useful in assessing exposure trends and in quantifying pesticide exposure and dose. In this chapter we have highlighted that many biomarkers of current-use pesticides may not be sufficiently sensitive to be useful biomarkers. Therefore, there is a need to improve our analytical capabilities, and to develop new methods or alternative approaches to measure biomarkers of pesticide exposure in biological matrices; these new approaches should consider the measurement of biologically relevant analytes. Additionally, the increased use of specific biomarker measurements is warranted to better understand aggregate pesticide exposures. For

non-specific biomarkers, more exposure data is needed to determine the relative contribution from each parent compound. For biomarkers that are affected by environmental degradates in addition to biologic metabolites, there is a need to better characterize exposure to the parent compounds vs. exposure to the environmental degradates.

One of the biggest challenges facing pesticide biomonitoring research is the interpretation of measurements of non-persistent current-use pesticides (i.e., OP, carbamate, and pyrethroid insecticides, and herbicides) in samples of human urine. Urinary measurements of non-persistent pesticide biomarkers are difficult to interpret in terms of exposure and dose because of their highly variable nature (reflecting variable exposure levels), and other factors that can influence their measurement (e.g., rate of urine production and excretion, time of sample collection). Therefore, studies are needed to show that urinary biomarker levels can accurately reflect exposure and dose after making adjustments for the aforementioned sources of variability. Presumably, this should first be demonstrated using 24-hour individual urine samples. Data can then be compared to values adjusted for creatinine concentration, specific gravity, or time of previous urination and volume of the current void. Comparisons between dose estimates fashioned from 24-hours voids vs. adjusted estimates will allow us to evaluate the suitability of using refined measurement techniques to accurately decipher exposure and dose. Positive findings from these studies could be used to improve large-scale population-based studies, in which dosimetry approaches could be performed using appropriate and cost-effective techniques.