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Article

A Preliminary Study of Biomonitoring for Bisphenol-A in Human Sweat

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Measurement of human exposure to the endocrine disruptor bisphenol-A (BPA) is hampered by the ubiquitous but transient exposure for most individuals, coupled with a short metabolic half-life which leads to high inter- and intra-individual variability. We investigated the possibility of measuring multiday exposure to BPA in human sweat among volunteer participants with the goal of identifying an exposure assessment method less affected by temporal variability. We recruited 50 participants to wear a sweat collection patch (PharmChek®) for 7 days with concurrent collection of daily first-morning urine. Urines and sweat patch extracts were analyzed with quantitative LC–MS-MS using a method we previously validated. In addition, a human volunteer consumed one can of commercially available soup (16 oz, 473 cm^3) daily for 3 days and collected urine. Sweat patches (n = 2, 1 per arm) were worn for the 3 days of the study. BPA was detected in quality control specimens prepared by fortification of BPA to sweat patches, but was only detected at 5× above average background on three participant patches. Although the highest measured urine BPA concentration was 195 ng/mL for an individual with deliberate exposure, no BPA was detected above background in the corresponding sweat patches. In this preliminary investigation, the use of sweat patches primarily worn on the upper-outer arm did not detect BPA exposures that were documented by urine monitoring. The absence of BPA in sweat patches may be due to several factors, including insufficient quantity of specimen per patch, or extremely low concentrations of BPA in naturally occurring sweat, among others.

Introduction

Exposure to endocrine disrupting chemicals such as bisphenol-A (BPA) via plastics is ubiquitous and subject to great concern among scientists and the public. BPA exposure in humans has been associated with impaired health outcomes including immune dysfunction (1), implantation failure in women undergoing *in vitro* fertilization (2), male sexual dysfunction (3) and altered hormone levels and reduced sperm quality in males undergoing infertility evaluation (4, 5). Experimental animal evidence suggests a relationship between exposure to endocrine disrupting chemicals including BPA and reproductive endpoints including lactation, but the relationship in humans has been less explored (6). The rapid metabolism of BPA (half-life ~6 h) (7) means that it is reasonable to expect daily variation in measures of urinary exposure (8–11). In humans, between 84–97% of BPA is eliminated in urine 5–7 h after exposure and can increase to 100% after 24 h (7, 12).

An innovative study at Harvard University recruited participants for a cross-over study in which they were randomly assigned to eat either fresh or canned soup (blinded) for lunch daily for 5 days, and urinary BPA was measured in the afternoons of the fifth and sixth days (13). The measured BPA levels were over 1,000% higher during the canned soup ingestion week compared with the same individual's fresh soup consumption week, demonstrating the impact of transient BPA exposures on levels measured in human subjects.

Exposure to BPA is typically measured in human studies via analysis of urine specimens. The type of urine specimen has varied from decades-old stored samples to prospectively collected single specimens (most common) to total urine collections for some period of time, usually 24 h (10). In at least one study, participants collected all urine excreted for a 7-day period (9). The choice of sample type and frequency is determined not only by appropriateness of the matrix for the analyte of interest, but also by logistical considerations and what participants are willing or able to do within the timeline or budget of the study. Urine is relatively easy to collect, non-invasive, and large sample volumes are generally available, making it an attractive specimen for biomonitoring studies.

Given the rapid metabolism of BPA, researchers are concerned about variability and accurate exposure assessment of BPA in urine. One study compared 24- and 48-h urine collection with spot urine collection, and determined that spot measures could be used to characterize population distribution of intakes, but did not support spot measures for individual-level exposure classification (10). A small study had eight adults collect all urine for a week in order to quantify the between- and within-person variability, and found that for spot collections, there was considerable within-day variance (9). The authors noted that 24-h urine collections accurately reflect exposure that day but do not capture day-to-day variation (9). Measuring BPA in human sweat collected continuously over a period of 7 days could help address this variability.

