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ORIGINAL ARTICLE

Interpreting variability in population biomonitoring data: Role of elimination kinetics

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Biomarker concentrations in spot samples of blood and urine are implicitly interpreted as direct surrogates for long-term exposure magnitude in a variety of contexts including (1) epidemiological studies of potential health outcomes associated with general population chemical exposure, and (2) cross-sectional population biomonitoring studies. However, numerous factors in addition to exposure magnitude influence biomarker concentrations in spot samples, including temporal variation in spot samples because of elimination kinetics. The influence of half-life of elimination relative to exposure interval is examined here using simple first-order pharmacokinetic simulations of urinary concentrations in spot samples collected at random times relative to exposure events. Repeated exposures were modeled for each individual in the simulation with exposure amounts drawn from lognormal distributions with varying geometric standard deviations. Relative variation in predicted spot sample concentrations was greater than the variation in underlying dose distributions when the half-life of elimination was shorter than the interval between exposures, with the degree of relative variation increasing as the ratio of half-life to exposure interval decreased. Results of the modeling agreed well with data from a serial urine collection data set from the Centers for Disease Control. Data from previous studies examining intra-class correlation coefficients for a range of chemicals relying upon repeated sampling support the importance of considering the half-life relative to exposure frequency in design and interpretation of studies using spot samples for exposure classification and exposure estimation. The modeling and data sets presented here provide tools that can assist in interpretation of variability in cross-sectional biomonitoring studies and in design of studies utilizing biomonitoring data as markers for exposure.

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INTRODUCTION

Biomonitoring has been hailed as the "gold standard" of exposure assessment for environmental chemical exposures[1] because it demonstrates and measures markers for biologically absorbed chemical in the body. In this respect it is distinct from, and in many cases is arguably superior to, conventional exposure assessment methodologies, which rely on measures of chemicals in external media (air, water, and foods) and estimates of contact rates in order to estimate daily intake levels. Recognition of the value of biomonitoring data has led to widespread incorporation of biomonitoring in the design of studies that examine potential associations between health outcomes and chemical exposures and that evaluate population exposures to chemicals.

Implicit in the use of biomonitoring data in these frameworks is an assumption that the relative magnitude and variation of measured biomarker concentration in a biological medium such as blood or urine is directly reflective of magnitude and variation of external exposure to the chemical. Thus, for example, reverse dosimetry approaches have been used to estimate the distribution of daily intakes of phthalates, bisphenol-A, various pesticides, and other chemicals corresponding to the distribution of measured urinary biomarker concentrations observed in population surveys.[2–4] More sophisticated reverse dosimetry approaches have been described and demonstrated (reviewed in Clewell et al.),[5] but the simple urinary reverse dosimetry approach is the approach used most commonly.

Although not often discussed explicitly, this approach includes an implicit assumption that the measured concentration in a spot sample (a single urinary aliquot at a point in time) is a reasonable surrogate for 24-h average urinary concentration (either on a volume or creatinine-corrected basis). That is, urinary concentrations are assumed to be directly related in a mathematical sense to intake rates over the previous short time window. An intake dose (D, mg/day) is calculated based on a measured spot urinary concentration (C), assumptions regarding 24-h urinary volume or creatinine excretion (V_{24} or Cr_{24}), and data on the fraction of ingested parent compound excreted in urine (F_{UE}, either as parent or as the measured metabolite)[6]:

$$D = \frac{C * (V_{24} \text{ or } Cr_{24})}{F_{UE}} \quad (1)$$

Dose estimates are then normalized to bodyweight (measured or nominal) to obtain estimated daily doses in risk assessment-relevant units of mg/kg-day. This equation relies upon data from controlled human exposure studies that measure the total amount of parent compound or metabolite output in urine per unit of

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parent compound ingested (F_{UE}) over a time frame sufficient to account for full metabolism and elimination of the subject compound as well as an assumption of steady-state exposure. Even when no explicit calculation of intake doses is made, the variation in sample concentrations is often implicitly or explicitly assumed to be directly correlated with the variation in dose and is used, for example, as the exposure metric in linear regression analyses examining potential relationships to health outcome measures.

If the biomarker concentration is stable (as would occur for a compound with a long elimination half-life and frequent consistent exposures), the urinary reverse dosimetry calculation is appropriate; alternatively, collection of 24-h urine samples also provides appropriate concentration data for estimation of a daily dose (but is generally impractical). However, many of these compounds have very short half-lives relative to intervals between exposures, and substantial intra-individual, within-day variation in biomarker concentration has been demonstrated for many commonly examined analytes because of their rapid absorption and elimination.[7–10] Direct use of the spot urinary concentrations from an individual over the course of a single day for such analytes in reverse dosimetry approaches using Eq. 1 and related approaches would result in widely varying estimates of external dose for the same day and individual (variations by up to a factor of 1000; see, for example, Preau et al. 2010).[7]

This potential error in the assumption that a spot sample is an accurate surrogate for the 24-h average urinary concentration for an individual has generally not been considered, quantified, or evaluated in various reverse dosimetry exercises using population biomonitoring data (see, for example, references [2–4,6]–4,6). This within-individual, within-day variation in biomarker concentrations requires a more nuanced interpretation of the relationship between the distribution of spot urinary biomarker concentrations and the underlying distribution in daily doses in the population than that provided by reverse dosimetry, which assumes a direct correlation between the measured spot urinary biomarker concentration and the magnitude of recent external dose.

This analysis examines the relationships between first-order elimination half-life relative to the interval between exposures and the resulting degree of variation in biomarker concentrations using a framework of simple simulations. The simulations rely upon an assumption that biomarker concentration *vs* time profiles between and across exposure events can be modeled using a simple first-order decay function. The impact of variation in exposure rates across and within individuals is also examined, again based on a simple assumption that distributions of doses can be approximated as lognormal distributions with varying geometric standard deviations (GSDs), reflecting the often-observed pattern of lognormal distribution of environmental concentrations.[11]

The goals of the simulations are as follows:

(1) Characterize the degree of variation in spot urinary biomarker sample concentrations that can be attributed simply to within-individual, within-exposure interval variation resulting from the first-order elimination of analyte between exposure events. This degree of variation is characterized as a function of the half-life of elimination relative to the exposure interval, τ.
(2) Provide tools for examining variation in population biomonitoring data that allow insight into the degree of variation in the underlying dose rate distribution in the population, given information regarding the half-life of elimination and expected exposure interval.
(3) Characterize the degree to which application of urinary reverse dosimetry methods to urinary biomarker concentrations from spot sampling may misrepresent the degree of variation in underlying intake dose rates as a function of half-life of elimination and expected exposure interval.

