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

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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.

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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).

measurements, the concentrations were ln-transformed. The statistical software programme SPSS (PASW 18, SPSS Inc.) was used to calculate the within- and between-person variances and the ICCs based on a two-way random ANOVA. All other data handling and analyses were made in SAS version 9.1 (SAS Institute Inc. Cary, NC, USA).

2.3.2. Exposure classification analysis

We divided phenol concentrations into tertiles and examined the consistency of phenol concentration levels by counting how many times the repeated measurements from the same individual were classified within the same tertile (none, two or three times). The three 24-h urine samples and the corresponding three second day's first morning urine samples collected approximately 40 days apart were included. Tertile cut-off points were based on the distribution of the given phenol in all three sample types.

2.3.3. Analyses of samples collected within 24 h

Spearman's rank correlation coefficient was used to analyse the correlation between the osmolality adjusted concentrations measured in the three different types of urine samples (spot, first morning and 24-h urine) collected within 24 h (samples from second visit), of which the second day's first morning and the spot urine sample were subsets of the 24-h urine sample. In addition we computed the median osmolality adjusted concentrations in these samples collected within 24 h among the 31 men, who provided all three sample types. We used the non-parametric Wilcoxon signed rank test to test for pairwise differences between osmolality adjusted concentrations in the spot, first morning and 24-h samples collected within the same 24 h.

2.3.4. Estimation of BPA intake

Toxicokinetic studies of BPA have found that BPA is almost completely recovered in the urine within 24 h of oral administration (Völkel et al., 2002, 2005; Fisher et al., 2011). Estimations of the daily intake were therefore made on the 98 24-h urine samples from the 33 men using the equation

$$\text{daily intake (ng/kg/day)} = \frac{\text{excreted urinary BPA (ng/mL)} \times \text{24 hours urine volume (mL/day)}}{\text{body weight (kg)}}$$

3. Results

3.1. Phenol concentrations

BPA, TCS, BP-3 and ΣDCP were detectable in the majority of the samples (> 70%) with BP-3 and TCS being excreted in the highest median concentrations followed by BPA and ΣDCP (Table 2). 2-PP was detectable in more than 50% of the samples, whereas 4-PP and 2,4,5-TCP were detectable in 11–44% of the samples and TCC in 2–29% of the samples, depending on sample type (Table 2). The ranges of TCS and BP-3 concentrations were wide with maximum levels being, respectively, more than 900- and 1600-fold higher than the corresponding median concentration. In comparison the ranges of BPA and ΣDCP were much narrower. In the following analyses we only included the four compounds, which were detected in > 70% of samples.

3.2. Temporal variability

Table 3 shows the ICCs, the within- and between-person variance for repeated measurements of BPA, TCS, BP-3 and ΣDCP according to sample type with and without adjustment for osmolality, volume or creatinine. For all BPA measurements the within-person variance was higher than the between-person variance, resulting in ICCs of 0.10 for morning urine samples, 0.26 for 24-h samples and 0.42 for spot samples. For TCS the between-person variance accounted for more than 50% of the total variance with ICCs of the same magnitude for 24-h and morning urine samples and higher ICC for the two spot urine samples (Table 3). The highest ICCs for the 24-h and morning urine samples were found for BP-3. The ICCs for the three sample types of BP-3 were of the same magnitude. The ICCs for ΣDCP ranged from 0.39 in morning urine samples and 0.50 in 24-h urine samples to 0.72 in spot urine samples (Table 3).

The variability in phenol measurements is also illustrated in Fig. 1.

Adjustments for osmolality, volume and creatinine did not substantially change the ICCs for the repeated measurements (Table 3).

3.3. Classification analysis

The majority of men had two out of three repeated measurements categorised within the same tertile of phenol exposure

Table 2
Distribution of urinary phenol concentrations (ng/mL) in 33 Danish men according to the method of urine sampling.

