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24-hour human urine and serum profiles of bisphenol A: Evidence against sublingual absorption following ingestion in soup

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ABSTRACT

Extensive first-pass metabolism of ingested bisphenol A (BPA) in the gastro-intestinal tract and liver restricts blood concentrations of bioactive BPA to <1% of total BPA in humans and non-human primates. Absorption of ingested BPA through non-metabolizing tissues of the oral cavity, recently demonstrated in dogs, could lead to the higher serum BPA concentrations reported in some human biomonitoring studies. We hypothesized that the extensive interaction with the oral mucosa by a liquid matrix, like soup, relative to solid food or capsules, might enhance absorption through non-metabolizing oral cavity tissues in humans, producing higher bioavailability and higher serum BPA concentrations. Concurrent serum and urine concentrations of d6-BPA, and its glucuronide and sulfate conjugates, were measured over a 24 hour period in 10 adult male volunteers following ingestion of 30 μg d6-BPA/kg body weight in soup. Absorption of d6-BPA was rapid ($t_{1/2}$ = 0.45 h) and elimination of the administered dose was complete 24 h post-ingestion, evidence against any tissue depot for BPA. The maximum serum d6-BPA concentration was 0.43 nM at 1.6 h after administration and represented <0.3% of total d6-BPA. Pharmacokinetic parameters, pharmacokinetic model simulations, and the significantly faster appearance half-life of d6-BPA-glucuronide compared to d6-BPA (0.29 h vs 0.45 h) were evidence against meaningful absorption of BPA in humans through any non-metabolizing tissue (<1%). This study confirms that typical exposure to BPA in food produces picomolar to subpicomolar serum BPA concentrations in humans, not nM concentrations reported in some biomonitoring studies.

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Introduction

Bisphenol A (BPA[1]), 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). Worldwide, scientific review panels convened by regulatory bodies have concluded that BPA is safe as currently used (United States Environmental Protection Agency, 1993; European Food Safety Authority, 2007; U.S. Food and Drug Administration, 2008; Japanese National Institute of Advanced Industrial Science and Technology, 2011; World Health Organization and Food and Agriculture Organization of the United Nations, 2011; Health Canada, 2012). Such panels also consistently conclude that the preponderance of BPA exposure is via the diet (>90%) since dietary intake assessments consistently exceed biomonitoring-based assessments of aggregate daily intake and no other significant sources of exposure have been identified (World Health Organization and Food and Agriculture Organization of the United Nations, 2011; European Food Safety Authority, 2013). Dietary intervention studies demonstrating reduction in urinary BPA after a shift to fresh foods also specifically support these conclusions (Christensen et al., 2012). These findings have been challenged by some for failure to consider measured serum BPA concentrations in humans ~1 ng/ml (4 nM) and higher, as well as routes of exposure other than food (Hormann et al., 2014; Vom Saal and Welshons, 2014). The preponderance of data, findings from international regulatory agency reviews (U.S. Food and Drug Administration, 2008; World Health Organization and Food and Agriculture Organization of the United Nations, 2011; European Food Safety Authority, 2013), and assessments integrating exposure, biomonitoring and human pharmacokinetic data (J. Teeguarden et al., 2013) support the conclusion

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[1] Abbreviations: The following terms were used throughout the manuscript in reference to forms of BPA: BPA, the unmetabolized, bioactive parent form; BPAG, BPA-glucuronide metabolite; BPAS, BPA-sulfate metabolite; Total BPA, the sum of BPA and all BPA conjugated metabolites. d6-, isotopically (deuterium) labeled forms; $^{13}C_{12}$, ^{13}C isotopically labeled forms. NCTR, National Center for Toxicological Research; bw, body weight.

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that serum BPA concentrations in humans >1 ng/ml are not realistically achievable in populations exposed orally via food. Possible exceptions are rare exposure scenarios that have been proposed, including hospital patients undergoing invasive procedures with plastic medical devices (J. Teeguarden et al., 2013), holding BPA-containing receipts for extended periods against the palm of a hand wetted with ethanol-based hand sanitizer (Hormann et al., 2014), and plastic sex toys (ANSES, 2013). Alternatively, observations of serum BPA levels ≥1 ng/ml have been attributed to sample contamination, a well-documented problem affecting trace level analysis of BPA and many other ubiquitous environmental contaminants (Dekant and Völkel, 2008; Markham et al., 2010; Twaddle et al., 2010; Teeguarden et al., 2011; J. Teeguarden et al., 2013; Birnbaum et al., 2012; Calafat et al., 2013; Ye et al., 2013).

Post-exposure contamination of serum by BPA during either blood collection, sample processing, or storage, has been reported consistently by those monitoring for it (Dekant and Völkel, 2008; Markham et al., 2010; Twaddle et al., 2010; Teeguarden et al., 2011, 2013a). More recently, in the current study and in a NIEHS-supported round robin (Vandenberg et al., 2014), materials that were mostly free of BPA were identified through careful testing. In both cases, plastic materials commonly used in blood collection were found to introduce BPA into serum samples. The majority of studies reporting serum BPA levels ≥1 ng/ml were conducted in hospitals, where medical interventions and blood collection with plastics were common, and/or failed to implement or report one or more key procedures for monitoring and limiting BPA contamination in the sample chain (J. Teeguarden et al., 2013; Ye et al., 2013): 1) field blanks; 2) laboratory blanks; 3) storage blanks; 4) reporting of BPA and total BPA levels; 5) matched urine samples; and 6) direct measurement of BPA-glucuronide (BPAG) and BPA-sulfate (BPAS) to confirm the presence of metabolites. Recent reviews referencing evidence of anomalously high serum BPA levels in humans did not evaluate the studies according to these six criteria or any other objective, accepted criteria (Vandenberg et al., 2010, 2013; Vom Saal and Welshons, 2014). Large-scale, and federally funded biomonitoring programs have concluded that measurement of BPA and total BPA in serum are not appropriate means of evaluating human exposure (Koch and Calafat, 2009; Koch et al., 2012; Calafat et al., 2013), given the propensity for contamination of serum and the much higher concentrations found in urine (Teeguarden et al., 2011). The current study design used isotopically labeled BPA (d6) as the dosing material to simultaneously evaluate possible contamination by the unlabeled BPA native to the environment and blood collection equipment, and avoid any influence on the pharmacokinetic measurements.

A contrasting view holds that there is "little or no direct evidence" for the assertion that "high" plasma levels in humans are artifacts of sample contamination (Gayrard et al., 2013), that instead, it is a "manufactured controversy (Vom Saal and Welshons, 2014)." Gayrard et al. suggested an alternative hypothesis — higher plasma concentrations in humans could result from sublingual absorption of ingested BPA by the vascularized tissues of the oral cavity, which bypasses gastrointestinal (GI-tract) and hepatic first pass metabolism that would otherwise restrict blood BPA concentration to <1% of total BPA (the sum of BPA and all metabolites). Testing this hypothesis in anesthetized beagle dogs, Gayrard et al. reported bioavailability >90% when concentrated aqueous and ethanolic solutions of BPA were placed under the tongue for 10 min, a site of high absorptive potential. Concentrations of BPA and the BPA/BPAG ratio were measured in blood collected from the jugular vein, which directly drains the oral cavity, and were higher than those measured in a leg vein after oral bolus exposure. Based on these findings, Gayrard et al. speculated that ng/ml levels of BPA, which have ratios of BPA/BPAG > 0.01, are achievable in humans consuming food. They also concluded that absorption from the GI-tract and absorption from the oral cavity (sublingual) are readily distinguishable by the serum BPA/BPAG ratios. The pooling of BPA solutions in the mouth of the dog and the reporting of unmixed serum BPA concentrations local to the site of absorption present challenges to the interpretation of the study in the context of human oral exposures and published biomonitoring data. The implications of this research in dogs for human exposure and health required additional experimental evidence regarding the extent and frequency of sublingual absorption in orally exposed humans (J.G. Teeguarden et al., 2013).

The goal of the present study was to characterize the serum and urine concentration time-courses for BPA in humans following controlled oral cavity exposure in a manner that reflects typical human exposure to BPA in liquids, like soup or beverages. Ingestion of a common food item was selected since this is the predominant route of adult and child exposure (European Food Safety Authority, 2007; Chapin et al., 2008; Carwile et al., 2009; Food and Drug Administration, 2009; Schecter et al., 2010; Rudel et al., 2011; World Health Organization and Food and Agriculture Organization of the United Nations, 2011; Geens et al., 2012), accounting for 90–99% of exposure (Wilson et al., 2007; Morgan et al., 2011; World Health Organization, 2011). The vehicle, a commercial soup product, represents a liquid carrier of BPA capable of higher surface area contact and absorption, compared to solid foods. Ingestion of the soup also allowed for absorption across all surfaces of the oro-gastric tract, not just the oral cavity or GI tract. Exposure conditions, the amount of soup, and the period of ingestion were matched to normal human feeding behavior. By design, the study allowed direct evaluation of hypotheses regarding "high" internal exposure to BPA following absorption in the oral cavity, and also the plausibility of oral cavity or oro-gastric tract absorption leading to unconjugated BPA concentrations in human blood >1 ng/ml (4 nM) in biomonitoring samples of systemic venous blood.

