Assessing the Quantitative Relationships between Preschool Children's Exposures to Bisphenol A by Route and Urinary Biomonitoring

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Received Date XXXX (inserted by journal)

Title Running Head. Children's Exposures to Bisphenol A and Biomonitoring

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ABSTRACT

Limited published information exists on young children's exposures to bisphenol A (BPA) in the United States using urinary biomonitoring. In a previous project, we quantified the aggregate exposures of 257 preschool children to BPA in environmental and personal media over 48-h periods in 2000-2001 at homes and daycares in North Carolina and Ohio. In the present study for 81 Ohio preschool children ages 23 – 64 months, we quantified the children's urinary total BPA (free and conjugated) concentrations over these same 48-h periods in 2001. Then, we examined the quantitative relationships between the children's intakes doses of BPA through the dietary ingestion, nondietary ingestion, and inhalation routes and their excreted amounts of urinary BPA. BPA was detected in 100% of the urine samples. The estimated median intake doses of BPA for these 81 children were 109 ng/kg/day (dietary ingestion), 0.06 ng/kg/day (nondietary ingestion), and 0.27 ng/kg/day (inhalation); their estimated median excreted amount of urinary BPA was 114 ng/kg/day. Our multivariable regression model showed that dietary intake of BPA ($p=0.04$) and creatinine concentration ($p=0.004$) were significant predictors of urinary BPA excretion, collectively explaining 17% of the variability in excretion. Dietary ingestion of BPA accounted for >95% of the children's excreted amounts of urinary BPA.

KEYWORDS. Bisphenol A, preschool children, exposure, urinary biomonitoring, Ohio

INTRODUCTION

Bisphenol A (BPA) is a high production volume chemical that is commonly used in the manufacturing of polycarbonate plastics and epoxy resins, with annual production of over six billion pounds worldwide (*1-2*). In the United States (U.S.), polycarbonate plastics or epoxy resins can be found in a variety of consumer products, which include reusable food and beverage containers, baby bottles, plastic dinnerware, toys, compact disks, and metal food can linings (*3-7*). BPA can also be used in the processing of other types of plastics (e.g., polyvinyl chloride) and thermal paper (*8*).

For preschool aged children in the U.S, information on human exposure to BPA is rather limited (9-*10*). BPA has been detected in several media including food, air, and dust samples collected at residences and child care centers (*9, 11-12*). Moreover, Wilson et al. (*9*) showed that dietary ingestion through the consumption of both solid and liquid foods was probably the major route of exposure for 257 preschool children to BPA at their homes and daycare centers in North Carolina and Ohio. This information suggests that BPA may be migrating out of packaging materials and containers into food and beverage products that are being consumed by children (*13-16*).

Once ingested, BPA is efficiently absorbed (>95%) from the gut, and it primarily undergoes phase II metabolism by conjugation with glucuronic acid in the liver (*10,17*). BPA is mainly eliminated in the urine as BPA-glucuronide with an average elimination half-life of about six hours in humans (*17*). Several studies have recently reported detectable levels of BPA in urine samples of infants, toddlers (1 year olds), and school-aged children (>5 years old) in the U.S. (*6, 7, 18-23*). We are unaware, however, of any published information on the concentrations of BPA in the urine of U.S. preschool

children, ages 2-5 years. In addition, we are unaware of any published studies in the U.S. that have assessed the quantitative relationships between preschool children's exposures to BPA and urinary biomonitoring.

In the present work, the objectives were to quantify the concentrations of total BPA in a subset of the 257 preschool children's (ages 2-5 years) archived urine samples from the Children's Total (Aggregate) Exposure to Persistent Pesticides and Other Persistent Organic Pollutants (CTEPP) study and then to examine the quantitative relationships between the children's intake doses of BPA through the dietary ingestion, nondietary ingestion, and inhalation routes and their excreted amounts of urinary BPA. Aggregate exposure is defined as the combined exposures of a person to a single chemical from all sources, pathways, and routes.

MATERIALS and METHODS

In a previous publication by Wilson et al. (*9*), we quantified the aggregate exposures of 257 CTEPP preschool children to BPA in several environmental (i.e., soil, dust, and air) and personal media (i.e., solid food, liquid food, and hand wipes) at their homes and daycare centers in North Carolina and Ohio. In our present work, we had 81 CTEPP Ohio children's archived urine samples that were collected in 2001 quantified for concentrations of total BPA (free and conjugated) in 2006. Then we assessed the quantitative relationships between the 81 children's excreted amounts of urinary BPA, measured in the archived samples, and their intake doses of BPA through the dietary ingestion, nondietary ingestion, and inhalation routes previously measured for these 81 CTEPP participants and published by Wilson et al. *(9*).

Briefly in the CTEPP study, the 81 children were randomly recruited using a probability-based, multistage stratified random sampling plan from homes and daycare centers in six Ohio counties between January 2001 and November 2001. The children had environmental, personal, and biological (urine) samples collected over a 48-h period at their residences (home group) and/or at their homes and daycare centers (daycare group). Of these 81 children, 41 were from the home group and the rest were from the daycare group. The mean and median ages of these children were both 45 months, and their ages ranged between 23 and 64 months.

