Comparison of Bisphenol A and Bisphenol S Percutaneous Absorption and Biotransformation

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BACKGROUND: Bisphenol S (BPS) has been widely substituted for bisphenol A (BPA) on thermal papers, but little is known about its skin absorption.

OBJECTIVES: We compared the percutaneous absorption and biotransformation of BPS and BPA in vitro and in a controlled human trial.

METHODS: Absorption and biotransformation of BPS and BPA were monitored across reconstructed human epidermis at two environmentally relevant doses over 25 h. In the human trial, five male participants handled thermal receipts containing BPS and washed their hands after 2 h. Urine (0–48 h) and serum (0–7.5 h) were analyzed for target bisphenols, and one participant repeated the experiment with extended monitoring. BPS data were compared with published data for isotope-labeled BPA (BPA-d10) in the same participants.

RESULTS: At doses of 1.5 and 7.7 μg/cm² applied to reconstructed human epidermis, the permeability coefficient of BPS (0.009 and 0.003 cm/h, respectively) was significantly lower than for BPA (0.036 and 0.033 cm/h, respectively), and metabolism of both bisphenols was negligible. In participants handling thermal receipts, the quantities of BPS and BPA-d10 on hands was significantly correlated with maximum urinary event flux (µg), but the slope was lower for BPS than for BPA (β = 0.12 and 1.1, respectively). As a proportion of total urinary bisphenol, free BPS [mean ± standard deviation (SD): 6.9 ± 2.8%] was higher than for free BPA (2.7 ± 1.9%). Postexposure maximum urinary BPS concentrations (0.93 to 3.0 ng/mL; n = 5) were in the 93–98th percentile range of BPS in background Canadians (0.91–3.2 ng/mL; n = 467).

CONCLUSION: Both the in vitro and human studies suggested lower percutaneous absorption of BPS compared with BPA, but a lower biotransformation efficiency of BPS should also be considered in its evaluation as a BPA substitute. https://doi.org/10.1289/EHP5044

Introduction

Bisphenol A (BPA) is a weak estrogen that has been widely used for polycarbonate plastic manufacturing, for epoxy resin linings of food and beverage containers (Ballin et al. 2008), and as a developer in thermal paper, such as for retail shopping receipts and airport luggage tags (Liao and Kannan 2011). According to national surveys in Canada (2012–2013), ~90% of the general population have detectable urinary BPA (>0.2 ng BPA/mL) (Health Canada 2015). An exposure assessment by the European Food Safety Authority (EFSA 2015) estimated that dietary exposure contributed approximately 90% of total human BPA exposure (von Goetz et al. 2017) and, based on in vitro absorption studies, that dermal exposure to thermal papers contributed 10% for some age groups (von Goetz et al. 2017). However, a recent study showed an unexpectedly long elimination half-life for BPA after a single dermal exposure to simulated thermal receipt papers, and the resulting intake approached the average daily dietary intake of the general Canadian population (Health Canada 2012; Liu and Martin 2017). These data suggest that dermal intake of BPA may be higher than previously estimated.

Due to the health risks of BPA, alternative bisphenols, including bisphenol S (BPS), have been widely substituted as alternatives (Björnsmo dotter et al. 2017; Liao et al. 2012b), and BPS is now widely used as an alternative to BPA in paper products (Björnsmo dotter et al. 2017; Liao et al. 2012b). High concentrations of BPS were detected in paper products, including in thermal receipts and paper currencies (Liao et al. 2012b). We randomly collected 20 thermal receipts from local stores in Edmonton, Canada, and found that BPS was the main bisphenol in 9 of 20 receipts (Liu et al. 2018). A study in the United States similarly found high levels of BPS or BPA in 52% and 44% of thermal receipts, respectively, from 50 different vendors in the city of Columbia, Missouri, and in Southern Missouri (Hormann et al. 2014). Occupational exposure of cashiers to BPS from handling thermal receipts has been suggested by two studies. In one, higher urinary BPS was detected in a group of 32 cashiers after their work shift, compared with preshift urinary samples (Thayer et al. 2016). In another, significantly higher urinary BPS was detected among 17 cashiers relative to 15 controls (Ndaw et al. 2018).