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 # 2014-14). The participation of the National Center for Toxicological Research was reviewed and approved by the FDA Research Involving Human Subjects Committee (RIHSC#10-119T) and determined not to constitute engagement in human subjects research because it was limited to analysis of anonymized samples.

Protocol review. The protocol was reviewed by an international panel of academic and governmental scientists with expertise in human biomonitoring of BPA.

Volunteer selection and demographics. Ten randomly selected healthy adult non-smoking (no nicotine product use) male volunteers were recruited for the study from the Salt Lake City, UT metropolitan area in 2014. 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, negative in a urine drug and alcohol screen, and had not undergone dental procedures in the two days preceding the study. Clinical examination verified that all volunteers had a normal, non-diseased oral mucosa throughout the mouth, without injuries or conditions that affect oral drug uptake. The average age of volunteers was 28.5 years (range 21–45). The average weight of volunteers was 81.3 kg (range 64.1–111 kg). The average BMI for volunteers was 24.5 (range, 19–35). Demographic information for each volunteer is provided in the supplemental data (see Table 1 in Teeguarden et al., submitted for publication). All ten enrolled volunteers completed the study.

Protocol. Volunteers were admitted to the clinical facility (PRA, Salt Lake City) in the morning (Day 1). Volunteers were provided with breakfast 1 h prior to ingesting BPA. Volunteers were provided with 12 oz of a commercial tomato soup product prepared using label instructions

that contained a 30 μg/kg bw dose of deuterium labeled (d6) BPA (d6-BPA) and instructed to ingest the soup in their normal fashion. Volunteers were provided non-alcoholic liquids of their choice to maintain hydration. Individuals were free to ingest liquids in accordance with their own wishes, but were encouraged to ingest ~120 ml of water each hour of the 16 hour waking period (total 1.9 L). Volunteers were provided regular meals for the remainder of the clinical stay. Venous blood samples were drawn immediately prior to ingestion of the soup, immediately after ingestion of the soup, and at regular intervals throughout the 24 hour study period. All voided urine was collected at regular intervals for 13 h, then as volunteered for any additional voids, and again at the study conclusion, 24 h after ingestion. Within the minor time constraints imposed by the logistics of dual sampling, time-matched urine and blood samples were collected. Both serum and urine were analyzed using published procedures (see details below). Volunteers were housed overnight and the final sample collection at 24 h concluded the study.

Oral exposure. An ethanolic solution of d_6-BPA (99.7% purity) was prepared at a concentration of 10 mg/mL and the concentration was verified by LC/MS/MS as previously described (Twaddle et al., 2010). Ethanol solutions of d6-BPA are stable for >1 year at refrigerator temperature and this solution was used within 2 weeks of preparation. An aliquot corresponding to a dose of 30 μg/kg body weight (3 μL/kg bw) was dispensed into warmed tomato soup, mixed, and immediately provided to volunteers for ingestion. The dose of d6-BPA administered was verified by recording the volume (via syringe) and the weight of material delivered (before and after measurement of the syringe weight). Volunteers ingested the soup in their normal manner to assure that all characteristics of the exposure reflected typical exposure conditions for oral-route exposure to BPA. The average period of time for soup ingestion was 9 min (range, 5–11). To assure that the entire dose was ingested, any remaining soup adhering to the bowl was collected by the volunteer using a piece of bread, which was then ingested to complete the dosing.

Serum and urine collection. Serum. Pre-study and terminal blood samples were drawn using a straight needle, single-use vacutainer holder, and a BD gold-top serum collection tube. All other blood samples were collected using the following components: BD Nexiva catheter (BD 383516), Baxter Clearlink luer-activated valve (Baxter 2N8399), luer-lok access device (BD 364902), BD gold top vacutainer tubes (BD 368977), 10 mL saline flush syringe (BD 306546), 10 mL empty syringe (BD 309604), 12G × 1.25 inch blood collection needle (BD systems, 368607) (see selection of materials, below). After serum separation, serum was transferred through a polypropylene pipet tip to polypropylene cryovials (Globe Scientific 3004 3.6 mL cryovials) and stored in a freezer at −20 °C within ~2 h. Approximate blood sampling intervals were: pre-study, immediately after ingestion of the soup, then 10, 20, 30, 40, 60, and 90 min and 2, 3, 4, 5, 7, 9, 11, 13 and 24 h after ingestion of the soup. Actual sampling times for each volunteer are presented in graphical form in Figs. 2–5 (Teeguarden et al., submitted for publication) (serum) and Fig. 3A (urine). Samples were frozen before being shipped overnight on dry ice and stored at −60 °C until analyzed.

Urine. All expressed urine was collected over the course of the 24-hour study. Urine was collected into polypropylene urine hats (Kendall) and the volume, weight and time were recorded. Urine sampling occurred immediately prior to ingestion of the soup, then at regular intervals corresponding to the timing of the blood samples (within logistical limits). Urine samples were not taken at the 10 and 30 min time points. Aliquots of each sample were transferred to polypropylene cryovials (Globe Scientific 3004 3.6 mL cryovials) and stored in a freezer within ~2 h. Samples were shipped overnight on dry-ice and stored at −60 °C until analyzed.

Selection of materials. Pre- and post-study evaluations of blood collection materials were conducted to identify materials free of BPA (i.e., polycarbonate plastic), if possible. Extensive research was conducted on the material composition of available devices and components (i.e., needle/catheter system, syringes, luer-activated values, luer-lok access devices, vacutainer tubes) including direct contact with five major manufacturers: B. Braun Medical, Baxter, Becton Dickinson (BD), Terumo Medical Products and Tangent Medical Technologies. All complete sets of components for collecting repeat blood samples (e.g., indwelling cannulae) included at least one item made with polycarbonate plastic (BPA-containing). In most cases, the polycarbonate parts were external, with no direct contact with the collected blood. One complete set of blood collection materials was tested by collecting serum, and processing it according to the blood collection protocol detailed below. The items tested were: BD Nexiva catheter (BD 383516), Baxter Clearlink luer-activated valve (Baxter 2 N8399), luer-lok access device (BD 364902), BD gold top vacutainer tubes (BD 368977), 10 mL saline flush syringe (BD 306546), 10 mL empty syringe (BD 309604), 12G × 1.25 inch blood collection needle (BD systems, 368607). Serum was also collected using just the straight needle, a single-use vacutainer holder (BD 364815) and a BD serum collection tube. Rat serum free from detectable BPA, previously established to be BPA-free within the limits of detection, was used (Churchwell et al., 2014).

Serum and urine analysis of d6-BPA. Concentrations of total d6-BPA in serum and urine were quantified using LC/MS/MS as previously described (Twaddle et al., 2010) after enzymatic hydrolysis using 200 μg of a mixture of β-glucuronidase and arylsulfatase from *Helix pomatia* (Sigma #5926-5KU). The method detection limit for total d6-BPA, defined as a signal/noise ratio of greater than or equal to 3, was approximately 0.2 nM (0.05 ng/mL) in 10–100 μL aliquots of serum using negative ion electrospray LC/MS/MS. Concentrations of d6-BPA in serum and urine were determined using the derivatization procedure described in Patterson et al. (2013). For analysis of d6-BPA in 100 μL aliquots of serum, the limit of detection (LOD, S/N = 3) was 0.004 nM (0.001 ng/mL). Concentrations of BPA were quantified using LC/MS/MS as previously described (Teeguarden et al., 2011; Churchwell et al., 2014) and reported as the amount above the daily limit of blank (LOB), which was determined from reagent blanks analyzed in replicate as the mean ± 2 SD (range 0.02–0.96 ng/mL, n = 4).

Quality control measures were performed during every sample set, including the analysis of blank and d6-BPA-spiked serum samples, pooled human serum and urine samples containing primarily conjugated forms of d6-BPA, blank injections, and injections of authentic standards. Pre-dosing serum and urine samples contained undetectable levels of d6-BPA and metabolites.

Analysis of d6-BPA-glucuronide and d6-BPA-sulfate. Concentrations of d6-BPAG were quantified using LC/MS/MS and $^{13}C_{12}$-BPAG as an internal standard in a manner analogous to that previously described for BPAG (Churchwell et al., 2014). The d6-BPAG (and d6-BPAS, see below) was not available as a standard so the same transitions used for the unlabeled BPAG (BPAS) were adjusted to the d6 variant. This made it necessary to assume that the response factors vs. the $^{13}C_{12}$-labeled conjugate were also identical, but since the primary transition used for quantification involves loss of the charged conjugate moiety (glucuronic acid or sulfate) and does not occur at a site of isotopic substitution, the possibility for isotope effects in fragmentation was deemed negligible. For analysis of d6-BPAG in 10 μL aliquots of serum, the limit of detection (LOD, S/N = 3) was 7 nM (3 ng/mL) and in 1 μL aliquots of urine, the limit of detection (LOD, S/N = 3) was 70 nM (30 ng/mL).