Sample type	Compound	Limit of detection	N > LOD (%)	Percentiles						Geometric mean (95%CI)
			Minimum	5th	25th	50th	75th	95th	Maximum	
Spot urine	BPA	0.12	53 (82)		< LOD	0.35	1.75	3.90	6.40	14.2	1.19 (0.81;1.75)
N=65	TCS	0.06	65 (100)	0.10	0.18	1.05	3.86	22.4	611	1334	5.60 (3.14;10.00)
	TCC	0.01	19 (29)				< LOD	0.04	0.46	0.56	[a]
	BP-3	0.07	61 (94)		< LOD	1.90	4.37	18.0	37.6	7112	4.97 (2.98;8.29)
	ΣDCP	0.07	52 (80)		< LOD	0.19	0.82	1.66	5.56	9.26	0.57 (0.39;0.85)
	2,4,5-TCP	0.06	21 (32)				< LOD	0.40	1.64	5.55	[a]
	2-PP	0.12	41 (63)			< LOD	0.25	0.35	0.71	1.07	0.21 (0.17;0.25)
	4-PP	0.13	29 (44)				< LOD	1.14	3.36	4.84	[a]
Morning	BPA	0.12	84 (87)		< LOD	1.06	2.16	3.91	17.5	32.3	1.67 (1.25;2.24)
urine	TCS	0.06	93 (96)	< LOD	0.09	0.96	2.74	24.1	422	2168	4.85 (2.88;8.15)
N=97	TCC	0.01	15 (15)					< LOD	0.12	0.22	[a]
	BP-3	0.07	92 (95)	< LOD	< LOD	2.17	6.75	14.0	97.8	8029	5.84 (3.89;8.76)
	ΣDCP	0.07	86 (89)		< LOD	0.30	0.64	1.33	7.86	9.89	0.58 (0.44;0.76)
	2,4,5-TCP	0.06	21 (22)					< LOD	1.29	2.09	[a]
	2-PP	0.12	56 (58)			< LOD	0.18	0.33	0.81	6.20	0.19 (0.16;0.23)
	4-PP	0.13	34 (35)				< LOD	0.99	5.31	57.8	[a]
24-h	BPA	0.12	71 (72)			< LOD	1.48	3.46	9.36	31.5	0.93 (0.66;1.31)
urine	TCS	0.06	89 (91)		< LOD	0.67	2.04	27.1	293	2020	3.54 (2.07;6.06)
N=98	TCC	0.01	2 (2)						< LOD	0.29	[a]
	BP-3	0.07	81 (83)		< LOD	0.83	2.79	9.62	73.3	4144	2.38 (1.46;3.90)
	ΣDCP	0.07	72 (73)			< LOD	0.37	0.87	5.42	9.43	0.30 (0.22;0.40)
	2,4,5-TCP	0.06	11 (11)					< LOD	0.36	1.61	[a]
	2-PP	0.12	53 (54)			< LOD	0.15	0.27	0.44	0.75	0.16 (0.14;0.18)
	4-PP	0.13	38 (39)				< LOD	1.04	2.81	72.4	[a]

[a] Not calculated as the proportion of measurements below LOD was too high to provide a valid result. LOD: limit of detection.

Table 3
Intra-class coefficients (ICCs), 95% confidence interval (95% CI) and variance estimates (between- and within-person variance, σ^2) of ln-transformed repeated measurements of BPA, TCS, BP-3 and ΣDCP in 24-h urine samples[a] ($N=3$ repeated samples from 32 men), first morning urine samples[a] ($N=3$ repeated samples from 31 men) and spot urine samples1;[b] ($N=2$ repeated samples from 32 men).