Phthalates and BPA have been detected in human sweat (14, 15), when collected in a sauna or during exercise. There is evidence that BPA may bioaccumulate in adipose tissue and has a lipophilic affinity for fat with a fat : blood coefficient of 3.3 (16). Lipophilic compounds stored in adipose tissue can be excreted through human sebaceous glands into sebum that could be collected by a sweat patch. We hypothesized that the use of a sweat collection patch, similar to those used for monitoring drug exposure, worn for 7 consecutive days, could be used to estimate participant exposure to BPA within a cohort of volunteer participants.

We developed a protocol to recover and analyze total BPA (unconjugated BPA plus monoglucuronide conjugate and monosulfate conjugate) from the sweat patches, and tested it on patches fortified with BPA. Participants successfully completed the protocol, but we detected BPA at 5× above the average background on only three (6%) of the participant patches.

Methods

Participants

Participants were healthy males and females living in the greater Salt Lake City, Utah area. Recruitment began in July 2013 and concluded in October 2013. Participants were eligible if they were ≥18, able to respond to study questionnaires in English, and regular consumers of canned or prepackaged foods. The study was approved by the University of Utah Institutional Review Board, and participants were required to sign an informed consent document prior to participation.

Exposure assessment

Exposure assessment was performed for a single week, during which participants collected daily first-morning urine, kept a daily diary of all food, drink, exercise and medication consumption, and wore the sweat patch continuously. On Day 1, participants met with a member of the study staff who explained study procedures, provided biospecimen collection materials, obtained height and weight measurements, and applied the sweat patch. A spot urine sample was collected after enrollment on Day 1, and participants were instructed to collect first-morning samples for the next 6 days while wearing the patch. Urine was collected in BPA-free four ounce polyproprylene sample cups, then transferred to BPA-free 50 mL polypropylene tubes that were placed in the participants' home freezer until the end of the collection period. In cases where first-morning samples were not collected, participants were instructed to collect later in the day and mark the sample to indicate that it was not first-morning. On Day 8, a member of the study staff removed the sweat patch and transported both the urine samples and the sweat patch to the Center for Human Toxicology at the University of Utah for analysis. Patches were stored and transported in sterile, BPA-free, four ounce polyproprylene sample cups.

Sweat patches (PharmChek®, Pharmchem, Inc., Fort Worth, TX, USA) were applied according to the manufacturers' recommendations to skin cleaned with alcohol wipes, on either the upper-outer arm or the front or back midriff above the waist but below the ribcage. Patches feature a polyurethane film with an absorption pad and were additionally covered with an adhesive overlay to increase wear-time. PharmChek® patches have been used successfully to monitor drugs of abuse, neuroimmune biomarkers, nicotine and calcium in previous studies (17–19). Sweat patches have also been successfully worn for periods of 7 days in previous studies (20–22).

Statistical analysis

Statistical analysis was performed using the SAS software (version 9.3; SAS Institute, Inc., Cary, NC, USA). Descriptive statistics and distributions of urinary BPA concentrations were tabulated. A total of 46 (12%) urine samples were below the limit of quantification (0.75 ng/mL) and were assigned a value of LOQ/$\sqrt{2}$, or 0.53 ng/mL (23). Because the number of urine samples from each participant varied and within-person concentrations were log-normally distributed, geometric means (GMs) with 95% confidence intervals (CIs) are presented and these include clustering by individual to account for repeated measures.

Urinary BPA analysis

Urine samples were stored at −20°C upon arrival at the laboratory and again after processing. Total BPA (unconjugated BPA plus monoglucuronide conjugate and monosulfate conjugate) was measured in the urine samples using ultra high-performance liquid chromatography–tandem mass spectrometry. Analytical chemistry methods and quality control procedures have previously been described (24). Briefly, the method utilized liquid/liquid extraction with 1-chlorobutane and a human urine aliquot size of 800 μL. Chromatography was performed on an Acquity UPLC® system with a Kinetex® Phenyl-Hexyl column. Mass spectrometric analysis was performed with negative electrospray ionization on a Quattro Premier XE™. The internal standard was d_{16}-bisphenol A. Acceptance criteria for analytical standards and quality controls were ±20% of nominal concentration. Matrix stability in human urine was validated after 24 h at ambient temperature, after three freeze/thaw cycles and after frozen storage at −20°C and −80°C for up to 218 days. Laboratory glassware and consumables, reagent chemicals and biosample collection materials were verified to be BPA-free prior to use to prevent contamination.