$^{13}C_{12}$-BPA-Sulfate (Sodium salt) was purchased from Sigma Chemical Co. (St. Louis, MO) and received as a solid. Chemical concentrations were determined by LC-UV analysis (PDA eλ Detector, Acquity, Waters, Inc.) set to monitor 278 nm and calibrated against authentic unlabeled

BPAS (Sigma). Full scan LC/MS (ES-) and LC-UV (278 nm) analysis was performed (Quattro Premier XE, Waters, Inc., Milford, MA) to establish chemical and isotopic purity (75% and 99.5%, respectively), and to determine source and MS1 parameters for optimal ionization. Daughter scan analysis was performed to establish product ions, collision cell and MS2 parameters. Resolution for MRM analysis was set to 0.9 Th for precursor and product ions.

LC and MS parameters. The LC method for d6-BPAS was identical to that previously reported for BPAG (Churchwell et al., 2014) and the MRM transitions are listed in Table 2 (Teeguarden et al., submitted for publication).

A calibration curve was established by varying BPAS concentrations across a range while keeping the $^{13}C_{12}$-BPAS concentration constant and injecting each solution twice. The response factor was established across a range of 0.01×–10× internal standard concentration. The result was a linear calibration curve with a slope of 0.92 and r^2 value greater than 0.999.

Method validation, serum. Control serum was spiked with BPAS at 2.5, 5, 10, 25, 50, and 100 ng/mL and analyzed in quadruplicate over two separate days, in conjunction with buffer and serum blanks. The analysis consisted of using 10 μL serum and 100 pg $^{13}C_{12}$-BPAS internal standard. Briefly, 10 μL of serum was added to a 0.5 mL centrifuge tube and 100 μL of a 1 pg/μL internal standard solution prepared in 50/50 methanol/acetonitrile was added. The tube was capped, vortex mixed, centrifuged for 3 min at 16,100 ×*g* and the supernatant was transferred to a 2-mL 96-well plate. The samples were evaporated under a heated stream of nitrogen (SPEdry, Biotage, Charlotte, NC) and reconstituted in 20/80 methanol/water. Injection volumes were 35 μL. Validation results are shown in Table 3 (Teeguarden et al., submitted for publication).

Method validation, urine. Control urine (pre-dosing) was spiked with BPAS at 10, 100, and 1000 ng/mL and analyzed in quadruplicate over two separate days, in conjunction with buffer and urine blanks. Analysis consisted of using 1 μL (eq) of urine for the 10 and 100 pg/μL concentrations and 0.1 μL (eq) of urine for the 1000 pg/μL. Water was used as the diluent for all urine samples. 10 μL of the diluted urine solution was used for analysis and 100 μL of 1 pg/μL $^{13}C_{12}$-BPAS was used. Sample extraction was performed using the same method described for serum. Validation results are shown in Table S4 (Teeguarden et al., submitted for publication).

LOD/limit of quantification (LOQ). Limits of detection (S/N = 3) and quantification (S/N = 10) for d6-BPAS in serum (10 μL aliquot) and urine (1 μL aliquot) were: serum: LOQ, 0.4 ng/mL (1.3 nM) and LOD: 0.1 ng/mL (0.3 nM); urine: LOQ, 10 ng/mL and LOD, 3 ng/mL.

Pharmacokinetic analysis. Plots of serum concentrations of total and d6-BPA at each time point following oral administration were analyzed using model-independent pharmacokinetic analysis (PK Solutions 2.0 software, Summit Research Services, Montrose, CO). All data points collected were used for the graphical analyses described. Log-linear plots were fitted to up to three kinetic phases corresponding to elimination, distribution, and absorption processes. The contribution from slower processes was subtracted from the earlier phases of the curve (i.e., feathering). For example, the first-order distribution rate constant (k_D) was determined after subtracting the contribution from the terminal elimination phase (k_E). Similarly, contributions from the elimination and distribution phases were subtracted to obtain the absorption rate constant (k_A). The maximal concentration (C_{max}) values were obtained from visual inspection of the time–concentration profiles at the time the maximal concentration was obtained (T_{max}). The area under the time–concentration curve ($AUC_{0-\infty}$) was determined by using the trapezoidal rule. Serum pharmacokinetics of formation and elimination for d6-BPAG and d6-BPAS were similarly determined. Half-lives for all kinetic processes were determined from the rate constants using the relationship: half-life ($t_{1/2}$) = ln 2 / k.

Fractional uptake of BPA by non-metabolizing tissues and arterial blood levels. PBPK model simulations of BPA absorption in the GI tract vs. BPA absorption from the GI tract and through oral-cavity or other tissue surfaces without metabolism, were conducted to calculate what level of non-GI tract absorption could be experimentally observed in mixed systemic blood. Our second modeling objective was to simulate arterial blood concentrations following non-GI tract absorption as an alternative to measurement in humans, which is rarely done due to the higher risks of sampling at this site. A published human PBPK model (Fisher et al., 2011; Yang et al., 2013) calibrated to monkey and human oral-route PK data (Völkel et al., 2002; Doerge et al., 2010b) was used to simulate the BPA PK data reported here assuming either: 1) 100% absorption through the GI tract; 2) 99% absorption through the GI tract and 1% absorption through a non-metabolizing tissue surface; or 3) 90% absorption through the GI tract and 10% through a non-metabolizing tissue surface. Absorption occurring outside the GI tract was described as transfer directly to arterial blood at a rate equal to the dose divided by an absorption period of 9 min, the average period of time of soup ingestion.

Calculation of bioactive BPA serum concentrations in adults and children resulting from oral-cavity exposure to BPA. Serum concentrations in individuals and populations exposed orally via solid or liquid food were calculated directly from the relationship between total dose and measured C_{max} (Eq. (1)), and between serum and urine total BPA concentrations (Eq. (2)) measured in this study and others, as described previously (Teeguarden et al., 2011, Eq. (1)):

$$[BP_{serum}A] = \frac{\mu g\,BPA\,Exposure}{kg\,body\,weight} \times \frac{[BP^{serum}_{Total}A]}{\frac{\mu g\,BPA\,Exposure}{kg\,body\,weight}} \times UCF \quad (1)$$

$$[^{serum}BPA] = \left[^{Urine}_{Total}BPA_{sample}\right] \times \frac{[^{serum}_{Total}BPA]}{[^{serum}_{Total}BPA]} \times UCF \quad (2)$$

where UCF is the fraction of total BPA in the form of BPA and [] indicates concentrations. To assure that serum concentrations could not be lower than we calculate, we assumed the entire daily dose was ingested in one meal, and that 100% of the dose was absorbed.

Statistics. Differences between half-times for absorption and elimination were tested for significance using the paired t-test function or Mann–Whitney Rank Sum Test in SigmaPlot© (version 11).

Preliminary data from the NIEHS oral d6-BPA human PK study. Serum and urine d6-BPA and total d6-BPA concentrations from a pilot study in three human subjects administered 100 μg/kg bw d6-BPA on a cookie were kindly provided by Dr. Kris Thayer for inclusion in this manuscript (Thayer and Birnbaum, submitted for publication).

Results

Administered dose

The average delivered dose of d6-BPA was 30.2 μg/kg bw (Standard error of the mean, SEM, 0.1 range, 29.5–30.7). The administered dose, for volunteers 2, 5, 6, 8, 11, 14, 15, 16, 17, and 18 were 30.5, 30.0, 30.1, 30.7, 30.3, 30.7, 30.4, 29.5, 30.2, and 29.5 μg/kg bw. The ingestion period, measured as the time between the start and end of soup ingestion, was on average, 9.2 min (SEM 0.5, range 5–11).

Urine output

Volunteers ingested significantly more liquid during the study period than the 1.9 L total water intake specified in the protocol. The average total daily urine output, a proxy for liquid ingestion, was 6 L (STD, 1.7 L). The range was 2.8–8.9 L.

