Twenty-Four Hour Human Urine and Serum Profiles of Bisphenol A during High-Dietary Exposure

Justin G. Teeguarden, Antonia M. Calafat, Xiao yum Ye, Daniel R. Doerge, Mona I. Churchwell, Rudy Gunawan, and Morgan K. Graham

By virtue of its binding to steroid hormone receptors, bisphenol A (BPA, the unconjugated bioactive monomer) is hypothesized to be estrogenic when present in sufficient quantities in the body, raising concerns that widespread exposure to BPA may impact human health. To better understand the internal exposure of adult humans to BPA and the relationship between the serum and urinary pharmacokinetics of BPA, a clinical exposure study was conducted. Blood and urine samples were collected approximately hourly over a 24-h period from 20 adult volunteers who ingested 100% of one of three specified meals comprising standard grocery store food items for breakfast, lunch, and dinner. The volunteers' average consumption of BPA, estimated from the urinary excretion of total BPA (TOTBPA = conjugated BPA + BPA), was 0.27 μg/kg body weight (range, 0.03–0.86), 21% greater than the 95th percentile of aggregate exposure in the adult U.S. population. A serum time course of TOTBPA was observable only in individuals with exposures 1.3–3.9 times higher than the 95th percentile of aggregate U.S. exposure. The TOTBPA urinary concentration $T_{max}$ was 2.75 h (range, 0.75–5.75 h) post-meal, lagging the serum concentration $T_{max}$ by ~1 h. Serum TOTBPA area under the curve per unit BPA exposure was between 21.5 and 79.0 nM*h/kg/μg BPA. Serum TOTBPA concentrations ranged from less than or equal to limit of detection (LOD, 1.3 nM) to 5.7 nM and were, on average, 42 times lower than urine concentrations. During these high dietary exposures, TOTBPA concentrations in serum were undetectable in 83% of the 320 samples collected and BPA concentrations were determined to be less than or equal to LOD in all samples.

Key Words: bisphenol A; pharmacokinetics; exposure; biomonitoring; endocrine disruptors; urine; serum.

Bisphenol A (BPA the unconjugated bioactive monomer), a monomer used in the manufacture of polycarbonate plastics and epoxy can liners, is found in trace quantities in some consumer products and food containers (Chapin et al., 2008). By virtue of its binding to steroid hormone receptors, BPA is hypothesized to be estrogenic when present in sufficient quantities in the body. Results from rodent feeding studies across multiple generations consistently show minimal effects in adults and offspring (Chapin et al., 2008; Tyl et al., 2002), but results of other rodent studies raise the possibility of effects on prostate and mammary gland development and behavior (Chapin et al., 2008; Prins et al., 2011).

In humans, 84–97% of the administered dose of BPA is absorbed and eliminated as glucuronide or sulfate conjugates (conjugated BPA, CNJBPA) in urine over the 5–7 h following oral administration, increasing to as high as 100% after 24 h (Volkel et al., 2002, 2005). The complete or nearly complete renal elimination of absorbed BPA has made measurements of urinary concentrations of BPA a useful regularly used biomarker of human aggregate (all routes) exposure, not just the oral route exposure (Calafat et al., 2005, 2009; Lakind and Naiman, 2008, 2010).

Human exposure to BPA has been extensively studied by measuring the amount eliminated in urine, most often by single spot samples (Calafat et al., 2005, 2008; Chapin et al., 2008; Lakind and Naiman, 2010). Exposure is widespread; most adults (91–93%) surveyed in two industrialized nations, the United States and Canada, are exposed regularly to BPA (Calafat et al., 2008; Health-Canada, 2010; Lakind and Naiman, 2010). Two large nationally based urine biomonitoring studies with broad demographics, consisting of 2517 (National Health and Nutrition Examination Survey [NHANES] 2003–2004) and 2535 (NHANES 2005–2006) persons 6 years of age and older, concluded that median aggregate human BPA exposures in the United States from all routes of exposure were 0.034 μg/kg/day in 2005–2006 and 0.051 μg/kg/day in 2003–2004, with an overall range (25th–95th percentiles) of 0.017–0.274 μg/kg/day (Lakind and Naiman, 2008, 2010). These exposures raise concerns that...
et al. between 0.2 and 1.2% of the total BPA (TOTBPA). The urinary elimination profile of TOTBPA and the serum time course of TOTBPA and BPA by concurrently determining the 24-h concentration of BPA following typical daily exposures are similar to, above, or below blood/tissue concentrations causing demonstrably adverse effects in animal models. The main objective of this study was to characterize internal exposure to TOTBPA and BPA by concurrently determining the 24-h urinary elimination profile of TOTBPA and the serum time course of TOTBPA and BPA in a group of healthy adult humans on a controlled diet enriched in canned food items likely to be significant dietary sources of BPA. In addition, we sought to produce an accurate measure of human exposure to BPA before and after consuming a potentially BPA-rich diet and characterize hourly fluctuations in serum and urine BPA.