Table I
Characteristics of the Study Population

Characteristic	All subjects ($n = 50$) Mean ± SD, or n (%)	Male ($n = 20$) Mean ± SD, or n (%)	Female ($n = 30$) Mean ± SD, or n (%)
Age (years)	32.2 ± 8.2	30.9 ± 6.2	33.0 ± 9.2
BMI (kg/m^2)	25.9 ± 6.5	26.4 ± 5.9	25.7 ± 6.9
Race			
Caucasian	39 (78.0)	15 (75.0)	24 (80.0)
Other/multiracial[a]	10 (20.0)	5 (25.0)	5 (16.7)
Missing	1 (2.0)	0	1 (3.3)
Hispanic			
Yes	5 (10.0)	2 (10.0)	3 (10.0)
No	45 (90.0)	18 (90.0)	27 (90.0)
Annual income[b]			
<$19,000	5 (10.0)	1 (5.0)	4 (13.3)
$20,000–$39,000	14 (28.0)	5 (25.0)	9 (30.0)
$40,000–$74,999	20 (40.0)	8 (40.0)	12 (40.0)
$75,000–$99,000	8 (16.0)	6 (30.0)	2 (6.7)
≥$100,000	1 (2.0)	0	1 (3.3)
Prefer not to answer	2 (4.0)	0	2 (6.7)
Employment			
Employed for wages	33 (66.0)	12 (60.0)	21 (70.0)
Self-employed	3 (6.0)	1 (5.0)	2 (6.7)
Homemaker	3 (6.0)	0	3 (10.0)
Student	9 (18.0)	6 (30.0)	3 (10.0)
Unemployed/other[c]	2 (4.0)	1 (5.0)	1 (3.3)
Education			
High school	3 (6.0)	2 (10.0)	1 (3.3)
Some college (1–3 years)	11 (22.0)	3 (15.0)	8 (26.7)
College (>4 years, graduate)	36 (72.0)	15 (75.0)	21 (70.0)

SD, standard deviation; BMI, body mass index.
[a]Includes Asian, Black/African American, Pacific Islander and American Indian/Alaskan Native.
[b]US Dollars, combined household income.
[c]Includes out of work, retired and unable to work.

Sweat patch analysis

Sweat patches were extracted by folding the individual patches into quarters and placing them into polypropylene test tubes (16 × 100 mm). Methanol was added (6 mL) as the extraction solvent, and the tubes were placed on a reciprocal shaker for 20 min. The methanol extracts were transferred to fresh tubes for solvent evaporation in a Turbovap® at 40°C. The dried

Table II
Distribution of Urinary BPA Concentrations (ng/mL) and Intraclass Correlation Coefficients among 20 Males and 30 Females

Subjects	*n*	*n* > LOQ (%)	Percentiles							GM (95% CI)	ICC (95% CI)
			Min	5th	25th	50th	75th	95th	Maximum		
All	386	340 (88.0)	<LOQ	<LOQ	1.32	2.58	5.72	14.73	75.20	2.74 (2.23, 3.37)	0.21 (0.13, 0.33)
Male	150	142 (94.7)	<LOQ	<LOQ	1.88	3.32	6.93	16.60	75.20	3.59 (2.63, 4.90)	0.20 (0.10, 0.36)
Female	236	198 (83.9)	<LOQ	<LOQ	1.06	2.14	4.50	14.50	38.90	2.31 (1.76, 3.03)	0.19 (0.08, 0.40)

LOQ, limit of quantification; CI, confidence interval; ICC, intraclass correlation coefficient.

extracts were reconstituted with 100 μL of 50 : 50, 5 mM ammonium bicarbonate : acetonitrile (mobile phase) and transferred to conical, polypropylene autosampler vials for analysis. Sweat patch extracts were analyzed using the published UHPLC–MS-MS method for urine samples (24). Patches were analyzed in small batches, which included positive controls (0.5 ng/pad and 1 ng/pad fortified pads) and negative controls (one pad blank without internal standard and two pad blanks fortified with internal standard) in each batch. Pad results were expressed semi-quantitatively as the measured response (peak area of BPA/peak area of d_{16}− BPA) in each sample relative to the average measured response of the two pad blanks with internal standard.