Serum d6-BPA and metabolite kinetics

d6-BPA. The d6-BPA blood concentration time-course showed two distinct phases, absorption and distribution/elimination. d6-BPA was rapidly and completely (100% recovery of dose in urine, see below) absorbed, with an average half-life of 0.45 h (SEM, 0.03) (Table 1, Fig. 1A). The average peak concentration of 0.43 nM (SEM 0.14) occurred 1.6 h (SEM 0.17) after ingestion of the soup. d6-BPA was eliminated from serum with a terminal half-life of 5.5 h (SEM 0.51). The elimination half-life for d6-BPA was not statistically different from those for the metabolites or total d6-BPA using the paired t-test. The $AUC_{0-\infty}$ for d6-BPA was 2.5 nmol × h × L^{-1}, corresponding to 0.23% of the total d6-BPA $AUC_{0-\infty}$. The $AUC_{0-\infty}$ values varied over a 4-fold range (1.4–5.7 nmol × h × L^{-1}) within the group of subjects and a significant positive association with BMI was indicated by linear correlation analysis ($r^2 = 0.63$, $p = 0.006$). The C_{max} values varied from 0.3 to 0.7 nM but the linear correlation with BMI did not reach significance ($r^2 = 0.39$, $p = 0.055$). The percentage of BPA in total d6-BPA varied with time, rising from below 0.1% to 0.45% after an initial decline (Fig. 4). d6-BPA serum concentration time courses and pharmacokinetic parameters for individual volunteers can be found in Fig. 2 (Teeguarden et al., submitted for publication) and Table 5 (Teeguarden et al., submitted for publication), respectively.

d6-BPAG. The appearance half-life of this major metabolite of d6-BPA was 0.29 (SEM 0.04) hours, significantly (paired t-test, $p < 0.05$) faster than d6-BPA (Table 1, Fig. 1). The maximum d6-BPAG concentration of 286 nM (SEM 23) occurred 24 min earlier than the corresponding d6-BPA T_{max}. The area under the curve ($AUC_{0-\infty}$) was 680 (SEM 56) nmol × h × L^{-1}, 64% of the total BPA $AUC_{0-\infty}$. d6-BPAG serum concentration time course and pharmacokinetic parameters for all volunteers can be found in Fig. 3 (Teeguarden et al., submitted for publication) and Table 6 (Teeguarden et al., submitted for publication), respectively. The elimination half-life for d6-BPAG was not statistically different from d6-BPAS or total d6-BPA.

d6-BPAS. d6-BPAS represented 12.4% of the total BPA ($AUC_{0-\infty}$). The appearance half-life of d6-BPAS was 0.62 (SEM 0.13), two times slower than the appearance half-life of d6-BPAG, and 33% slower than d6-BPA (Table 1, Fig. 1A). The peak d6-BPAS concentration, 18 nM (SEM 1.9) occurred at 2.2 (SEM 0.19) hours, 1 h later than the d6-BPAG peak, and 36 min after the d6-BPA peak concentration. d6-BPAS serum concentration time courses and pharmacokinetic parameters for all volunteers can be found in Fig. 4 (Teeguarden et al., submitted for publication) and Table 7 (Teeguarden et al., submitted for publication), respectively. The elimination half-life for d6-BPAS was not statistically different from d6-BPAG or total d6-BPA.

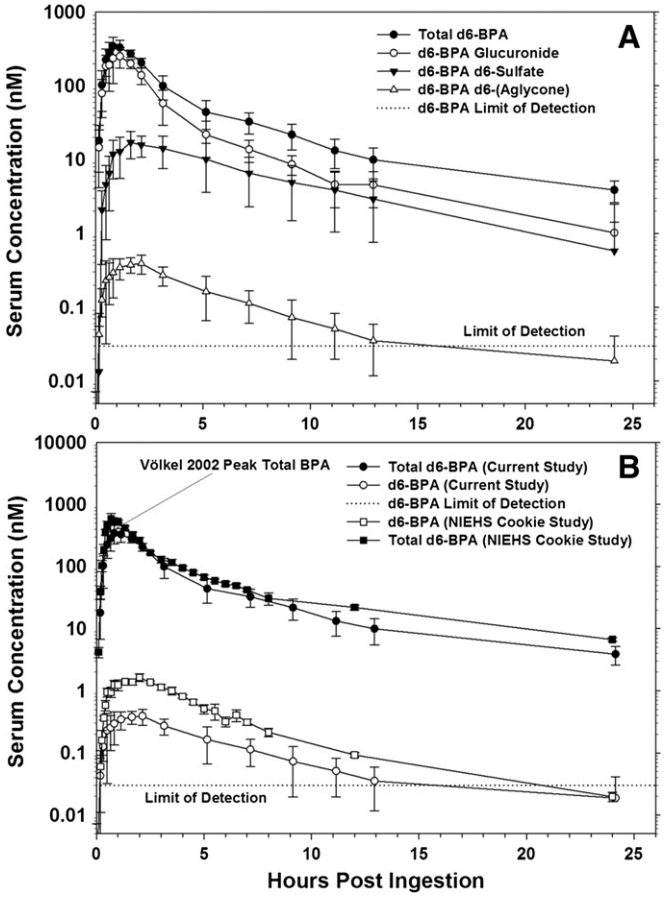

Fig. 1. Average serum concentrations of d6-BPA and metabolites. Panel A) Averaged serum concentration time courses for d6-BPA, d6-BPAG, d6-BPAS, and total d6-BPA are shown following administration of 30 μg/kg bw in soup. Panel B) Total d6-BPA (preliminary data) from an unpublished NIEHS human oral dosing study (Thayer and Birnbaum, submitted for publication), and the peak total d14-BPA concentration from the Völkel et al. (2002) study, both adjusted for dose, are plotted to demonstrate consistency across studies and exposure vehicles (gelatin capsule, cookie, soup).

Total d6-BPA. The pharmacokinetic parameters of total d6-BPA reflect the summation of the pharmacokinetics of d6-BPA, d6-BPAG, d6-BPAS and other metabolites (e.g., d6-diconjugates), but are dominated by the contribution of d6-BPAG. The appearance half-life of 0.28 (SEM 0.06) hours and T_{max} of 1.1 (SEM 0.14) hours were nearly identical to the corresponding d6-BPAG parameters (Table 1, Fig. 1A). The

Table 1
Pharmacokinetic parameters for d6-BPA, its metabolites, and total d6-BPA.

Compound	$T^{1/2}$ (h)			C_{max} (nM)	T_{max}(h)	$AUC_{0-\infty}$ (nmol × h × L^{-1})
	Elimination	Distribution	Absorption/Appearance			
d6-BPA	5.5 (0.5)	0.87 (0.3)	0.45 (0.03)[a]	0.43 (0.14)	1.6 (0.17)	2.5 (0.4)
d6-BPAG	7.3 (1.9)	–	0.29 (0.04)	286 (23)	1.2 (0.16)	680 (31)[b]
d6-BPAS	5.0 (1.5)	–	0.62 (0.13)	18 (1.9)	2.2 (.19)	131 (23)[b]
Total d6-BPA	7.8 (1.3)	–	0.28 (0.06)	365 (24)	1.1 (0.14)	1057 (47)

Mean and (SEM).
[a] Statistically different from d6-BPAG.
[b] Variability in d6-BPAG and d6-BPAS concentrations 11 to 24 h post dosing precluded calculation of an accurate $AUC_{0-\infty}$ for volunteers 8 and 18. Instead, an AUC_{0-t} was calculated directly from the data. The difference between AUC_{0-t} and $AUC_{0-\infty}$ was ~4% in the 8 volunteers where both could be calculated, supporting use of AUC_{0-t} in the two volunteers where $AUC_{0-\infty}$ could not be calculated. Elimination half-lives for d6-BPAG and total d6-BPA were calculated for 8 volunteers, excluding 8 and 18 where there were insufficient data to calculate an elimination half-life. The elimination half-life for d6-BPAS did not include a value from volunteer 8 due to insufficient data.

elimination half-life was 7.8 h. The $AUC_{0-\infty}$ was 1057 nmol × h × L^{-1} (SEM 47). Total d6-BPA serum concentration time course and pharmacokinetic parameters for all volunteers can be found in Fig. 5 (Teeguarden et al., submitted for publication) and Table 8 (Teeguarden et al., submitted for publication), respectively. Total d6-BPA concentrations were often higher than the sum of d6-BPAG + d6-BPAS + d6-BPA in all subjects, 77% of serum AUC, which suggested additional metabolites were present. A qualitative analysis using LC/MS/MS demonstrated the presence of d6-BPA-*bis*-sulfate and a mixed glucuronide/sulfate diconjugate in serum and urine samples (~1 h post-dosing but not in pre-dosing) from Subject 2 (not shown). The elimination half-life for total d6-BPA was statistically different from the half-life of d6-BPAS, but not d6-BPA or d6-BPAG.

Urinary elimination kinetics

Urinary elimination of total d6-BPA was rapid (i.e., the elimination half-life was ~2 h) and complete within 24 h after ingestion of soup containing d6-BPA. The average fraction of dose eliminated was 104% (SEM 2.6, Fig. 2). Urine total BPA concentrations varied as a function of time across 2.5 orders of magnitude (~20–6000 ng/mL) with a median value of 945 ng/mL, but largely paralleled serum total d6-BPA concentrations (Fig. 3A). Post-dosing urine samples, but not pre-dosing, consistently contained measurable levels of d6-BPA; however, the percentage was always well below 1% of the total d6-BPA present (GM, 0.03%, range 0.004%–0.423%).

