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Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples

Tina Harmer Lassen [a,*], Hanne Frederiksen [a], Tina Kold Jensen [a,b], Jørgen Holm Petersen [a,c], Katharina M. Main [a], Niels E. Skakkebæk [a], Niels Jørgensen [a], Selma Kløve Kranich [a], Anna-Maria Andersson [a]

[a] Rigshospitalet, Copenhagen University Hospital, Department of Growth and Reproduction, Blegdamsvej 9, 2100 Copenhagen, Denmark
[b] Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark
[c] Department of Biostatistics, University of Copenhagen, Copenhagen, Denmark

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ABSTRACT

Human exposure to modern non-persistent chemicals is difficult to ascertain in epidemiological studies as exposure patterns and excretion rates may show temporal and diurnal variations. The aim of this study was to assess the temporal variability in repeated measurements of urinary excretion of bisphenol A (BPA) and seven other phenols. All analytes were determined using TurboFlow-LC–MS/MS. Two spot, three first morning and three 24-h urine samples were collected from 33 young Danish men over a three months period. Temporal variability was estimated by means of intraclass correlation coefficients (ICCs). More than 70% of the urine samples had detectable levels of BPA, triclosan (TCS), benzophenone-3 (BP-3) and sum of 2,4-dichlorophenol and 2,5-dichlorophenol (ΣDCP). We found low to moderate ICCs for BPA (0.10–0.42) and ΣDCP (0.39–0.72), whereas the ICCs for BP-3 (0.69–0.80) and TCS (0.55–0.90) were higher. The ICCs were highest for the two spot urine samples, which were collected approximately 4 days apart, compared with the 24-h urine samples and the first morning urine samples, which were collected approximately 40 days apart. A consequence of the considerable variability in urinary excretion of BPA may be misclassification of individual BPA exposure level in epidemiological studies, which may lead to attenuation of the association between BPA and outcomes. Our data do not support that collection of 24-h samples will improve individual exposure assessment for any of the analysed phenols.

1. Introduction

Bisphenol A (BPA) and other phenolic compounds such as triclosan (TCS), benzophenone-3 (BP-3), 2,4-dichlorophenol (2,4-DCP) and 2,5-dichlorophenol (2,5-DCP) are non-persistent compounds with suspected endocrine disrupting properties (Krause et al., 2012; Ogawa et al., 2006; Richter et al., 2007; Gee et al., 2008; Kumar et al., 2009; Takahashi et al., 2011; Ma et al., 2012), which humans are commonly exposed to (Hill, et al., 1995; Calafat et al., 2008a, 2008b; Vandenberg et al., 2010).

Diet is considered to be the primary source of exposure to BPA through leaching from food packaging, but non-food sources may also contribute through dermal exposure or through inhalation (Geens et al., 2012). Exposure sources of the antimicrobial agents TCS and triclocarban (TCC) include personal care products such as toothpaste and soaps (Chen et al., 2008; Dann and Hontela, 2011), while BP-3 is a commonly used sunscreen agent, for which exposure may occur through dermal application of sunscreens and cosmetics (Krause et al., 2012). 2,4-DCP is used in the synthesis of phenoxy acid herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D), and general population exposure to 2,4-DCP may be through inhaling contaminated air or ingesting contaminated water. In addition, 2,4-D can upon intake be metabolised back into 2,4-DCP (Centers for Disease Control and Prevention, 2012a). 2,5-DCP is a metabolite of 1,4-dichlorobenzene (1,4-DCB), which is used in the production of insecticides and in products such as mothballs and toilet deodorant blocks. General population exposure occurs mainly through breathing vapours of 1,4-DCB containing products (Yoshida et al., 2002; ATSDR, 2006). The phenylphenols 2-phenylphenol (2-PP) and 4-phenylphenol (4-PP) are used as agricultural fungicides and sanitisers and exposure may occur through ingestion of contaminated food (Centers for Disease Control and Prevention, 2012b).

After exposure, these compounds are rapidly metabolised; primarily by phase II metabolism (conjugation) to increase their

Abbreviations: BPA, bisphenol A; TCS, triclosan; TCC, triclocarban; BP-3, benzophenone-3; 2,4-DCP, 2,4-dichlorophenol; 2,5-DCP, 2,5-dichlorophenol; ΣDCP, sum of 2,4-dichlorophenol and 2,5-dichlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol; 2-PP, 2-phenylphenol; 4-PP, 4-phenylphenol.

