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Inter- and intra-individual variation in urinary biomarker concentrations over a 6-day sampling period. Part 1: Metals

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HIGHLIGHTS

- Over a 6 day period, all individual urine samples were collected for 8 individuals.
- Four metals (As, Cd, Mn, Ni) were quantified in each individual urine sample.
- ICCs were low, but increased with creatinine and specific gravity (SG) adjustment.
- SG-adjustment was consistently highest correlated with metal excretion rates.

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ABSTRACT

The aim of the current HBM-study is to further the understanding of the impact of inter- and intra-individual variability in HBM surveys as it may have implications for the design and interpretation of the study outcomes. As spot samples only provide a snapshot in time of the concentrations of chemicals in an individual, it remains unclear to what extent intra-individual variability plays a role in the overall variability of population-wide HBM surveys. The current paper describes the results of an intensive biomonitoring study, in which all individual urine samples of 8 individuals were collected over a 6-day sampling period (a total of 352 unique samples). By analyzing different metals (As, Cd, Mn, Ni) in each individual sample, inter- and intra-individual variability for these four metals could be determined, and the relationships between exposure, internal dose, and sampling protocol assessed. Although the range of biomarker values for different metals was well within the normal range reported in large-scale population surveys, large intra-individual differences over a 6-day period could also be observed. Typically, measured biomarker values span at least an order of magnitude within an individual, and more if specific exposure episodes could be identified. Fish consumption for example caused a twenty- to thirty-fold increase in urinary As-levels over a period of 2–6 h. Intra-class correlation coefficients (ICC) were typically low for uncorrected biomarker values (between 0.104 and 0.460 for the 4 metals), but improved when corrected for creatinine or specific gravity (SG). The results show that even though urine is a preferred matrix for HBM studies, there are certain methodological issues that need to be taken into account in the interpretation of urinary biomarker data, related to the intrinsic variability of the urination process itself, the relationship between exposure events and biomarker quantification, and the timing of sampling. When setting up HBM-projects, this expected relationship between individual exposure episode and urinary biomarker concentration needs to be taken into account.

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1. Introduction

Over the last decade, human biomonitoring (HBM) has gained a significant amount of attention from the scientific community, environmental health risk managers, and policy makers as a tool to increase understanding of the use of internal chemical dose in environmental health impact assessments. Due to an increase in sensitivity of analytical methods, analytical capacity and a change in social awareness toward pollution exposure, there has been a rapid increase in the development and application of internal dose as a metric to evaluate human exposure. In many cases, HBM data have been proven to be a valuable addition to, or have even surpassed, estimates of exposure based on environmental measurements. As HBM provides insight into the presence of a chemical substance (or respective metabolites; biomarkers) in a person's body, it takes into account often poorly understood processes such as bioaccumulation, excretion, metabolism, and aggregate uptake variability through different exposure pathways. Hence, these data can be more relevant for health risk assessment than extrapolations from chemical concentrations in soil, air, and water (Bevan et al., 2012; Manno et al., 2010; Pirkle et al., 1995; Smolders et al., 2008).

Additionally, HBM procures a change in perception from the general public, as "pollution gets personal" when HBM data are being collected (Stokstad, 2004). Not only does this relate to a change of perception toward exposure and potential adverse health effects in the general public, it also integrates environmental exposure in a way that is more likely to be consistent with an individual's potential adverse health status.

Urine is probably the most frequently used matrix to quantify the degree of environmental or occupational exposure to pollutants, especially for substances with short biological half-lives (Esteban and Castaño, 2009; Barr et al., 2005). The collection and analysis of urine samples carries no associated risk, and large volumes can at once be gathered per individual (Aylward et al., 2013; Polkowska et al., 2004; Smolders et al., 2009). Typically, spot collection of urine samples is most frequently used in biomonitoring programs, especially for surveys where large numbers of participants are involved because of its ease of collection, minimal expertise and logistic requirements, and the non-invasive character of sample collection.