Urine:blood total BPA ratios

Determining the quantitative relationship between concurrent concentrations of total d6-BPA in serum and urine after a defined dosing event was an important design element of this study. Urine:blood total d6-BPA ratios allow estimation of blood concentrations from the more commonly measured urine total BPA concentrations (i.e., reverse dosimetry (J. Teeguarden et al., 2013)). The urine:blood total d6-BPA ratio varied with time (Fig. 4) with a mean value of 40 for the whole exposure period and geometric mean 41 for the 2–24 hour post-dosing period (Table 2). The average urine:blood ratio calculated as the ratio of the observed 24-hour urine total d6-BPA AUC/blood total d6-BPA AUC was 35. Urine:blood total d6-BPA ratios were correlated ($R^2 = 0.65$) with total urine volume (Fig. 5), which was ~3 times higher in this study than under normal conditions (2 L/day). The mean regression line would predict urine:blood total d6-BPA ratios of ~65 for individuals with a more typical urinary output of 2 L per day for a 70 kg person. The urine:blood total d6-BPA ratios reported here are thus expected to be a lower bound estimate of values in the normal human population based on higher than normal fluid intake during sample collection during the current study.

PBPK modeling of sublingual absorption

PBPK model simulations described the oral pharmacokinetics of d6-BPA in this study accurately and precisely when 100% absorption through the GI tract was used (Fig. 6). Importantly, this assumption led to a distinct oral absorption phase characterized by a 0.45 hour oral absorption half-life, which is significantly slower than the corresponding appearance of d6-BPAG (0.29 h) or total d6-BPA (0.28 h) in serum. Simulations with even 1% absorption through a non-metabolizing tissue surface showed a much faster rise in initial serum BPA concentrations, which is a consequence of direct introduction into the central compartment (e.g., by i.v. injection), but inconsistent with the experimental data. Under conditions of 1 to 10% non-GI tract absorption, the initial venous serum concentrations of BPA were predicted to be 12–120 times higher than the initial measured value at the 10 minute time point.

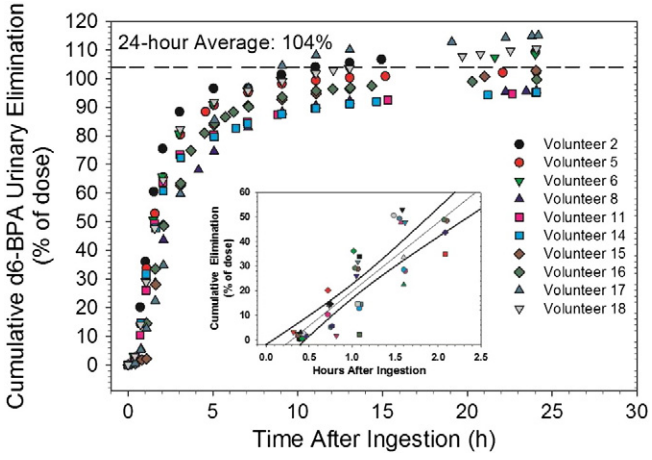

Fig. 2. Cumulative urinary elimination of total d6-BPA in all volunteers. Complete elimination of the administered dose is evidence against a physiological or anatomical depot or accumulation of d6-BPA in any tissue. Inset: Regression analysis on the linear portion of the curve was used to determine the mean and range of d6-BPA elimination half-times in urine.

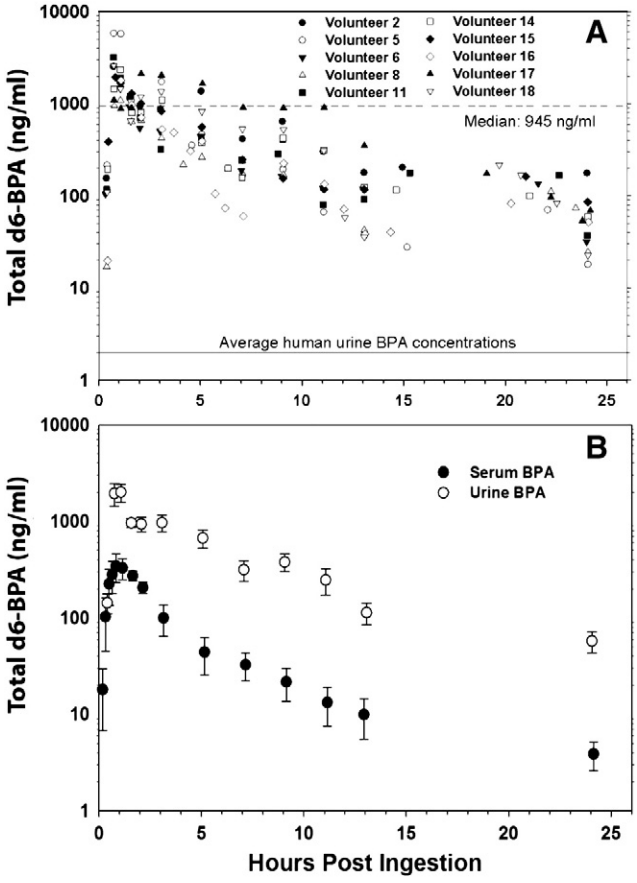

Fig. 3. Urine and serum total d6-BPA concentrations for all volunteers and sampling times. Panel A). Urine total d6-BPA concentrations vary considerably, but follow a pattern consistent with rapid absorption, metabolism and excretion of d6-BPA following oral cavity exposure. The median concentration was 500 times higher than the corresponding mean of urine samples from large scale, federally funded human biomonitoring studies (~2 ng/ml), indicative of the relatively low typical exposures in humans. Panel B) Serum and urine concentrations time courses were similar. Linear regression analysis of concurrent time points showed a high degree of correlation ($R^2 = 0.8$, $p < 0.001$).

Arterial blood BPA concentrations during sublingual exposure

A validated PBPK model was used to simulate serum concentrations of BPA at the site of sampling mixed venous blood and arterial concentrations of BPA after oral exposure to the total average and 95th upper percentile of total daily human exposure to BPA. Simulations assumed the upper bound on sublingual absorption in humans in the current study (1%). Arterial blood concentrations transiently exceeded venous blood concentrations for the period of absorption and then were no different than venous blood concentrations (Fig. 7). Arterial blood concentrations did not exceed 22 pM after high exposure and 4 pM at median exposures. Concentrations would be lower under typical human exposures where the total daily dose is ingested in multiple meals.

Bioactive BPA serum concentrations in adults and children resulting from oral-cavity exposure to BPA

The maximum expected serum BPA concentrations were calculated from a tabulation of total daily BPA exposures for all life-stages, including pregnancy (Table 3). Maximum blood BPA concentrations were calculated by multiplying total exposure by the ratio of the peak total BPA serum concentration to dose ratio (12 nM/μg/kg body weight) and then multiplying by the time-weighted fraction of total BPA present as BPA (0.0023). The median of the mean values was 1.9 pM (range 0.414–39). The median of the upper bound values was 7.7 pM (range, 1.9–116). Instead, if we use the BPA C_{max}/dose ratio (0.0012 nM/μg/kg bw) from the current study, the corresponding peak concentration for the adult U.S. population at the upper 95th percentile of exposure would be ~3.9 pM (~1 pg/mL), two orders of magnitude below levels of detection in serum.

Monitoring for contamination of serum samples by BPA

The current study used controlled dosing with d_6-BPA, which precluded interference by sample contamination with BPA. Detailed investigation of the sources and magnitude of possible sample contamination during sample collection and storage were needed to support a subsequent study that will report BPA levels in human samples designed to address the continuing issue of sample contamination. BPA-free rat serum was passed through blood collection devices to test for solubilization of BPA in the apparatus under study. Eight serum samples were collected in duplicate using four complete repeated blood sampling apparatuses. All (100%) of the second draws from each device, and one of the first serum samples from the devices contained detectable BPA (0.06 ng/mL–0.18 ng/mL, Fig. 1 (Teeguarden et al., submitted for publication)) consistent with contamination. Water passed through the full repeated collection apparatus extracted lesser amounts of BPA than serum (not shown).

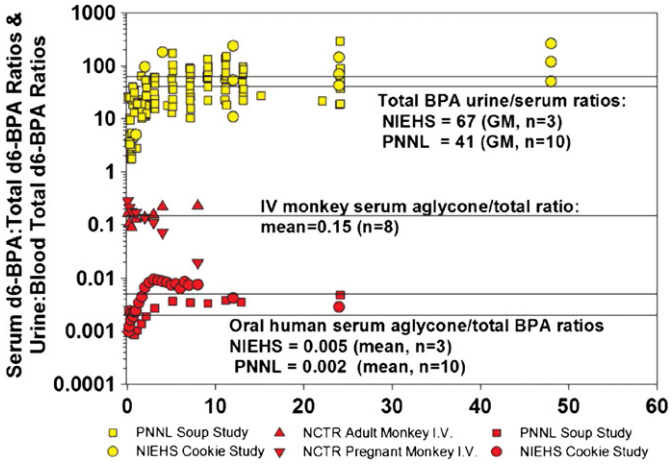

Fig. 4. Comparison of serum d6-BPA fraction and urine:blood total d6-BPA ratios across studies using controlled dosing with d6-BPA in human subjects. Serum d6-BPA fractions (red symbols) were consistently ≤0.01 (Mean, 0.002–0005) following oral exposure, and ≤0.15 following i.v. administration in non-human primates (Doerge et al., 2010b; Patterson et al., 2013). Urine to blood ratios (yellow symbols) averaged 67 during normal hydration and 41 during elevated hydration, which reduces BPA concentrations.