**MATERIALS AND METHODS**

All human subject research activities were conducted in accordance with protocols approved by the Pacific Northwest National Laboratory Institutional Review Board (IRB # 2008-23-EXP, FWA IRB00000285). The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was limited to analysis of anonymized samples and determined not to constitute engagement in human subjects research. Similarly, participation by the National Center for Toxicological Research (NCTR) laboratory was reviewed by the U.S. Food and Drug Administration (FDA) Research Involving Human Subjects Committee and determined not to constitute engagement in human subjects research.

**Volunteer selection and demographics.** Twenty randomly selected healthy adults (age 18–55 years) nonsmoking (no nicotine product use) male (10) or nonpregnant female (10) volunteers were recruited for the study in 2009 from the Tacoma, WA, metropolitan area. Volunteers had normal GI tract, kidney, and liver function, as determined by clinical laboratory tests, medical history, and physical examination, were not taking medications that alter hepatic glucuronidation/sulfonation or renal elimination, were HIV and hepatitis free, and had not undergone dental procedures in the 2 days preceding the study. The average age of males and female volunteers was 34 years (20–54) and 28.3 years (18–53), respectively. The average weight of male and female volunteers was 83.7 kg (63.4–107.23) and 80.17 kg (60.1–110.72), respectively. Demographic information for each volunteer is provided in the Supplementary Table S1. Volunteers were housed in a clinical facility (Charles River; Northwest Kinetics, Tacoma, WA) for ~36 h and provided with one of three specified meals comprising standard food items—purchased in 2009 from grocery stores in the Tacoma WA area—for breakfast, lunch, and dinner. The diet was rich in canned foods and juices to represent a potentially high BPA dietary exposure scenario. All voided urine was collected at regular intervals over a 24-h period and matching serum samples were taken until 10 P.M. of the first study day. Both serum and urine were analyzed as described before (see details below) (Ye et al., 2005, 2008) for TOTBPA and BPA at the CDC using solid-phase extraction coupled with isotope dilution-high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), considered the “gold standard” for biomonitoring studies (Vandenberg et al., 2010).

**Protocol.** Volunteers were admitted to the clinic in the evening of day 0 and fasted overnight with access only to BPA-free water (verified analytically; all water blanks were below the limit of detection [LOD] of 1.8 nM) provided in glass bottles to clear the majority of any BPA ingested earlier in the day from their system. On day 1, volunteers emptied their bladders immediately before the start of the study at the breakfast meal. Breakfast (7 A.M.), lunch (1 P.M.), and dinner (7 P.M.) comprised three predefined selections each (e.g., breakfast 1, 2, and 3) (see “Meals” section below). At each meal, volunteers selected and ingested 100% of one of the meals. This study design balanced the goals of assessing pharmacokinetic parameters and variability in diet and blood and urine concentrations. To ensure hydration for blood sampling and sufficient liquid to support regular urine sampling, volunteers ingested 500–600 ml of liquid at each meal and 200 ml of BPA-free water every hour between meals. Blood samples were collected each hour from 10 P.M. to 10 P.M. Urine samples were collected every hour until 10 P.M. and then only during voluntary urination between 10 P.M. and the first morning void, which completed the study. Urine volumes and time of collection were recorded for each void.

