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# Short term variability in urinary bisphenol A in Australian children

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ABSTRACT

Used frequently in food contact materials, bisphenol A (BPA) has been studied extensively in recent years, and ubiquitous exposure in the general population has been demonstrated worldwide. Characterizing within- and between-individual variability of BPA concentrations is important for characterizing exposure in biomonitoring studies, and this has been investigated previously in adults, but not in children. The aim of this study was to characterize the short-term variability of BPA in spot urine samples in young children. Children aged ≥2–<4 years ($n = 25$) were recruited from an existing cohort in Queensland, Australia, and donated four spot urine samples each over a two day period. Samples were analysed for total BPA using isotope dilution online solid phase extraction–liquid chromatography–tandem mass spectrometry, and concentrations ranged from 0.53 to 74.5 ng/ml, with geometric mean and standard deviation of 2.70 ng/ml and 2.94 ng/ml, respectively. Sex and time of sample collection were not significant predictors of BPA concentration. The between-individual variability was approximately equal to the within-individual variability (ICC = 0.51), and this ICC is somewhat higher than previously reported literature values. This may be the result of physiological or behavioural differences between children and adults or of the relatively short exposure window assessed. Using a bootstrapping methodology, a single sample resulted in correct tertile classification approximately 70% of the time. This study suggests that single spot samples obtained from young children provide a reliable characterization of absolute and relative exposure over the short time window studied, but this may not hold true over longer timeframes.



## 1. Introduction

Biomonitoring provides an aggregate measure of exposure via all sources and pathways and has been hailed as the 'gold standard' for environmental exposure assessment (Sexton et al., 2004). Bisphenol A (BPA) is used as a plasticizer in polycarbonate plastics, particularly for food-contact items, and in sealers and liners for canned foods. BPA exposure was identified as being of potential concern due to evidence of estrogenic activity, and it has been studied extensively over the last decade. BPA has been linked to a range of human health outcomes including behavioural effects, disruption of endocrine function, and alteration of reproductive structure and function (reviewed in Rochester (2013)). BPA has a relatively short half-life in the range of 2–4 h, with near-complete urinary excretion in the 24 h following exposure (Christensen et al., 2012b; Shin et al., 2004; Teeguarden et al., 2011; Volkel et al., 2002, 2008). Urinary biomonitoring is performed routinely and ubiquitous exposure has been demonstrated in non-occupationally exposed populations worldwide, including Canada and North America, Europe and the Asia-Pacific region (reviewed in Vandenberg et al. (2010)).

For BPA and many other environmental chemicals, exposure levels are often characterized based on a single spot urine sample. This can be useful in cross-sectional studies, such as the National Health and Nutrition Environmental Survey (NHANES) conducted in the United States, but has limited usefulness when trying to study a subtle endpoint, such as a potential health-outcome, or for longitudinal or long-term studies. Within- and between-individual variation in urinary concentrations is expected due to exposure differences related to diet and other lifestyle factors (Casas et al., 2011; Geens et al., 2012), and this variation is commonly what researchers aim to characterize in population-wide biomonitoring studies. However, for chemicals with short half-lives and rapid elimination kinetics such as BPA, the timing of sample collection relative to the exposure event will also significantly influence the measured concentration (Aylward et al., 2012). This can result in exposure misclassification (Lassen et al., 2013) which has

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consequences for epidemiological evaluations and for risk and health assessments, so this variability should be accounted for.

A number of studies have investigated temporal variability of urinary measures of BPA. Most have focussed on adults from the general population (Arakawa et al., 2004) (Christensen et al., 2012a; Lassen et al., 2013; Mahalingaihah et al., 2008; Nepomnaschy et al., 2009; Teitelbaum et al., 2008; Ye et al., 2011) and report large within- and between-individual variation in urinary measures. Few studies have looked at variability during pregnancy (Braun et al., 2011a, 2012; Casas et al., 2013; Meeker et al., 2013), and none have investigated potential variability in children <6 years. Understanding exposures during critical windows of development such as childhood is particularly important because their physiology and unique susceptibility mean that children are disproportionately exposed to environmental chemicals compared with adults; and early life exposures facilitate longer latency periods for the development of chronic disease in adulthood (Scheuplein et al., 2002; WHO, 2004, 2011). Providing a framework for understanding biomonitoring data from children can assist in accurate risk assessment of such exposures. The aim of this study was to (1) characterize the short term variability in spot urine samples in young children; and (2) provide guidance for prospective cohort studies on the type, number, and frequency of collection for specimens for assessing short term environmental exposures to BPA in children.