Discussion

Cross-study consistency of human BPA pharmacokinetics. The pharmacokinetics of d6-BPA and total d6-BPA observed following non-bolus oral-cavity/oro-gastric tract tissue exposures in soup are quantitatively consistent with the pharmacokinetics following: 1) bolus GI tract-only absorption following administration of a hard gelatin capsule (Völkel et al., 2002, 2005); 2) oral-cavity/oro-gastric tract tissue exposure via normal foods including soup, canned fruit in liquid, canned vegetables in liquid, and canned meat in liquid (Teeguarden et al., 2011); and 3) preliminary data from oral-cavity/oro-gastric tract tissue exposure in a cookie (Thayer and Birnbaum, submitted for publication). The extensive excretion of total administered dose in the urine (mean 104%) is consistent with essentially complete absorption and urinary elimination of the administered dose following both bolus oral and dietary exposure in humans (Völkel et al., 2002; Thayer and Birnbaum, submitted for publication). The serum d6-BPA C_{max} was ~4 times higher in the cookie study compared to the soup study, but showed similar T_{max} and elimination kinetics (Fig. 1). In both studies, conjugated d6-BPA (total d6-BPA) appeared more rapidly than d6-BPA, consistent with metabolism and then active transport of the metabolites from GI tract to the blood, and inconsistent with uptake through non-metabolizing tissues of the oro-gastric compartments. The key empirically derived parameters directly applicable to human biomonitoring were quantitatively similar between the oral bolus and three oral-dietary exposure studies. The total d6-BPA C_{max} value adjusted for dose was 12 nM per μg/kg body weight following exposure in soup (current study). This values lies between the values measured in our previous controlled study of human oral exposure in food (17 nM per μg/kg body weight) and the value reported by Völkel et al. after exposure to d14-BPA in a gelatin capsule (11 nM per μg/kg body weight), but lower than the preliminary value reported following oral exposure via a cookie of ~20 nM/μg/kg body weight (Thayer and Birnbaum, submitted for publication). The values from all four oral exposure studies fall within a factor of two of each other. The exposure-normalized AUC for total BPA was also similar for the three studies it was reported in: 35 nM * h/μg/kg body weight in the current study, 29 nM * h/μg/kg body weight in the Völkel et al. study, and 33–42 nM * h/μg/kg body weight in our previous food exposure study (Teeguarden et al., 2011). The exposure-normalized C_{max} for d6-BPA in the current study was 0.014 nM per μg/kg body weight. The corresponding exposure-normalized C_{max} for d6-BPA in the preliminary data from the NIEHS cookie study (Thayer and Birnbaum, submitted for publication) was ~4× higher at 0.054 nM/μg/kg body weight.

The unadjusted mean value of the urine:blood total d6-BPA ratio reported in the current study, 40, compared well to the mean value, 42, in our previous study with similarly high (5.2 L) liquid ingestion (Teeguarden et al., 2011). The higher than normal liquid ingestion, estimated by monitoring total urine output, was an unexpected consequence of ad libitum access to liquids during the study period. In order to compare urine:blood ratios of total BPA from the current study to previous studies, we characterized the influence of hydration status and urine concentration of BPA on urine:blood total BPA ratios by fitting a simple regression line to the urine:blood ratio vs. daily urine output curve (Fig. 5). From the regression, the urine:blood total BPA ratio calculated for normal hydration status (2 L/day) was 65. This value is nearly identical to the value measured in humans following

exposure in a cookie, 67 (preliminary data; Thayer and Birnbaum, submitted for publication). Overall, there was remarkable consistency between the pharmacokinetic parameters reported in the current study and two previous human oral BPA exposure studies reporting concurrent measurement of blood and urine concentrations, when accounting for hydration status.

Consistency of evidence against significant sublingual absorption from human studies. This is the second study from our laboratories that characterized contemporaneous blood and urine concentrations of total BPA following ingestion of typical foods (Teeguarden et al., 2011). This study confirms and extends the findings of four previous human studies (Völkel et al., 2002, 2005; Teeguarden et al., 2011; Thayer and Birnbaum, submitted for publication), as well as quantitative analysis of literature urine biomonitoring data (J. Teeguarden et al., 2013) that reject the plausibility of ng/mL levels of BPA in serum from the general adult U.S. population exposed orally to BPA, even at 95th percentile levels of aggregate exposure. The two studies conducted in our laboratories exposed volunteers to BPA in common food items, including soup in both studies, under normal feeding conditions, which allowed for oral-cavity tissue exposure during drinking and chewing, or extensive oral cavity liquid exposure during ingestion of soup. Serum BPA levels were below detection limits in all samples in the first, high exposure (95th percentile) study and total BPA was only observed in serum for a small number of subjects (Teeguarden et al., 2011). Scaling the d6-BPA blood concentration data in the current study by dose, maximal blood BPA concentrations following oral exposure to upper bound (95th percentile) on total daily human exposure (0.27 μg/kg bw/day) would be 0.0038 nM, approximately 100 times lower than current detection limits, substantiating the findings of the first study. Further, d6-BPA was on average 0.2% of total d6-BPA in this study that was optimized for any possible oral-cavity absorption.

The rapid and extensive appearance of a glucuronide conjugate in serum is consistent with pre-systemic Phase II metabolism in the GI tract and liver that limits internal exposures to the parent BPA to below a percent of the total BPA present in the C_{max} or AUC. The significantly faster appearance of d6-BPAG and total d6-BPA, compared with d6-BPA, in the systemic circulation (serum, Fig. 1) and BPA percentages below 1% of the total (Table 1) are inconsistent with significant oral-cavity absorption despite administration of d6-BPA in a liquid food item that makes intimate contact with the oral mucosa before ingestion.

All these human pharmacokinetic data stand in stark contrast to the much higher bioavailability, serum BPA concentrations, and BPA fractions (90%) reported in a model of oral-cavity exposure in beagle dogs (Gayrard et al., 2013). Differences between the findings in humans and the dog model can be readily attributed to differences in study design. Placing concentrated BPA-containing solutions onto the buccal cavity of a supine anesthetized dog created a long, physiologically abnormal residence time over these absorptive tissues. The residence time for BPA in the oral cavity in the current human study was consistent with normal periods of liquid ingestion, swallowing, and oral cavity clearance. Second, serum BPA concentrations in the dog study were measured in the descending jugular vein, which drains the oral cavity. This unusual and inappropriate (Sohlberg et al., 2013) site for pharmacokinetic analysis is not the site of blood sampling used for any of the published human biomonitoring studies, and brings into question selected attribution of the dog results to humans (Hormann et al., 2014). By sampling systemic blood from the antecubital vein, the site of systemic sampling used in all published human biomonitoring studies, the current study enabled direct comparison of findings to other adult human serum biomonitoring studies. The current study assessed serum oral absorption pharmacokinetics in an upright and alert person with normal cardiac output, rather than the reduction expected in a supine anesthetized dog. Gayrard et al. (Gayrard et al., 2013) reported that the supine anesthetized dog data provided evidence that sublingual absorption in humans could lead to the "ng/mL levels" of BPA and high BPA fractions reported in some human biomonitoring studies; however, the studies cited did not control or monitor for sample contamination by BPA (reviewed in J. Teeguarden et al., 2013 and Dekant and Völkel, 2008). Vom Saal and Welshons (Vom Saal and Welshons, 2014) recently reinforced Gayrard et al.'s predictions. We find that these hypotheses of sublingual absorption of BPA are inconsistent with serum data from humans after various kinds of oral exposure, including solid and liquid foods, and supporting PBPK modeling.

Evidence against anatomical or physiological BPA depots in humans. The parallel elimination profiles and similar half-times for elimination of d6-BPA, d6-BPAG, d6-BPAS, and total d6-BPA in humans with BMI from 19 to 35 (Fig. 1, Table 1) and essentially quantitative urinary excretion within approximately 24 h are inconsistent with any depot for d6-BPA (Fig. 3). Controlled dosing pharmacokinetic studies with d6-BPA in mouse adipose tissue (Doerge et al., 2012) and pregnant monkeys similarly showed the absence of a tissue- or pregnancy-related depot (e.g., fetus or placenta; Patterson et al., 2013).