The results of the simulations are compared with data collected and recently published by researchers from the Centers for Disease Control and Prevention (CDC) in an observational study of eight volunteers who collected each urine void for a week[7,8,10] and are used to examine population data from the National Health and Nutrition Examination Survey (NHANES). The simulations and data sets allow examination of the impact of within-day temporal variability on how representative spot sample biomarker concentrations are of longer-term biomarker concentrations and underlying exposure levels.

METHODS

Simple Pharmacokinetic Simulations

In practice, the ultimate elimination of most chemicals can be modeled as first-order processes, even when the underlying processes are more complex, providing an estimate of the "half-life" of elimination. Although such models do not capture the full range of complexity of the underlying biological processes or tissue-specific distribution, they are used here for the sake of simplicity in examining temporal patterns in chemical biomarker concentrations. If a first-order elimination rate for urinary elimination of a chemical is assumed, the theoretical profile of biomarker concentration over time, $C(t)$ following an exposure has the shape of an exponential decay curve with a rate constant, k, related to the chemical half-life, HL, decaying from an initial concentration of C_0 following a bolus exposure event:

$$k = \ln(2)/\mathrm{HL} \tag{2}$$

And

$$\frac{dC}{dt} = D(t) - k * C \tag{3}$$

Equation 3 describes the rate of change in concentration for the theoretical blood concentration *vs* time curve for a compound administered intravenously and which is eliminated (or metabolized) with a simple first-order process.

For exposures occurring via the oral route with biomarker concentrations examined in the urine, additional processes alter the concentration *vs* time curves, including particularly the oral absorption rate and the collection of excreted compound in the bladder followed by periodic voiding. A simple compartmental model that includes these processes was used to simulate urinary biomarker concentrations; this model is depicted in Figure 1 and the parameter distributions used in the modeling are described in Table 1. The model was implemented in Microsoft Excel 2011. The equations for rate of change of the amount of analyte in the GI tract (A_{GI}, Eq. 4), central compartment (A_{CC}, Eq. 5), and the bladder (A_B, Eq. 6) are as follows (see Table 1 for symbol definitions):

$$\frac{dA_{GI}}{dt} = D(t) - k_a * A_{GI} \tag{4}$$

$$\frac{dA_{CC}}{dt} = k_a * A_{GI} - k * A_{cc} \tag{5}$$

$$\frac{dA_B}{dt} = k * A_{cc} \tag{6}$$

Analyte accumulates in the bladder until voiding. The concentration of analyte in a given sampled void (C_{SV}) was calculated as:

$$C_{SV} = \frac{\int_{t_{PV}}^{t_{SV}} k^* A_{CC}(t)dt}{\frac{(t_{SV}-t_{PV})}{24} * V_{24}} \tag{7}$$

Where t_{SV} and t_{PV} are the times of the sampling void and previous void, respectively; V_{24} is the average 24-h volume of urine, and $A_{cc}(t)$ is the time-dependent amount of analyte in the central compartment.

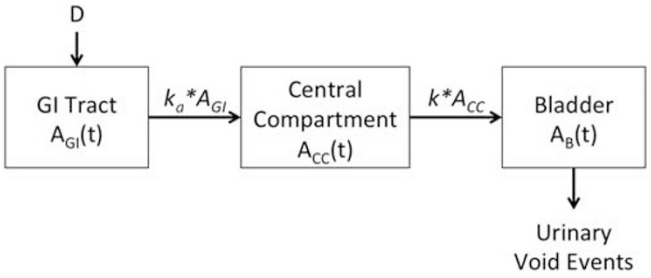

Figure 1. Compartmental model used to simulate urinary elimination kinetics. Symbols are defined in Table 1.

Table 1. Parameters used in urinary biomarker modeling.

Parameter symbol	*Definition*	*Value or distribution*
D	Oral dose	Lognormal distribution; GM = 1, GSDs of 1, 1.5, 2, 2.5, 3, 3.5, and 4
HL	Half-life of elimination	Various values relative to exposure interval from 0.083 to 2
T	Exposure interval	1
k_a	Oral absorption rate	Lognormal distribution, GM = 0.8, GSD = 2.8, truncated below 0.1 and above 5 per hour[a]
K	Elimination rate	Calculated using equation 2
V_{24}	24-h urinary volume	1.7 l[b]
P_V	Period since last void	Custom distribution from CDC data set[7]
t_{SV}	Time of sampled void	Uniform distribution between exposure event 5 and 6
t_{PV}	Time of void previous to sampled void	Calculated as t_{SV}–P_V
A_{GI}	Amount in gastrointestinal tract	Calculated (eq. 4)
A_{CC}	Amount in central compartment	Calculated (eq. 5)
A_B	Amount in bladder	Calculated (eq. 6)
C_{SV}	Concentration in sampled void	Calculated (eq. 7)

Model illustrated in Figure 1 and described in equations 4–7 in text.
[a]Distribution estimated based on oral absorption rates observed for >100 drugs.[24]
[b]Average value for adults from Van Haarst et al.[18]

The following simulations were conducted:

Simulation 1: simple simulations assuming repeated exposures to a unit dose of a chemical followed by first-order decay of the resulting biomarker concentration (theoretical relative concentrations in blood following a bolus intravenous exposure, using Eq. 3) were conducted for a range of half-lives of elimination (represented as a fraction of the exposure interval, τ). Half-life is expressed as a fraction of τ because the relative degree of change in concentration over the course of an exposure interval is directly related to this fraction. The simulations incorporated repeated exposure at the regular exposure interval τ for five exposure events (sufficient to achieve approximately 97% of quasi-steady-state). These simulations resulted in a set of relative concentration *vs* time curves.

Simulation 2: using the simple urinary model (Figure 1, Eqs. 4–7), simulations employing distributions of dose rates were conducted. Doses were selected randomly from lognormal distributions with different GSDs. Oral absorption rates and time since previous urinary void were varied according to distributions described in Table 1. Monte Carlo simulations were conducted in Crystal Ball (Oracle, version 11.1.2.1). A standard exposure interval was assumed, and separate simulations were conducted for each of several values for half-lives of elimination relative to exposure interval, τ. The variation in the HL/τ ratio can be used to examine both uncertainty and variability in HL as well as varying exposure intervals. For example, a value of HL/τ of 0.5 applies to a chemical with a half-life of 6 h and estimated exposure interval of 12 h, as well as to a chemical with a 12-h half-life and estimated exposure interval of once per day. Similarly, if individuals in the population are expected to have variations in HL, results for a range of values of the HL/τ can be examined to assess the impact of this variability.

