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SOURCES OF VARIABILITY IN BIOMARKER CONCENTRATIONS

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Human biomonitoring has become a primary tool for chemical exposure characterization in a wide variety of contexts: population monitoring and characterization at a national level, assessment and description of cohort exposures, and individual exposure assessments in the context of epidemiological research into potential adverse health effects of chemical exposures. The accurate use of biomonitoring as an exposure characterization tool requires understanding of factors, apart from external exposure level, that influence variation in biomarker concentrations. This review provides an overview of factors that might influence inter- and intraindividual variation in biomarker concentrations apart from external exposure magnitude. These factors include characteristics of the specific chemical of interest, characteristics of the likely route(s) and frequency of exposure, and physiological characteristics of the biomonitoring matrix (typically, blood or urine). Intraindividual variation in biomarker concentrations may be markedly affected by the relationship between the elimination half-life and the intervals between exposure events, as well as by variation in characteristics of the biomonitored media such as blood lipid content or urinary flow rate. Variation across individuals may occur due to differences in time of sampling relative to exposure events, physiological differences influencing urinary flow or creatinine excretion rates or blood characteristics, and interindividual differences in metabolic rate or other factors influencing the absorption or excretion rate of a compound. Awareness of these factors can assist researchers in improving the design and interpretation of biomonitoring studies.

Biomonitoring in human biological matrices—usually blood or urine—has been called the "gold standard" for assessing human exposure to chemicals in the environment and through product use (Sexton et al., 2004). While human biomonitoring (HBM) has a long history of application in the realm of occupational exposure, the last 10 years have seen an expansion of application of biomonitoring to assessments of general population chemical exposures at generally far lower exposure levels. HBM has become a primary tool for exposure characterization in a wide variety of contexts, including population monitoring and characterization at a national level, assessment and description of cohorts, and individual exposure assessments in the context of epidemiological research into potential adverse health effects of chemical exposures.

This growth is due at least in part to improvements in analytical chemistry, including growing lab capacity and reductions in cost,

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coupled with the increasing focus on more subtle exposure levels that involve more complex exposure sources and routes of exposure. Focus on these lower level exposure contexts has resulted in increasing recognition of limitations of conventional exposure assessment approaches in characterizing relative exposure levels within and among different populations and, increasingly, individual subjects. The ability to capture a marker of internal exposure level through sampling of blood or urine presents a powerful alternative or supplemental tool for exposure assessment in these contexts. However, this shift to measuring internal exposures through biomonitoring also poses new challenges for interpretation (Angerer et al., 2011; Aylward et al., 2012; Bevan et al., 2012).

In general, measured biomarker concentrations (in the context of this review these are defined as biomarkers of exposure—primarily measures of chemicals and/or metabolites and reaction products in blood and urine) are interpreted as direct surrogates for exposure level, and biomarker concentrations are often implicitly assumed to be linearly related to external exposure levels. However, as with any method of characterizing exposure, there are numerous factors that influence variation in biomarker concentrations in addition to exposure magnitude. As use of HBM as an exposure characterization tool grows in the evaluation of population exposure levels and in increasingly complex and subtle studies of exposure and response, a more detailed consideration of these sources of variation grows in importance. A more in-depth understanding of the sources of variation may enable enhancements in study design to address some factors and enhance the relevance and accuracy of the collected HBM data as an exposure measure in the context of the goals of a specific study.

Any discussion of the application of HBM to chemical exposure characterization and assessment of variability in biomarkers requires some definition of terms. Exposure can be characterized in terms of external media concentrations such as milligrams per liter in water, milligrams per cubic meter in air, or milligrams per kilogram in diet. Exposure can also be characterized in terms of intake dose (usually rationalized to body weight: mg/kg bw-d) or absorbed dose (in mg/kg bw-d). In some cases absorbed dose is highly correlated to intake dose; however, some chemical-specific, physiological, or pathway-specific factors may result in significant differences between intake and absorbed dose. Finally, exposure may be characterized in terms of the peak concentration at a sensitive time point or integrated area under the curve or amount of a chemical at the target tissue of interest over a day, a week, or a lifetime. Each of these definitions has relevance and application in the environmental health arena. A regulator with responsibility for control of pollution sources may be most interested in the external environmental media concentration; an epidemiologist may be most interested in characterization of intake or absorbed dose; and a toxicologist may be most interested in concentration at a target tissue. Factors affecting variation in biomarker concentrations may influence the relationship between biomarker concentration and the various exposure definitions in different ways.

Similarly, the terms “variability” and “variation” require some consideration. Biomarker concentrations vary both within and between individuals. Within individuals, there are likely to be both within- and across-day variations in biomarker concentrations. Thus, the variation in biomarker concentrations observed in a population biomonitoring study reflects both within- and between-individual variation.

Variation in external exposure magnitude, both between individuals and for an individual within and across days, is the most obvious factor directly influencing variation in biomarker concentrations. However, the focus of this review is on factors that affect variation in biomarker concentrations apart from variations in external exposure magnitude, with a particular focus on biomonitoring for environmental exposures. These factors fall broadly into three main categories:

- Variation related to timing of sample collection relative to exposure events.

- Variation related to the physiological characteristics of the biological matrix (blood, urine).
- Variation related to inter individual variation in chemical toxicokinetics.

Our goals are to describe the factors influencing variation in biomarker concentrations and characterize the magnitude of variation attributable to a given factor. In the process, an attempt to identify factors that may lead to systematic, rather than random, error in relating biomarker concentrations to exposure magnitude or that may lead to over- or underestimation of exposure levels based on biomarker concentrations was also undertaken. This information can inform design and analysis of data from biomonitoring studies.