Sample type		BPA			TCS			BP-3			ΣDCP		
		Variance estimate		ICC (95% CI)	Variance estimate		ICC (95% CI)	Variance estimate		ICC (95% CI)	Variance estimate		ICC (95% CI)
		Between-person σ^2	Within-person σ^2		Between-person σ^2	Within-person σ^2		Between-person σ^2	Within-person σ^2		Between-person σ^2	Within-person σ^2	
24 h urine samples	Unadjusted (ng/mL)	0.74	2.05	**0.26** (0.05;0.50)	3.98	3.28	**0.55** (0.35;0.73)	4.26	1.94	**0.69** (0.52;0.82)	1.07	1.08	**0.50** (0.29;0.69)
	Osmolality adjusted ($ng/mL_{(osm)}$)	0.72	2.21	**0.25** (0.03;0.48)	4.30	3.19	**0.57** (0.38;0.74)	4.28	2.02	**0.68** (0.51;0.81)	1.12	1.16	**0.49** (0.28;0.68)
	Total daily excretion (ng/day)	0.70	2.24	**0.24** (0.03;0.48)	4.50	3.17	**0.59** (0.39;0.75)	4.70	1.80	**0.72** (0.57;0.84)	1.27	1.14	**0.53** (0.32;0.71)
	Creatinine adjusted (μg/g creatinine)	0.60	1.75	**0.26** (0.04;0.49)	4.05	3.11	**0.57** (0.36;0.74)	3.94	1.63	**0.71** (0.54;0.83)	0.96	0.93	**0.51** (0.30;0.70)
First morning urine samples	Unadjusted (ng/mL)	0.22	1.88	0.10 (0;0.35)	3.71	2.92	**0.56** (0.36;0.74)	3.04	1.20	**0.72** (0.55;0.84)	0.77	1.19	**0.39** (0.17;0.61)
	Osmolality adjusted ($ng/mL_{(osm)}$)	0.22	1.86	0.11 (0;0.35)	3.73	2.93	**0.56** (0.36;0.74)	3.03	1.24	**0.71** (0.54;0.83)	0.75	1.18	**0.39** (0.17;0.61)
Spot urine samples	Unadjusted (ng/mL)	1.04	1.45	**0.42** (0.09;0.67)	4.79	0.53	**0.90** (0.81;0.95)	3.47	0.85	**0.80** (0.64;0.90)	1.78	0.68	**0.72** (0.51;0.86)
	Osmolality adjusted ($ng/mL_{(osm)}$)	0.90	1.36	**0.40** (0.07;0.65)	4.97	0.35	**0.93** (0.87;0.97)	3.30	0.83	**0.80** (0.63;0.90)	1.74	0.62	**0.74** (0.53;0.86)

ICCs: intraclass correlation coefficients=between person variance divided by the total variance (sum of between- and within-person variance). ICCs presented in bold: p-value < 0.05. σ^2=variance.

[a] 24-h and first morning urine samples were collected approximately 40 days apart.

[b] Spot urine samples were collected approximately 4 days apart.

(Table 4). Only three men (10%) had all three morning urine measurements of BPA in the same tertile, as opposed to 13 (42%), 12 (39%) and 8 (26%) for TCS, BP-3 and ΣDCP measurements, respectively. Likewise, the consistency of the categorical exposure assessments in 24-h urine samples was slightly higher for TCS, BP-3 and ΣDCP than for BPA.

3.4. Correlation between 24-h, first morning and spot urine samples collected within 24 h

Osmolality adjusted BPA concentrations in spot, first morning and 24-h urine samples were pairwise moderately correlated (Table 5). In contrast, the BP-3 and TCS concentrations of the three different sample types collected within 24 h were highly correlated ($r > 0.80$). Likewise moderate to high pairwise correlations between the concentrations of ΣDCP in the three sample types were observed.

3.5. Median differences in samples from the same day

In samples collected on the same day (second visit), the 24-h urine samples had the lowest median osmolality adjusted concentration for BPA, BP-3 and ΣDCP, which was statistical significantly lower than the corresponding concentration in the first morning urine samples ($p < 0.05$). The median osmolality adjusted concentration of spot urine samples was between the 24-h and the first morning urine samples (rank order: 24-h < spot < first morning urine sample) for these three compounds. For TCS no differences in osmolality adjusted concentrations were observed between the three methods of sample collection (Fig. 2; unadjusted median concentrations are shown in Supplementary materials Fig. S1).

3.6. Estimated BPA intake

The estimated median daily intake of BPA was 27.4 ng/kg/day (maximum 536 ng/kg/day).

4. Discussion

Our study revealed that it is challenging to estimate 'true' individual human exposure levels to non-persistent chemicals such as phenols by means of urine samples. Overall, serial measurements of TCS and BP-3 were more consistent than those of BPA and ΣDCP irrespective of urine sample type. The high variability in BPA measurements both over time and within the same day may be due to variation of exposure through diet and other lifestyle factors (Geens et al., 2012). The higher consistency in the TCS and BP-3 measurements compared with BPA and ΣDCP could indicate more stable exposure sources to these two compounds. The data was collected during late spring and summer and sun lotion application may possibly be one of the BP-3 exposure sources.