For each value of HL/τ, seven separate populations of 10,000 individuals were simulated, each corresponding to a hypothesized lognormal dose rate distribution with geometric mean 1 and GSD equal to 1 (constant dose rate across all individuals in the population, P95:P50 of dose rate distribution equal to 1), 1.5, 2, 2.5, 3, 3.5, and 4 (P95:P50 of dose rate distribution equal to approximately 10). This population size was chosen for the simulation because it was large enough to result in relatively stable estimates of the P95:P50 measure given the multiple varied parameters in the simulations. The parameters varied in the simulation as well as the distributions assumed for each parameter are described in Table 1. Two simulations were conducted for each combination of HL/τ and dose distribution GSD, with each simulation run on a population of 10,000 individuals:

- Case 1: each individual was assumed to experience a constant, repeated, dose (drawn from the lognormal distribution) for six exposure events with exposure interval τ. A biomarker ''sample'' was drawn from the individual's concentration *vs* time curve at a randomly selected time with uniform distribution between exposure events 5 and 6; or
- Case 2: each individual was assumed to experience varying doses, with each dose drawn independently from the dose rate distribution for six exposure events with exposure interval τ. Biomarker "samples" were again drawn from the individual's concentration *vs* time curve at a randomly selected time with uniform distribution between exposure events 5 and 6.

These two cases correspond generally to two conceivable situations: exposure levels for an individual are relatively consistent over time, but individuals in the population differ from one another (to greater or lesser degrees) in their consistent levels of exposure (case 1); or exposure levels within and between individuals vary substantially over time without long-term consistent inter-individual differences in exposure levels (case 2).

A gross measure of the population variation in biomarker concentrations, the ratio of the 95th percentile to the 50th percentile in a population sample (P95:P50 ratio), was defined as a metric to reflect variation in simulated population biomonitoring data. This metric is a crude measure of variation, but one that can be easily examined in the summary reporting of real-world population biomonitoring data as well as in the simulations presented here. This metric addresses the usual interest in examining the impact and sources of higher-end exposure levels. Finally, examination of the lower percentiles of exposure distributions using biomarkers in real-world data sets is often difficult because of analytical sensitivity issues that may result in the inability to detect or quantify biomarkers at the lower percentiles. If the biomarker concentrations are approximately lognormally distributed, the P50:P05 ratio will be similar to the P95:P50 ratio. Using the P95:P50 ratio metric, the impact of relative half-life of elimination compared with exposure interval on variation in biomarker concentrations can be illustrated across a range of HL/τ values.

Data Sets for Evaluation of Urinary Simulation Predictions

The results from the simulations above are compared with a data set collected by the CDC and described in recent publications.[7,8,10] The CDC data set was generated from a 1-week urine collection effort. Briefly, eight volunteers (4 men and 4 women) collected each urinary void over the course of a week, recording the time and volume of each void before preserving an aliquot for analysis. Concentrations of monoethyl phthalate (MEP, the monoester metabolite of diethyl phthalate, DEP), mono-2(ethylhexyl) phthalate (MEHP, the primary monoester metabolite of di-2(ethylhexyl) phthalate (DEHP)), and mono-(ethylhydroxy) phthalate (MEHHP, one of the major secondary oxidative metabolites of DEHP),[7]

BPA[8] were measured in each sample aliquot. The analytes were detected with frequencies of 100%, 99.8%, and 91% for MEHHP, MEP, and BPA, respectively. Bodyweights and heights were not recorded and are not available. We obtained the data set from the CDC.

For each CDC data set subject, the daily mass of urinary analyte (M_A, μg/day) excreted was calculated for MEHHP (a principal metabolite of DEHP), MEP (the monoester metabolite of DEP), and BPA, as the sum of the products of the concentration in each spot sample (C_{A_i}) and the corresponding urinary void volume (V_i) for that day, for all voids, *i*, within a day from 1 to *n*:

$$M_A = \sum_{i=1}^{n} C_{A\_i} * V_i \quad (8)$$

Estimates of the mass fraction of parent compound excreted as specific analytes is available for MEHHP (0.17 of parent compound DEHP mass),[12] MEP (0.64 of parent compound DEP mass),[3] and BPA (1.0, recovered as free and conjugated parent compound).[13] Using this mass excretion fraction, the estimated daily intake mass of parent compound (M_{parent}, μg/day) corresponding to the daily elimination mass of analyte in urine can be calculated:

$$M_{Parent} = \frac{M_A}{F_{UE}} \quad (9)$$

For the purposes of this analysis, we calculated the daily mass of analyte eliminated for each participant (Eq. 4) and converted to estimated daily dose of parent compound (Eq. 5), resulting in a distribution of 44 daily dose estimates (omitting participant-days with missing urinary voids). As bodyweights were not measured or reported in the CDC data set, these estimates were not normalized to bodyweight, but rather remain in total mass per day units.

Finally, the simulation results generated here were used to examine data from the NHANES survey for a number of analytes that have estimates of half-life of elimination available. Based on hypothesized exposure intervals and the half-lives identified in the literature, estimates of the general range of variation in population dose rates were made based on the observed P95:P50 biomarker concentration ratios.

RESULTS

Simulations

Figure 2 illustrates theoretical biomarker concentration *vs* time curves for a repeated unit dose assuming different values for the half-lives of elimination relative to the exposure interval, HL/τ, using the simplest exponential decay model (Eq. 3), which corresponds to repeated bolus intravenous dosing with subsequent first-order elimination of analyte. Within an individual, between exposure events, the degree of variation in biomarker concentrations depends upon the relationship between the half-life of elimination and the interval between exposures. Under this conceptual framework, variation in the distribution of randomly sampled biomarker concentrations from a population with concentration *vs* time curves illustrated in Figure 2, will be a function of the half-life of elimination of the chemical relative to the exposure interval: chemicals with long half-lives relative to the interval between exposures will show little variation in randomly collected spot sample concentrations, while those with half-lives that are short relative to the exposure period will show larger variation.

The results from the more biologically realistic urinary biomarker sampling simulations (model illustrated in Figure 1 and described in Eqs. 4–7) using variable dosing rates drawn from different distributions are presented in Table 2. The results illustrate the theoretical relationship between the biomarker P95:P50 and both the half-life as a fraction of exposure interval and of the GSD of the distribution of dose levels. The results are presented for both case 1 as described above in Methods, which in real-world terms would correspond to the extreme case in which between-individual variation in exposure was much greater than within-individual variation in exposure levels in a given time frame, and for case 2, in which doses vary comparably both within and between individuals.