Results

A total of 50 participants were recruited including 30 females and 20 males with a mean age of 32.2 ± 8.2 years (Table I). Participants were primarily Caucasian (39, 78.0%) and non-Hispanic (45, 90.0%). Most participants were college educated (36, 72.0%), with an annual income ranging from $40,000–$74,999 (20, 40.0%). The mean body mass index among all participants was 25.9 ± 6.5 kg/m^2. Sweat patches were retrieved from all 50 participants without any loss to follow-up. The majority of participants chose to wear the patch on the upper-outer arm (34, 68%), followed by the back midriff (11, 22%) and the front midriff (5, 10%). Two patches were removed prior to the end of the 7-day collection period because the adhesive had lost integrity and was no longer properly adhered, but the remainder of patches was intact at the end of the collection period.

The majority (44, 88%) of participants collected all eight possible urine samples, with three (6%) participants collecting only seven, one (2%) collecting only five and two (4%) collecting only four urine samples. A total of 386 urine samples were collected, of which 366 (94.8%) were first-morning samples (Table II). Among male participants, urinary BPA concentrations ranged from <LOQ to 75.20 ng/mL and the GM was 3.59 ng/mL (95% CI 2.63, 4.90). Among female participants, urinary BPA concentrations ranged from <LOQ to 38.90 ng/mL and the GM was 2.31 ng/mL (95% CI 1.76, 3.03). The GM for all samples combined was 2.74 ng/mL (95% CI 2.23, 3.37) and had an Intraclass correlation coefficient (ICC) of 0.21 (95% CI 0.13, 0.33), indicating poor reliability of urine samples (25, 26). ICC is a measure of the reliability of repeated measures over time, defined as the ratio of the between-subject variance and total variance. ICC ranges from 0 to 1, with values near 0 indicating poor reliability and values near 1 indicating high reliability (26).

Extraction and analysis of the participant sweat patches yielded only three (6%) detections of BPA greater than five times (5×) the average background values (5.1×, 9.0× and 9.9×). Each of the three positive patches belonged to female participants, and none of the individuals had high urinary BPA concentrations relative to the overall distribution of urinary BPA among all female participants (Table III). Two of the positive detection patches were worn by the participant on the upper-outer arm and one was worn on the front midriff. BPA was recovered appropriately from the quality control patches and positive and negative controls for each analytical run.

Table III
Urinary BPA Concentrations (ng/mL) and Sweat Patch Results for Positive Patches[a]

	GM and 95% CI	Sweat patch result	Patch location
Participant 1	3.67 (3.06, 4.27)	5.1× background	Upper-outer arm
Participant 2	1.45 (1.20, 1.70)	9.0× background	Front midriff
Participant 3	12.0 (11.5, 12.5)	9.9× background	Upper-outer arm

GM, geometric mean; CI, confidence interval.
[a]Only patches >5× the average background were considered positive detections.

To assess BPA sweat patch detectability after a known BPA exposure, a human volunteer consumed one can of commercially available soup (16 oz, 473 cm^3) daily for 3 days and collected urine 2 h later. Sweat patches (n = 2, 1 per arm) were worn for the 3 days of the study. Although the highest measured urine BPA concentration was 195 ng/mL for this individual, no BPA was detected at 5× above the average background in the corresponding sweat patches.