When comparing urine sampling method, 24-h urine samples did not provide higher consistency in the repeated measurements of TCS and BP-3 compared with first morning urines. For BPA and ΣDCP only slightly higher ICCs were found for 24-h urine samples compared with first morning urines. The collection of 24-h urine samples in epidemiological studies is logistically much more difficult than those of morning or spot samples. Our data do not support that such an effort is justified for improving individual exposure assessments. For all phenols included in the ICC-analysis, we observed higher ICCs for spot urine samples compared with the two other sample types. The spot urine samples were, however, collected only approximately 4 days apart and are therefore not directly comparable with the first morning and

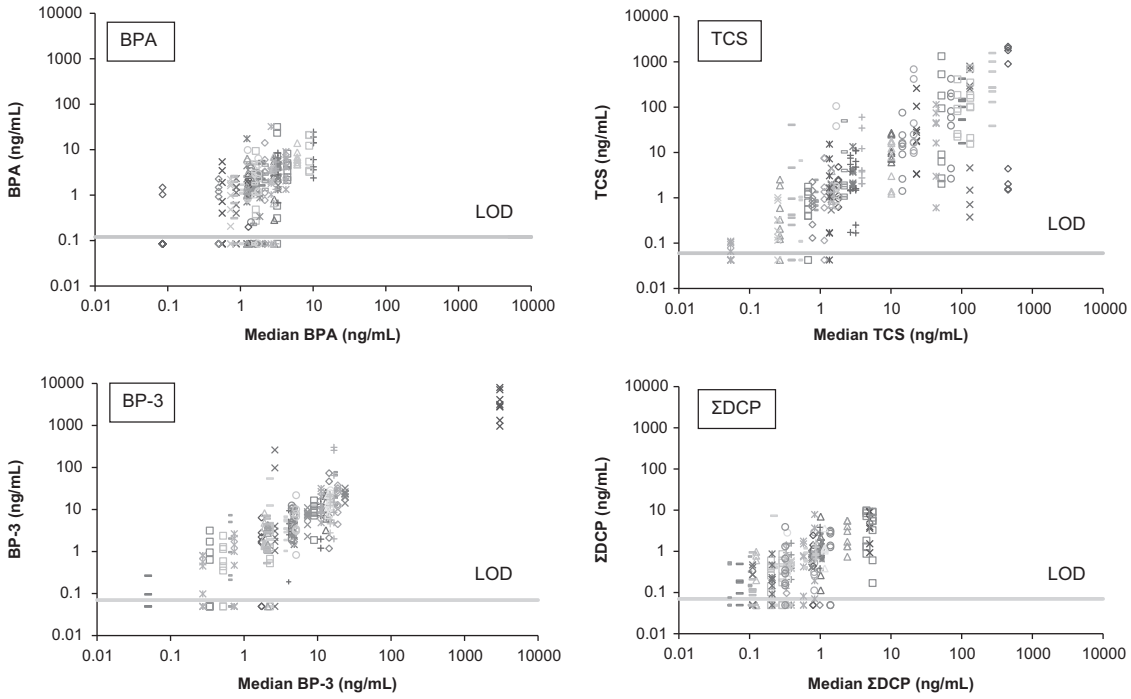

Fig. 1. Individual median phenol concentration of all eight repeated samples plotted against the concentrations from the separate samples of each individual ($N=33$). Limit of detection (LOD) is displayed as a horizontal line. Note that the axes are logarithmic and fixed at the same level in order to better illustrate differences in excretion ranges and variability of the four phenols.

Table 4
Number (%) of men with morning urines and 24 h urine samples in the same tertile concentration within 3 months. Restricted to men with three measurements of first morning urine ($N=31$) and 24-h urines ($N=32$).