VARIATION RELATED TO TIMING OF SAMPLE COLLECTION RELATIVE TO EXPOSURES

As with many other methods of measuring exposure, biomonitoring may represent a snapshot in time. In most studies, biomarkers are measured in a single biological sample, typically blood or urine, collected at a specific time point. In biomonitoring studies in the occupational environment, there is often some information about the timing, duration, and perhaps magnitude of external exposure. However, in studies of general population groups, usually there is little or no information on when the previous exposure(s) to the chemical(s) of interest occurred. The interplay between the chemical-specific toxicokinetics and the timing of previous exposure(s) relative to sampling time dictates the measured concentration in a spot sample after a given dose. Temporal variation due to changes in biomarker concentrations associated with the kinetics of absorption, distribution, metabolism, and elimination (ADME) within an individual is reflected in varying spot sample concentrations in that individual at various times. Similarly, spot sampling from individuals in a population at random times since last exposure results in varying measured concentrations even if each person is exposed to a similar amount of chemical (Figure 1).

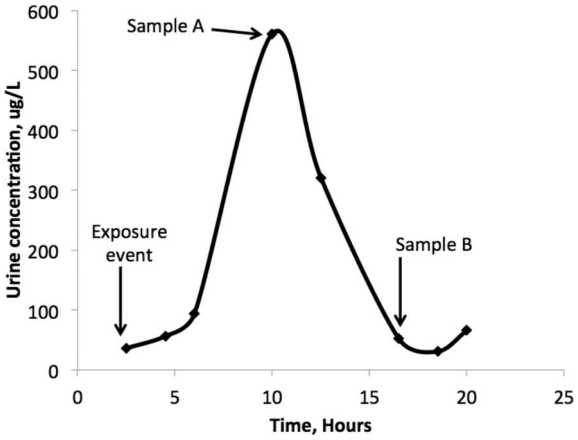

FIGURE 1. Concentration versus time curve for a chemical in urine following a single exposure event (actual data from a serial urine collection effort). If two people who have an identical exposure have urine samples taken at two different times following the exposure event (A and B in figure), the concentration of chemical in urine (or blood) can vary significantly. The results would likely be interpreted as indicating that Subject A had experienced higher exposure than Subject B.

The degree to which this temporal variation contributes to within- and between-individual variation in measured spot sample concentrations depends upon the relationship between the exposure interval and the half-lives of various pharmacokinetic processes relevant to the biomarker measurement (Aylward et al., 2012). In practice, the most important of these is typically the biomarker elimination half-life. Figure 2 illustrates conceptually the temporal variability in biomarker concentrations as a function of the exposure interval and half-life of elimination. For a given repeated constant external exposure dose, variability in biomarker concentration is greatest when the half-life is short and exposures are infrequent, and variability is lowest when half-life is long and/or exposures are frequent.

The impact of the relationship between the half-life of elimination and exposure interval on the variation in predicted biomarker concentrations was evaluated using simple first-order pharmacokinetic models by Aylward et al. (2012). In the simplest case, exposure events were simulated as instantaneously absorbed and distributed doses, with sampling occurring

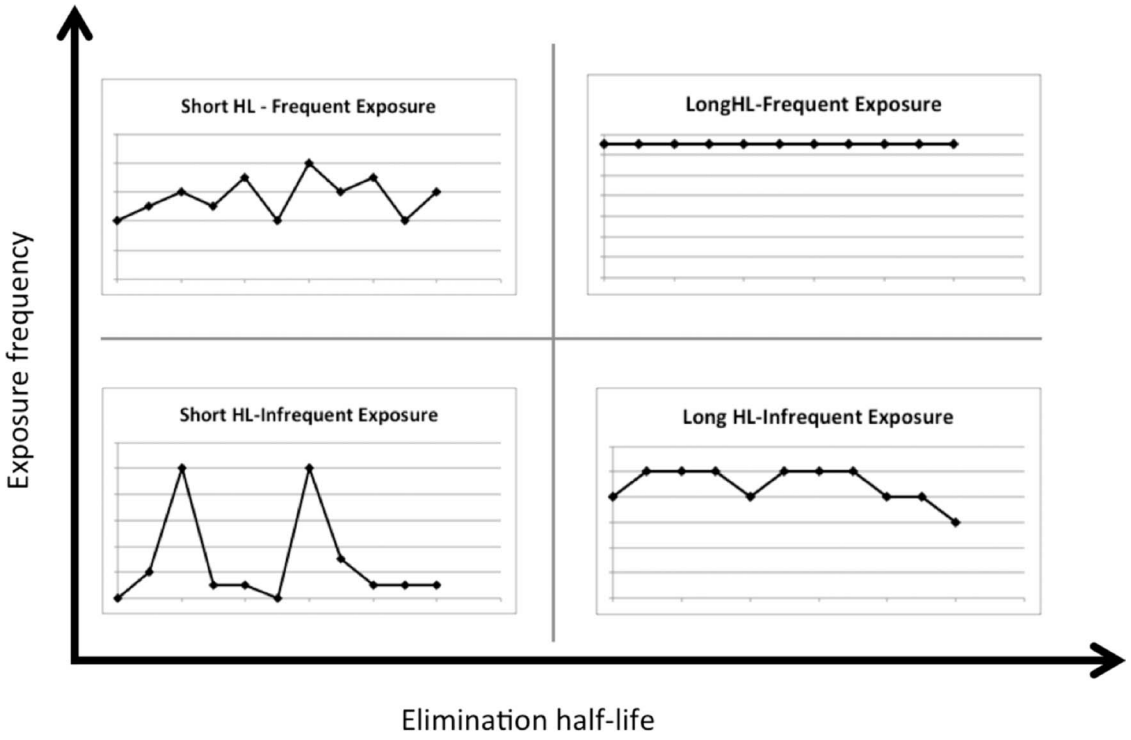

FIGURE 2. Conceptual schematic of the degree of variability in chemical concentration in a biological matrix as a function of half-life in the matrix and frequency of exposure. Concentrations are presented as relative concentrations, without units.

randomly in time from the resulting exponential decay curves. Predicted biomarker concentrations varied by more than two orders of magnitude when the half-life of elimination was one-eighth of the exposure interval (e.g., a 3-h half-life for a chemical with a once daily exposure pattern) (Figure 3). Inclusion of more realistic modeling of time-dependent absorption and a urinary compartment reduced the extremes of variation somewhat, but in practice, when the half-life of elimination was less than one-half of the exposure interval, variation in biomarker concentrations predicted for random spot sampling from such exponential decay curves exceeded the variation in underlying dose distributions.