Field Sampling

For this subset of 81 Ohio children, field sampling occurred at 81 homes and 16 child daycare centers. The sampling methodology for the collection of the environmental and personal samples over a 48-h period by field staff at these locations has been described earlier (*9, 24*) and is summarized in the Supplemental Information. For the biological samples, up to six spot urine voids (i.e., morning, after lunch, and before bedtime) were collected from each child by their primary caregiver over the 48-h sampling period. Spot urine samples were collected from the children using bonnets placed under toilet seats; then transferred to polypropylene containers with lids (Fisher Scientific). All urine samples were kept in chilled coolers by the adult caregivers at the homes or daycare centers over the 48-h monitoring period. Then, the urine samples were transported in coolers with blue ice by field staff to the laboratory and stored in freezers ($\leq -10^{o}C$) until analyses.

Human Subjects Review

This was an observational research study, as defined in 40 Code of Federal Regulations (CFR) Part 26.402. The study protocol and procedures to obtain the assent of the children and informed consent of their parents or guardians were reviewed and approved by an independent institutional review board and complied with all applicable requirements of the Common Rule regarding additional protections for children (Subpart D). The Centers for Disease Control and Prevention (CDC) investigators did not have access to any identifiable information from the study participants. As a result, the involvement of the CDC laboratory was limited and determined not to constitute engagement in human subjects research.

Sample Analyses

Detailed extraction and analytical procedures used to quantify BPA levels in the environmental and personal media can be found in Wilson et al. (*9*). For the biological samples, all of the urine voids for each child at their home and/or daycare center over the 48-h monitoring period were pooled in 2001 by laboratory technicians at Battelle in Columbus, Ohio, aliquots were analyzed for target compounds, which at that time did not include analysis for urinary BPA or its metabolites. The remaining amount of urine in the samples was placed into individual 120 mL high density polyethylene plastic bottles with lids and stored in freezers ($\leq$ -10^{o}C) at Battelle in Columbus, Ohio.

In 2004, the pooled urine samples were shipped on dry-ice via overnight from Battelle to the US Environmental Protection Agency's (US EPA) National Exposure Research Laboratory located in Research Triangle Park, NC and stored in freezers (-80^{o}C) for approximately two years. In January 2006, a US EPA technician selected the first 97 urine samples from the 81 Ohio children that contained an adequate amount ($\geq$15

mL) of urine. The urine samples were thawed at room temperature in a laboratory fume hood overnight. Then, each sample was vigorously shaken by hand and a 2 mL aliquot of urine was pipetted into a 5 mL polypropylene cryovial with a screw cap lid. All aliquots were blinded and shipped on dry ice via overnight to the CDC laboratory in Atlanta, GA in January 2006. The urine samples were kept in freezers at -80^{o}C at CDC until analyses for total BPA (free plus glucuronide and sulfate conjugates) using the published method by Ye et al. (*25*). All urine samples were analyzed by April 2006. The limit of detection (LOD) for BPA in urine was 0.4 ng/mL. The estimated limit of quantification (LOQ) was approximately three times the reported LOD.

Quality Control Procedures for Urine

To quantify the concentration of total BPA in urine, two low-concentration (~2.5 ng/mL) and two high-concentration (~16 ng/mL) quality control materials, prepared at CDC with pooled human urine spiked with BPA, were analyzed in each batch with standard, reagent blank, and study samples (*25*). The study samples were analyzed in approximately 11 weeks in 10 separate batches. QC concentrations were evaluated by use of standard statistical probability rules (*26*). The inter-batch RSD of these QCs was 18.8% at 2.4 ng/mL and 13.7% at 16.2 ng/mL. BPA concentrations in the laboratory blanks analyzed with the study samples were <LOD.

Duplicate samples (aliquots of each individual urine sample) were also prepared in 2006 by the US EPA for about 25% of the urine samples. Thirty-one duplicate samples were analyzed by the CDC for total BPA with the study samples (CDC researchers were blinded to the presence and identity of these duplicate samples). The mean±std of the

absolute difference between duplicates total BPA concentrations was 1.15±1.0 ng/mL and the mean relative percent difference was 28%, considerably larger than the RSD of the laboratory QCs (see above). Furthermore, the mean relative percent difference for BPA was similar to that obtained for other phenols measured concurrently with BPA and also for phthalate metabolites which were measured at CDC using a different analytical method and instrumentation. All of these findings taken together suggest that the mean relative percent differences for BPA in the duplicate urine samples are not associated with the analytical measurement of BPA, but may be due to sample preparation procedures (e.g., inadequate mixing of the urine during aliquotting) at the US EPA laboratory.

Statistical Analyses

All BPA concentrations that were less than the LOD were assigned a value of LOD divided by the square root of two. Descriptive statistics (median, range, and select percentiles [25^{th}, 75^{th}, and 95^{th}]) were calculated for BPA in each environmental and personal medium at the 81 children's homes and daycare centers in Ohio. Descriptive statistics were also computed for total BPA in the urine samples as unadjusted (ng/mL), creatinine-adjusted (ng/mg), and specific gravity-adjusted (ng/mL) values for these children overall and by group (home and daycare). Results from the duplicate urine samples were not included in the above analyses. Each urine sample was also analyzed for creatinine (range 16.6 – 232.8 mg/dL) and specific gravity (range 1.01 – 1.03), and all of these values were used for the descriptive statistics (*27-28*). Creatinine and specific gravity are

common correction methods for adjusting for variable dilutions in adult urine samples, however, creatinine may not be a reliable adjustment measure for children (*27-28*).