No. of samples in the same tertile	BPA	TCS	BP-3	ΣDCP
Morning urines				
0	8 (26)	2 (6)	0 (0)	4 (13)
2	20 (65)	16 (52)	19 (61)	19 (61)
3	3 (10)	13 (42)	12 (39)	8 (26)
24-h urines				
0	5 (16)	3 (9)	2 (6)	2 (6)
2	20 (63)	21 (66)	19 (59)	21 (66)
3	7 (22)	8 (25)	11 (34)	9 (28)

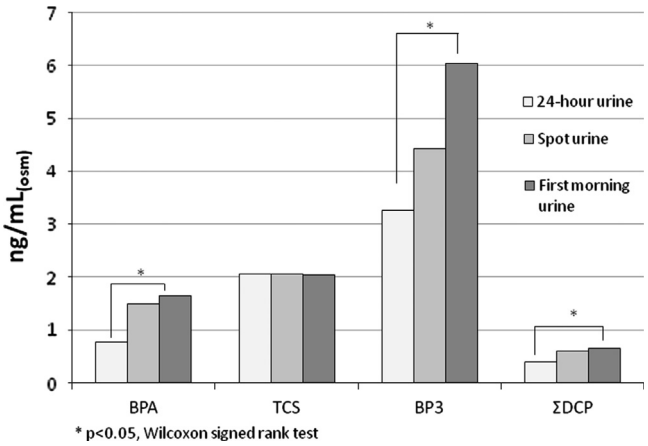

Fig. 2. Osmolality adjusted median concentrations ($ng/mL_{(osm)}$) of samples collected within the same day (second visit, $N=31$).

24-h urine samples collected approximately 40 days apart. Although the half-lives of the phenols are less than 24 h, e.g. approximately 4–6 h for BPA (Hissink et al., 1997; Völkel et al., 2002, 2005; Sandborgh-Englund et al., 2006; Pascal-Lorber et al., 2012), the higher ICC for spot urine samples could indicate higher similarity in phenol exposure sources within a short term than long term interval. None of the methods used to adjust for urinary dilution (osmolality, volume and creatinine) changed the consistency of repeated measurements. Thus, urinary dilution may not be a major contributing factor to the observed variation, which rather reflects true temporal variations in exposure patterns. Exclusion of one man with extremely and consistently high BP-3 concentrations did not substantially change the results (data not shown).

In urine samples collected within the same 24 h, the moderate within-day correlations in BPA samples indicate that BPA excretion varies considerably within the day in an individual, while the moderate to high correlations of ΣDCP samples indicate less variation within the day than for BPA. For TCS and BP-3 the high within-day correlations indicate that classification of exposure level by means of either sample type will be equally good. However, for BPA, BP-3 and ΣDCP the levels of osmolality adjusted concentrations observed in the three methods of sample collection differed in general in the same direction with the highest median

Table 5
Spearman correlation coefficients between concentrations of BPA, TCS, BP-3 and ΣDCP in samples collected within the same 24 h.

	BPA	TCS	BP-3	ΣDCP
24-h urine vs. next day's first morning urine	0.56**	0.95**	0.89**	0.56**
24-h urine vs. spot urine	0.44*	0.94**	0.93**	0.75**
Spot urine vs. next day's first morning urine	0.46*	0.90**	0.88**	0.75**

* $p<0.05$.
** $p<0.001$.

concentrations in first morning urines and the lowest in the 24-h urine samples. This finding of difference in phenol concentration levels according to method of sample collection has potential implications for risk assessments based on human biomonitoring, as calculations of average daily intake will yield different results depending on the method of urine sampling. That the osmolality adjusted median concentrations of the second day's first morning and spot urine samples exceeded that of the 24-h urine sample, even though these samples are subsets of the 24-h urine samples, indicates that in general lower concentrations could be expected in spot samples collected later in the day compared to samples collected in the morning.

4.1. Future design and interpretation of epidemiological studies

Our results can be used for interpretation and future design of epidemiological studies examining the effect of phenol exposure on health related outcomes. The considerable variability of phenol urinary excretion over time within one person, in particular for BPA (ICCs ranging from 0.10 to 0.42), hampers exposure classification in epidemiological studies and potentially leads to misclassification when the exposure measures are intended to reflect exposure through a longer period of time than just the hours immediately prior to urine sampling. For studies investigating the effect of, for example, BPA on some health outcome, an important consequence of exposure misclassification of BPA, is that the estimated effect of BPA generally will be attenuated, making it more difficult to detect statistically significant associations with an outcome. Thus when statistically significant associations are observed in spite of misclassification on phenol exposure, the actual effects may be even larger than estimated. The results from the repeated measurements, which we present in this study, can be used to correct for this measurement error in studies examining the association between urinary phenol concentration and a health outcome. In the simplest case, when conducting a linear regression analysis in which BPA is the only explanatory variable, the attenuation factor is equal to the ICC (Andersen and Skovgaard, 2010). One would therefore expect that the effect of BPA (e.g. measured in 24-h samples) would be underestimated by approximately a factor of 4, because ICC equals 0.26. Statistical methods for correction of measurement errors in e.g. logistic regression analysis and in more complex statistical models with several covariates are described in e.g. Fuller (1987), Rosner et al. (1992), Buonaccorsi (2010) and Armstrong (1990).