The impact of rapid elimination half-life on the representativeness of spot biomarker samples can be seen in a recent study by researchers from the U.S. Centers for Disease Control and Prevention (CDC). Each urinary void over a week was captured for eight volunteers. The volume and timing of each void

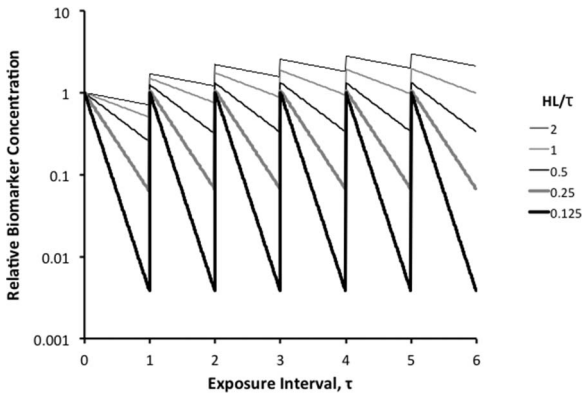

FIGURE 3. Simulated biomarker concentration vs time curves for a repeated untit dose at a consistent interval assuming different values for half-life of elimination as a fraction of the exposure interval, τ. Figure adapted from Aylward et al. (2012).

was recorded, and the urine samples were analyzed for bisphenol A (BPA), metabolites of two phthalates, and several polycyclic aromatic hydrocarbons (PAH) metabolites (Ye et al., 2011; Preau et al., 2010; Li et al., 2011). The impact of rapid elimination half-life on

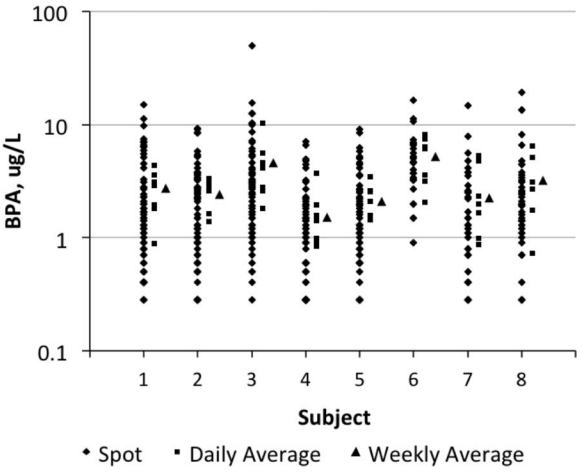

FIGURE 4. Distribution of spot sample, daily average, and weekly average concentrations of BPA for 8 volunteers in the CDC 1-wk urinary collection study (Ye et al., 2011). Figure adapted from Aylward et al. (2012).

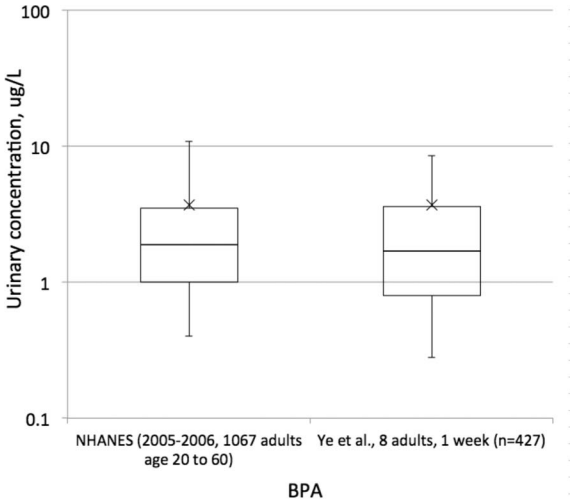

FIGURE 5. Distribution of measured spot sample urinary BPA concentrations in 8 volunteers in the CDC 1-wk collection effort (Ye et al., 2011) compared to the distribution of spot sample concentrations in the NHANES 2005–2006 survey for adults in the same age range. Boxes extend from the median to the 25th and 75th percentile and whiskers extend to the 5th and 95th percentiles for each data set. X indicates arithmetic mean.

variation in spot sample concentrations can be examined for BPA, which is eliminated rapidly and completely in urine, with elimination half-life of approximately 4 to 6 h (Volkel et al., 2005, 2008). Figure 4 shows the distributions of spot sample concentrations, the volume-weighted average daily concentrations, and the weekly average concentration for each of the eight individuals. While spot sample concentrations within and between individuals varied by approximately two orders of magnitude, daily average concentrations within individuals varied by less than a factor of 3, as did variation in weekly average concentrations across all 8 individuals. The distribution of spot sample concentrations from these eight individuals over the course of the one week period replicates almost exactly the distribution of spot sample concentrations in the NHANES 2005–2006 survey for adults of the same age range (Figure 5). These data suggest that range of average urinary concentrations over longer time periods in the population and, by extension, the distribution of intake doses in the population are less variable than suggested by the distribution of spot sample concentrations for compounds with rapid elimination behavior relative to exposure frequency.

This data set and other recent similar studies (Bradman et al., 2012; Frederiksen et al., 2012; Wielgomas, 2013) demonstrate that single spot samples may not allow accurate exposure characterization over even a short time frame (1 wk) for chemicals with short half-lives. Frederiksen et al. (2012) reported low intraclass correlation coefficients (ICC; the ratio of between-person variation to total variance in the studies population) for spot samples for characterizing longer term concentrations of various phthalate metabolites in urinary samples in young men in Denmark. Bradman et al. (2012) found that concentrations of organophosphate metabolites in urinary spot samples varied widely both within and between children over the course of a week. Single spot samples provided moderate sensitivity but high specificity for detecting high or elevated relative exposure level (top 20 or 40% of week-long average concentrations, respectively). That is, if an elevated concentration was measured in a single spot sample, it was likely that the individual had experienced an elevated week-long average exposure level. However, such exposures were not necessarily detected through reliance on a single spot sample. This is consistent with the exponential decay curve

for short-lived compounds when the interval between exposures is greater than twofold the half-life of elimination: On a time-weighted average basis, most of the decay curve will be at concentrations below the time-weighted average, while the period in which increased concentrations are present will be relatively short. Bradman et al. (2012) also reported large variation between days in exposure levels for individuals, indicating sporadic rather than consistent exposure rates for an individual, even over a short time period such as a week. This raises once again the question of what true exposure measure is relevant for the health outcome or health risk of interest in a given study—peak concentrations, short-term average exposure levels, or longer term average levels (which will also be chemical dependent)?