Discussion

We sought to develop an innovative method for BPA exposure assessment, which would mitigate the temporal variability inherent in urine collection methods due to population-level ubiquitous exposure and the short metabolic half-life of BPA. Collection of sweat is non-invasive, generally available in quantities sufficient for analysis, and has been used within the contrived environment of a sauna to measure BPA (14). We were able to detect BPA in each urine sample, but were able to detect BPA above 5× the average background on only 3 of the 50 sweat patches worn by participants for 7 days. It may be that the volumes of sweat excreted during activities of daily living are insufficient to accumulate detectable quantities of BPA. Sweat patch location may also be a factor. There is evidence that xenobiotics are stored disproportionately in lipid compartments in the human body (27, 28); therefore, excretion of toxicants may also differ depending on location. In our study, two positive detection patches were worn by the participant on the upper-outer arm with the third positive detection patch located on the front midriff. However, the participant with known exposure and high urinary concentrations also wore the patches on the

upper-outer arm, but neither patch detected BPA. Other sites with moderate-to-high sebum secretion, such as axilla and groin, may better capture released BPA and should be studied in the future. However, sites such as axilla and groin are typically covered with significant body hair and are located in jointed areas, which may prevent the polyurethane film of the patch from forming a seal against the epidermis.

Future studies could examine volumes of sweat collected on the patch and BPA among individuals with varying occupations, body compositions, in different climates, and with patches placed on other sites on the human body. A previous study which reported detection of BPA in sweat (14) had induced sweating via confinement in a sauna or vigorous exercise and then used a metal scraper to collect the sweat. In a stress situation, perhaps, a greater proportion of BPA is excreted via sweat than during usual activities of daily living.

The analytic method successfully recovered BPA introduced onto sweat patches in laboratory research and development, but these methods need further study. First, a matrix effect was present on participant sweat patch samples when compared with the sweat patch controls, based on the internal standard responses. Second, analysis was limited by the small volumes of sweat collected by the passive sampling mechanism of the patch. To detect BPA sufficiently above the background, greater volumes of sweat are likely required.

Conclusion

Based on analysis of this pilot study, the use of sweat patches for monitoring exposure to BPA in community-based studies requires further study.