Knowledge about the variation in phenol excretion can also be used in the design of new studies. When calculating the required number of participants needed to be able to detect a certain difference in e.g. a health outcome measure as an effect of phenol exposure, the phenol ICC can be used in power calculations in order to take into account the above mentioned effect attenuation, which consequently leads to an increase in the estimated required sample size.

The observed variability in serial BPA measurements is in agreement with levels observed in other studies (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; Meeker et al., 2013) with ICCs ranging between 0.11 (Braun et al., 2011) and 0.43 (Nepomnaschy et al., 2009). Ye et al. have examined the urinary concentrations of BPA in spot, first morning and 24-h samples among eight adults, who collected all urine samples during a week. In agreement with our findings, they found considerable variation in creatinine-corrected urinary BPA concentrations in all three sample types. The ICCs were 0.23 for first morning urines and 0.12 for simulated 24-h urines. The within-day variation was the main contributor to the total variance of the spot samples (70%), followed by between-day (21%) and between-person variability (9%) (Ye et al., 2011).

Our estimates of temporal variability in TCS, BP-3 and ΣDCP are also in agreement with a study of 105 pregnant women from Puerto Rico providing three spot urine samples during pregnancy, for which ICCs of 0.47, 0.62, 0.38 and 0.49, respectively, for TCS, BP-3, 2,4-DCP and 2,5-DCP were observed (Meeker et al., 2013). The findings from Teitelbaum et al. of ICCs of 0.36 for 2,4-DCP and 0.40 for 2,5-DCP in repeated creatinine corrected phenol urinary measurements in 29 children aged 6–10 years, who provided between 2 and 7 spot samples over a period of 6 months, are also at comparable levels with our results (Teitelbaum et al., 2008). However, compared with our findings of high ICCs for TCS and BP-3, somewhat lower ICCs for creatinine corrected TCS and BP-3 (0.39 and 0.46, respectively) were found among the children in the study from Teitelbaum et al. Children may, however, have different patterns of exposure and metabolism from those of adults, and the results may therefore not be directly comparable.

The estimated median daily intake of BPA of approximately 27 ng/kg/day among 33 Danish men is of the same magnitude as found in an American population with data from 2005 to 2006 (33.7 ng/kg/day in the overall population and 37.3 ng/kg/day among the male population) (Lakind and Naiman, 2011). Somewhat higher estimates of daily intake were calculated on the basis of data from an American population in 2003–2004: overall median 50.5 ng/kg/day and 57.2 ng/kg/day among the male population only (Lakind and Naiman, 2008).

The tolerable daily intake (TDI) for BPA is estimated to be 0.05 mg/kg body weight (EFSA, 2010). The estimated maximum intake among the 33 Danish men was 536 ng/kg/day, thus 90 times lower than the TDI. This TDI level is currently heavily debated as adverse effects of BPA have been observed in animals at doses below the level, which the current TDI is based on (vom Saal et al., 2007; Vandenberg et al., 2012; Beronius and Hanberg, 2012).

In conclusion, we found low to moderate degree of temporal consistency for serial measurements of urinary BPA and ΣDCP excretion levels, while serial measurements of BP-3 and TCS showed higher consistency. In comparison with first morning urine samples, 24-h urine samples of TCS and BP-3 did not provide more consistent measurements and the sample types collected within the same day were highly correlated. Our data indicate that first morning urine samples provide as good exposure classification as those of 24-h urine samples. The considerable individual variability in urinary excretion over time of BPA, regardless of sample type, is likely to disguise or underestimate potential health hazards because of exposure misclassification, when using urinary excretion as proxy of exposure in epidemiological studies.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.07.001.

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