When dealing with exposures to non-persistent chemicals, one has to be aware of the often intermittent nature of exposure and the generally rapid metabolic conversion and excretion of these chemicals. Consequently, these characteristics are reflected in urine as the preferential matrix in large-scale human biomonitoring studies. Additionally, urine volume–contrary to blood as the preferred matrix for persistent chemicals – is subject various factors influencing renal clearance. Spot urine samples convey an inherent variability in terms of time since previous void, sample volume and concentration/dilution of a sample. To correct for the dilution of spot urine samples, two different methods are typically used for standardization:

- By expression per gram of creatinine: The World Health Organisation (WHO) has developed guidelines which stipulate that samples with creatinine concentrations <30 or >300 mg/dL are regarded as either too diluted or too concentrated. However, these guidelines have been questioned recently based on detailed assessment of the role of age, gender and ethnicity and may not be appropriate for pregnant women or children (Barr et al., 2005; Polkowska et al., 2004; WHO, 1996).
- By taking account of the gravity or relative density of urine (Polkowska et al., 2004; Miller et al., 2004; Cone et al., 2009; Koch et al., 2014). Also here, cut-off values have been identified outside of which a sample is classified as either too diluted or concentrated (DHHS 2004; EWDTS 2002; Ikeda et al., 2003).

For the efficiency evaluation of risk management options and efficacy of environment and health policies, repeated or even routine biomonitoring may be desirable to improve an understanding of the presence and levels of rapidly absorbed and eliminated compounds. Particularly for short-lived chemicals such as volatile organic compounds, agricultural pesticides, plasticizers, or compounds present in personal care products, a single sample may not reflect peak exposures arising through infrequent exposure episodes. Repeated sampling of high-exposure subjects provides more insight into the true nature of these episodes and of their toxicological consequences (Anderson et al., 1993 Aylward et al., 2014). For this reason, the appropriateness of using single spot samples to quantify exposure, particularly for later application in long-term epidemiological follow-up studies, has recently been questioned (Aylward et al., 2012 Chaumont et al., 2013).

Typically, large-scale human biomonitoring surveys gather a single spot sample from each participating individual, often first morning voids (FMVs) or convenience spot samples. The timing of these convenience samples, both in terms of time of day and time relative to any exposure, is not generally controlled, and hence may represent an additional source of variability. Twenty-four hour urine samples represent a better approximation of the true biomarker excretion, and have successfully been sampled in for example the German Environmental Specimen Bank for several decades (Wiesmüller et al., 2007) and were used for exposure and risk assessment (Koch et al., 2012; Schütze et al., 2014; Wittassek et al., 2007). However, collecting 24 h-samples is laborious and incomplete samples might pose problems (Akerstrom et al., 2012; Aylward et al., 2012). Therefore, most of the large scale biomonitoring programs like NHANES in the USA (Calafat, 2012), GerES in Germany (Kolossa-Gehring et al., 2012), ENNS in France (Fréry et al., 2011), FLEHS in Flanders (Steunpunt Milieu and Gezondheid, 2011) and the (DEMO)COPHES study on harmonised HBM in Europe (Joas et al., 2012) resort to spot urine samples. In order to ascertain that spot urine samples are relevant for longer term conditions, more or less steady-state conditions, including stable biokinetics, a relatively constant rate of exposure, and a dynamic equilibrium among different body tissues, are required. Simply assuming that biomarker values are representative for a steady-state concentration in the measured matrix may not be a justified assumption and requires additional investigation.

Therefore, there are several factors that may be relevant in understanding the representativeness of HBM samples, and hence assessing the inter- and intra-individual variability in biomarker levels (Aylward et al., 2012, 2014):

- Biological half-life: this chemical-specific parameter is critical for understanding the representativeness of single spot samples. Inter-individual variation in half-life also plays an important role as, at best, only the 'population average' half-life will be known. Consequently, the unknown inter-individual variations in half-life mean that any single biomonitoring value can support a range of long-term exposures.
- Exposure pattern and intensity: this is related to occurrence of chemicals in different environmental compartments, the frequency with which humans get in contact with these compartments through different uptake routes, and the level of resulting exposure. E.g., exposure through drinking water may be very different than exposure through dermal contact;
- Sampling parameters: factors related to the sampling scheme such as the timing of sample collection, time elapsed between urination or the method used to calculate urinary dilution