The estimated intake doses (ng/kg/day) of BPA by the dietary ingestion, nondietary ingestion, and inhalation routes for each child and their estimated excreted amounts (ng/kg/day) of unadjusted urinary BPA were calculated based on equations reported in an earlier publication (*9*). These equations and the variables defining these equations are also presented in Table S1. We used a 100% absorption rate for the dietary and nondietary ingestion routes based on a study conducted by Volkel et al. (*17*), in which six adult (human) volunteers were orally administered 5 mg of d_{16}-BPA by capsule and completely eliminated 100% of the dose (118± 21%) as d_{16}-BPA glucuronide in their urine. This 100% absorption rate for adults was used as no published data currently exist on the toxicokinetics of BPA in children. For the inhalation rate we assumed a default 50% absorption rate since no human studies exist in the published literature (*10, 29*).

Pearson correlation coefficients were used to examine the pairwise relationships between the response variable (i.e., the natural logarithm [ln] of the excreted amounts of urinary BPA (ng/kg/day)) and the independent variables (i.e., ln creatinine concentration (mg/dL), ln specific gravity, and ln of dietary ingestion, nondietary ingestion, and inhalation doses (ng/kg/day) of BPA). Furthermore, these correlations were used to examine the potential collinearity between the independent variables prior to the modeling process. Multivariable regression analysis was performed using a sequential, step-wise backward elimination process using the PROC REG procedure in SAS. The step-wise procedure was used to generate the "best" model and at each step, we looked at the level of significance of the independent variable and its effect on the r^2 of the model.

There were two criteria for keeping independent variables in our model: a variable that had a p $\leq$0.10, or a variable that when excluded from the model changed the r^2 by 10% or more. All statistical analyses were performed using SAS version 9.1 (SAS Cary, NC).

RESULTS

Table 1 presents the distributions for BPA in the environmental and personal media for the 81 children at their homes and daycare centers in Ohio. Results showed that for these children BPA was detected the most often in the solid food (100%), hand wipe ($\geq$90%), and liquid food ($\geq$73%) samples at both their homes and daycare centers. The median levels of BPA were similar for each type of medium at both locations. However, the median levels of BPA were at least seven times higher in the solid food samples (3.5-3.6 ng/g) than in the liquid food samples (0.4-0.5 ng/mL) at both locations (*assuming the density of the liquid food is ~1 at 21^o C; ng/g=ng/mL*).

Table 2 presents the distribution of unadjusted, creatinine-adjusted, and specific gravity-adjusted urinary BPA concentrations for these 81 children. BPA was detected in 100% of the children's urine samples. The median unadjusted urinary BPA concentrations was 5.2 ng/mL for the children overall. The median unadjusted urinary concentration of BPA was slightly higher for the children in the home group (5.2 ng/mL) compared to children in the daycare group (4.9 ng/mL). The maximum unadjusted urinary BPA concentration was 211 ng/mL for one child in the home group.

Table 3 presents the distributions of the children's estimated intake doses of BPA by route and their excreted amounts of urinary BPA. The children's estimated median intake doses of BPA were 109 ng/kg/day, 0.06 ng/kg/day, and 0.27 ng/kg/day for the

dietary ingestion, nondietary ingestion, and inhalation routes, respectively. The children's estimated median excreted amount of urinary BPA was 114 ng/kg/day. The children's estimated intake doses by route compared to their excreted urinary amounts of BPA are also presented in Figure 1.

Pearson correlation coefficients for BPA by route are given in Table 4. The data show that excreted amounts of urinary BPA were significantly correlated with the children's creatinine levels in urine (r=0.31, p=0.006) and marginally significant with the children's specific gravity levels in urine (r=0.21, p=0.06) and potential dietary ingestion doses of BPA (r=0.23, p=0.07). Pearson correlations indicated potential collinearity existed between creatinine and specific gravity (p<0.0001) and dietary BPA and inhalation BPA (p<0.01). Since the Pearson correlation coefficient showed that creatinine and specific gravity measurements were highly correlated (r=0.43, p=<0.0001), these two independent variables were separately evaluated to determine the "best model" to characterize the relationship between the ln of the excreted urinary BPA and the ln of the intakes doses of BPA by each route. Our preliminary modeling results showed that ln creatinine (r^2=0.17) compared to ln specific gravity (r^2=0.11) was the better independent variable of the two to use in our subsequent regression modeling.

The results from our full regression model for BPA show an r^2=0.12 which suggested that approximately 12% of the variability of the excreted amounts of BPA was explained by the children's estimated intake doses of BPA through the dietary ingestion, nondietary ingestion, and inhalation routes and by creatinine levels (Table S2). Our final reduced model showed that dietary intake of BPA (p=0.04) was a significant predictor and creatinine concentration (p=0.004) was a highly significant predictor of urinary BPA,

collectively explaining 17% of the variability of the excreted amounts of BPA in the children's urine samples (Table 5). It is important to mention that at step 1 of the stepwise regression, BPA dietary intake was considered a non-significant variable; however, BPA dietary intake reduced the r^2 by 30% when it was excluded from this model. Therefore, this variable was retained in our regression model. Additional regression modeling showed that solid food (p=0.04) compared to liquid food (p=0.78) was significantly contributing to the children's dietary doses of BPA.