* Corresponding author.
E-mail address: tina.harmer.lassen@rh.regionh.dk (T.H. Lassen).

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water solubility, and excreted in urine with elimination half-lives of less than 24 h (Hissink et al., 1997; Völkel et al., 2002, 2005; Sandborgh-Englund et al., 2006; Pascal-Lorber et al., 2012). Due to the rapid urinary excretion and the diversity in exposure sources, the excreted concentration may vary over the day and between days. This variability in a person's urinary phenol concentration level may lead to misclassification of individual exposure levels in epidemiological studies, when using a single measurement of urinary phenol excretion as a biomarker of exposure. Data on the variability of the urinary excretion of environmental phenols may therefore be a valuable tool in designing and interpreting epidemiological studies, which use urinary phenol concentrations as a biomarker of exposure.

Individual variability in repeated measurements of urinary BPA concentrations has been investigated in different populations. Generally, only a low to moderate reproducibility over time was found (Arakawa et al., 2004; Teitelbaum et al., 2008; Mahalingaiah et al., 2008; Nepomnaschy et al., 2009; Ye et al., 2011; Braun et al., 2011, 2012; Christensen et al., 2012; Meeker et al., 2013). To our knowledge, only two studies have examined the individual variability in repeated measurements of other phenols than BPA: one study conducted among children, who may have different excretion patterns than adults (Teitelbaum et al., 2008), and another study among pregnant women in Puerto Rico (Meeker et al., 2013).

The aim of this study was to assess the temporal variability of urinary excretion of the following phenols present in the environment: BPA, TCS, TCC, BP-3, the sum of 2,4-DCP and 2,5-DCP (ΣDCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2-PP and 4-PP. The study was based on repeated collection of 24-h, first-morning and spot urine samples from 33 men collected over a 3 months period.

2. Materials and methods

2.1. Study population and data collection

The study population consisted of 33 men, who delivered urine samples four times over a period of approximately 3 months. Data collection took place from April 2008 to September 2008. The study design is outlined in Table 1. The participants in this study were recruited from an ongoing study of reproductive health in young men described in detail in Jørgensen et al. (2002, 2012). Totally eight samples were collected from each man for this study. At the first visit a spot urine sample was collected. At a median of 4 days thereafter following samples were collected: a spot urine sample, and the total amount of urine during the following 24 h, including the second days first morning urine collected separately. The 24-h urine from the second visit thus includes all voids starting with the spot urine sample to and inclusive of the next day's first morning urine. At the third visit (median: 44 days, min–max: 31–71 days after first visit) and the fourth visit (median: 87 days, min–max: 67–105 days after first visit), all voids during 24 h were collected, including the second day's first morning urine collected separately (see Table 1). Four samples were missing, leaving 260 samples eligible for analyses (for further details on study design see Frederiksen et al. (2013b)). Median anthropometric characteristics of the participants: height 180 cm (5th and 95th percentiles: 173 and 194 cm), weight 77.1 kg (5th and 95th percentiles: 60.0 and 93.1 kg) and Body Mass Index (BMI: weight in kg/(height in m)^2) 22.7 kg/m^2 (5th and 95th percentiles: 18.5 and 29.1 kg/m^2).

All participants received written and oral information about the study and provided a written acceptance to participate. The study was approved by the ethical committee for the Copenhagen municipality (ref. nos.: KF 01-117/96 and KF 01-292/98 with amendment of January 19, 2006).

2.2. Chemical analyses

The urinary content of total (free and conjugated) BPA, TCS, TCC, BP-3, the sum of 2,4-DCP and 2,5-DCP (ΣDCP), 2,4,5-TCP, 2-PP and 4-PP was analysed by a newly developed method for simultaneous quantitative determination using isotope dilution TurboFlow-liquid chromatography–tandem mass spectrometry (LC–MS/MS) with preceding enzymatic deconjugation by adding a mixture of β-glucuronidase and sulfatase and incubating for 2 h at 37 °C. Further details on chemical analyses are available in Frederiksen et al. (2013a). In short, samples were analysed in 13 batches over a period of 3 weeks. Each batch included standards for calibration curves, about 25 unknown samples, two blanks, two urine pool controls and two urine pool controls spiked with phenol standards at low and high levels. The inter-day variation, expressed as the relative standard deviation (RSD), was ≤12% for most analytes in both spiked samples except for TCC (17%) and 4-PP (<14%). The recovery of spiked samples was >95% for all analytes except for TCS (87%).