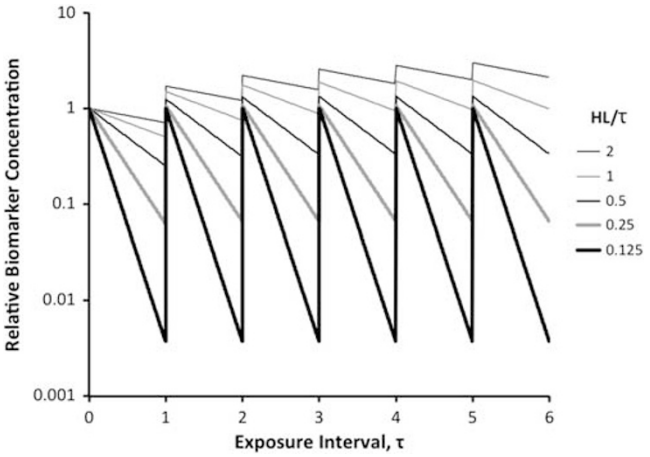

Figure 2. Simulated biomarker concentration *vs* time curves for a repeated unit dose at a consistent interval assuming different values for half-life of elimination as a fraction of the exposure interval, τ. Curves were generated using Eq. 3

Table 2 also allows comparison of the predicted degree of variability in the biomarker concentration (as represented by the P95:P50 ratio of the biomarker concentration distribution) can be compared with the variability in the underlying dose distribution (as represented by the P95:P50 ratio of the lognormal dose distribution, column 2 in Table 2). The ratio of these two measures provides an indication of the degree of over- or under-prediction of variation in doses provided by the variation in the biomarker concentrations.

For both cases, in situations in which the half-life of elimination is less than one-quarter to one-half of the exposure interval, the simulated distribution in randomly sampled biomarker concentrations as represented by the P95:P50 ratio is wider than the corresponding variation in dose rates in the population (Table 2). For example, in the extreme case of a short-lived compound (HL of 2 h) with exposure interval, τ, of 24 hours (i.e., once per day, HL/τ = 0.08), even in a population of individuals all experiencing repeated identical daily exposure rates (GSD = 1, P95:P50 ratio of dose distribution = 1), the predicted P95:P50 ratio exceeds 8; the result would be the same for a compound with a half-life of 6 h but exposure interval of 72 h (e.g., following occasional ingestion of a specific food with the compound present as a residue). For values of HL/τ approaching 1, the variability in the distribution of predicted biomarker concentrations approaches the variability in the underlying dose distribution.

For case 2, for values of HL/τ at or exceeding 0.5, the predicted variation in biomarker concentrations is actually lower than the variation in the underlying dose rate distribution. This reflects the "averaging out" of biomarker concentrations because of varying dose rates within the individual over time when elimination is slow enough (relative to exposure interval) to provide an integrated reflection of exposure events including those preceding the most recent event.

Comparison of Simulation Results to CDC Serial Urine Collection Data Set

The simulations presented here can be compared with the CDC serial urine collection data set to assess the validity of the simulations. Figure 3 illustrates the subject-specific spread in measured concentrations for each of the three analytes in each

Table 2. Simulated population P95:P50 ratios of spot urinary biomarker concentrations as a function of the half-life of elimination (as a fraction of exposure interval, τ) and as a function of the GSD of the population lognormal dose rate distribution for a simulated population of 10,000 individuals under two cases.

GSD (dose)	P95:P50 ratio of dose distribution	Simulated P95:P50 ratio of biomarker concentrations HL/τ							P95:P50 biomarker concentrations / P95:P50 dose distribution HL/τ						
		0.083	0.125	0.167	0.25	0.5	1	2	0.083	0.125	0.167	0.25	0.5	1	2
Case 1: inter-individual variation in dose rate, but constant dose rate within individual															
1	1	8.2	4.5	3.3	2.3	1.5	1.2	1.1	8.2	4.5	3.3	2.3	1.5	1.2	1.1
1.5	2	9.7	5.7	4.2	3.1	2.3	2.0	2.0	4.8	2.8	2.1	1.5	1.1	1.0	1.0
2	3.1	12.7	7.6	5.7	4.4	3.4	3.2	3.1	4.1	2.5	1.8	1.4	1.1	1.0	1.0
2.5	4.5	16.8	10.2	8.0	6.1	4.9	4.6	4.6	3.7	2.3	1.8	1.4	1.1	1.0	1.0
3	6.1	20.6	12.8	10.0	7.8	6.5	6.2	6.1	3.4	2.1	1.6	1.3	1.1	1.0	1.0
3.5	7.8	24.4	15.1	11.7	9.6	8.1	7.9	7.8	3.1	1.9	1.5	1.2	1.0	1.0	1.0
4	9.8	29.6	18.8	14.7	12.2	10.3	9.9	9.8	3.0	1.9	1.5	1.2	1.1	1.0	1.0
Case 2: dose rate varies both within and between individuals															
1	1	8.2	4.5	3.3	2.3	1.5	1.2	1.1	8.2	4.5	3.3	2.3	1.5	1.2	1.1
1.5	2	9.4	5.5	4.1	2.9	2.0	1.6	1.4	4.7	2.7	2.0	1.5	1.0	0.8	0.7
2	3.1	12.1	7.4	5.5	4.0	2.7	2.1	1.9	3.9	2.4	1.8	1.3	0.9	0.7	0.6
2.5	4.5	16.2	9.9	7.6	5.4	3.6	2.7	2.3	3.6	2.2	1.7	1.2	0.8	0.6	0.5
3	6.1	19.2	11.8	9.0	6.8	4.4	3.3	2.9	3.2	1.9	1.5	1.1	0.7	0.5	0.5
3.5	7.8	24.5	14.7	11.1	8.4	5.3	3.9	3.4	3.1	1.9	1.4	1.1	0.7	0.5	0.4
4	9.8	28.4	17.3	13.1	9.6	6.2	4.6	4.0	2.9	1.8	1.3	1.0	0.6	0.5	0.4

The right half of the table presents the degree to which the variability in the biomarker distribution over- or under-predicts the variability in the dose rate distribution. Shaded cells denote regions of dose distribution and HL/τ ratio where biomarker variability is equal to or greater than dose rate variability.