**Meals.** Meals were designed to include the major food groups with the condition that canned meats, fruits, and vegetables—all potential sources of BPA—were present in each meal (Schecter et al., 2010). Measurement of BPA levels in the foods was outside the scope of the current study for logistical and financial reasons. It was also unnecessary. Because ~100% of absorbed BPA is eliminated in urine, urinary BPA can be considered equal to internal exposure. Breakfast 1: one hardboiled egg, one 6 oz. yogurt, 3 oz. canned pears in light syrup, 3 oz. canned tuna on toast, and one #7 bottled water. Breakfast 2: 4 oz. canned peaches in light syrup, 3 oz. Vienna sausages, and one canned apple juice. Breakfast 3: one hardboiled egg, 3 oz. canned fruit cocktail in light syrup, 3 oz. spam, one 6 oz. yogurt, and one canned apple juice. Lunch 1: 3 oz. canned turkey, one plain bagel (white), one packet cream cheese, 2 oz. canned asparagus, 2 oz. canned mandarin oranges, one pudding cup, and one canned apple juice. Lunch 2: 2 oz. canned carrots, one pudding cup, 2 oz. canned pears in light syrup, 1.5 cups canned chicken noodle soup, four saltine crackers, and one bottle water. Lunch 3: 3 oz. canned chicken, one pita pocket (white), one packet cream cheese, one pudding cup, 2 oz. canned peaches in light syrup, 2 oz. canned green beans, and one canned apple juice. Dinner 1: 3 oz. canned roast beef, one plain bagel (white), one packet cream cheese, 3 oz. canned green beans, 3 oz. canned pears in light syrup, one pudding cup, and one bottle water. Dinner 2: 3 oz. com, 4 oz. canned peaches in light syrup, 1.5 cups canned clam chowder, four saltine crackers, one pudding cup, and one canned apple juice. Dinner 3: 3 oz. canned peas, 3 oz. canned pineapple in light syrup, 1.5 cups chicken noodle soup, four saltine crackers, one pudding cup, and one bottle water. The water consumed during and between all meals was stored in glass bottles and was determined analytically to be BPA free. The meal choices made by each volunteer can be found in Supplementary Table S2.

**Urine and serum handling.** Urine and serum samples did not come into contact with any plastic materials other than polypropylene. Urine samples were collected and stored refrigerated (4°C) in polypropylene containers for up to 23 h and then frozen at −20°C. Blood samples were taken by trained phlebotomists via implanted cannulae and samples were allowed to clot at room temperature for 2 h, centrifuged, and then serum was stored at −20°C. Five to

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ten milliliter aliquots of serum and urine were prepared and shipped for analysis on dry ice. Five field blanks, comprised of BPA-free water, were collected using the same procedures that were used for urine collection and serum collection (passed through same apparatus).

**Serum and urine analysis.** Analysis of serum samples was conducted with and without enzymatic deconjugation for TOTBPA and BPA, respectively, and urine was analyzed only for TOTBPA at CDC by automated online solid-phase extraction-HPLC–isotope dilution tandem mass spectrometry (Ye et al., 2005, 2008). The limits of detection were 1.8 nM (0.4 ng/ml [urine]) and 1.3 nM (0.3 ng/ml [serum]) for analysis of 100 μl samples. To ensure data accuracy and precision, each batch of samples included quality control (QC) materials, analytical standards, and reagent blanks. The QC concentrations were evaluated using standard statistical probability rules (Caudill et al., 2008). For urine, the method accuracy, expressed as a spiked recovery percentage, ranged from 98 to 113% at four different spiking levels (Ye et al., 2005). Each batch of samples included two high-concentration (~10 μg/ml) and two low-concentration (~3 μg/ml) quality control samples (QCH and QCL, respectively), prepared by spiking different levels of BPA to urine pool as described previously (Ye et al., 2005). The method precision, determined by calculating the coefficient of variations (CVs) of 22 repeated measurements of the QCL and QCH materials over 6 months, was 7.4% (QCL) and 5.3% (QCH). Results were similar for serum analyses. A more detailed description of the analytical method, including performance and precision measures, and sample chromatograms are provided as Supplementary Method S3. BPA concentrations in all five field blanks were below LOD, evidence that background or sample chromatograms are provided as Supplementary Method S3. BPA concentration (expressed as a spiked recovery percentage, ranged from 98 to 113% at four different spiking levels (Ye et al., 2005). 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The following criteria were used for identifying the T max for TOTBPA urine concentration and urine amount. Urine concentration and urine amount profiles for TOTBPA were considered separately because the latter integrates concentration and urine volume to give a unique profile for the amount leaving the systemic compartments via the urine. Urine concentration criteria: TOTBPA urine concentration ≥ 2×LOD, greater than the previous time point, and measured during a period of regular – 1-h urine collection intervals (i.e., not after 10 p.m). There were a total of 28 observations used to calculate the concentration T max. Urine amount criteria: TOTBPA urine concentration ≥ 2×LOD, urine amount greater than the previous time point, and measured during a period of regular – 1-h urine collection intervals (i.e., not after 10 p.m). There were a total of 32 observations used to calculate the T max for urine amount.