DISCUSSION

In the U.S, limited information exists on the potential exposure of children to BPA using biomonitoring. Urinary concentrations ranging from 0.4 to 211 ng/mL showed that all 81 CTEPP preschool children in Ohio were exposed to BPA at their homes and daycare centers in 2001. We believe this is the first study that has published urinary BPA concentrations for young children, ages 2-5 years, in the U.S. A few other studies in the U.S. have also reported detectable levels of BPA in urine samples from premature infants (1.6 - 946 ng/mL), 1 year old toddlers (0.04 - 17 ng/mL), and school-aged children > 5 years old (0.3 - 54 ng/mL) (*6, 7, 18-23*). The 2003-2004 National Health and Nutrition Examination Survey (NHANES, (*6)*), a U.S. general population study that included 314 children, ages 6 to 11 years, reported slightly lower geometric mean urinary BPA concentrations (3.6 ng/mL) compared to the preschool children in our study (4.8 ng/mL). Other studies from Canada, Germany, and Spain have also reported measureable levels of BPA in urine samples from infants 1 to 5 months old (0.2 -17.4 ng/mL), preschool-aged children 3-5 years old (0.3 – 205 ng/mL), and school-aged

children > 5 years old (0.2 – 110 ng/mL) (*30-33*). The above studies confirm that there is widespread exposure of children to BPA worldwide.

Assuming steady-state absorption of BPA, our children's estimated maximum aggregate intake dose (4.7 ug/kg/day) was 10 times lower than both the established oral reference dose (RfD) of 50 ug/kg/day by the US EPA's Integrated Risk Information System (*34*) or the Tolerable Daily Intake (TDI) of 50 ug/kg/day by the European Food Safety Authority (*35*). Our maximum aggregate intake dose value was calculated by multiplying the highest unadjusted urinary BPA concentration (211 ng/mL) for one child by an estimated daily urine excretion rate of 22.4 mL/kg body weight (*12*, *36-37*). In addition, the RfD and TDI values above correspond to the recently derived biomonitoring equivalent (BE) value of 2 mg/L of BPA in human urine (*38*). This information suggests that the children's measured urinary BPA concentrations (maximum value of 211 ng/mL) were well below the current BE for BPA. Limited information currently exists in the published literature on the exposure levels and potential health risks of children to BPA using urinary biomonitoring.

Very few studies have been conducted that have estimated the aggregate exposures of children to BPA (*1*, *9*, *12*). In a previous publication by Wilson et al. (*9*) on the CTEPP study, we showed that dietary ingestion of solid and liquid foods was probably the major route of exposure for 257 preschool children (130 North Carolina and 127 Ohio children) to BPA at their residences and daycare centers in 2000-2001. However in the CTEPP study, we did not measure the BPA concentrations in the children's urine samples in 2003. In our current work, we quantified the urinary BPA concentrations for 81 of the Ohio preschool children from the CTEPP study recruited in 2001 and then assessed the quantitative relationships between the children's excreted

amounts of BPA and their estimated intake doses of BPA by route (using a subset of exposure data in Wilson et al. (*9*)). The current biomonitoring results have supported our earlier publication (*9*) showing that the dietary intake of BPA through the consumption of both solid and liquid foods was the major exposure route, and this route accounted for > 95% of the children's excreted amounts of BPA in urine. In addition, our final reduced regression model showed that only creatinine concentration and dietary doses of BPA were significant predictors of urinary BPA excretion, collectively explaining 17% of the variability in the urinary BPA data. Additional regression modeling also showed that solid food ($p=0.04$) compared to liquid food ($p=0.78$) was significantly contributing to the children's dietary intake doses of BPA.

The 81 CTEPP Ohio children's consumption of both solid and liquid foods contributed to their exposures to BPA through the dietary ingestion route. However, an interesting observation was that the median levels of BPA were at least seven times higher in the solid food samples compared to the liquid food samples at both the homes and daycare centers of these 81 Ohio preschool children which is agreement with another previously published study by Braunrath et al. (*13*). This information suggests that packaging materials and/or containers were the major source of the BPA found in the CTEPP Ohio children's composited food samples (*13-16*). Several studies (*13-16*) have also detected BPA in different types of canned foods (i.e., vegetables, fruits, and meats) and bottled beverages (i.e., infant formulas and soft drinks). In addition, research has shown that the types of liquids stored in polycarbonate containers (i.e., water, oils, and salt solution) and/or physical conditions such as temperature (heating) can increase the migration of BPA into food and beverage products (*39-43*). This suggests that these

CTEPP Ohio preschool children were likely being exposed to BPA from many different types of food and beverage products that they normally consume. Limited information is currently available in the published literature on the physical and chemical conditions that increase the tendency for BPA to migrate from food packaging and containers.