Compared with BPA, BPS has a similar molecular size and structure and seems to offer no advantages with respect to endocrine-disrupting activities (Chen et al. 2002; Kitamura et al. 2005; Rochester and Bolden 2015), aquatic toxicity (U.S. EPA 2014), persistence (U.S. EPA 2014; Ike et al. 2006), or bioaccumulation potential (U.S. EPA 2014). To our knowledge, the percutaneous absorption of BPS has not been studied in vitro, and no study has compared the percutaneous absorption of BPA and BPS. Nevertheless, BPS was detectable in 59% of urine samples (>0.10 ng/mL) from Canadian pregnant women (Liu et al. 2018) and in 81% of urine samples (>0.02 ng/mL) from the United States and seven Asian countries (Liao et al. 2012a). In part due to higher dietary intake of BPA than BPS (Liao and Kannan 2013, 2014), average urinary concentrations of BPS were 3–12 times lower than for BPA among American adults between 2009 and 2013 (Ye et al. 2015), and 8 times lower than for BPA among pregnant Canadian women between 2010 and 2012 (Liu et al. 2018). Nevertheless, an increasing trend of urinary BPS concentrations among American adults between 2000 and 2014 indicated recent increasing exposure to BPS (Ye et al. 2015).

Here we compared the percutaneous absorption of BPA and BPS using an in vitro skin model derived from human keratinocytes. We also evaluated the dermal exposure of BPS from native thermal receipts in four volunteer participants and compared this to an identical trial, with the same participants, where isotope-labeled BPA (BPA-d10) was handled on simulated thermal receipt papers (Liu and Martin 2017). Concentrations of free and

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total bisphenols were analyzed in samples to evaluate their comparative dermal absorption and metabolism.

Materials and Methods

Chemicals and Reagents

BPA-(diphenyl-13C12) (BPA-13C12, 98%; 100 μg/mL acetonitrile) and BPS-(diphenyl-13C12) (BPS-13C12, 98%; 100 μg/mL methanol) were from Cambridge Isotope Laboratories. BPS (98%, solid) and Lucifer Yellow dilithium salt were from Sigma-Aldrich. β-glucuronidase and sulfatase from Helix pomatia (≥100,000 units/mL β-glucuronidase; ≤7,500 units/mL sulfatase) were from Sigma-Aldrich. A lactate dehydrogenase (LDH) cytotoxicity kit was from TaKaRa. TRITON® X-100, PROTEIN GRADE® Detergent (10% solution, sterile filtered) was from Sigma-Aldrich. Liquid chromatography (LC)–grade water, methanol, and acetonitrile were from Fisher Scientific. LC-grade ammonium acetate and analytical grade formic acid (98%) were from Sigma-Aldrich.

In Vitro Skin Model

The EpiDerm™ EPI-212-X kit and permeation device (Figure S1) were from MatTek Corporation. According to the manufacturer, the EpiDerm™ EPI-212 tissue constructs are a three-dimensional tissue model consisting of normal human epidermal keratinocytes on tissue culture inserts (https://www.mattek.com/products/epiderm/). The tissue structure and cellular morphology consists of organized and proliferative basal cells, spindles and granular layers, and cornified epidermal layers that are mitotically and metabolically active. According to instructions from the company, the thickness of the EpiDerm™ tissue is approximately 120 μm, and 20–30% of the total thickness is stratum corneum. Although substances such as caffeine and testosterone have been shown to permeate the EpiDerm™ skin model better than pig skin, permeation rates between the EpiDerm™ and porcine models were correlated (Schäfer-Korting et al. 2008), suggesting that this model has relevance for contrasting the percutaneous penetration of two bisphenols. The kit includes 12 EpiDerm™ skin model samples, maintenance medium, and phosphate-buffered saline (pH 7.4). The EpiDerm™ EPI-212-X kit was delivered cold within 24 h of production, and the percutaneous absorption tests were completed within 36 h after receiving the kit.

In Vitro Permeability Experiments

To avoid interference from potential contamination of native BPA and BPS, pure stable isotope–labeled BPA (BPA-13C12) and BPS (BPS-13C12) were used for percutaneous absorption studies in vitro. A mixture of BPA-13C12 and BPS-13C12 was prepared in LC-grade water as donor solution (Figure S1) at 1 μg/mL (low dose) and 5 μg/mL (high dose). As the chemical concentration at the skin–donor interface could affect the permeation rate, we used the same weight concentration for BPA-13C12 and BPS-13C12 in the same donor solvent, although molar concentrations were slightly different due to the different molecular weights (i.e., 240 g/mol for BPA-13C12; 262 g/mol for BPS-13C12). The selected concentrations were much lower than water solubility limits for BPA (i.e., 120 μg BPA/mL at 20°C)(Robinson et al. 2009) or BPS (380 μg BPS/mL at 25°C, pH 7) [from SciFinder, calculated using Advanced Chemistry Development Software (version 11.02; ACD/Labs)]. The applied low exposure level (6.3 nmol/cm² for BPA-13C12 and 5.7 nmol/cm² for BPS-13C12) can be compared with the average dose of BPA (5.0 nmol to a single finger) from handling of thermal paper (Biedermann et al. 2010).