**Statistics.** Grubbs test was used to identify outliers using a p value of <0.05.

**RESULTS**

**Total Daily BPA Exposure**

The average total daily exposure to BPA based on the 24 h sum of TOTBPA eliminated in urine from the three meals was 21 μg (SD = 18.6), with a range of 3.3–73 μg (Table 1). Using individual body weights (bws) and assuming that 100% of BPA in the meal was absorbed and eliminated in urine, the average daily exposure was 0.27 μg/kg bw (range, 0.03–0.86, SD = 0.23). This group exposure level is 21% greater than the most recently measured upper 95th percentile of aggregate (all routes) exposure in the general U.S. population (>6 years old) (Lakind and Naiman, 2010) (Table 1). Exposures were greater than or equal to the 72nd percentile of aggregate U.S. exposure for all but one volunteer and were greater than or equal to the 89th percentile in 65% of volunteers. Nine volunteers had exposures that exceeded the 95th percentile of aggregate U.S. exposure. BPA exposures in this study can therefore be viewed as representative of, or exceeding, the high end of the U.S. BPA exposure distribution. Negative findings regarding urine and serum BPA therefore cannot be viewed as the result of low exposures. Most importantly, a serum time course of TOTBPA was observable only in those individuals with exposures 1.3–3.9 times higher than the 95th percentile of aggregate U.S. exposure.

**Urine BPA Concentrations**

All volunteers eliminated quantifiable amounts of TOTBPA in the urine following one or more meals (Figs. 1 and 2). Urine TOTBPA concentrations and amounts were strongly influenced by meal type (breakfast, lunch, or dinner) and when the meal was
ingested relative to the sampling time, varying considerably within individuals over the study period and between individuals. The average TOTBPA concentration of the 385 urine samples (12–23 per volunteer) was 25.8 nM (SD = 55 nM) with a range of 1.8 nM (LOD) to 548 nM or in units of nanograms per milliliter, 5.9 (SD = 12.6) with a range of 0.40 nM (LOD) to 125 nM.

Detectable amounts of TOTBPA were commonly observed in urine in the first sample collected after a meal, 1 h after the meal was provided. The urine amount time course showed an approximately 1 h lag behind TOTBPA serum concentrations (Fig. 3). The TOTBPA urine concentration \( T_{\text{max}} \) was 2.75 h (range, 0.75–5.75 h) post-meal. The average time to maximum amount of TOTBPA (nanograms) eliminated in a given urine void was similar: 2.2 h after ingestion of the meal (range, 0.82–4.75 h).

There was substantial intermeal variability in the daily TOTBPA eliminated in urine (Fig. 4). Fifty-one percent of urine samples taken between the breakfast and lunch meals had undetectable levels of TOTBPA. Based on urinary elimination data, dinners 1, 2, and 3 and lunch 3 contained higher amounts of TOTBPA than the other meals. By contrast, very little TOTBPA was eliminated after ingestion of all study breakfasts and lunches 1 and 2, evidence that these meals contained very little TOTBPA relative to the other meals. Therefore, use of the LOD to represent the maximum possible urine concentration had a considerable effect on the estimate of TOTBPA eliminated after the breakfast meal, but much less so for the lunch and dinner meals, and for the daily total (Table 1).

Intrameal variability on the TOTBPA excreted in urine was also observed. For individuals ingesting the same meal, the CV in the calculated amount of BPA eliminated during the intermeal period for lunches 1, 2, and 3 and dinner 3 was between 15 and 27%; however, the CV was even larger for dinners 1 and 2, between 41 and 60% (single values identified as outliers were not used in calculating the mean or CV) (Fig. 4).

### Serum BPA and CNJ/BPA Concentrations

Between the initiation of the study at breakfast and completion of sample collection at 10 P.M., 15 blood samples