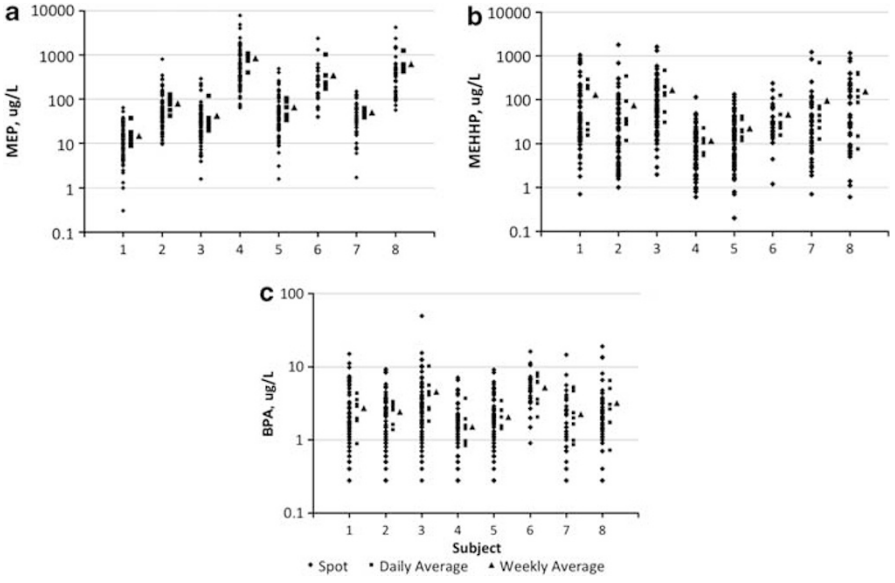

Figure 3. Concentrations of (**a**) MEP, (**b**) MEHHP, and (**c**) BPA in urine samples from eight participants in the CDC serial urinary collection data set. For each participant, the individual spot sample concentrations, as well as the daily and weekly average concentrations, are shown.

spot sample, for the daily average concentration, and for the weeklong average concentration. These data allow estimation of actual daily doses for DEP, DEHP, and BPA for the eight individuals over the course of a week based on the complete information regarding urinary volume and concentrations and Eqs. 8 and 9. Based on the known half-lives, an estimate of the interval between exposures, and the observed distribution in urinary spot sample analyte concentrations (represented by the P95:P50 biomarker concentration ratios), a rough estimate of the GSD of the underlying dose distribution can be made using Table 2. This estimate of the GSD of the underlying dose distribution can be compared with the GSD of the distribution of actual daily doses calculated from the person-days with complete urinary excretion data.

The estimated dose distribution GSDs based on the elimination half-lives and observed biomarker P95:P50 ratios compare well with the actual distributions in calculated doses for all three analytes (Table 3), although the estimated and observed GSD for DEP doses exceed the range considered in the simulations presented here. This suggests that the basic framework presented here in which biomarker concentrations within an individual, within an exposure interval, are modeled using random sampling from a simple first-order urinary elimination model is a reasonable way to examine the impact of relatively short elimination half-lives on population biomarker concentration variation.

In Table 3, the P95:P50 ratios for each of the three analyte biomarker spot concentration distributions (which, through use of

Table 3. Application of Table 2 to estimation of dose distributions using the CDC serial urinary void collection data.

Analyte (parent)	*Observed P95:P50 biomarker ratio*	*Half-life and exposure interval*		*Simulation-based predicted distribution of underlying doses*[b]		*Observed distribution of underlying daily doses*[c]		*Degree of mis-estimation of P95:P50 ratio of doses based on reverse dosimetry on spot samples*[d]
		Estimated HL, h	*HL/τ*[a]	*GSD*	*P95:P50 ratio*	*GSD*	*P95:P50 ratio*	
MEHHP (DEHP)	16.5	2–3[e]	0.08–0.125	~2.5–3.5	4.5–8	2.8	4.8	3.5-Fold overestimate
MEP (DEP)	28.6	2–3[e]	0.08–0.125	~4	~10	4.9	21.1	1.3-Fold overestimate
BPA (BPA)	5.6	5.4[f]	0.225	~2	3	1.7	2.5	2.2-Fold overestimate

The observed P95:P50 ratios of the analyte concentrations in the data set on person-days of complete collections (328 collections) and information about the elimination half-lives of the analytes were used to predict the GSD (and corresponding P95:P50 ratio) of the underlying distribution of daily doses in the eight participants over the course of the week of observation. This predicted distribution of doses is compared to the actual distribution of daily doses calculated from the full analyte elimination data for 44-person days of complete observations.
[a]Exposure interval τ for these calculations was estimated as 24 h based on inspection of void concentration *vs* time curves and because doses were estimated on a daily basis.
[b]From Table 2.
[c]Daily doses calculated from daily mass of analyte eliminated using equations 8 and 9 for 44 person-days with complete urine collections.
[d]Calculated as the ratio of the spot biomarker P95:P50 ratio (column 2) to that of the observed distribution of daily doses (column 8).
[e]Initial elimination phase for MEHHP (Koch et al.[25]); also applied to MEP, by analogy.
[f]Half-life from Volkel et al.[13]

Eq. 1, are equal to the P95:P50 ratios for the calculated underlying dose estimates based on spot samples) can be compared with the P95:P50 ratios for the observed daily doses (based on Eqs. 8 and 9) for 44 person-days in the CDC data set. For DEHP and BPA, reliance on the spread of underlying biomarker concentrations to estimate the spread of doses overestimates the actual variation in doses, by factors of 3.5- and 2.2-fold, respectively. For DEP, the magnitude of overestimation is smaller (1.3-fold), reflecting the wide inter-individual variation in dose rates. Figure 4 presents the relative distributions of estimated daily dose rates based on spot samples using reverse dosimetry (Eq. 1) to the estimated dose distributions on a daily mass excretion basis (Eqs. 8 and 9) and further to the distribution of average daily doses over the week across the eight individuals. The relative spreads of the different methods for examining exposure illustrates the degree to which the reliance on distributions of spot sample concentrations exaggerates the actual distribution of doses, particularly when average dose rates over a period of time are of interest.

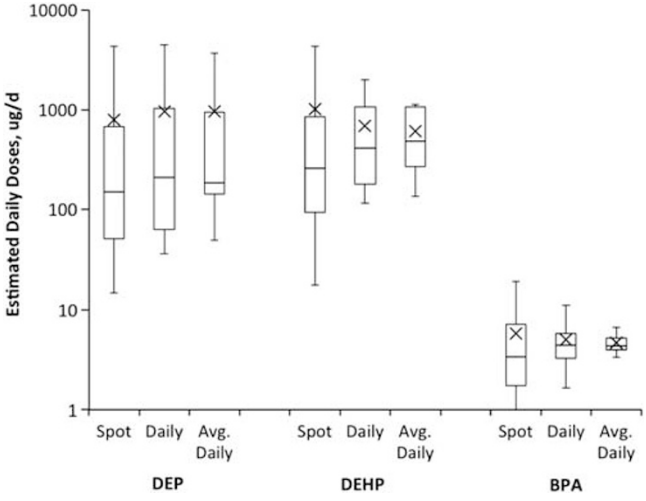

Figure 4. Calculated daily doses of parent compounds for the samples from the CDC serial urinary collection data set for DEHP, DEP, and BPA. Boxes represent the 25th to median and median to 75th percentiles, while whiskers extend to the 5th and 95th percentiles; X symbols denote arithmetic means. In each graph, the distributions represent the doses calculated through application of reverse dosimetry to measured spot sample concentrations, the calculated actual daily doses for 44 person-days with complete urine collection data based on the 24-h mass excretion of analyte (Eqs. 8 and 9 in text), and the calculated average daily dose over the week for the eight individuals ($n = 8$).