The experimental procedure is shown in Figure S2. After receiving the kit, the 12 skin tissues were immediately incubated in 6-well plates containing 1 mL of maintenance medium. All incubation conditions were 5 ± 1% carbon dioxide at 37 ± 1°C. After 1 h of incubation, the tissues were clamped into permeation devices and put into 6-well plates containing 5 mL phosphate-buffered saline in each well (Figure S1). Then 0.4 mL of donor solution was added on the 0.26 cm² skin surface, and the dermis side was kept in contact with phosphate-buffered saline as receiver solution (Figure S1). The integrity of the skin tissue and permeation chamber were tested by dye diffusion in negative controls (see “Analysis of Lucifer Yellow in Negative Controls”). We also tested the viability of skin cells during the experiment using the LDH cytotoxicity detection kit (see “Skin Cell Viability”).

The entire receiver solution was collected in 9-mL cryovials at each time point after adding the donor solution (Figure S2), and the permeation device with tissue was placed into another well with fresh receiving buffer. After recording the volumes, collected receiver solutions were stored at 4°C until analysis. At the end of the experiment, the donor solution was collected, and the surface of the skin tissue was rinsed using 3 × 0.4 mL water. The collected donor solution and rinses were combined (approximately 1.6 mL total) for evaluating unabsorbed test compound. The tissue was removed from the permeation device and extracted with 3 × 2 mL methanol, each time with 15 min of ultrasonication to extract target analytes.

Analysis of Lucifer Yellow in Negative Controls

Diffusion of Lucifer yellow in the negative controls was used to evaluate the integrity of the skin tissue and permeation chamber. Lucifer yellow was tested at excitation/emission wavelengths of 485/535 nm using a multimode microplate reader (FilterMax F5) from Molecular Devices. The calibration curves of Lucifer yellow were prepared in PBS and water from 0.5 μM to 50 μM (six points, including 0 μM), and the regression coefficients of the curves were >0.999.

Skin Cell Viability

The LDH cytotoxicity detection kit was used to measure the viability of skin cells during the experiment based on the measurement of LDH activity in each receiver solution. The increase in LDH activity is directly proportional to the number of damaged cells; thus, higher LDH activity in receiver solution can be correlated with lower skin cell viability. LDH activity was measured in every receiver solution collected over five time points for low and high doses of bisphenols, and was measured in receiver solutions collected at 1 h from three vehicle controls and in receiver solution collected at 25 h from one vehicle control containing 1% TRITON® X-100. An aliquot (100 μL) of receiver solution (three replicates for each time point, thus 15 samples for each treatment) was combined with 100 μL of reaction mixture (mixture of catalyst and dye solution from the kit) in 96-well plates. After incubation at room temperature (protected from light) for 30 min, the absorbance of the samples was measured at 450 nm, with the reference wavelength at 620 nm, using a multimode microplate reader (FilterMax F5) from Molecular Devices. According to the instructions of LDH cytotoxicity detection kit, the viability of the EpiDerm™ cells was evaluated by the cytotoxicity (%) calculated by the following equation:

\[ \text{Cytotoxicity} = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{low control}}}{\text{Abs}_{\text{high control}} - \text{Abs}_{\text{low control}}} \times 100 \]

where \( \text{Abs}_{\text{sample}} \) is the absorbance value of the receiver solution from bisphenol exposed samples. The absorbance of receiver
Dermal Exposure to BPA-d16 and Bisphenol S in Humans

This human study was reviewed and approved by the Health Research Ethics Board of the University of Alberta, and all participants provided informed consent before participating. Experimental details were previously described for study of the dermal absorption of BPA-d16 from simulated thermal receipt papers (Liu and Martin 2017), and here we describe related methods in the same participants to study the dermal absorption of native BPS from authentic receipt papers collected during purchases in local grocery stores. In the 24-h period prior to arriving at the clinic, and also during the monitoring period, participants were asked to avoid contact with thermal receipt papers and not to consume alcoholic beverages, medications, or canned food.