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**TABLE 1**

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Breakfast (μg)</th>
<th>Lunch (μg)</th>
<th>Dinner (μg)</th>
<th>Corresponding percent of U.S. exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76 (150)</td>
<td>0.92 (21)</td>
<td>1.61 (0)</td>
<td>45</td>
</tr>
<tr>
<td>2</td>
<td>0.60 (439)</td>
<td>17.5 (0)</td>
<td>12.7 (0)</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>1.52 (11)</td>
<td>2.07 (0)</td>
<td>17.1 (0)</td>
<td>97</td>
</tr>
<tr>
<td>4</td>
<td>1.36 (708)</td>
<td>19.8 (0)</td>
<td>20.1 (0)</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>1.08 (0)</td>
<td>1.74 (0)</td>
<td>4.38 (0)</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>0.51 (512)</td>
<td>2.23 (4)</td>
<td>2.53 (0)</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>0.83 (88)</td>
<td>1.28 (13)</td>
<td>6.78 (0)</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>1.16 (1546)</td>
<td>1.79 (0)</td>
<td>5.55 (0)</td>
<td>89</td>
</tr>
<tr>
<td>9</td>
<td>0.65 (12)</td>
<td>1.18 (12)</td>
<td>4.06 (0)</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>0.89 (100)</td>
<td>1.62 (9.3)</td>
<td>6.07 (0)</td>
<td>82</td>
</tr>
<tr>
<td>11</td>
<td>0.83 (776)</td>
<td>1.19 (0)</td>
<td>15.5 (0)</td>
<td>97</td>
</tr>
<tr>
<td>12</td>
<td>1.32 (123)</td>
<td>1.55 (34)</td>
<td>11.0 (0)</td>
<td>93</td>
</tr>
<tr>
<td>13</td>
<td>1.11 (165)</td>
<td>23.3 (0)</td>
<td>48.9 (0)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>14</td>
<td>0.98 (37)</td>
<td>1.45 (8)</td>
<td>5.18 (0)</td>
<td>84</td>
</tr>
<tr>
<td>15</td>
<td>1.09 (687)</td>
<td>21.9 (0)</td>
<td>21.9 (0)</td>
<td>99</td>
</tr>
<tr>
<td>16</td>
<td>2.71 (0)</td>
<td>26.2 (0)</td>
<td>11.9 (0)</td>
<td>98</td>
</tr>
<tr>
<td>17</td>
<td>0.84 (2288)</td>
<td>1.10 (4)</td>
<td>8.90 (0)</td>
<td>92</td>
</tr>
<tr>
<td>18</td>
<td>0.80 (222)</td>
<td>1.36 (21)</td>
<td>6.40 (0)</td>
<td>79</td>
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<tr>
<td>19</td>
<td>1.18 (311)</td>
<td>1.81 (9)</td>
<td>17.2 (0)</td>
<td>96</td>
</tr>
<tr>
<td>20</td>
<td>0.60 (45)</td>
<td>18.3 (0)</td>
<td>22.5 (0)</td>
<td>99</td>
</tr>
<tr>
<td>Mean</td>
<td>1.04 (53)</td>
<td>7.41 (1)</td>
<td>12.5 (0)</td>
<td>&gt;95</td>
</tr>
<tr>
<td>SD</td>
<td>0.48</td>
<td>9.38</td>
<td>10.8</td>
<td>0.23 (1.3)</td>
</tr>
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</table>

*The sum of BPA eliminated in the period between the stated meal and the next meal or the end of the study period in the case of dinner. Urinary concentrations below the LOD were assigned a value equal to the LOD. The values in parentheses represent the percent difference between the reported value and the value calculated using zero instead of the LOD for concentrations less than LOD.

*Volunteer daily exposure is placed in the context of aggregate adult exposure in the United States (NHANES 2005–2006) by indicating where the exposure falls in the percentiles of BPA exposure reported by Lakind (Lakind and Naiman, 2010). One hundred percent absorption and elimination of ingested BPA were assumed in equating urinary elimination with exposure.
were taken from each volunteer, a total of 320 serum samples when the prestudy sample from each volunteer is included. Though the average total daily intake of BPA (0.27 µg/kg) was greater than the 95th upper percentile of aggregate exposure in the general U.S. population (>6 years old) (Lakind and Naiman, 2010), 83% of the serum samples had TOTBPA levels that were undetectable (Fig. 5). When serum concentrations and urine concentrations of TOTBPA were both above the LOD, serum concentrations ranged from 3- to 250 (mean = 42) -fold below urine concentrations at the nearest time point (Table 2). Only volunteers eliminating greater than approximately 12 µg (range 12–49 µg) or 0.13 µg/kg bw (range 0.13–0.57 µg/kg bw) of BPA in urine for any between-meal interval (volunteers 2, 3, 4, 11, 13, 15, 16, and 20) had consistently (greater than LOD for more than one consecutive sample) quantifiable concentrations of TOTBPA in serum over the same interval (Fig. 2 and Supplementary Fig. 1). These volunteers had daily exposures 1.3–3.9 times higher than the 95th percentile of aggregate U.S. exposure.