Examination of NHANES Data

The P95:P50 ratios of biomarker concentrations reported in the CDC National Exposure Report[14] based on biomonitoring data collected during the NHANES survey can be examined in light of the results of the simulations presented here and information on half-lives of elimination and hypothesized exposure frequencies or intervals. As a result of the use of the P95:P50 metric for examination of variation, only data for compounds for which detection rates exceeded 50% can be examined. In addition, data on half-life of elimination are not available for all compounds.

A subset of NHANES analytes with both information on half-life and sufficient detection rates is presented in Table 4. The general trend of increasing variation in the distribution of spot biomarker concentrations with decreasing half-life is clearly demonstrated in the data from NHANES (Figure 5). Based on the simulation results from Table 2, a general estimation of the likely variation in underlying daily (24-h) dose distributions indicated by the variation in biomarker concentrations can be made based on assumptions regarding likely exposure interval(s). This exercise demonstrates application of the simple simulations presented here to population biomonitoring data. The estimates of variation in underlying daily dose distributions can be used to inform the interpretation of the population biomonitoring data in a risk assessment context, albeit this approach does not provide estimates of distributions in longer-term averages of daily doses, which is of interest for most risk assessment paradigms.

DISCUSSION

Chemical compounds with short half-lives of elimination relative to likely exposure frequency are included in many current

Table 4. P95:P50 ratios from the NHANES 2003–2004 biomarker concentration data for selected chemicals with detection rates exceeding 50% and available information on half-life of elimination (CDC 2009).

Analyte	*HL (d)*	*P95:P50 biomarker ratio*	*Estimated exposure frequency*	*HL/τ*	*Predicted distribution of underlying doses*[a]		*HL reference*
					GSD[a]	*P95:P50 ratio*	
Chloroform	0.17	5.0	Multiple per day	0.50	2.5–3	4.5–6	Reviewed in Aylward et al.[26]
MEHHP	0.1	12.5	Daily	0.1	2.5–3.5	4.5–8	Koch et al.[12]
Enterodiol	0.18	8.2	Daily	0.20	2.5–3	4.5–6	Kuijsten et al.[27]
BPA	0.23	5.7	Daily	0.23	2	3	Volkel et al.[12]
Bromodichloromethane	0.25	6.8	Multiple per day	0.50	3–4	6–10	Reviewed in Aylward et al.[26]
Perchlorate	0.31	3.9	Daily	0.30	1.5–2	2–3	Lorber[28]
Daidzein	0.33	17.3	Daily	0.33	>4	>10	Shelnutt et al.[29]
Equol	0.42	8.1	Daily	0.40	3.5–4	8–10	Metzner et al.[30]
Genistein	0.42	20.3	Daily	0.40	>4	>10	Metzner et al.[30]
Triclosan	0.46	50.1	Once per week to one per day	0.1 to 0.5	>4	>10	Sandborgh-Englund et al.[31]
Enterolactone	0.53	8.9	Daily	0.50	3.5->4	8->10	Kuijsten et al.[27]
Thallium	20	2.6	Unknown	>2	2–3	2–6	Chandler et al.[32]
Acrylamide hemoglobin adducts, nonsmokers only	60	1.8	Daily	>2	1.5–2	2–3	Hays and Aylward[33]
HxCDD (ages 30–40)	3650	2.2	Daily	>2	1.5–2.5	2–4	Flesch-Janys et al.[34]
PFOA	>1000	2.4	Daily	>2	1.5–2.5	2–4	Seals et al.[35]
PCB 180 (ages 30–40)	>2500	3.3	Daily	>2	2–3.5	3–8	Brown et al.[36]

Based on an estimate of the exposure interval and known half-life of elimination, the predicted P95:P50 ratio of dose rates underlying the observed distribution of biomarker concentration was estimated using Table 2. The pattern of biomarker P95:P50 concentrations from NHANES as a function of chemical half-life is also presented in Figure 5.
[a]Derived from examination of Table 2.

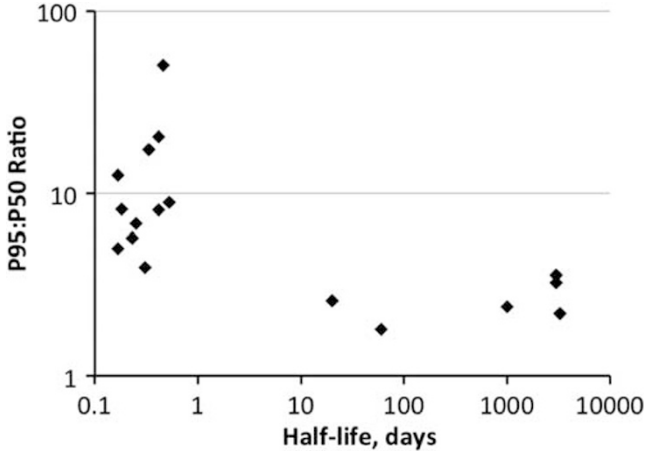

Figure 5. P95:P50 ratio of biomarker concentrations from the NHANES 2003–2004 survey for selected chemicals with detection rates >50% and available information on half-life of elimination (Table 4). P95:P50 ratios tend to decrease as half-life of elimination increases.

biomonitoring programs such as NHANES. Reverse dosimetry approaches for urinary analytes, which assume a direct relationship between the urinary biomarker concentration and the intake dose leading to that concentration, have been widely used to characterize both central tendency and upper bound exposure rates in terms of mg/kg-d (for example, see references [2–4]). However, because of the wide intra-individual variation in biomarker concentrations that occur for such compounds over time following an exposure event because of the properties of first-order elimination curves, the degree of variation in underlying population dose rates is not equal to degree of variation in spot biomarker concentrations observed. The data sets and simulations presented here demonstrate that while urinary reverse dosimetry calculations based on central tendency estimates of biomarker concentrations (i.e., median, geometric means, or interquartile ranges) may provide relatively accurate estimations of population average exposure levels, reliance on tails of the distributions to estimate distributions of underlying dose rates or characterize upper bound exposure rates will overestimate the degree of variation in underlying dose rates when the elimination half-life is short compared with the exposure interval.