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References

1. Clayton, E.M., Todd, M., Dowd, J.B., Aiello, A.E. (2011) The impact of bisphenol A and triclosan on immune parameters in the US population, NHANES 2003–2006. *Environmental Health Perspectives*, **119**, 390–396.
2. Ehrlich, S., Williams, P.L., Missmer, S.A., Flaws, J.A., Berry, K.F., Calafat, A.M. *et al.*,(2012) Urinary bisphenol A concentrations and implantation failure among women undergoing in vitro fertilization. *Environmental Health Perspectives*, **120**, 978–983.
3. Li, D., Zhou, Z., Qing, D., He, Y., Wu, T., Miao, M. *et al.*, (2010) Occupational exposure to bisphenol-A (BPA) and the risk of self-reported male sexual dysfunction. *Human Reproduction*, **25**, 519–527.
4. Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T. *et al.*, (2010) Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reproductive Toxicology*, **30**, 532–539.
5. Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R. (2011) Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. *Environmental Health Perspectives*, **119**, 252–257.
6. Tharp, A.P., Maffini, M.V., Hunt, P.A., VandeVoort, C.A., Sonnenschein, C., Soto, A.M. (2012) Bisphenol A alters the development of the rhesus monkey mammary gland. *Proceedings of the National Academy of Sciences United States of America*, **109**, 8190–8195.
7. Volkel, W., Colnot, T., Csanady, G.A., Filser, J.G., Dekant, W. (2002) Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. *Chemical Research in Toxicology*, **15**, 1281–1287.
8. Mahalingaiah, S., Meeker, J.D., Pearson, K.R., Calafat, A.M., Ye, X., Petrozza, J. *et al.* (2008) Temporal variability and predictors of urinary bisphenol A concentrations in men and women. *Environmental Health Perspectives*, **116**, 173–178.
9. Ye, X., Wong, L.Y., Bishop, A.M., Calafat, A.M. (2011) Variability of urinary concentrations of bisphenol A in spot samples, first morning voids, and 24-hour collections. *Environmental Health Perspectives*, **119**, 983–988.
10. Christensen, K.L., Lorber, M., Koch, H.M., Kolossa-Gehring, M., Morgan, M.K. (2012) Population variability of phthalate metabolites and bisphenol A concentrations in spot urine samples versus 24- or 48-h collections. *Journal of Exposure Science and Environmental Epidemiology*, **22**, 632–640.
11. Lassen, T.H., Frederiksen, H., Jensen, T.K., Petersen, J.H., Main, K.M., Skakkebaek, N.E. *et al.*, (2013) Temporal variability in urinary excretion of bisphenol A and seven other phenols in spot, morning, and 24-h urine samples. *Environmental Research*, **126**, 164–170.
12. Volkel, W., Kiranoglu, M., Fromme, H. (2008) Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. *Toxicology Letters*, **179**, 155–162.
13. Carwile, J.L., Ye, X., Zhou, X., Calafat, A.M., Michels, K.B. (2011) Canned soup consumption and urinary bisphenol A: a randomized crossover trial. *JAMA*, **306**, 2218–2220.
14. Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A. (2012) Human excretion of bisphenol A: blood, urine, and sweat (BUS) study. *Journal of Environmental and Public Health*, **2012**, 185731.
15. Genuis, S.J., Beesoon, S., Lobo, R.A., Birkholz, D. (2012) Human elimination of phthalate compounds: blood, urine, and sweat (BUS) study. *ScientificWorldJournal*, **2012**, 615068.
16. Csanady, G.A., Oberste-Frielinghaus, H.R., Semder, B., Baur, C., Schneider, K.T., Filser, J.G. (2002) Distribution and unspecific protein binding of the xenoestrogens bisphenol A and daidzein. *Archives of Toxicology*, **76**, 299–305.
17. Kintz, P., Henrich, A., Cirimele, V., Ludes, B. (1998) Nicotine monitoring in sweat with a sweat patch. *Journal of Chromatography B: Biomedical Sciences and Applications*, **705**, 357–361.
18. Rianon, N., Feeback, D., Wood, R., Driscoll, T., Shackelford, L., LeBlanc, A. (2003) Monitoring sweat calcium using skin patches. *Calcified Tissue International*, **72**, 694–697.
19. Concheiro, M., Shakleya, D.M., Huestis, M.A. (2011) Simultaneous analysis of buprenorphine, methadone, cocaine, opiates and nicotine metabolites in sweat by liquid chromatography tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, **400**, 69–78.
20. de la Torre, R., Pichini, S. (2004) Usefulness of sweat testing for the detection of cannabis smoke. *Clinical Chemistry*, **50**, 1961–1962.
21. Chawarski, M.C., Fiellin, D.A., O'Connor, P.G., Bernard, M., Schottenfeld, R.S. (2007) Utility of sweat patch testing for drug use monitoring in outpatient treatment for opiate dependence. *Journal of Substance Abuse Treatment*, **33**, 411–415.
22. Brunet, B.R., Barnes, A.J., Choo, R.E., Mura, P., Jones, H.E., Huestis, M.A. (2010) Monitoring pregnant women's illicit opiate and cocaine use with sweat testing. *Therapeutic Drug Monitoring*, **32**, 40–49.
23. Hornung, R.W., Reed, L.D. (1990) Estimation of average concentration in the presence of nondetectable values. *Applied Occupational and Environmental Hygiene*, **5**, 46–51.
24. Anderson, D.J., Brozek, E.M., Cox, K.J., Porucznik, C.A., Wilkins, D.G. (2014) Biomonitoring method for bisphenol A in human urine by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B Analytical Technologies in Biomedical Life Sciences*, **953–954**, 53–61.

25. Hankinson, S.E., Manson, J.E., Spiegelman, D., Willett, W.C., Longcope, C., Speizer, F.E. (1995) Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. *Cancer Epidemiology, Biomarkers and Prevention*, **4**, 649–654.
26. Rosner, B.A. (2010) *Fundamentals of Biostatistics*. Brooks/Cole: Boston, MA.
27. Schnare, D.W., Denk, G., Shields, M., Brunton, S. (1982) Evaluation of a detoxification regimen for fat stored xenobiotics. *Medical Hypotheses*, **9**, 265–282.
28. Yu, G.W., Laseter, J., Mylander, C. (2011) Persistent organic pollutants in serum and several different fat compartments in humans. *Journal of Environmental and Public Health*, **2011**, 417980.