There were some limitations to the interpretation of our data. The estimated aggregate exposures only apply to the 81 CTEPP Ohio preschool children examined in 2001 and may not reflect the everyday BPA exposures of other preschool children in Ohio, in the U.S. or in other countries. Since the urine samples were pooled for each child, we cannot ascertain the variability of BPA urinary concentrations over the 48-h study period. Changes in diet, fluid intake, individual toxicokinetics, and/or urine output would likely affect the variability of BPA in urine over time. Furthermore, it is unclear whether these children had steady-state or intermittent exposures to BPA. Steady-state exposures to BPA would likely result in relatively constant urinary biomarker concentrations over time (e.g., daily, monthly). In contrast, intermittent exposures to BPA like dietary ingestion of foods would likely significantly increase the urinary concentrations of BPA within a few hours because of the short biological half-life (<6-h) and rapid urinary elimination of BPA in humans (*10, 44*). In addition, the children's food and urine samples were collected during the same 48-h period, therefore some measurement error would likely occur as the urine collection period could partly reflect BPA intake from the prior day. Further research is needed to understand the important factors that may impact the temporal variability of BPA in children's urine.

In conclusion, our current work showed that dietary ingestion through the consumption of both solid and liquid foods was the major route of these 81 CTEPP Ohio

preschool children's exposures to BPA, and this route likely contributed to >95% of their excreted amounts of urinary BPA.

Literature Cited

(1) World Health Organization (2010). Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A: Summary Report including Report of Stakeholder Meeting on Bisphenol A. Ottawa, Canada, World Health Organization: Available: http://www.who.int/foodsafety/chem/chemicals/BPA_Summary2010.pdf

(2) Vandenberg, L. N.; Maffini, M. V.; Sonnenschein, C.; Rubin, B. S.; Soto, A. M. Bisphenol A and the great divide: A review of controversies in the field of endocrine disruption. *Endocr. Rev.* **2009**, 30, 75-95.

(3) Tsai, W.T. Human health risk on environmental exposure to bisphenol A: A review. *J. Environ. Sci. Heal. C.* **2006**, 24, 225-255.

(4) Vandenberg, L. N.; Hauser, R.; Marcus, M.; Olea, N.; Welshons, W.V. Human exposure to bisphenol A (BPA). *Reprod Toxicol* **2007**, 24, 139-177.

(5) LaKind, J. S.; Naiman D. Q. Bisphenol A (BPA) daily intakes in the United States: Estimates from the 2003-2004 NHANES urinary BPA data. *J. Expo. Sci. Environ. Epidemiol.* **2008**, 18, 608-615.

(6) CDC (Centers for Disease Control and Prevention). Fourth national report on human exposure to environmental chemicals. 2009. http://www.cdc.gov/exposurereport/

(7) Fourth National Report on Human Exposure to Environmental Chemicals. Updated Tables, February 2011. Atlanta, GA: Centers for Disease Control and Prevention; National Center for Environmental Health; Division of Laboratory Sciences Available: http://www.cdc.gov/exposurereport/pdf/Updated_Tables.pdf

(8) National Toxicology Program. NTP Brief on Bisphenol A [CAS NO. 80 – 05 – 07]. 2008.

(9) Wilson, N.; Chuang, J.; Lordo, R.; Morgan, M.; Sheldon, L. An observation study of the potential exposures of preschool children to pentachlorophenol, bisphenol A, and nonylphenol at home and daycare. *Environ. Res.* **2007**, 103(1), 9-20.

(10) Dekant, W.; Volkel, W. Human exposure to bisphenol A by biomonitoring; Methods, results and assessment of environmental exposures. *Toxicol. Appl. Pharmacol.* **2008**, 228, 114-134.

(11) Wilson, N. K.; Chuang, J. C.; Lyu, C. Levels of persistent organic pollutants in several child day care centers. *J. Expo. Anal. Environ. Epidemiol.* **2001**, 11, 449-458.

(12) Wilson, N. K.; Chuang, J. C.; Lyu, C.; Menton, R.; Morgan, M. K. Aggregate exposures of nine preschool children to persistent organic pollutants at daycare and at home. *J. Expo. Anal. Environ. Epidemiol.* **2003**, 13, 187-202.

(13) Braunrath, R.; Podlipna, D.; Padlesak, S.; Cichna-Markl, M. Determination of bisphenol A in canned foods by immunoaffinity chromatography, HPLC, and fluorescence detection. *J. Agric. Food Chem.* **2005**, 53, 8911-8917.

(14) Yonekubo, J.; Hayakawa, K.; Sajiki, J. Concentrations of bisphenol A, bisphenol A diglycidyl ether, and their derivatives in canned foods in Japanese markets. *J. Agric. Food Chem.* **2008**, 56, 2041-2047.

(15) Cao, X.; Corriveau, J.; Popovic, S. Levels of bisphenol A in canned soft drink products in Canadian markets. *J. Agric. Food Chem.* **2009**, 57, 1307-1311.

(16) Ackerman, L. K.; Noonan, G. O.; Heiserman, W. H.; Roach, J. A.; Limm, W.; Begley, T. H. Determination of bisphenol A in U.S. Infant formulas: Updated methods and concentrations. *J. Agric. Food. Chem.* **2010**, 58, 2307-2313.

(17) Volkel, W.; Colnot, T.; Csanady, G. A.; Filser, J. G.; Dekant, W. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chem. Res. Toxicol.* **2002**, 15, 1281-1287.