Pre-exposure urine and serum samples were collected from each participant (n = 6), as well as hand wipe samples collected onto moistened Kimwipes. Each participant was first asked to handle a simulated receipt containing isotope-labeled BPA (25 mg BPA-d16/g paper, equivalent to 102 μmol BPA-d16/g paper) for 5 min, and then to immediately handle a native receipt paper which contained, on average, 21 mg BPS/g paper (equivalent to 84 μmol BPS/g paper or 0.48 μmol BPS/cm², range: 68–140 μmol BPS/g paper, or 0.37–0.80 μmol BPS/cm²) with the same hand for another 5 min. The start time of receipt handling was defined as time 0 for purposes of data analysis. To prevent unintended oral exposure, the participants then put a single nitrile glove on the exposed hand for 2 h, during which time they read or worked casually in an office. After removing the nitrile glove, the exposed hand was carefully wiped with a water-moistened Kimwipe, and participants were asked to wash their hands with soap and water.

Three postexposure serum samples were collected from each participant at 2.5, 5, and 7.5 h following the dermal exposure. All urine samples after the dermal exposure were collected for up to 48 h. Due to low levels of bisphenols on postexposure hand wipes and nondetectable BPA-d16 in urine from one participant (P6) (Liu and Martin 2017), their data was excluded from analysis.

One month after the above dermal exposure study, one participant agreed to return for a repetition of the exposure study exactly as above, except with a longer sampling period for urine (9 d) and serum (at 22 h and 51 h postexposure).

Sample Analysis

Free and total BPS, BPA-13C12, and BPS-13C12 in urine, serum, receiver solution, and the skin tissue were detected by online solid phase extraction coupled to LC and an Orbitrap Elite™ hybrid mass spectrometer (Thermo Fisher Scientific). The analytical method was described previously. To semi-quantitatively evaluate transfer of BPS to the hands from thermal receipts, the pre- and postexposure hand wipes were extracted with 2 × 20 mL methanol and sonicated for 15 min each time.

BPA-13C12 was used as the internal standard for quantification of BPS in the human study, while samples from the BPA-13C12 and BPS-13C12 in vitro permeability experiments were quantified using six-point external standard calibration curves constructed from these isotope-labeled standards. Recovery of the target bisphenols in urine, serum, and receiver solution was in the range of 92% to 116%, with relative standard deviation (SD) between 4% and 14% (Table S1). The regression coefficients of the six-point standard curves of target analytes were always >0.99. BPS and isotope-labeled bisphenols were not detectable in any sample containers, blank Kimwipes, or in procedural blanks (LC-grade water) extracted and analyzed with every batch of up to 20 samples.

Limits of detection (LODs) were defined by an instrumental signal-to-noise ratio of 3 (Table S1).

Statistical Analysis

Statistical analysis was performed with Stata software (version 12.0; Stata), and the level of significance for all of statistical tests was p < 0.05. For the in vitro study, the absorption parameters of BPA-13C12 and BPS-13C12 were compared using the paired t-test. For the human trial, nondetect results were substituted by one-half the LOD for data analysis, and all nondetects were censored using black circles in associated figures. The sampling times of maximum excreted total BPA-d16 and BPS (Tmax) following dermal exposure were compared using the paired t-test. The paired t-test was also used to compare the maximum urinary event flux, divided by mass of test substance on hands, between total BPA-d16 and BPS. Spearman correlation coefficients and linear regression model were used to evaluate the association between mass of bisphenols on postexposure hand wipes and their maximum urinary event flux.

Results

Percutaneous Absorption of BPS-13C12 and BPA-13C12 in Vitro

The in vitro experiments with isotope-labeled BPS and BPA showed excellent mass balance, with 96–107% of the applied dose recovered after 25 h in the skin tissue or donor/receiving solutions (Table 1). In negative controls, 95 ± 5.6% (mean ± SD) of dosed Lucifer yellow was recovered in the dosing solution at the end of the experiment, with 1.7 ± 0.04% present in skin