The results of the simulations presented here provide a basis for predicting the potential degree of the overestimation of variation in dose rates (Table 2). These results also provide a tool for estimating population dose rate distributions based on biomarker distributions given information regarding approximate half-life of elimination and estimated exposure intervals. This discussion focuses on upper-end tails because that is generally the principal concern in use of biomonitoring data for public health applications, but this mis-estimation of doses occurs at the lower tails of the distribution as well. This analysis has focused on urinary biomarkers because of the availability of the CDC serial urinary collection data set for examination of the simulations and because urinary biomarkers are collected more often than blood-based biomarkers because of the less invasive nature of the sampling. However, the analysis of variation because of the first-order decay curves presented here is generally applicable to blood-based biomarkers as well.

Many additional factors (other than variation in dosing rates, oral uptake rates, and urinary void timing) not simulated here can contribute to or modify the degree of variation in spot sampling results that is attributable to properties of first-order decay curves

as predicted from the simulations. For example, inter-individual variation in elimination half-life for various chemicals has been observed and appears to be relatively lower for compounds with shorter biological half-lives than for those with longer biological half-lives (reviewed in Spaan et al.).[15] The impact of such variation can be examined using the simulations presented here by varying the HL/τ parameter to an appropriate degree for the compound of interest.

In addition, for many compounds, elimination occurs through a biphasic or multi-phasic process, with a shorter initial elimination half-life followed by a longer terminal half-life. More elaborate efforts to reconstruct external exposure, which rely on fully developed chemical- or class-specific physiologically based pharmacokinetic models and constructed exposure scenarios have been developed for a few compounds (reviewed in Clewell et al.),[5] but the approach here provides a more generalizable and simpler tool that can be used to guide initial interpretation and design of studies with a general estimate of expected biomarker variations. Depending on model and data availability and study needs, more resource-intensive approaches can then be applied.

Physiological factors including variations in urinary volume because of hydration status, or variations in creatinine excretion rates within individuals within or between days, or between individuals, can influence variations in measured urinary spot sample concentrations (on a volume or creatinine-corrected basis, respectively). Although substantial effort has been devoted to development of equations for prediction of creatinine excretion rates based on age, gender, body weight, and height (Mage et al.[16,17]), less information is available about within-individual variation in creatinine excretion rates. In the CDC data set, the coefficients of variation (CV) across the eight individuals for creatinine excretion rate in mg/h on a sample-by sample basis ranged from 24% to 50%. The CVs for urinary volume rates in ml/h for the eight individuals ranged from 50% to 115% over the course of the week. On a daily basis, the CVs for creatinine excretion rates in g/day ranged from 2% to 33% across the eight individuals, while the CVs for daily urinary volume in l/day ranged from 20% to 27%. The degrees of variation in urinary volumes in the CDC data set per void and per day are consistent with those previously reported for urinary volume in adults.[18] Although the CDC data demonstrate that creatinine excretion occurs at a more constant rate than urinary volume, there remains some intra-individual variation in creatinine excretion rate both between urinary voids and across days that may influence variation in biomarker concentrations when those concentrations are normalized to creatinine. Finally, other factors such as analytical imprecision can contribute to variation in measured concentrations, although relative errors in high quality analytical work should be relatively small compared with many of the other factors discussed here.

Environmental epidemiology studies increasingly include biomonitoring as a primary means of exposure characterization. Although biomonitoring is also used in occupational studies, where the timing of sampling relative to exposures is generally known, interpretation in the environmental exposure context is more challenging. For compounds with short half-life relative to exposure interval, characterization of individual exposure levels in such studies should consider both intra- and inter-day variation in biomarker concentrations. A number of studies have examined potential intra-individual variation in biomarker concentrations either over short or longer time periods relevant to the health outcomes of interest in the studies. Table 5 provides an overview of available studies that examined intra-individual variation in biomarker concentrations in environmental epidemiological study settings. The objective of most of these studies was to investigate how reliable spot samples were as measures of exposure or indicators of longer-term average concentrations. These studies have predominantly focused on phthalates, phenols, and polycyclic aromatic hydrocarbon metabolites. Many studies reported results in terms of intra-class correlation coefficients (ICCs), the fraction of total variation explained by between-subject variation.[19] ICC values below 0.5 indicate that within-subject variation is greater than between-subject variation. ICC values below 0.4 are generally considered to indicate that single samples will not provide reliable classification of subjects with respect to exposures, while ICC values approaching or exceeding 0.75 are desirable for good to excellent reliability in exposure classification.[19]

With some exceptions, these studies found that intra-individual variability in repeated spot samples for the examined analytes exceeds inter-individual variability for compounds with relatively short elimination half-lives. Exceptions included mono benzyl phthalate (MBzP)[20,21] and MEP[7] as well as summed inorganic-derived arsenic species (but not individual inorganic-derived arsenic metabolites[22,23]). These findings suggest that exposure profiles for these compounds within individuals may be more stable because of more consistent and frequent exposure events. For example, Adibi et al.[20] found the highest ICC among phthalate metabolites for MBzP (0.66) and that MBzP in urine was highly correlated with repeated measurements of the parent compound, butylbenzylphthalate (BBzP), in indoor air (which also showed a high ICC of 0.83). Exposure via indoor air would result in an exposure profile that is characterized by short exposure interval (nearly continuous exposures) and a correspondingly larger value for HL/τ. Similarly, for MEP, exposures may be related to individuals' consistent use patterns of consumer products containing DEP.[7] These studies suggest that for BBzP and DEP, exposures vary relatively more across than within individuals, with some individuals experiencing consistently higher exposure levels than others in the populations and time frames studied.