(18) Liu, Z.; Wolff, M.S.; Moline, J. Analysis of environmental biomarkers in urine using an electrochemical detector. *J. Chromatogr. B.* **2005**, 819, 155-159.

(19) Calafat, A. M.; Ye, X.; Wong, L. Y.; Reidy, J. A.; Needham. L. L. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ. Health Perspect.* **2008**, 116(1), 39-44.

(20) Teitelbaum, S. L.; Britton, J. A.; Calafat, A. M.; Ye, X.; Silva, M. J.; Reidy, J. A.; Galvez, M. P.; Brenner, B. L.; Wolff, M. S. Temporal variability in urinary concentrations of phthalate metabolites, phytoestrogens and phenols among minority children in the United States. *Environ. Res.* **2008**, 106, 257-269.

(21) Calafat, A. M.; Weuve, J.; Ye, X.; Jia, L. T.; Hu, H.; Ringer, S.; Huttner, K.; Hauser, R. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. *Environ. Health Perspect.* **2009**, 117(4), 639-644.

(22) Wolff, M. S.; Teitelbaum, S. L,; Pinney, S.M.; Windham, G.; Liao, L.; Biro, F.; Kushi, L. H.; Erdmann, C.; Hiatt, R. A.; Rybak, M. E.; Calafat, A. M. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. *Environ. Health Perspect.* **2010**, 118(7), 1039-1046.

(23) Wolff, M. S.; Teitelbaum, S. L,; Pinney, S.M.; Windham, G.; Liao, L.; Biro, F.; Kushi, L. H.; Erdmann, C.; Hiatt, R. A.; Rybak, M. E.; Calafat, A. M. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. *Environ. Health Perspect.* **2010**, 118(7), 1039-1046.

(24) Wilson, N.K.; Chuang, J. C.; Iachan, R.; Lyu, C.; Gordon, S. M.; Morgan, M. K.; Ozkaynak, H.; Sheldon, L. Design and sampling methodology for a large study of preschool children's aggregate exposures to persistent organic pollutants in their everyday environments. *J. Expo. Anal. Environ. Epidemiol.* **2004**, 14, 260-274.

(25) Ye, X. Y.; Kuklenyik, Z.; Needham, L. L.; Calafat, A. M. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. *Anal. Chem.* **205**, 77, 5407-5413.

(26) Caudill, S. P.; Schleicher, R. L.; Pirkle, J. L. Multi-rule quality control for the age-related eye disease study. *Stat. Med.* **2008**, 27, 4094-4106.

(27) O'Rourke, M.K.; Lizardi, P.S.; Rogan, S.P.; Freeman, N.C.; Aguirre, A.; Saint, C.G. Pesticide exposure and creatinine variation among young children. *J. Expo. Anal. Environ. Epidemiol.* **2000**, 10, 672–681.

(28) Barr, D. B.; Wilder, L.C.; Caudill, S.P.; Gonzalez, A.J.; Needham, L.L.; Pirkle, J.L. Urinary creatinine concentrations in the U.S. Population: Implications for urinary biologic monitoring measurements. *Environ. Health Perspect*. **2005**, 113 (2), 192-200.

(29) Ross, J. H.; Driver, J. H.; Cockran, R. C.; Thongsinthusak, T.; Kreiger, R. I. Could pesticide toxicology studies be more relevant to occupational risk assessment? *Ann. Occup. Hyg.* **2001**, 45(1001), S5-S17.

(30) Becker, K.; Goen T.; Seiwert, M.; Conrad, A.; Pick-Fub, H.; Muller, J.; Wittassek, M.; Schulz, C.; Kolossa-Gehring, M. GerES IV: Phthalate metabolites and bisphenol A in urine of German children. *Int. J. Environ. Health*. **2009**, 212, 685-692.

(31) Volkel, W.; Kiranoglu, M.; Fromme, H. Determination of free and total bisphenol A in urine of infants. *Environ. Res*. **2011**, 111, 143-148.

(32) Health Canada. Report on human biomonitoring of environmental chemicals in Canada; Results of the Canadian health measures survey cycle 1 (2007-2009). 2010.

(33) Casas, L.; Fernandez, M.F.; Llop, S.; Guxens, M.; Ballester, F.; Olea, N.,; Irurzun, M.B.; Rodriguez, L.S.; Riano, I.; Tardon, A.; Vrijheid, M.; Calafat, A.M.; Sunyer, J. Urinary concentrations of pthalates and phenols in a population of Spanish pregnant women and children. *Int. Environ*. **2011**, 37, 858-866.

(34) IRIS (US EPA's Integrated Risk Information System). Bisphenol A. (CASRN 80-05-7). 1988. http://www.epa.gov/IRIS/index.html

(35) European Food Safety Authority (EFSA). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food. On request from the commission related to 2,2-bis(4-hydroxyphenyl)propane (bisphenol A). Question number EFSA-Q-2005-100. Adopted on November **2006**. The EFSA Journal 428: 1-75.

(36) Miller, L. A.; Stapleton, F.B. Urinary volume in children with urolithiasis. *J. Urol*. **1989**, 141 (4), 918–920.

(37) Szabo, L.; Fegyverneki, S. Maximum and average urine flow rates in normal children—the Miskolc nomograms. *Br J Urol* **1995**, 76 (1), 16–20.