| Table 1. Comparison of percutaneous absorption flux (F, cm²/h) and permeability coefficient (Kp, cm/h) of BPA-13C12 and BPS-13C12 in donor solution, skin tissue extracts, and receiver solutions 25 h after dosing in EpiDerm human skin models. | Low dose (1.5 μg/cm²) | High dose (7.7 μg/cm²) |
|---|---|---|
| BPA-13C12 (6.3 nmol/cm²) | BPS-13C12 (5.7 nmol/cm²) | BPA-13C12 (32 nmol/cm²) | BPS-13C12 (29 nmol/cm²) |
| Recovery of bisphenols in donor solution | 14 ± 2.0% | 16 ± 1.0% | 13 ± 0.7% | 17 ± 0.4% |
| Recovery of bisphenols in skin tissue | 14 ± 2.0% | 16 ± 1.0% | 13 ± 0.7% | 17 ± 0.4% |
| Cumulative recovery in receiver solution (25 h) | 43 ± 7.0% | 8.0 ± 8.0% | 46 ± 1.4% | 6.4 ± 3.3% |
| Sum of recovery (mass balance) | 96 ± 11% | 98 ± 16% | 97 ± 0.7% | 107 ± 3.7% |
| Fmax (nmol/cm²/h) | 0.15 ± 0.044 | 0.036 ± 0.056 | 0.068 ± 0.055 | 0.064 ± 0.047 |
| F11-25h (nmol/cm²/h) | 0.089 ± 0.007 | 0.013 ± 0.004 | 0.052 ± 0.012 | 0.065 ± 0.020 |
| Kp (cm²/h) | 0.036 ± 0.011 | 0.009 ± 0.015 | 0.033 ± 0.003 | 0.003 ± 0.002 |

Note: Results are expressed as mean ± standard deviation (n = 3). Using paired t-tests, the Kp of BPA-13C12 was significantly higher than for BPS-13C12 at both doses (p < 0.01). The simple linear model was used for calculating Fmax, and F11-25h, using the following equation: y = F × T + intercept; y is cumulative absorbed bisphenols in receiver solution divided by surface area of skin tissue (unit: nmol/cm²), and T is the sampling time of receiver solution. Time is from 0 to 7 h for calculating Fmax and is in the range of 11 to 25 h for F11-25h. Kp was calculated from Fmax divided by the concentration (unit: nmol/cm²) of bisphenols in donor solution.
tissue. Thus, skin tissue integrity was acceptable, and the permeation chamber functioned appropriately. Moreover, cytotoxicity throughout exposure was <15% (Table S2), suggesting that most skin cells were viable throughout the exposures.

There was little difference in total BPA-\textsuperscript{13}C\textsubscript{12} or BPS-\textsuperscript{13}C\textsubscript{12} recovered in the skin tissue (14–17%) after 25 h, but strong and consistent differences in the amount of test substance accumulating in receiver solution. At both doses, <10% of total BPS had migrated into the receiver solutions, whereas 43–46% of total BPA was recovered in receiver solutions (Table 1). The time trends throughout the experiment show the higher rate of percutaneous absorption of BPA compared with BPS at both doses (Figure 1). The slope of all absorption curves declined throughout the experiment; thus, the maximal absorption flux ($F_{\text{max}}$; nmol/cm\textsuperscript{2}/h) was calculated from the slope of the absorption curve between 0 and 7 h using simple linear regression. At the low dose, mean $F_{\text{max}}$ of total BPA was 0.15 nmol/cm\textsuperscript{2}/h, significantly ($p < 0.01$) higher than for BPS, 0.036 nmol/cm\textsuperscript{2}/h. At the higher dose, the difference between BPA and BPS was also significant ($p < 0.01$) and even greater, with $F_{\text{max}}$ of total BPA (0.68 nmol/cm\textsuperscript{2}/h) approximately 11-fold higher than for total BPS (0.064 nmol/cm\textsuperscript{2}/h). The absorption flux of total BPA from 11–25 h ($F_{11-25\text{h}}$) was calculated from the slope of the absorption curve between 11 and 25 h using simple linear regression, and was also significantly higher than for BPS (Table 1).

Permeability coefficients ($K_p$) were calculated from $F_{\text{max}}$ divided by applied dosage. As shown in Table 1, the $K_p$ of BPA calculated at low and high doses were similar. The average $K_p$ of BPS at the higher dose (0.003 cm/h) was threefold lower than at the lower dose (0.009 cm/h), but not significantly different ($p = 0.59$). The average $K_p$ of BPA was fourfold and 11-fold higher for BPS at low and high doses, respectively, and the differences were statistically significant ($p < 0.01$).