For chemicals with long elimination half-lives relative to intervals between exposures, different factors influence the variation in biomarker concentrations. For example, for chemicals with extremely long half-lives such as persistent organochlorine (OC) compounds, population variation in biomarker concentrations is strongly influenced by the historical patterns of changes in exposure levels in the population (Ritter et al., 2009, 2011). Thus, for some persistent compounds such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD), there are strong age-related trends in population biomarker concentrations, reflecting a steep decline in exposures over the past 40 years. For example, individuals exposed during the periods of highest general population dioxin exposures in the United States (late 1960s and early 1970s) display markedly higher serum lipid TCDD concentrations than younger persons, even as biomarker concentrations across all age groups fall due to declining external exposures (Hays and Aylward, 2003).

VARIATION RELATED TO BIOLOGICAL MATRIX

The biological media selected for sampling in biomonitoring studies also exhibit variations that affect biomarker concentrations independent of other factors. Urine and blood are the most common matrices used for biomonitoring studies. However, human milk has also been frequently used particularly for persistent organic pollutants (Berlin et al., 2005; LaKind et al., 2005). Hair and saliva are also candidate matrices for selected analytes (Esteban and Castano, 2009). This review focuses on physiological factors that may influence variability in analytes measured in blood and urine. However, analogous issues, as well as media-specific issues, need to be considered if other matrices are used. The information presented here does not address issues related to the potential impact of disease states such as renal or hepatic diseases on biomarker variation. However, such conditions may of course alter the relationships between external exposure and biomarker concentrations, and may therefore contribute to variation in biomarker concentrations observed in the population.

Some of the physiological variations related to urine and blood that impact biomarker concentrations can be characterized. Urine and blood differ in fundamental ways as biological matrices of relevant biological exposure. Urinary concentrations generally are not necessarily directly informative about concentrations at target tissues of interest in the body (e.g., concentration of hippuric acid in urine has no direct relevance to concentration of toluene in brain). In contrast, blood biomarker concentrations provide a measure of exposure that is more closely relevant to target tissue concentrations. However, even in the case of blood biomarkers, consideration of the relationship between the biomarker being measured and the toxicologically active compound is required. That is, if the biomarker is a deactivated metabolite, the relationship between the blood concentration of that metabolite and the active compound at the target site of interest may not be straightforward. However, as urine collection is a noninvasive procedure that is easily applicable for almost all population (sub)groups, it remains an essential matrix in all population HBM surveys, or for studies that require repeated sampling (Smolders et al., 2009a).

Urine as the Biomonitored Matrix

Variations in the urinary flow rate, that is, volume of urine produced per unit time, due to variations in hydration status both within and between individuals, may result in substantial variations in the concentration of biomarkers within this matrix when the mechanism of biomarker excretion is independent of urinary flow rate (which is common for most chemicals). Such variation occurs on several levels: Longer term average urine flow rates vary with age and may differ between genders (van Haarst et al., 2004). However, substantial variation within an individual occurs both within and across days.

In particular, spot sample urinary flow rates vary tremendously. The U.S. NHANES 2009–2010 survey cycle collected information on urinary flow rates for participants by measuring the void volume and asking participants the time of previous void. Figure 6 shows the distribution of urinary flow rates for the collected spot samples from nearly 7800 individuals from ages 6 to 80 years. The flow rates show a gradual increase with age, peaking in mid-adulthood, then declining gradually with age (Figure 6a). When rationalized to body weight, the pattern is somewhat different: Children exhibit higher flow rates per kilogram body weight than adults, suggesting that for the same intake of analyte on a micrograms per kilogram per day basis, the urinary concentrations are more dilute, appearing to be lower, than in adults (Figure 6b). The NHANES survey did not collect flow rate information for children younger than 6, and the literature is limited on this age group. However, the trend toward higher flow rates on a body-weight-adjusted basis appears to continue, with infants having the highest flow rates per kilogram body weight (Goellner et al., 1981; Heffernan et al., 2013).

In addition, urinary flow rates for spot samples among individuals of the same age vary from the central tendency by more than an order of magnitude, and this variation is greater than the variation in average rate across ages (see Figure 6). Further, within individuals, urinary flow rates vary greatly within and across

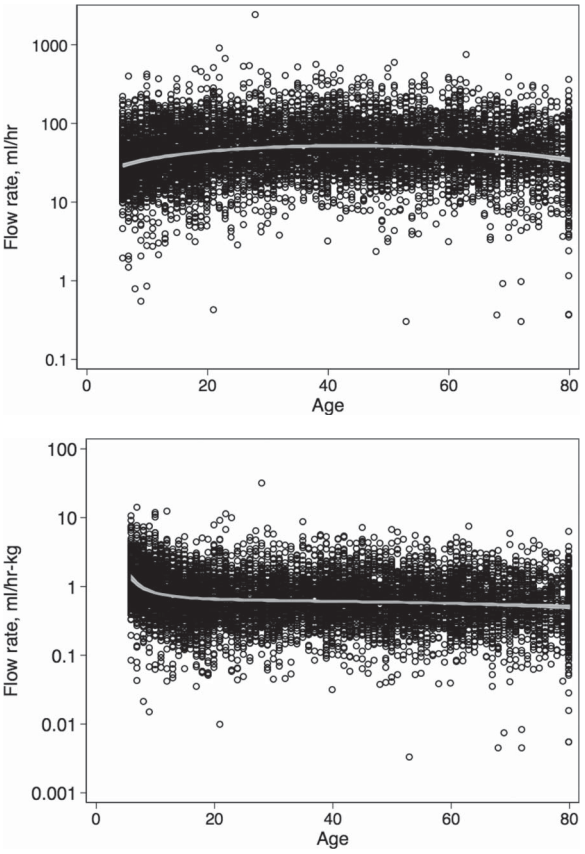

FIGURE 6. Spot sample flow rates as a function of age from NHANES 2009–2010. Flow rates were calculated as the volume of the collected void divided by the time since previous void: (A) absolute flow rates, ml/h; (B) flow rates per kg body weight.