(38) Krishnan, K.; Gagne M.; Nong, A.; Aylward L.L.; Hays S.M. Biomonitoring equivalents for bisphenol A (BPA). Regul Toxicol and Pharm. **2010**, 58(1), 18-24.

(39) Schecter, A.; Malik, N.; Haffner, D.; Smith, S.; Harris, T.; Paepke, O.; Birnbaum, L. Bisphenol A (BPA) in U.S. Food. *Environ. Sci. Technol*. ePub ahead of print (October 2010).

(40) Kang, J. H.; Kito, K.; Kondo, F. Factors influencing the migration of bisphenol A from cans. *J Food Prot* **2003**, 66(8), 1444-1447.

(41) Le, H. H.; Carlson, E. M.; Chua, J. P.; Belcher, S. M. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicol. Lett.* **2008**, 176, 149-156.

(42) Lim, D. S.; Kwack, S. J.; Kim, K. B.; Kim, H. S.; Lee B. M. Potential risk of bisphenol A migration from polycarbonate containers after heating, boiling, and microwaving. *J. Toxicol. Env. Heal. A.* **2009**, 72, 1285-1291.
(43) Nam, S. H.; Seo, Y. M.; Kim, M. G. Bisphenol A migration from polycarbonate baby bottle with repeated use. *Chemosphere* **2010**, 79, 949-952.
(44) National Research Council (NRC) of the National Academies of Sciences. Human Biomonitoring for Environmental Chemicals. 2006, p 215.

Acknowledgements: We thank Amber Bishop and Jack Reidy for the measurements of BPA. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. *Disclaimer: The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here under Contract #68-D-99-011 to Battelle. It has been subjected to Agency review and approved for publication.*

Table 1. Concentrations of BPA in environmental and personal media collected in 2001 at the homes and daycare centers of 81 CTEPP preschool children in Ohio[ab]

Medium	Unit	Location	N	% Detect[c]	Mean	SD[d]	GM[e]	Min.	Percentiles 25th	50th	75th	95th	Max.
Environmental Media													
Indoor air	ng/m^3	Home	81	68	2.5	4.2	1.5	0.6	0.9	1.1	1.9	7.9	30.3
		Daycare	22	73	1.4	1.4	1.1	0.6	0.8	0.9	1.5	1.8	7.4
Outdoor air	ng/m^3	Home	81	40	1.3	2.2	1.0	0.7	0.9	0.9	0.9	1.7	19.0
		Daycare	16	44	1.2	1.6	0.8	0.6	0.6	0.6	0.8	6.9	6.9
Dust	ng/g	Home	76	43	55.9	75.3	40.4	19.6	21.1	40.0	50.9	200	589
		Daycare	23	70	47.3	32.1	38.9	20.0	21.7	35.9	71.5	100	124
Soil	ng/g	Home	81	1	----	----	----	----	----	----	----	----	----
		Daycare	16	0	----	----	----	----	----	----	----	----	----
Personal Media													
Hand wipes	ng/cm^2	Home	61	100	0.02	0.03	0.01	0.001	0.01	0.01	0.02	0.05	0.3
		Daycare	29	90	0.01	0.03	0.01	0.001	0.01	0.01	0.01	0.04	0.1
Solid food	ng/g	Home	81	100	5.7	9.8	3.7	0.9	2.1	3.6	5.5	14.3	84.1
		Daycare	29	100	4.4	3.7	3.4	1.0	2.2	3.5	4.5	11.9	18.6
Liquid food	ng/mL	Home	81	73	0.9	1.9	0.5	0.2	0.2	0.4	0.7	2.0	16.3
		Daycare	28	82	0.9	0.9	0.6	0.2	0.4	0.5	1.1	1.8	5.0

[a]A subset of the environmental and personal media data at the homes and daycare centers collected for 257 preschool children as described in Wilson et al., (*9*) was used to calculate the environmental and personal media concentrations of BPA for the 81 Ohio children examined in the present study.
[b] For this subset of CTEPP Ohio preschool children, field sampling occurred at 81 homes and 16 child daycare centers.
[c]Percentage of samples with detectable levels of BPA
[d]Standard deviation
[e]Geometric mean

Table 2. Urinary BPA concentrations in 81 CTEPP preschool children in Ohio over a 48-h monitoring period in 2001[a].

Urine	N	Mean	SD[b]	GM[c]	Min.	Percentiles				Max.
						25th	50th	75th	95th	
ng/mL										
All Children	81	8.9	23.6	4.8	0.4	2.6	5.2	7.5	20.8	211
Home Group	41	10.7	32.4	4.8	0.6	2.6	5.2	7.0	20.8	211
Daycare Group	40	7.1	8.0	4.7	0.4	3.0	4.9	7.8	26.6	38.6
ng/mg-creatinine										
All Children	78	13.1	38.7	6.6	0.5	4.0	5.9	9.1	21.8	334
Home Group	41	17.0	52.5	7.2	1.7	4.0	7.3	9.1	20.3	334
Daycare Group[d]	37	8.7	10.3	6.1	0.5	4.2	5.4	9.0	21.8	62.3
ng/mL-specific gravity										
All Children	80	9.8	22.5	5.3	0.5	3.0	5.7	8.0	24.6	186
Home Group	41	10.6	28.5	5.3	0.5	3.1	5.3	7.6	16.9	186
Daycare Group[e]	39	9.0	14.1	5.3	0.5	3.0	5.8	8.1	48.5	79.6

[a]These are unadjusted urinary BPA concentrations; total BPA (free and conjugated) was measured in the urine samples.
[b]Standard deviation
[c]Geometric mean
[d]Creatinine could not be quantify in three of the children's urine samples
[e]Specific gravity could not be quantified in one child's urine sample

Table 3. The preschool children's estimated intake doses to BPA by route and their excreted urinary amounts of total BPA[a].