The extent of test substance biotransformation was evaluated by comparing free and total bisphenol concentrations in skin tissue as well as in receiver solutions. At 25 h, free BPS was significantly lower than total BPS in the skin tissue following both low and high dosage, with approximately 20% of BPS present as conjugated

Figure 1. Cumulative penetration of isotope-labeled bisphenol A (BPA-\textsuperscript{13}C\textsubscript{12}) and bisphenol S (BPS-\textsuperscript{13}C\textsubscript{12}) over time in the percutaneous in vitro absorption tests at two doses. Low exposure levels of BPA-\textsuperscript{13}C\textsubscript{12} and BPS-\textsuperscript{13}C\textsubscript{12} were 6.3 nmol/cm\textsuperscript{2} and 5.7 nmol/cm\textsuperscript{2}, respectively. High exposure levels were 32 nmol/cm\textsuperscript{2} for BPA-\textsuperscript{13}C\textsubscript{12} and 29 nmol/cm\textsuperscript{2} for BPS-\textsuperscript{13}C\textsubscript{12}. Data are expressed as mean ± standard deviation.
metabolite (Figure 2). In contrast, no significant difference was observed between free and total BPA in the skin tissue, thereby indicating no significant proportions of conjugated metabolites. There was also little evidence for any BPA or BPS metabolites crossing into the receiver solutions; an exception was for BPA at 3 h in the high-dose treatment, whereby free BPA was significantly lower (71%) than total BPA (Figure 2).

**Figure 2.** Mean concentrations (± standard deviation; n = 3) of free and total isotope-labeled bisphenol S (BPS-\(^{13}\)C\(_{12}\)) (A: low-level exposure; C: high-level exposure) and bisphenol A (BPA-\(^{13}\)C\(_{12}\)) (B: low-level exposure; D: high-level exposure) in receiver solutions and skin tissue after dosing of bisphenols in the EpiDerm™ human skin model. *p < 0.05 for comparison of free bisphenol and total bisphenol by the paired t-test.

Transfer from Receipt Papers to Hands
Low amounts of native BPS (0.006–0.049 µg) were detected in pre-exposure hand wipes of all participants (Table S3), whereas BPA-\(^{13}\)C\(_{12}\) was not detected (Liu and Martin 2017). After handling the receipt papers, the average mass of total BPS in postexposure hand wipes was 3.9 µg (range: 0.71–10 µg) (Table S3), which is five times higher than the mean mass of BPA-\(^{13}\)C\(_{12}\) in the same hand wipes (0.77 µg, range: 0.07–3.0 µg) (Liu and Martin 2017).

Urinary Excretion
Concentrations of total BPS in preexposure urine samples were generally low, ranging from <LOD–0.23 ng/mL in P1, P2, P4, and P5 (Table S4), but much higher in one individual (P3, 8.8 ng/mL). Thus, interference from background native BPS exposure complicated interpretation of urinary BPS in the experiment; unlike for BPA-\(^{13}\)C\(_{12}\), which was not detected in pre-exposure urine samples (Liu and Martin 2017). Nevertheless,
Figure 3. Associations between mass of deuterated bisphenol A (BPA-$d_{16}$) and bisphenol S (BPS) on postexposure hand wipes (2 h after handling the receipts) and their maximum urinary event flux over 48 h after handling of receipts. Data are plotted by participant: P1 (●), P2 (●), P3 (▲), P4 (○) and P5 (×).

urinary total BPS concentrations followed a similar temporal pattern as total BPA-$d_{16}$ (Figure S3). Furthermore, the sampling time of maximum excreted BPS ($T_{max}$) was the same as the $T_{max}$ for BPA-$d_{16}$ among most participants, except P4, and there was no significant difference between mean $T_{max}$ of BPA-$d_{16}$ and BPS (Table S4).

As shown in Figure 3, maximum urinary event flux (μg) of BPA-$d_{16}$ within 48 h following the dermal exposure (i.e., peak values in Figure S3) was significantly correlated with the mass of BPA-$d_{16}$ (μg) in postexposure hand wipes of the five participants ($p < 0.01$; Spearman’s ρ = 0.97), and the intercept of the linear model was close to zero. For BPS, the correlation was also significant ($p < 0.05$; Spearman’s ρ = 0.90), but the intercept of its linear model was >zero (Figure 3). The maximum urinary event flux (μg) of BPA-$d_{16}$ (within 48 h), divided by the mass of BPA-$d_{16}$ in postexposure hand wipes, was significantly higher than for BPS among five participants ($p < 0.01$); despite the small number of participants, the power of this test was above 0.95, based on 5% type I error.

Linear regression models (Figure 3) indicated that an increase of 1 μg BPA on the skin led to a mean increase of 1.1 μg BPS in maximum urinary event flux within 48 h, whereas 1 μg BPS on the skin only led to a mean increase of 0.12 μg BPS in maximum urinary event flux within 48 h (Figure 3). Figure S4 also demonstrates lower relative urinary BPS, compared with BPA-$d_{16}$, after normalizing to mass of test substance on hands for each individual.