days for spot samples. In a data set collected by researchers at the Centers for Disease Control and Prevention (CDC; Preau et al., 2010) in which each urine void over a week was collected for each of 8 individuals, the coefficient of variation for 24-h urine volumes for the each of the 8 individuals ranged from 18 to 27%; however, the coefficient of variation for the urinary flow rate in the spot samples for each of the individuals ranged from 50 to 115% (Table 1). In a recent study of similar design with collection of each urine void from 8 adults in Belgium over 6 d, similar coefficients of variation for 24-h urine volumes and urinary flow rates for spot samples were observed (respectively 17 to 34 % and 44–96%; data not shown). Historical changes in hydration status have also been noted. Twenty-four-hour

urine samples collected within the German Environmental Specimen Bank program suggest that adults are better hydrated today (urine output of 1600 ml/d on average) compared to 10 years ago (urine output of 900 ml/d on average) (Koch et al., 2012).

Creatinine correction has been used widely in biomonitoring studies as a method to adjust for variations in hydration status. In this approach, the measured biomarker concentration is divided by the measured creatinine concentration in the sample, resulting in a measure of mass of analyte per mass of creatinine. This approach relies upon the observation and assumption that for an individual, creatinine excretion occurs at a more constant rate than urinary flow. If the creatinine concentration is relatively low in a sample, this indicates a more dilute sample (higher urinary flow rate), while a higher creatinine concentration reflects a more concentrated sample (lower urinary flow rate). The CDC eight-subject study confirms that creatinine excretion rates are more constant for individuals than urinary flow rates; however, there remains substantial sample-to-sample variation in creatinine excretion rates within and between individuals (Table 1). However, again, creatinine correction is appropriate only when examining biomarkers whose rate of excretion is independent of the rate of urine production by the kidney. An example of a compound for which excretion is directly related to urinary flow rate is ethanol. In this case, adjustment using creatinine is inappropriate (Jones, 2006).

In addition, there are substantial age-related changes in creatinine excretion rates (Mage et al., 2008; Barr et al., 2005; Remer et al., 2002). Children excrete less creatinine per kilogram body weight than adults. If the creatinine correction approach is applied in studies in which samples are collected from children and from adults, children's creatinine-corrected concentrations will appear higher than those from adults for a given external exposure rate simply because the creatinine excretion rate per kilogram body weight is lower. Similarly, women excrete lower amounts of creatinine per kilogram body weight than

TABLE 1. Urine Flow Rates and Creatinine Excretion Rates From CDC 1-wk Study

	Urinary flow rates						Creatinine excretion rates					
	Spot samples, ml/h			24-h composite, ml/d			Spot samples, mg/h			24-h composite, mg/d		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
S1	106	72	0.68	2146	470	0.22	54.3	12.9	0.24	1266	73	0.06
S2	223	167	0.75	2410	*	*	54.7	15.9	0.29	814	*	*
S3	76	58	0.76	1477	270	0.18	48.4	23.8	0.49	1065	85	0.08
S4	219	195	0.89	3610	959	0.27	56.8	18.6	0.33	1274	332	0.26
S5	189	218	1.15	2191	431	0.20	90.9	40.1	0.44	2091	204	0.10
S6	67	34	0.51	1507	278	0.18	68.9	11.3	0.16	1630	34	0.02
S7	141	112	0.79	2289	460	0.20	79.0	20.3	0.26	1777	594	0.33
S8	100	106	1.06	1408	360	0.26	67.0	17.7	0.26	1475	197	0.13

Note. Study described by Preau et al., 2010; data requested and obtained from CDC; see Aylward et al. (2012) for further discussion of this data set).
*Only one day of complete urine collection for this subject.

men, and creatinine excretion declines with age in adults. Finally, creatinine excretion does not increase linearly with body mass, and thus systematic differences in creatinine excretion for individuals with elevated body mass index compared to lean subjects will also affect the calculated analyte excretion rates in terms of micrograms analyte per gram creatinine (Huber et al., 2011). Thus, application of the creatinine-correction approach to populations of varying ages, genders, and body mass indices results in differences in apparent exposure levels, resulting from differences in creatinine excretion rates, even if exposure levels in micrograms per kilogram per day are identical.

Urine specific gravity has also been used as a marker for assessing hydration status and variation in urinary flow rates (Boeniger et al., 1993; Dai et al., 2011; Heavner et al., 2006). The degree of variation in urine specific gravity, factors affecting that variation, and correlation to urinary flow rates have not been thoroughly studied. For example, Moriguchi et al. (2005) found that urine specific gravity declines with age in women over age 30. Specific gravity adjustment has been much less commonly used than creatinine correction to adjust for urinary flow rate in biomonitoring studies. Similarly, urine osmolality has been used as a method for adjusting for hydration status; however, few studies examined the factors other than urinary flow rate that may influence urinary osmolality (Perrier et al., 2013).

Urinary concentrations of biomarkers are also influenced by variations in urinary flow rate related to time since last void. As chemicals or their metabolites are cleared from the blood via the kidney, they are excreted into the urinary bladder at rates that are influenced both by the biomarker concentration in the blood and by the capacity of the kidney to remove the compound from the circulation. For compounds with rapid elimination half-lives, the rate of excretion from the kidney may change sharply over a period of a few hours. However, the urinary bladder acts as a holding reservoir for compounds that are actively excreted and not passively resorbed via osmosis (e.g., volatile organic compounds, or VOC). Thus, the concentration in urine at the time of voiding reflects a volume-weighted average of the excretion rate from the kidney, rather than the peak rate of excretion over the time period covered. Data from the NHANES 2009–2010 data set also demonstrate that there is a time-dependent decrease in urinary flow rates (see Figure 7). The combination of these two factors makes interpretation of a measured analyte concentration (which is the quotient of the mass of analyte excreted during the time covered by the void and the volume of urine produced during that same time period) in terms of exposure rate less straightforward.