Urinary osmolality, which is a measure of urinary dilution, was measured by the freezing point depression method using an automatic cryoscopic osmometer (Osmomat® 030 from Gonotec, Berlin, Germany). For each nine samples measurement, a standard urine pool was measured. Mean urinary osmolality for this standard pool (N=42) was 0.341 Osm/kg with a relative standard deviation (RSD) of 0.68%. The median (range) osmolality of all urine samples included in this study was 0.799 (0.081–1.238) Osm/kg, which is within the normal range.

Creatinine was measured in 24-h urine samples by colorimetric enzymatic assay (Roche Diagnostics GmbH, Mannheim, Germany)

2.3. Statistical methods

Descriptive statistics of phenol urinary concentration were computed for each type of urine sampling. In order to correct for urinary dilution, we adjusted phenol concentrations for the urinary osmolality, normalised to the median osmolality of all samples (0.8 Osm/kg). This was done for all samples with a measured phenol concentration above the phenol specific LOD by the following equation:

$$\text{osmolality adj. concentration}_i\ (\text{ng/mL}_{(\text{osm})}) = \frac{C_i\ (\text{ng/mL}) \times \text{Osm}_\text{M}\ (\text{Osm/kg})}{\text{Osm}_i\ (\text{Osm/kg})},$$

where C_i (ng/mL) is the excreted urinary phenol concentration for the ith sample, Osm_M (Osm/kg) is the median osmolality of all samples and Osm_i (Osm/kg) is the osmolality of sample i. Adjustment for creatinine is another commonly used method to adjust for urinary dilution. To allow for comparison with results from other studies using this approach, we have also presented results of 24-h urine samples corrected for creatinine by

$$\frac{\mu\text{g analyte}}{\text{g creatinine}} = \frac{\text{urinary phenol concentration}\ (\mu\text{g/L}) \times 1000\ (\text{mg/g})}{\text{creatinine concentration}\ (\text{mmol/L}) \times 113.12\ (\text{mg/mmol})},$$

where 113.12 mg/mmol is the molecular weight of creatinine. Twenty-four hours excretion (ng/day) was calculated for 24-h urine samples by multiplying the volume (mL/day) with the phenol concentration (ng/mL). Samples with concentrations below LOD were not adjusted for osmolality, creatinine or volume, but were substituted by the phenol specific value of LOD/$\sqrt{2}$ (Hornung and Reed, 1990).

2.3.1. Intraclass correlation coefficient analysis

The temporal variability was assessed by calculating the within- and the between-person variances as well as the intraclass correlation coefficients (ICCs) for the serial measurements of urinary excretion of BPA, TCS, BP-3 and ΣDCP, as these compounds were most frequently detected in urine samples (>70% of samples above LOD). The ICC takes a value between 0 and 1 and reflects the relationships between the within- and between-person variances (calculated by dividing the estimated between-person variance by the total variance). For example, an ICC of 0.55 means that 55% of the observed variation in the measurements is due to between-person variation and 45% is due to within-person variation. The variances and corresponding ICCs were calculated for the two spot urine samples, which were collected approximately 4 days apart, as well as the three 24-h urine samples and the three first morning urines, which were collected approximately 40 days apart. Due to the asymmetric distribution of the chemical

Table 1
Study design. Sample types collected at the four visits.

1st visit	2nd visit, 4 days[a]	3rd visit, 44 days[a]	4th visit, 87 days[a]
Spot urine sample	Spot urine sample[b]		
	24-h urine sample	24-h urine sample	24-h urine sample
	Next day's 1st morning urine sample[b]	Next day's 1st morning urine sample[b]	Next day's 1st morning urine sample[b]

[a] Median number of days since first visit.
[b] Collected as a separate sample and subsequently pooled with the 24-h urine sample (see also the Materials and methods section).