## 2. Study population and methods

### 2.1. Study population

The study population consisted of 25 children aged ≥2–<4 years (16 males, 9 females) who donated 4 urine samples over a 2 day period. The participants were recruited from an ongoing study of iodine status and thyroid function in South-East Queensland, Australia. Descriptive information about each specimen was limited to age, postal code and sex. Care-givers of participants were instructed to collect one morning and one afternoon spot sample (first morning void and first urine following the midday meal, respectively) on two consecutive days, to give a total of 4 samples per participant. Samples were collected from toilet-trained children in standard polyethylene urine specimen containers and frozen until analysis. Specimens were collected from May 2012 to March 2013, and analysed in April–May 2013. No measures of creatinine or specific gravity were available. This study was approved by the University of Queensland ethics committee (approval number 2011000125).

### 2.2. Chemical analysis

Urine samples were analysed for total BPA (free plus conjugated species) at the National Research Centre for Environmental Toxicology, University of Queensland, Australia using an online solid-phase extraction liquid chromatography tandem mass spectrometry method. Briefly, 50 μl urine was diluted, cleaved enzymatically and injected directly into the online system using a GX-271 liquid handler. Quantitation was performed by isotope dilution using $^{13}$C-BPA (Cambridge Isotope Laboratories). More details can be found in Heffernan et al. (2013). Synthetic urine (Calafat and Sampson, 2009) was used for quality control. Fortified synthetic urine (1 ng/ml) was used to monitor instrument performance (0.91 ± 0.24 ng/ml, $n = 13$), and background contamination was monitored by repeated measures of un-fortified synthetic urine (0.18 ± 0.021 ng/ml, $n = 11$).The limit of detection (LOD) was 0.062 ng/ml (calculated as 3 × standard deviation of the blank). As blank levels were significantly higher than the LOD, the limit of reporting (LOR) was calculated as 3 × average blank, and set as 0.53 ng/ml. No blank subtraction was performed.

### 2.3. Exposure estimation

Using model-predicted, age specific urinary flow, $F$ (ml/kg $d^{-1}$) constructed from published literature values (Ballauff et al., 1988; Ebner and Manz, 2002; Goellner et al., 1981; Magos, 1987; Maguire et al., 2007; Martins et al., 2011; Pratt et al., 1948; Roberts and Lucas, 1985) described previously (Heffernan et al., 2013), and measured pool concentrations, $C$ (ng/ml), daily urinary excretion of BPA, $E$ (ng/kg-d), was calculated for each pool according to the following:

$$E = F * C \quad (1)$$

Under a steady-state assumption, daily urinary excretion of BPA will be equal to daily intake (Volkel et al., 2002); therefore estimated daily BPA excretion can be taken as estimates of daily BPA intake in the population covered by this study. This approach explicitly assumes that the BPA urinary excretion rate during the time period covered by the urinary void sampled is consistent over a 24-hour period, and the flow rate, excretion and exposure data are presented as estimates only, and for comparison to guideline intake values.

### 2.4. Statistical analysis

Intraclass correlation coefficients (ICC) were calculated using Stata IC12 (Stata Corp., College Station, TX, USA). A mixed-effect model was implemented to assess the within- and between-individual variance, and ICC values were reported as the ratio of the between-individual variance to the total variance.

Using a bootstrapping exercise, the dataset of four samples per individual over a two-day period was used to assess the relative reduction in accuracy in exposure classification resulting from collection of fewer than 4 samples over the same period. The dataset was resampled to simulate collection of 1, 2, 3, or 4 samples from each individual. For each iteration a new geometric mean (GM) value was calculated for each individual based on the resampled simulated dataset. The Spearman rank correlation coefficient (rho, ρ) between the simulated GM and the 'true' or observed GM (from the original dataset) was calculated for each participant for each iteration. In addition, each individual was categorized into tertile by simulated GM, and the resulting categorization was compared to that based on the 'true' GM; the fraction that was correctly categorized was recorded. The simulation was conducted for 1000 iterations for each number of spot samples per individual (1 through 4). The resulting median Spearman rank correlation coefficients and the fraction of participants correctly categorized from the 1000 iterations are reported, along with confidence intervals estimated at the 2.5th and 97.5th percentiles of the results from the 1000 iterations (Table 2)

## 3. Results

BPA was detected in all samples, with 5% ($n = 5$) being <LOR. Concentrations ranged from <LOR to 74.5 ng/ml with a GM of 2.70 and geometric standard deviation (GSD) of 2.94 ng/ml (Table 1). One individual (participant nine) was clearly an outlier. Of the four samples from this individual, three were the highest in the dataset and the fourth sample from that individual was the sixth highest in the dataset. If that individual is omitted from the dataset, the GM and GSD are 2.45 and 2.64 ng/ml.

A multivariate regression model was used to assess the influence of sex and time of sample collection (classified as morning or afternoon) on BPA concentration. Neither variable was found to be a significant predictor of measured BPA concentrations. Between-individual variance for the repeat sampling was approximately equal to within-individual variance (ICC = 0.51; 95% CI 0.32–0.70). To test the sensitivity of the assessment, the individual with the most extreme values (participant nine) was omitted and the calculation repeated. The ICC