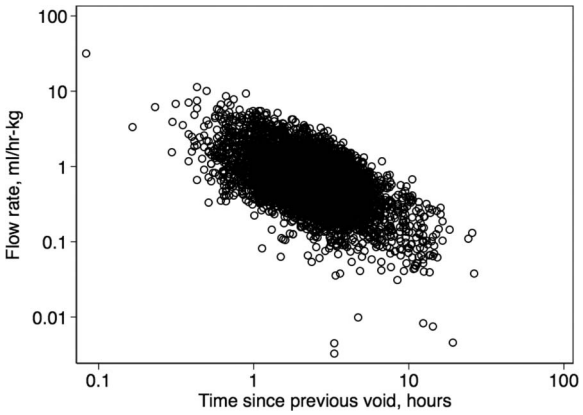

FIGURE 7. Flow rate per kg body weight as a function of time since previous void in the NHANES 2009–2010 database for adults ages 20 to 80 yr.

Further, urinary concentrations of biomarkers may also be influenced by the logistics behind a large-scale HBM survey. Traditionally, participants are asked to bring first-morning voids in predistributed containers, yet the representativeness of first morning voids in comparison to the "average void" throughout a day depends upon the exposure characteristics and pharmacokinetic properties of the chemical. In a current study conducted by the authors with collection of each urinary void over 6 d from 8 adults in Belgium, first morning voids generally were significantly larger, and may represent very different urine flow rates than the average daily void (data not shown; R. Smolders et al., personal communication). Taking into account that many chemicals that are the subject of HBM studies are taken up

through diet or through the use of personal care products, the discrepancy between timing of exposure (during the day) and collection of first morning voids may result in additional variability, depending upon the kinetics of the elimination of the compounds.

Finally, urinary flow rates may also be influenced by other physiological characteristics. For example, body-weight-adjusted flow rates appear to be negatively associated with BMI (Figure 8) in the spot samples collected during the NHANES 2009–2010 survey cycle. This indicates that, for a consistent dose in micrograms per kilogram per day across individuals of a compound that is eliminated in urine, the urinary concentrations will be higher (more concentrated in urine) in persons with higher BMI compared to persons of lower BMI. This may confound attempts to examine associations between BMI and chemical exposure as reflected in urinary concentrations (e.g., recently reported associations between urinary BPA concentrations and BMI in Trasande et al., 2012), since observed associations may be in part due to reverse causation: Higher BMI leads to lower urinary flow rates per kilogram body weight, resulting in more concentrated urine. Because information on flow rates such as that recently collected by the NHANES survey has not been routinely collected previously, such associations have not been recognized or analyzed. Additional efforts to evaluate and account for this phenomenon need to be undertaken.

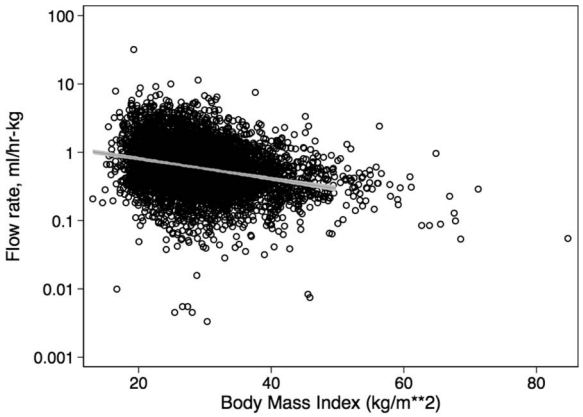

FIGURE 8. Spot sample flow rate (ml/h-kg BW) versus body mass index (BMI) for adults ages 20 to 80 yr in the NHANES 2009–2010 data set. Similar trends are evident in data for children and adolescents (data not shown).

Blood as the Biomonitored Matrix

Characteristics of blood or serum also influence the variation in measured blood biomarkers of exposure. The relationship between serum lipid and the measured wet-weight concentration (e.g., mass of chemical per volume of blood; μg/L) of highly lipophilic OC compounds is well known (Phillips et al., 1989). Serum lipid concentrations vary widely, both between individuals and within an individual over the course of a day following consumption of meals. Phillips et al. (1989) showed that wet-weight concentrations of lipophilic compounds varied directly with serum lipids, with wet-weight serum concentrations rising dramatically following consumption of a meal. However, normalization to an estimate of total serum lipid content resulted in a stable estimate of the concentrations of lipophilic substances (Phillips et al., 1989). Patterson et al. (1988) showed that for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, serum lipid-adjusted concentrations provide an accurate estimator for adipose tissue concentrations, reflecting the equilibrium in concentration among body lipid stores. As a result of these and other studies, lipid adjustment is a standard approach for examining the concentration of persistent highly lipophilic compounds in serum or blood.

However, lipid concentration in serum may influence the measured concentrations of other compounds that are lipophilic, even if they are not highly persistent. For example, serum concentrations of toluene are also positively associated with serum lipid content. In the NHANES 2003–2004 biomonitoring survey, serum concentrations of toluene in adults were positively associated with total serum lipids calculated using the formula of Phillips et al. (1989), with an approximately threefold rise in serum concentration associated with a 10-fold change in serum lipid concentration (Figure 9). However, lipid adjustment has not been routinely applied to evaluation of VOC in blood.

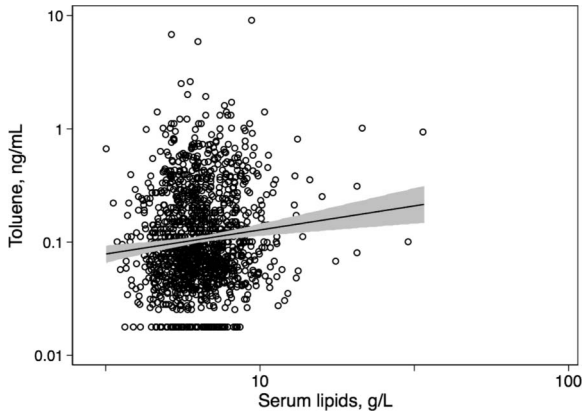

FIGURE 9. Serum toluene concentration vs. total lipids (calculated by the method of Phillips et al., 1989) in adults aged 20 to 60 yr in the NHANES 2003–2004 survey data. Line and shaded area are the linear regression and 95% CI.