	N[b]	Mean	SD[b]	GM[c]	Min.	Percentiles				Max.
						25th	50th	75th	95th	
Intake Dose of BPA (ng/kg/day)										
Dietary ingestion	66	156.5	181	105.6	22.2	59.0	109	172	480	1102
Nondietary ingestion	64	0.11	0.13	0.07	0.02	0.04	0.06	0.11	0.37	0.71
Inhalation	62	0.4	0.45	0.30	0.09	0.17	0.27	0.41	1.34	2.87
Urinary Output of BPA (ng/kg/day)										
Excreted Amount	81	196.8	520	104.6	8.8	57.2	114	164	458	4642

[a]The exposure data were calculated by route for 81 out of 257 preschool children using the environmental and personal media data reported in Wilson et al. (9) and the urinary biomonitoring data reported in the present study.
[b]A valid measurement did not exist for some children.

Table 4. Pearson correlations for estimating the 81 Ohio CTEPP preschool children's intake doses of BPA by route and excreted amounts of urinary BPA[a,b]

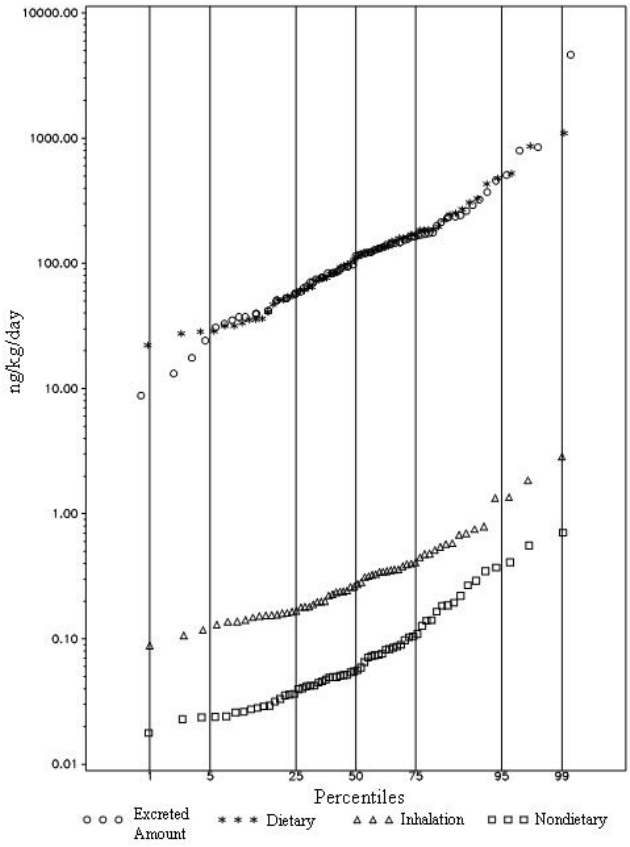
	Excreted Urinary BPA	Creatinine	Specific Gravity	Dietary BPA	Nondietary BPA	Inhalation BPA
Excreted Urinary BPA		0.31[c] 0.006[d]	0.21 0.06	0.23 0.07	-0.12 0.33	0.14 0.29
Creatinine			0.43 <0.0001	-0.12 0.34	-0.31 0.01	-0.11 0.38
Specific Gravity				-0.21 0.09	-0.09 0.49	-0.03 0.84
Dietary BPA					0.18 0.20	0.39 0.005
Nondietary BPA[e]						0.02 0.87

[a]The natural logarithm was used for each independent variable and for the outcome variable (excreted amounts of urinary BPA).
[b]Units for all variables are in ng/kg/day, except for creatinine concentration (mg/dl) and specific gravity (unitless)
[c]Pearson correlation coefficients
[d]Level of significance (p value)
[e]Nondietary ingestion is defined here as exposure to BPA through soil and dust intake.

Table 5. Final reduced regression model for urinary BPA[a,b,c].

	$\hat{\beta}$	SE($\hat{\beta}$)	p-value
Intercept	-0.56	1.5	0.71
Dietary BPA	0.29	0.13	0.04
Creatinine	0.95	0.32	0.004

[a]The natural logarithm was used for each independent variable and for the outcome variable (excreted amounts of urinary BPA (ng/kg/day))
[b]Units for dietary BPA and creatinine concentration are ng/kg/day and mg/dl, respectively
[c]r^2 =0.17



[a]The exposure data were calculated by route for 81 out of 257 preschool children using the environmental and personal media data reported in Wilson et al. (*9*) and the urinary biomonitoring data reported in the present study.

Figure 1. The estimated Ohio preschool children's intake doses of BPA by route compared to their excreted amounts of total urinary BPA.