In design of such studies, information on half-lives of the chemicals of interest can be used to predict a likely magnitude of intra-individual variation using Table 2 above, particularly if information is available on likely exposure pathways that allows estimation of likely exposure intervals (e.g., occasional encounter of residues of a pesticide in specific agricultural products *vs* inhalation of a common indoor air contaminant). The time frame of exposure characterization should be matched to the health outcomes of interest: if the health outcome is a chronic disease, single measurement of a transient biomarker is unlikely to provide accurate characterization of an individual's exposure during the time period relevant to disease development. If little or no information on pharmacokinetics and likely exposure pathways and frequencies are available, a pilot study similar to the CDC serial urine study, in which all urinary voids are collected over a period of time for a few individuals, will allow examination of intra-individual variability in biomarker concentrations to inform exposure characterization strategies and sample size requirements for the study.[10]

Biomonitoring provides powerful information on chemical exposure, but this information differs from that provided by external exposure estimates, which tends to focus on estimates of long-term average and upper bound exposure rates. In most cases, biomarkers are measured on a spot basis, capturing a biomarker concentration measure at a single point in time, usually with no knowledge of likely time of last exposure. Understanding of the factors in addition to variations in exposure level that influence the temporal variability in biomarker concentrations within and across individuals as illustrated in the simulations presented here can increase the value of collected biomonitoring data and inform design of such studies. Biomonitoring will be most useful when paired with knowledge regarding the pharmacokinetic characteristics of the compound of interest and complementary information on external exposure pathways, and both short-term and long-term temporal patterns in exposure, where possible. The analyses and tools presented here, while based on simple simulations, can be used to further evaluate population biomonitoring data, to construct hypotheses regarding

Table 5. Studies examining variation in repeated biomarker measurements in the context of environmental epidemiology studies.

Author	*Chemical(s)*	*Sample type*	*#Subjects*	*Frequency, duration*	*Samples/ subject*	*Findings*
Adibi et al.[20]	Phthalates	Spot	28	Sampled over 6 weeks	2–4	ICCs for DEHP and DEP metabolites $\leqslant$0.25; for DnBP and DiBP, ICCs ranged from 0.41 to 0.55; for MBzP, ICC = 0.65
\Arakawa et al.[37]	BPA	24-h	5	Daily for 5 days	5	Intra-individual variation (91%) greater than inter-indiviual variation (84%)
Baird et al.[38]	Phthalates	FMV	60	Every 2 weeks for 6 weeks	3	ICCs ranged from 0.21 to 0.53. Highest ICCs were for mono-benzyl phthalate (ICC = 0.53) and mono-ethyl phthalate (ICC = 0.48). Other phthalates had ICCs ranging from 0.21 to 0.37
Braun et al.[39]	BPA	Spot	389	Samples at gestation weeks 16 and 26 and at birth	3	Low reproducibility in urinary BPA concentrations within individuals (ICC = 0.1)
Fromme et al. [40]	Phthalates	Spot	50	Daily for 8 days	8	ICCs ranged from 0.2 to 0.57 (creatinine adjusted). Highest ICC was for mono-benzyl phthalate (0.57)
Hauser et al.[41]	Phthalates	Spot	11	Random over 3 months	$\leq$9	ICCs ranged from 0.21 to 0.71
Hoppin et al.[42]	Phthalates	FMV	46	Daily for 2 days	2	ICCs ranged from 0.53 to 0.80 (creatinine adjusted)
Kile et al.[22]	Arsenic	Spot	196	Every 3 months over 2 years	$\sim$8	ICCs ranged from (0.35 to 0.47) depending upon arsenic species (two exposure groups; high and low). Intra-individual variability for each metabolite was much greater than inter-individual variability
Kissel et al.[43]	Organo-phosphates	Spot	13	Up to 4 times in 1 day in each of two seasons	$\sim$ 8	First morning voids displayed less variability than collection times later in the day. Degree of variability was inversely related to half-life
Li et al.[10]	PAH metabolites	Spot	8	Every urine void over a week	27–74	ICCs ranged from 0.30 to 0.55 for spot samples, 0.33 to 0.65 for FMVs, 0.44 to 0.77 for 24-h voids
Mahalingaiah et al.[44]	BPA	Spot	31	Randomly over 14 to 482 days	3	Greater specificity, sensitivity and positive predictive power for tertile of exposure when using two versus one spot sample
Meeker et al.[45]	Insecticides	Spot	10	Approximately weekly for 3 months	9	Intra-individual variance exceeded inter-individual variance for TCP-y. Inter-individual variance exceeded intra-individual variance for 1-naphthol
Nepomnaschy et al.[46]	BPA	Spot	60	Every 2 weeks over a month	3	Within-subject variance (0.53) greater than between-subject variance (0.38)
Peck et al.[47]	Phthalates	Spot	25	Approximately weekly over a month	$\sim$3	ICCs ranged from 0.13 to 0.64 depending upon phthalate metabolite (creatinine adjusted). Highest ICCs were for MBzP and MEP
Preau et al.[7]	Phthalates	Spot	8	Every urine void over a week	27–74	Intra-individual variance greater than inter-individual variance for MEHHP in spot samples. Inter-individual variance was greater than intra-individual variance for MEP in spot samples
Rivera-Nunez et al.[23]	Arsenic	FMV, spot	131	Not reported	2	ICCs $>$0.88 for correlations between a single FMV and spot sample taken an unspecified or unknown duration of time between collections
Scher et al.[48]	Pesticides	24-h, spot, FMV	40	Every urine void, but composited into 24-h analyses over 5 days	$\sim$5	Fairly good relationship in concentration of analyte in FMV and spot sample, but authors caution that bias (positive and negative) is introduced when relying solely on spot samples
Sexton et al.[49]	Volatile organic compounds	Blood	150	Twice per year over 2 years	4	Within-child variability exceeded between-child variability for most VOC except 1,4-dichlorobenzene and tetrachloroethylene (inter- greater than intra-child variability) and ethylbenzene and 1,1,1-trichloroethane (variances approximately equal)
Sexton and Ryan[50]	Phthalates, OP metabolites, organochlorines, lead, mercury, VOCs	Spot urine, blood	65–91 (urine) 106–123 (blood)	Sampling in two seasons over 2 years	2–4	Between-child variance greater than or comparable to within-child variance for most analytes
Teeguarden et al.[9]	BPA	Spot urine, blood	20	Blood samples collected approximately hourly, every urine void collected over 24-h period.	$\sim$24	Within-day urinary total BPA concentrations in urine and blood concentrations varied by $>$10- and 5-fold, respectively, within a day in individuals following single exposure
Teitelbaum et al.[21]	Phthalates, phenols, phyto-estrogens	Spot	35	0, 1, 2, 3, and 6 months, plus one additional random sample 2 weeks after one of the other collection times	6	Substantially greater within-child variability than between-child variability for all 21 analytes measured except MBzP. ICCs were $<$0.3 for most analytes
Ye et al.[8]	BPA	Spot	8	Every urine void over a week	27–74	Within-person, within-day variance accounted for 70% of total variation

Sample matrix is urine unless otherwise specified.

exposure patterns, and in design of biomonitoring strategies in exposure and epidemiological evaluations of specific populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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