Serum TOTBPA rose rapidly after ingestion, with a mean time to maximum concentration of 1.63 h (SD = 0.47 h). When serum concentrations were significantly and consistently above detectable limits, the serum concentration time course and urinary elimination time course for TOTBPA were closely aligned, with a short lag time for urinary elimination (Fig. 3). Rapid absorption of BPA from food is consistent with previous reports of rapid absorption in humans (T\text{max}, 1.3 h) observed after administration of BPA in a gelatin capsule (Volkel et al., 2002). A single serum sample had a TOTBPA concentration above 4.4 nM (1 ng/ml) (Fig. 5). This sample was collected from volunteer 13 and corresponded to the period of highest exposure during any meal period for a volunteer (26.2 µg TOTBPA excreted). For the five volunteers with the most complete serum and urine time courses over the post-lunch period (volunteers 2, 13, 15, 16, and 20), the serum TOTBPA AUCs were 14.5, 9.9, 10.7, 10.3, and 6.4 nM h, respectively. For these same volunteers, after normalizing the serum TOTBPA AUCs to the meal-associated exposure (amount eliminated in the intermeal period per kilogram bw), the serum TOTBPA AUCs per micrograms BPA exposure were 79.0, 36.3, 40.0, 32, and 21.5 nMh/kg/µg TOTBPA (x = 41.8, SD = 21.9) for volunteers 2, 13, 15, 16, and 20, respectively.
For all 320 serum samples, BPA was below the detection limit (1.3 nM) when analyzed by the CDC laboratory. At the highest observed TOTBPA concentration of 5.7 nM for this study, the expected BPA serum concentration range would be expected to be between 0.007 (assumes 0.1% of TOTBPA is BPA) and 0.14 nM (assumes 2% of TOTBPA is BPA) based on reports of the fraction of TOTBPA present as BPA in serum following oral exposure in humans and nonhuman primates (Doerge et al., 2010; Taylor et al., 2011; Tominaga et al., 2006; Volkel et al., 2002). The subset of 55 samples with detectable TOTBPA was also analyzed by an independent laboratory (NCTR) for BPA to confirm these findings. Fifty-two of 55 serum samples had BPA concentrations that were below the daily determination of LOD (range

FIG. 3. Combined TOTBPA serum concentration and urinary elimination time courses for volunteers 16, 15, and 13 (left panel) and 2, 11, and 20 (right panel), a subset of volunteers with consistently detectable concentrations of TOTBPA in serum. Red arrows indicate the meal time (B, breakfast; L, lunch; and D, dinner). The amount of BPA eliminated through each intermeal period is provided. Urinary elimination was closely correlated to the serum time course, showing a consistent lag time of ~1 h.
After reanalysis, three serum samples had BPA concentrations above the LOD (0.5–0.9 nM). It was concluded that contamination was the most likely cause for these results for the following reasons: (1) When the samples were reanalyzed with an enzymatic hydrolysis step, the fraction of TOTBPA as BPA in these samples was higher than expected (up to 50% rather than the consistently reported 0.2–2%) for BPA reaching the blood via the oral route and (2) in two cases, serum samples from the same volunteer collected at different times had similar or higher TOTBPA but BPA was less than LOD. Contamination by BPA in the laboratory was also observed during method validation involving replicate analysis of samples in which only one of three replicates showed BPA concentrations greater than LOD (data not shown). Thus, infrequent contamination by BPA in this study was successfully detected and interpreted consistently with previous publications (Markham et al., 2010; Twaddle et al., 2010). The negative findings for serum BPA could not have been the result of BPA contamination in the laboratory, which would have biased the study toward more frequent BPA detection.

**DISCUSSION**

Resolution of the public health debate surrounding human exposure to BPA rests largely on determining if human blood and tissue concentrations of biologically active BPA are similar to or much lower than concentrations measured in test species responding adversely to exposure. The interpretation of reported levels of human internal exposure to BPA has, however, been controversial. Results from more than 20 studies...
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Predicted BPA concentration is approximately 0.057 nM. Assuming instead that the BPA/TOT BPA fraction was near values reported for nonhuman primates, 0.1 or 2%, serum BPA concentrations in our study would be between 0.001 nM at the LOD to 0.11 nM at the peak concentration.