Similarly, serum characteristics other than lipid content may influence the concentrations of other compounds with different physical/chemical properties. Perfluorinated compounds bind to protein components in the blood (D'eon et al., 2010). In the NHANES 2007–2008 survey, serum concentrations of perfluorooctanoic acid are associated with serum albumin concentrations in adults (Figure 10). Again, adjustment for serum protein content is not routinely included in assessment of serum concentrations of perfluorinated compounds.

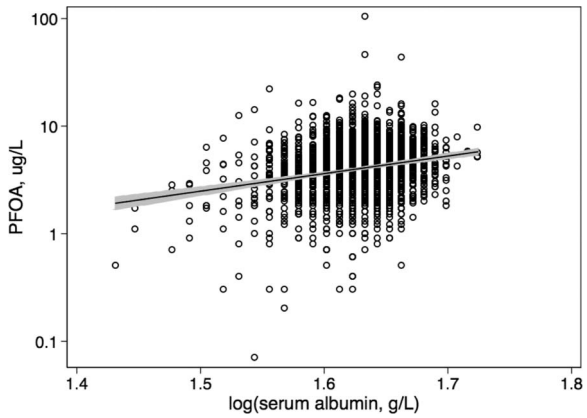

FIGURE 10. Serum PFOA concentration versus serum albumin in adults aged 20 to 60 yr in the NHANES 2003–2004 survey data. Line and shaded area are the linear regression and 95% CI.

VARIATION RELATED TO CHEMICAL TOXICOKINETICS (ADME)

The pharmacokinetic processes that influence biomarker concentrations of a given chemical (ADME) are all subject to interindividual variations due to variation in fundamental physiological parameters. For example, differences between individuals in age, body weight, body fat content, or other body composition parameters may substantially impact the effective volume of distribution for a compound or partitioning among tissues. Variations in respiration rates or cardiac output due to differences in physical activity may also influence uptake and distribution of compounds. Variations in metabolic capability due to genetic polymorphisms for key enzymes, developmental phase, or aging or disease could obviously influence both the extent and pattern of metabolism. Inhibition or induction of metabolic enzymes due to coexposure to other chemicals may alter metabolic rates within an individual. Finally, differences in clearance mechanisms including kidney function might impact efficiency of clearance of chemicals or metabolites from the body.

While each of these factors may vary substantially, the overall impact on biomarker concentrations may be less pronounced than the variation in individual parameters due to the fact that many processes are not rate-limiting and because of compensatory responses that may occur. For example, elevated respiration rate results in increased uptake of volatile compounds from air; however, the increased respiration rate also results in enhanced clearance via exhalation. Similarly, variations in enzyme expression levels and polymorphisms in enzymes may impact the rate of metabolism within a hepatocyte (Dorne, 2007). However, the variation in enzyme alleles and/or expression levels may not directly translate into variations in levels of parent compounds or metabolites circulating in blood or within tissues. This is because of limitations in delivery of compound to the site of metabolism, namely, resulting from limitations in delivery of compound to the liver, most often resulting from blood flow limitations,

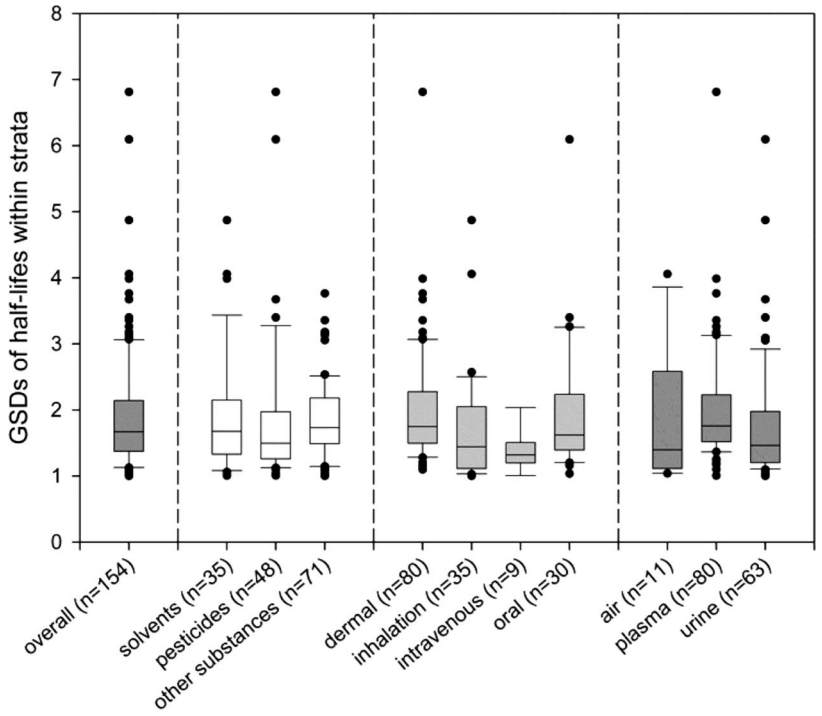

FIGURE 11. Variations (GSDs) in calculated half-lives for different strata, separate for several subgroups: (A) type of substance; (B) exposure route; (C) medium of analysis. Figure adapted from Spaan et al. (2010).

particularly at low environmental exposure levels (Kedderis, 1997; Kedderis and Held, 1996). The relative importance of metabolism versus flow limitations for compound clearance is also impacted by dose level (Dorne, 2007; Kedderis, 1997; Kedderis and Held, 1996). Physiologically based pharmacokinetic (PBPK) models provide a useful way of accounting for the various factors controlling overall compound elimination and how variations in the various factors (including rate of metabolism) are translated into whole-body clearance and concentrations of compound/metabolite in blood and tissues (Gentry et al., 2002; Nong et al., 2006; Kedderis, 1997; Kedderis and Held, 1996).