In the follow-up dermal exposure study with one returning participant, the mass of BPS in the postexposure hand wipe was three times higher than BPA-$d_{16}$, but excreted total BPS was still lower than total BPA-$d_{16}$ in most urine samples, with the exception of at 69 h, when total BPS increased to 3.4 μg (17 ng/mL) (Figure S3, P1-II). Nevertheless, even for this highest excreted BPS event, when normalized to mass of BPS in the postexposure hand wipe, the excreted BPS was still lower than BPA-$d_{16}$ at the same time (Figure S4, P1-II).

Over 2 d after handling the receipt papers, total BPS was detectable in 78% of postexposure urine samples (n = 60) from the five participants, of which 13% of samples had detectable free BPS. Among the postexposure urine samples with detectable free BPS, the average proportion of free BPS was 6.0 ± 2.7% (SD) (n = 6). In the follow-up dermal exposure study in one participant, the average proportion of free BPS was 7.4 ± 2.7% (n = 9). Altogether, the proportion of free BPS was, on average, 6.9 ± 2.8% in all postexposure urine samples with detectable free BPS. Moreover, urine samples with higher total BPS also had higher free BPS (Figure S5). Compared with BPA-$d_{16}$ studied previously in the same participants (Liu and Martin 2017), urinary free BPS as a proportion of total BPS was higher (Figure 4), suggestive of less efficient biotransformation for BPS.

**BisphenolS in Serum**

BPS was not detectable (i.e., <0.10 ng/mL) in serum collected over 7.5 h following the dermal exposure, consistent with the results for BPA-$d_{16}$ in the same participants (Liu and Martin 2017). In the follow-up dermal exposure study with one participant, no total BPS and no free BPS were detectable in serum collected at 22 or 51 h. As only two serum samples were collected, we may have missed the true peak concentration of BPS in serum. In the same serum samples, low total and free BPA-$d_{16}$ were detected (Liu and Martin 2017). However, the concentrations of total and free BPA-$d_{16}$ in serum (i.e., 0.018–0.030 ng/mL) were lower than current LOD for BPS in serum (i.e., 0.10 ng/mL).

**Discussion**

As an alternative to BPA, BPS has been widely substituted and is detectable in various paper products (Björnsdotter et al. 2017; Liao et al. 2012b). It was the main bisphenol in approximately half of thermal receipts sampled in the United States (n = 50) (Hormann et al. 2014), Italy (n = 50) (Russo et al. 2017), and Canada (n = 20) (Liu et al. 2018). Here, evidence was that the percutaneous absorption of BPS was consistently lower than BPA, both in vitro and in vivo. The $K_{ow}$ of BPS was, respectively, fourfold and 11-fold higher than that of BPS at low and high doses in vitro, which may partly be explained by differences in their physical properties. For example, the predicted log octanol–water partition coefficient (Log $K_{ow}$) of BPS (1.65) (http://www.chemspider.com/Chemical-Structure.6371.html?rid=7887c503-018) is lower than BPA (3.64) (http://www.chemspider.com/Chemical-Structure.6374.html?rid=8d98f412-b2d1-476d-92ed-3df564290c0a).
d2e4-4bb3-a058-f9e4644c8615), based on predictions from ChemSpider using the U.S. Environmental Protection Agency’s EPISuite™ software, thereby indicating that BPS is much less lipophilic. The barrier functions of the epidermis are mainly localized to the stratum corneum (Elias 2007), which consist of corneocytes embedded in lipid matrix (Mitra et al. 2015). Thus, the lower predicted lipophilicity of BPS compared with BPA may explain its lower percutaneous absorption.

We further examined the biotransformation of the two bisphenols in the EpiDerm™ skin model. In this model, the activities of phase II enzymes, including UDP-glucuronosyltransferase, have been shown to be comparable with activities in human skin (Götz et al. 2012). More than 70% of total BPS and total BPA were unconjugated in the skin tissue and in receiver solutions (Figure 2), suggesting limited biotransformation for both bisphenols in skin. The metabolism of BPA in the skin has been evaluated in previous in vitro studies. One study using human skin samples concluded that absorbed BPA was not biotransformed (i.e., <3%) (Marquet et al. 2011), while another study with viable human skin showed 27% conjugation to glucuronide and sulfate (Zalko et al. 2011). Although there are inconsistencies in these two study results, both of these experiments demonstrated a low metabolism efficiency of BPA in human skin. No previous in vivo or in vitro study that we are aware of has evaluated the biotransformation efficiency of BPS in skin. As glucuronidation and sulfation are the major metabolic processes for both BPA and BPS in humans following oral exposure (Oh et al. 2018; Thayer et al. 2015), the low biotransformation efficiency of BPA in skin may also suggest a low dermal biotransformation efficiency for BPS.