Most importantly, the estimates of peak BPA serum concentrations in this study were 1–3 orders of magnitude below levels recently associated with histological changes in a sensitive experimental model of rat prostate intraepithelial neoplasia (PIN) (1.1–7.8 nM BPA) (Prins et al., 2011). The concurrent collection of toxicity data and corresponding serum BPA concentration in the study by Prins et al. (2011) are a critical experimental design element often used to interpret endocrine toxicity studies (Chang et al., 2000; Doerge et al., 2002; Twaddle et al., 2003). Taken together, our findings are evidence that on the basis of internal exposures to BPA, the active form of the compound, and the Prins et al. (2011) rat model of histological changes from neonatal exposure to BPA, that BPA promotion of PIN would not be expected in the general human population unless: (1) Humans are far more sensitive—10 to 10,000 times—to the endocrine effects of BPA than in the sensitized neonatal rat or (2) human fetal/neonatal blood concentrations of BPA are much higher than were observed in the adult population of this study. Such levels of BPA in human fetal/neonatal blood do not seem likely, with the possible exception of occupational or clinical subpopulations with a much higher burden of exposure (Calafat et al., 2009), or much higher nonoral exposure than the general population. Testing this hypothesis directly in a cohort of pregnant women through daily monitoring of serum, TOT BPA and BPA over an extended period of days would seem to be a logical next step. However, recently reported mean urine concentrations in pregnant women (1.3–4.5 ng/ml) (Braun et al., 2009; Ye et al., 2009) are low enough that blood and serum levels, which in our study average 42 times lower than urine levels, are likely to be below current detection limits.

The characteristics of the serum concentration time course of TOT BPA following dietary exposure (volunteers 2, 13, 15, 16, and 20) in our study were consistent with a previous human study where BPA was administered in a hard gelatin capsule and at a much higher dose than the estimated exposure range for volunteers with detectable serum TOT BPA concentrations in the present study (0.18–0.32 μg/kg bw). The serum TOT BPA T max following dietary exposure in our study, 1.6 h, was slightly longer than the 1.3 h T max observed by Volkel et al. (2002), possibly representing the difference between oral bolus (capsule) and slower ingestion during a regular meal. The average peak to dose ratio was 10.7 nM/kg/μg in the Völkel study compared with 17.1 nM/kg/μg (SD = 3.5) in the present study (range, 12.8–21.5 nM/kg/μg). The exposure-normalized AUC was 29.2 nM·h/kg/μg BPA in the Völkel study, about 43% lower than the mean value for volunteers 2, 13, 15, 16, and 20, 41.8 nM·h/kg/μg BPA, but only 11% lower than the average of the volunteers 13, 15, 16, and 20 (32.5 nM·h/kg/μg (some of them relying on the use of archived specimens that had not been specifically collected for measuring BPA) comprising both small (~20) and larger numbers (~200 to 400) of hospitalized and nonhospitalized adults show BPA spot blood or serum concentrations between 1.4 and 19.2 nM. Such BPA blood concentrations would require oral exposure to BPA 2–3 orders of magnitude greater than aggregate exposure levels derived from urinary BPA concentrations (Dekant and Volkel, 2008; Lakind and Naiman, 2008, 2010) and estimates of dietary intake (Chapin et al., 2008; World Health Organization, 2010), unless the exposures occurred almost immediately prior to sampling before rapid conversion to the glucuronide could take place and/or by a nonoral route of administration. These data contrast to a strong and growing body of evidence from studies specifically designed to control for and monitor contamination by BPA, including studies in humans (Volkel et al., 2002, 2005) and nonhuman primates (Doerge et al., 2010; Taylor et al., 2011; Tominaga et al., 2006), which consistently show that peak or average serum BPA concentrations following oral administration are approximately 0.2–1% of TOT BPA and not more than 2% of the TOT BPA (Taylor et al., 2011).

In the present study, hourly monitoring of serum BPA in adult humans confirms these findings by showing that after eating meals rich in canned food items, and using highly sensitive analytical methodology, TOT BPA was undetectable in 83% of serum samples. More importantly, BPA concentrations were below the LOD, 1.3 nM, when the analyses were conducted at CDC. When confirmatory analyses were conducted at NCTR, 3/55 samples analyzed had detectable BPA concentrations (0.5, 0.6, and 0.9 nM) albeit in a range similar to the daily LODs (0.2–0.7 nM). The evidence presented here and elsewhere for low-level contamination (Markham et al., 2010; Twaddle et al., 2010), even in the face of extraordinary attention to this problem, suggests that these infrequent positive determinations near the detection limit should be suspect.

Thus, some attributions of high blood BPA concentrations from oral exposure seem implausible, though we believe specific hypotheses about the source of the BPA detected in these samples—e.g., dermal exposure, leaching from plastic tubing used in field collection—should be formulated and tested objectively.