The impact of physiological variability between adults and children on predicted steady-state blood concentrations of VOC was assessed by Pelekis et al. (2001) using PBPK models. The simulations showed that while body weight, rate of ventilation, fraction of cardiac output flowing to the liver, blood:air partition coefficient, and hepatic extraction ratio all influenced the predicted blood concentrations resulting from a given external air concentration, the overall variation predicted between adults and children was less than a factor of three across a wide range of VOC. Similarly, Nong et al. (2006) examined the impact of the ontogeny of CYP2E1 capability in infants and children on the predicted blood concentrations of toluene in children and found overall variation of less than a factor of two between infants and adults (slightly higher differences were found between susceptible neonates and adults—a factor of less than four) as the enzyme capability and other physiological factors matured.

Examination of real-world data on variations in elimination rates across chemicals demonstrates significant interindividual variation, even among relatively homogeneous population strata. Spaan et al. (2010) used data from volunteer studies to assess the biological contribution to interindividual variability in biological monitoring responses. Volunteer studies were considered particularly useful as

individuals typically receive the same dose, which minimizes the influence of variation in external exposure, and sampling is typically conducted at similar time points across all individuals, which also reduces another potential source of variation. A comprehensive literature search of the published and grey literature was conducted to collect original data from human volunteer studies utilizing biological monitoring. Ultimately 41 studies were included in the analysis, comprising a total of 6747 observations for one or more biomarkers from 223 volunteers (Figure 11). Data from these studies were grouped on the basis of study, substance under investigation, exposure route, biological matrix, exposure duration, and dose. As many studies were either multidose or had collected samples in multiple matrices, such as urine and plasma, or more than one metabolite, this grouping resulted in 278 experimental data sets (strata) for statistical analysis.

Interindividual variability was assessed in two ways. First, estimates of biomarker half-life were calculated for each individual based upon an assumed exponential form of elimination, thereby allowing the estimation of interindividual variability in half-lives within each experimental setup. Second, variation across individuals in biomarker concentrations at a given time point was estimated.

As would be expected with a wide range of biomarkers, the estimated half-lives in different strata ranged considerably from 15 min to 1019 h. Within strata the distribution of half-lives across individuals was right skewed, so the geometric standard deviation (GSD) was taken as the measure of interindividual variability. Expressed this way the variability of half-lives ranged from 1 to 6.8, with a median of 1.67 and an interquartile range of 1.37–2.14. No systematic differences in variability of half-lives were found between the different types of substances, different dosing routes (inhalation, dermal and oral), or analysis matrix (plasma or urine). To put this in context, based upon a GSD of 1.67, a biomarker with a (median) half-life of 10 h would have an interquartile range (across individuals) of 7–14 h, with the biomarker having a half-life longer than 20 h in approximately 10% of individuals.

For concentrations at a given time point the average GSD within strata ranged from 1 to 5.6 with a median of 1.4 and an interquartile range of 1.21 to 1.75. This again suggests that approximately a two- to threefold variation from the median biomarker concentration would be expected across individuals at a given time point following a given exposure. Converting these estimates to an estimate of interindividual variance on a log scale gave a median variance of 0.11 (interquartile range 0.04–0.31). These latter values provide a useful benchmark for interpreting estimates of interindividual variation in observational studies of biomarkers in general or occupational populations, which typically express variability using the same metric (Morton et al., 2010, 2011; Symanski and Greeson, 2002; Sobus et al., 2010; Pleil, 2009). The variability was observed to be slightly higher for solvents than for pesticides and other substances. Dermal and oral administration of substances also seemed to result in more variability compared to administration via inhalation, possibly reflecting less well-understood uptake patterns via these routes as an additional source of interindividual variation.

DISCUSSION AND CONCLUSIONS

Exposure assessment is a critically important tool for risk assessment and public health evaluations. With advancements in analytical technologies, biomonitoring has become a preferred form of exposure assessment for many types of evaluations. While biomonitoring reduces many forms of uncertainties associated with an external exposure assessment (no need to make assumptions about how much food or water is consumed, how much air is inhaled, how often a consumer product is come in contact with, how much of the chemical is resorbed through the skin, etc.) (Hays et al., 2012), issues associated with variability need to be accounted for and/or considered when designing a sampling strategy. The

purpose of the risk assessment, epidemiology study, or public health evaluation, in combination with knowledge about the expected intra- and interindividual variability in biomonitoring levels should inform the biomonitoring exposure assessment sampling strategy.

As with any exposure assessment technique, biomonitoring offers advantages and disadvantages. The advantages are well known and include reducing the number of assumptions that have to be made regarding contact and consumption rates with media/consumer products and food/water. Further, HBM as an integral measure over all routes and sources may elucidate potential exposure magnitudes that have not been anticipated or have been neglected in external aggregate exposure assessments and/or models. In other words, aggregate exposure approaches are designed to model exposures that might occur (often in a combination with worst case assumptions), while HBM provides valuable information on the extent of total exposure that actually occurred without per se giving any information on routes and sources. Integrating these two perspectives is the focus of many current research efforts (Koch et al., 2013; Scher et al., 2008; Smolders et al., 2009b; Tan et al., 2012).

A common criticism of biomonitoring is that it represents only a snapshot in time of a person's internal exposure. However, conventional exposure assessments also rely on exposure data that is generated by gathering concentrations of a chemical in a media/product at a snapshot in time (e.g., sampling a batch of food products at a specific point in time). In addition, modeling dermal absorption, which is highly relevant for many consumer products, is complex and often governed by default assumptions that in many cases can only be verified by means of human biomonitoring. Understanding issues related to biomonitoring variability and representativeness will assist exposure assessors and those who rely on them in the accurate interpretation of study findings.

While there are several sources of variability and uncertainty that will impact interpretation of biomonitoring data, these sources of variability are often less than the uncertainties associated with conventional external dose-based exposure assessments (Hays et al., 2012). With the appropriate understanding and appreciation of the relevant factors and corresponding enhancements to study design and data interpretation, some of the sources of variability that may exist for biomonitoring for a specific chemical can be accounted for in the interpretation of study findings. The sources of variability in biomonitoring data highlighted in this review may assist researchers in improving the design and interpretation of biomonitoring studies to achieve the intended goals, while accounting for and/or compensating for sources of variability.

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