Implications for BPA biomonitoring. The presence of diconjugated metabolites in serum and urine reported in this study emphasizes the value of measuring total BPA following quantitative hydrolysis of all conjugated forms, further validating the enzymatic hydrolysis method, which has been in use for decades. Measurement of BPA, BPAG, and BPAS, but not the diconjugated metabolites, would underestimate total BPA concentrations in serum and urine. It is noteworthy that the more accurate approach of measuring total BPA after enzyme hydrolysis has been consistently used for large scale, federally funded urinary biomonitoring

Table 2
Daily average total d6-BPA urine:blood ratios.

Volunteer	Observations	Urine:blood ratio Average (SEM)
2.0	14	75 (22)
5.0	15	36 (7)
6.0	13	31 (6)
8.0	12	24 (3)
11	14	35 (6)
14	14	29 (5)
15	12	34 (8)
16	9.0	34 (8)
17	14	53 (10)
18	14	45 (10)
Overall average	131	40 (3.4)
2–24 hour geometric mean	131	41

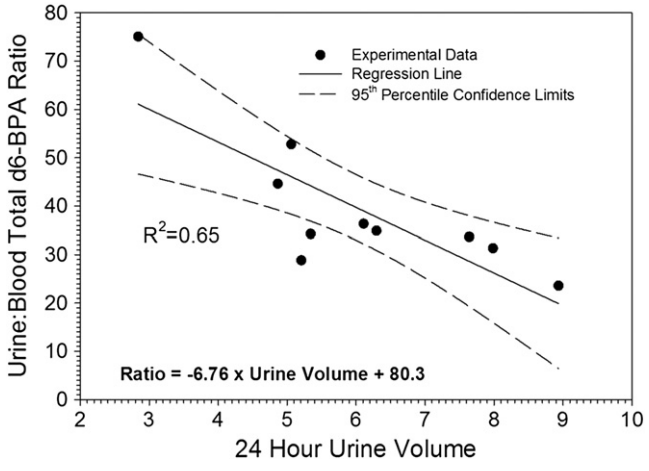

Fig. 5. The influence of urine output (liquid ingestion) on urine:blood total d6-BPA ratios. Linear regression of urine:blood total d6-BPA ratio and urine output showed a good correlation between the two ($R^2 = 0.65$, $P = 0.005$). The correlation was stronger ($R^2 = 0.77$, $P = 0.001$) for the 12–24 hour period after exposure (data not shown).

measurements of aggregate daily intake of BPA in the United States, Canada and Germany (Calafat et al., 2008; Koch et al., 2012; Arbuckle et al., 2014).

The comparison of serum and urinary concentration–time profiles and their consistency across several human oral pharmacokinetic studies emphasizes the inter-relationship of concentrations in these two compartments (Fig. 3B), and supports the use of the urine:blood ratios from controlled human studies, such as the one reported here, for purposes of interpreting human urine biomonitoring studies. Teeguarden et al. (2013) applied a geometric mean urine:serum ratio of 23 to calculate the corresponding BPA concentrations in a cohort of more than 28,000 people, across multiple life-stages, including children and pregnant women, and multiple countries. The resulting mean serum concentrations were in the low to sub pM range. Applying the urine:blood ratio adjusted to normal daily liquid ingestion in the current study (65), or the value reported by Birnbaum (62) would result in values even lower (~60%).

The consistency of all pharmacokinetic parameters, urine:blood ratios, BPA to total BPA ratios, and ratios of total BPA C_{max}/dose and AUC/dose across all reported human oral exposure studies further validates previously reported methods for estimating adult human serum concentrations for all types of oral-exposure from urine spot sampling, 24-hour urine biomonitoring, total daily exposure, or serum total BPA data (J. Teeguarden et al., 2013). Each of these methods is not susceptible to blood-derived sample contamination by BPA. The conclusion that serum concentrations of BPA in non-pregnant and pregnant adults and children following oral exposure in food and liquids (soup, canned meats, fruits and vegetables in liquid) fall in the range low pM to sub-pM range is substantiated by the data in the current study and PBPK modeling of total dietary intake estimates reported previously (J. Teeguarden et al., 2013).

Revised criteria for identification of contaminated blood samples. The quantitative relationships between serum BPA — serum total BPA and urine total BPA — serum total BPA reported in this study, and the consistency with previous human oral pharmacokinetic studies and non-human primate pharmacokinetic studies, provide quantitative criteria for identification of serum samples contaminated by BPA: 1) following ingestion, serum BPA is <1% of the total BPA, so that typical serum samples (i.e., randomly collected) containing >1% BPA are likely contaminated. Similarly, since BPA concentrations in urine were consistently well below 1% of the total, urine containing BPA above 1% should be considered contaminated; and 2) for all non-oral exposures, for example dermal exposure, or mixed i.v. and dermal exposures that might occur during medical interventions, an upper bound estimate of 15% BPA in serum can be estimated from the mean monkey i.v. exposure data (Doerge et al., 2010a; Patterson et al., 2013). Thus, because no route of exposure examined to date yields serum BPA concentrations >15% in the mixed serum sampled in human studies, any reports of serum BPA greater than 15% of the total BPA present in adult mixed serum likely represent contamination. These quantitative criteria extend previously published study design criteria (Ye et al., 2013) that are not met by those human biomonitoring studies reporting ng/mL levels of BPA in serum: (1) field blanks; (2) laboratory blanks; (3) storage blanks; (4) reporting of BPA and total BPA levels; and (5) matched urine samples.

We also found that drawing water through blood collection apparatus was less effective than use of serum for evaluating BPA contamination from sample collection materials. BPA-free serum samples pulled through blood collection materials in the current study showed clear evidence of contamination by BPA (Fig. 1; Teeguarden et al., submitted for publication), while BPA-free water samples pulled through the same apparatus solubilized lesser amounts of BPA. While initial screening with water is appropriate to identify some level of contamination, field blanks and routine validation of blood collection apparatus should use blood/serum, not water, to verify the absence of BPA contamination in the matrix for analysis. This is the recommended approach of the recent NIEHS-funded BPA analytical round robin (Vandenberg et al., 2014), although it has not been applied in subsequent studies (Hormann et al., 2014) by laboratories participating in and advocating for findings of this round robin (Vom Saal and Welshons, 2014).

Arterial BPA concentrations. Venous blood concentrations in the adult human population exposed normally through the diet have been shown, experimentally and computationally, to be below levels expected to have estrogen receptor-mediated biological effects (J. Teeguarden et al., 2013). Differences between venous and arterial blood concentrations of drugs and chemicals absorbed by the tissues of the oral cavity (e.g., nicotine) have been reported in humans (Benowitz et al., 2009; Mishina and Hoffman, 2014). Safety and logistical challenges preclude collection of arterial blood in human BPA biomonitoring and pharmacokinetic studies, including the current study. If oral-cavity absorption of

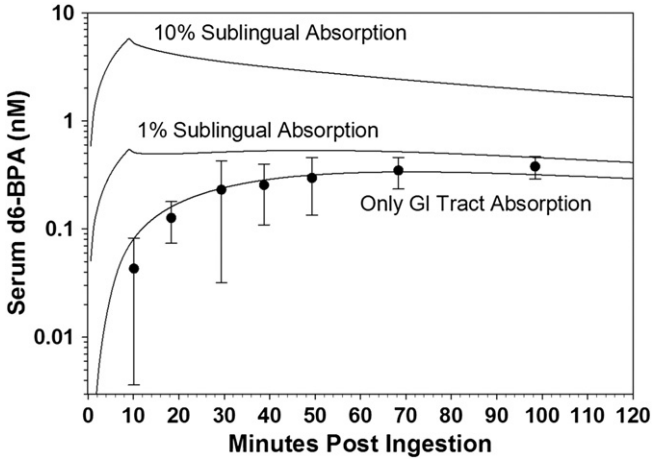

Fig. 6. Physiologically based pharmacokinetic model simulation of serum d6-BPA concentrations. A validated PBPK model (Yang et al., 2013) was used to simulate serum concentrations of BPA resulting from oral exposure to BPA with 10%, 1% or no BPA absorbed through a non-metabolizing tissue of the oro-gastric compartment (e.g., buccal). The experimental data (means ± SEM) were consistent with complete absorption through tissues with metabolic capacity, like the gastro-intestinal tract and liver, and inconsistent with even 1% absorption through a non-metabolizing tissue.

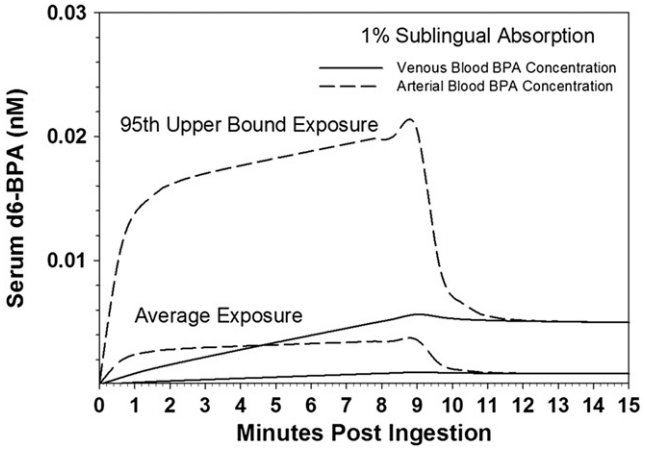

Fig. 7. Venous and arterial blood concentrations of BPA following sublingual absorption. A validated PBPK model (Yang et al., 2013) was used to simulate serum concentrations of BPA at the site of sampling mixed venous blood and arterial concentrations of BPA after oral exposure to the total average and 95th upper percentile of total daily human exposure to BPA. Simulations assumed the upper bound on sublingual absorption in humans in the current study (1%).