In the dermal exposure study with five participants, receipt papers used in the exposures contained similar concentrations of BPA and BPS (≈ 90 µmol/g paper), but the mass of BPS in postexposure hand wipes was, on average, five times higher than BPA-d16. This data should not necessarily be taken to mean that more BPS than BPA will transfer to skin from thermal receipts in the real world. This is because the BPA-d16 was spiked to the papers in solvent and allowed to penetrate the paper and dry, whereas it is believed that bisphenols in native receipts are present on the surface of the paper as a developer (U.S. EPA 2014). Nevertheless, the current hand wipe data are useful estimates of the applied dermal dose and were used to normalize urinary data for each participant.

After participants handled thermal receipts containing BPS collected from local grocery stores, maximum urinary event flux of BPS within 48 h was significantly correlated with the mass of BPS recovered on hand wipes after 2 h. Although the background exposure to BPS before and during the study period was uncontrolled and led to a positive intercept in the linear model between maximum urinary event flux and mass of BPS on hand wipes, the significant positive correlation suggested that the thermal receipts were the major source of the excreted BPS. Moreover, maximum urinary concentrations of BPS ranged from 0.93 to 3.0 ng/mL (n = 5), which approach or exceed the 95th percentile of urinary BPS in American adults (1.0–1.8 ng/mL; 2009–2014; n = 42–141) (Ye et al. 2015) and are in the range of the 93–98th percentiles of urinary BPS in pregnant Canadian women (0.91–3.2 ng/mL; 2010–2012; n = 467) (Liu et al. 2018). Thus, it is possible that thermal receipts are a major exposure source of BPS for a subpopulation who may handle the thermal receipts multiple times per day, but...
the small sample size here limits extrapolation. We previously showed that canned food consumption was significantly correlated with urinary BPA in pregnant women, whereas neither canned food nor meat consumption were correlated with urinary BPS (Liu et al. 2018). Thus, despite the lower absorption efficiency of BPS through skin, thermal receipts may still be the major source of BPS exposure for background populations.

Following dermal exposure, the relative time trends of BPA-\text{d}_{16} and BPS in urine appeared similar, but proportion of free BPS in the current study was higher than for BPA-\text{d}_{16} in the same participants (Figure 4), suggesting that the overall biotransformation efficiency of BPS was lower than BPA. Consistent with this result, human pharmacokinetic studies following oral administration showed that approximately 3% of total urinary BPS was unconjugated (Oh et al. 2018), compared with only 0.1% of total urinary BPA (Thayer et al. 2015). These differences may be due to less efficient glucuronidation of BPS, as human liver and intestinal microsomes were less effective for glucuronidation of BPS than BPA at environmentally relevant concentrations (Karrer et al. 2018). Given that only the unconjugated bisphenols bind to estrogen receptors (Matthews et al. 2001; Snyder et al. 2000), the higher relative amounts of free BPS are a concern for endocrine disruption.

Some limitations of the current studies are acknowledged. First, although the EpiDerm™ skin model consists of normal human epidermal keratinocytes, permeation may still be higher than in human skin (Schafer-Korting et al. 2008). Thus, permeation data from the present in vitro study should be regarded with some uncertainty if extrapolating to in vivo conditions. Nevertheless, the EpiDerm™ skin model is appropriate for comparing the relative percutaneous penetration of BPA and BPS. Secondly, in the human study, the wearing of a nitrile glove on the exposed hand may have influenced permeation of bisphenols, but unlikely affected absorption of one analyte more than the other. Third, the sample size of the human exposure study was quite small, as is common for studies of this nature (Oh et al. 2018; Thayer et al. 2015; Volkel et al. 2002). Nevertheless, statistical differences in the percutaneous absorption of BPA and BPS were observable; thus, statistical power was satisfactory for the current study objectives.

**Conclusion**

The percutaneous absorption of BPS was consistently lower than BPA in vitro, and a controlled human trial showed less absorption of BPS than BPA after handling thermal receipt papers. These data suggest that replacing BPA with same quantity of BPS in thermal receipts could reduce the total internal dose of bisphenols following skin exposure. However, the limited metabolic capability of skin should be considered in evaluation of the relative risks for bisphenols, and additional studies are needed to evaluate the biotransformation efficiency and distribution of BPS compared with BPA following dermal exposure of humans.

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