Though below the LOD in this study, serum BPA concentrations can be estimated from TOT BPA concentrations by applying the reported ratio of BPA/TOT BPA in human serum following oral exposure to BPA. The upper bound (i.e., LOD/ C max) of the BPA/TOT BPA ratio at C max in humans after oral exposure to BPA is 0.01 (1%) (Volkel et al., 2002), which is between the range reported for nonhuman primates (0.1–2% at C max) (Doerge et al., 2010; Taylor et al., 2011; Tominaga et al., 2006). Using the upper bound value in humans, the maximum possible concentration of BPA in 86% of the serum samples (those with TOT BPA less than or equal to LOD) from the 20 human volunteers in our study would be 0.01 nM (~2.3 pg/ml). In the highest of all 320 serum samples collected for this study where the TOT BPA concentration was 5.7 nM, the corresponding
BPA). These bw adjusted AUC/dose and $C_{\text{max}}$/dose ratios can be used to estimate population distributions of serum $\text{TOT} \text{BPA}$ $C_{\text{max}}$ and AUC from oral route exposure estimates made from urine biomonitoring studies. Similarly, estimates of the corresponding peak concentration and AUCs for BPA can be made by simply multiplying by the fraction of $\text{TOT} \text{BPA}$ present as BPA (0.1–2% for nonhuman primates).

The remarkable consistency between the serum pharmacokinetics of BPA in our high-dietary exposure study and the higher dose controlled exposure to encapsulated deuterated BPA is strong evidence that absorption and elimination kinetics are linear in humans across this range of administered doses encompassing the lowest estimated oral exposure (the mean from volunteers with observable serum $\text{TOT} \text{BPA}$ profiles) in our study (0.29 $\mu$g/kg bw) to the one time ingestion of 5 mg of BPA (the mean value of 64.1 $\mu$g/kg bw) of the Völkel et al. study. The consistency in the pharmacokinetics of BPA in humans in these two independent reports confirms the importance of both studies for understanding human oral route pharmacokinetics of BPA and supports the direct use of controlled human studies for estimating plausible levels of BPA in human blood following dietary exposure. Furthermore, the current results obtained using analytical methodology 10–45 times more sensitive than the previous human study by Völkel et al. suggest that reported BPA concentrations in human blood of 1.4–19.2 nM (Vandenberg et al., 2010) are highly unlikely in the general population exposed orally to amounts as much as approximately four times greater than the 95th upper percentile of aggregate exposure in the general U.S. population.

The rapid absorption and elimination kinetics of BPA observed in this and other human and nonhuman primate studies (Doerge et al., 2010; Taylor et al., 2011; Tominaga et al., 2006; Völkel et al., 2002) clearly demonstrate that spot urine samples reflect exposure in the prior meal, or prior 4- to 6-h period, but not the full day’s exposure. Moreover, the timing of a spot sample relative to the prior meal and, just as importantly, relative to prior urine voiding which eliminates accumulated BPA has a large impact on the accuracy of a single-spot samples (Ye et al., 2011). For example, on average, use of the first morning void spot sample collected after consuming the BPA-containing foods overpredicted measured BPA exposure by a factor of 3.6. In contrast, use of the first morning void collected prior to study initiation under-predicted BPA exposure during our exposure study by 27%. First morning void is a common sampling time for BPA biomonitoring (Nepomnaschy et al. 2009; Völkel et al., 2008).

The volunteers in the current study ingested large volumes of water to ensure the feasibility of regular urine collections, raising the possibility that high urine volumes could shift $\text{TOT} \text{BPA}$ concentrations toward undetectable levels in urine. However, $\text{TOT} \text{BPA}$ was detected in 74% of urine samples, providing more than sufficient information on the urinary time course for correlation with $\text{TOT} \text{BPA}$ serum concentrations and estimation of total daily exposure. Even with higher water ingestion distributed throughout the day, blood volumes would not be expected to affect the concentration of BPA in serum. The renal system is highly effective at maintaining intravascular fluid volumes and would rapidly compensate for increased ingestion of water with increased urine output (Sugaya et al., 2007; Tonstad et al., 2006), as was observed in this study (average urine output, 5.1 liters, SD 1.5 liters).

If objective scientific judgments are to be made regarding the implications of exposure to BPA, there must be continued focus on concurrent collection of internal exposure data for experimental models of toxicity. It will be similarly important to incorporate additional refinements in the ability to collect and interpret human BPA biomonitoring data through improved survey data and use of pharmacokinetic and reverse dosimetry models to calculate internal exposures where experimental measurements are not feasible.

**SUPPLEMENTARY DATA**

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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