Table 3
Summary of urine biomonitoring-based human BPA exposure measures.

Study description	N	Country	Citation	External exposure (μg/kg/day)[1]			Serum BPA (pM)		
				Lower	Mean	Upper	Lower	Mean	Upper
Spot urine biomonitoring based exposure assessments									
World Health Organization			a						
1–5 months	47	Germany	b	–	0.07	1.61	–	1.9	44
3–5 years	599	Germany	c	–	0.12	0.78	–	3.3	22
6–11 years	314	U.S.	d	–	0.07	0.31	–	1.9	8.6
NHANES 2003–4	2517	U.S.	e	0.024	0.047	0.27	0.66	1.3	7.5
NHANES 2005–6	2548	U.S.	f	0.017	0.035	0.22	0.47	0.97	6.0
CHMS	5472	Canada	g		0.021	0.089	–	0.58	2.5
Table 15, NTP Report			h				–	–	–
Japanese adults	22	Japan	i	0.008	0.028	0.075	0.22	0.77	2.1
Japanese adults	36	Japan	j	0.004	0.02	0.23	0.11	0.55	6.3
Males	5.0	Japan	j	0.01	0.022	0.22	0.28	0.60	6.0
Pregnant	56	Japan	k	–	–	0.16	–	–	4.4
College students	48	Japan	l	0.002	0.015	0.24	0.06	0.41	6.6
Girls 6–8 years old	90	U.S.	m	0.012	0.07	2.17	0.33	1.9	60
24 hour urine biomonitoring based exposure assessments									
Adult[2]	596	Germany	n	0.0002	0.04	0.17		1.0	4.7
Adult[2]	8.0	Germany	o	–	0.07	–		1.9	
Adult[2]	20	U.S.	p	0.03	0.27	0.86	0.8	7.5	24
Adult[2]	8.0	U.S.	q	0.05	0.07	0.09	1.4	1.9	2.5
Pre-school Children[3]	81	U.S.	r	0.057	0.105	0.458	1.6	2.9	13
Food survey exposure assessments									
World Health Organization			a						
Infants 0–6 months[4]	NA	MN		–	0.5	1.9		14	52
Infants 6–36 months[4]	NA	MN		–	0.1	1.5		3.0	41
Children ≥ 3 years	NA			–	0.7	1.9		19	52
Adults	NA			–	1.4	4.2		39	116
FDA		U.S.	s						
Infants 0–1 year	1758	U.S.		–	0.3	0.6		8.0	17
Infants 1–2 years	10,189	U.S.		–	0.5	1.1		14	30
Infants >2 years	31,257	U.S.		–	0.1	0.3		3.0	8.0
Food survey	NA	Sweden	t	0.038	0.052	0.07	1.0	1.0	2.0
Food survey	NA	Korea	u	–	0.03	–	–	1.0	0.0
Food survey	NA	New Zealand	u	–	0.008	0.29	–	–	8.0
Food survey	NA	Spain	u	–	0.0018	0.078	–	–	2.0
Food survey	NA	Belgium	u	–	0.015	0.086	–	–	2.0

[1]Lower is the lower bound (minimum or 25th percentile), mean is one measure of central tendency (geometric mean, median or mean, whichever was reported), upper is the upper bound (75th, 90th or 95th upper percentile or maximum, whichever was reported). CHMS, Canadian Health Measures Survey; NHANES National Health and Nutrition Examination Survey. [2]24 hour total urinary excretion of BPA. [3]Total excretion estimated from 6 spot samples over 48 h. [4]Formula diet, no use of polycarbonate bottles since they have been phases out of use. –, Exposures calculated for a population exposure, not for a population of a given size. [a]World Health Organization and Food and Agriculture Organization of the United Nations (2011); [b]Völkel et al. (2011); [c]Becker et al. (2009); [d]Calafat et al. (2008); [e]Lakind and Naiman (2008); [f]Lakind and Naiman (2010); [g]Lakind et al. (2012); [h]Chapin et al. (2008) [i]Tsukioka et al. (2004); [j]Arakawa et al. (2004); [k]Fujimaki et al. (2004); [l]Ouchi and Watanabe (2002); [m]Wolff et al. (2007); [n]Koch et al. (2012); [o]Moors et al. (2007); [p]Teeguarden et al. (2011); [q]Ye et al. (2011); [r]Morgan et al. (2011); [s]Food and Drug Administration (2009); [t]Gyllenhammar et al. (2012); [u]Geens et al. (2010).

BPA were sufficient under normal exposure conditions (e.g., food, liquids) to produce arterial concentrations of BPA that reach biologically active concentrations for the general population, these higher arterial concentrations would have to be considered in assessing human risks. Using the human BPA PBPK model fitted to the d6-BPA serum concentration data from this study, we simulated venous and arterial blood concentrations of BPA by following the maximum possible fractional absorption (<1%) identified in this study. Exposures equal to mean and 95th upper percentile total daily adult human exposure were simulated. Arterial blood concentrations were ~4–9 times higher than venous blood concentrations for period of ~9 min, after which they rapidly equilibrated with venous blood. Peak arterial blood concentrations never exceeded 22 pM at the 95th percentile human exposure level and 4 pM at median exposure. Both are below levels consistently shown to have effects in either cells or animal models (J. Teeguarden et al., 2013). In the adult human population, after normal ingestion of liquids containing BPA, arterial blood concentrations would likely be lower than the values we calculate here because: 1) it is unlikely the entire daily intake of BPA occurs in a single meal; 2) we used the highest possible fractional oral-cavity absorption that was consistent with our data, but the value is more likely much lower; and 3) 95th percentile exposures were used.

Hormann et al. (2014) reported serum BPA concentrations in the range of 6–10 ng/mL, 15–30 min after volunteers pressed a BPA-containing thermal paper receipt against one palm, that had been wetted with an ethanol-containing hand sanitizer, followed by ingestion of French fries using the hand that held the receipt. The purported mixed oral–dermal exposure, the simultaneous extraction of the receipt on the palm wet with ethanol, the absence of measures of total absorbed dose, the absence of measures of total BPA after enzymatic hydrolysis, and the absence of 24 hour urine and concurrent urine and blood measures in the volunteers each make the results difficult to interpret in the context of existing biomonitoring studies and normal human exposure during typical receipt handling and eating. However, the results of the current study are useful in this regard, to the extent that they demonstrate that sublingual absorption of BPA on French fries, or from licking fingers, did not contribute to the serum BPA levels.

Conclusions. We found evidence against, and no evidence for, meaningful sublingual absorption in humans eating soup in a typical manner. Moreover, the concordance of human pharmacokinetic data from BPA ingestion through either liquid food (this study), solid food (Thayer and Birnbaum, submitted for publication), or a gelatin capsule (Völkel et al., 2002) suggests that bolus administration models accurately

represent typical ingestion of BPA in foods. Extrapolation of findings from the supine anesthetized dog model to normal human oral exposures, as articulated by (Gayrard et al., 2013), were found to be inconsistent with the experimental human data. Instead, we conclude that consistency across several human oral pharmacokinetic studies reinforces and validates the direct use of available human data for biomonitoring, and for placing in vitro cell culture along with in vivo rodent and non-human primate toxicity studies in the context of human exposure for purposes of assessing risk, for example see European Food Safety Authority (2007, 2013), U.S. Food and Drug Administration (2008) and J. Teeguarden et al. (2013). The body of human pharmacokinetic data provides further support for use of exposure-normalized C_{max} (C_{max}/dose), and AUC (AUC/dose) values, urine:blood ratios, and PBPK modeling for predicting human venous and arterial blood concentrations for all human oral-exposure scenarios (J. Teeguarden et al., 2013; Yang et al., 2013). We introduce new criteria for identification of contaminated serum samples: serum BPA percentages above 1% for typical oral exposure scenarios and above 15% for atypical parenteral exposures; urine: blood ratios of total BPA less than 20. Finally, our PBPK model simulations show that even arterial blood concentrations of bioactive BPA reaching tissues would be below 22 pM when oral exposures equivalent to the 95% upper percentile total daily exposure occur in a single meal because negligible sublingual absorption through a non-metabolizing tissue is observed (<1%).

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Conflict of interest

The authors declare no conflict of interest: Funding was provided as an unrestricted grant. The granting organization did not review the study design, data, data analyses, the manuscript or conclusions prior to publication.

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