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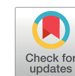
Toxicokinetics of bisphenol-S and its glucuronide in plasma and urine following oral and dermal exposure in volunteers for the interpretation of biomonitoring data



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A B S T R A C T

The measurement of bisphenol-S (BPS) and its glucurono-conjugate (BPSG) in urine may be used for the biomonitoring of exposure in populations. However, this requires a thorough knowledge of their toxicokinetics. The time courses of BPS and BPSG were assessed in accessible biological matrices of orally and dermally exposed volunteers. Under the approval of the Research Ethics Committee of the University of Montreal, six volunteers were orally exposed to a BPS-d8 deuterated dose of 0.1 mg/kg body weight (bw). One month later, 1 mg/kg bw of BPS-d8 were applied on 40 cm^2 of the forearm and then washed 6 h after application. Blood samples were taken prior to dosing and at fixed time periods over 48 h after treatment; complete urine voids were collected pre-exposure and at pre-established intervals over 72 h postdosing. Following oral exposure, the plasma concentration–time courses of BPS-d8 and BPSG-d8 over 48 h evolved in parallel, and showed a rapid appearance and elimination. Average peak values (± SD) were reached at 0.7 ± 0.1 and 1.1 ± 0.4 h postdosing and mean (± SD) apparent elimination half-lives ($t_{½}$) of 7.9 ± 1.1 and 9.3 ± 7.0 h were calculated from the terminal phase of BPS-d8 and BPSG-d8 in plasma, respectively. The fraction of BPS-d8 reaching the systemic circulation unchanged (*i.e.* bioavailability) was further estimated at 62 ± 5% on average (± SD) and the systemic plasma clearance at 0.57 ± 0.07 L/kg bw/h. Plasma concentration–time courses and urinary excretion rate profiles roughly evolved in parallel for both substances, as expected. The average percent (± SD) of the administered dose recovered in urine as BPS-d8 and BPSG-d8 over the 0–72 h period postdosing was 1.72 ± 1.3 and 54 ± 10%. Following dermal application, plasma levels were under the lower limit of quantification (LLOQ) at most time points. However, peak values were reached between 5 and 8 h depending on individuals, suggesting a slower absorption rate compared to oral exposure. Similarly, limited amounts of BPS-d8 and its conjugate were recovered in urine and peak excretion rates were reached between 5 and 11 h postdosing. The average percent (± SD) of the administered dose recovered in urine as BPS-d8 and BPSG-d8 was about 0.004 ± 0.003 and 0.09 ± 0.07%, respectively. This study provided greater precision on the kinetics of this contaminant in humans and, in particular, evidenced major differences between BPA and BPS kinetics with much higher systemic levels of active BPS than BPA, an observation explained by a higher oral bioavailability of BPS than BPA. These data should also be useful in developing a toxicokinetic model for a better interpretation of biomonitoring data.

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1. Introduction

Bisphenol S, bis(4-hydroxyphenyl) sulfone (BPS), is a substance synthesized from the sulfonating of phenols (Johannes, 2014). It is ubiquitous in the environment (Liao et al., 2012; Wan et al., 2018). BPS was synthesized for the first time in 1869, and it began to be used as a substitute to its analogue bisphenol A (BPA) in the years 2000 s (Glausiusz, 2014). BPS has a widespread commercial and consumer use. BPS is used as an intermediate for the production of epoxy resins and polycarbonate plastics; it is present in a variety of industrial products (*e.g.* cleaning products), food (*e.g.* meat, dairy products) and personal care products (*e.g.* body and hair products) (Rochester and Bolden, 2015; Viñas et al., 2010; Wu et al., 2018). It is also used as a developer in thermal papers (tickets), providing thermosensitive properties (ANSES, 2013). The general population can therefore be exposed through ingestion of contaminated food but also dermal contact (Chen et al., 2016; Wu et al., 2018).

Given that BPA has been shown to have endocrine disrupting properties, some authors have also assessed the estrogenic potencies of BPS *in vitro* and *in vivo* in animals (Chen et al., 2016; Rochester and Bolden, 2015). As observed for BPA, the latter reviews highlight that BPS is hormonally active, showing estrogenic activities in rats, zebrafish and Daphne magna *in vivo*, and oestrogenic and antiandrogenic potencies *in vitro*. Furthermore, according to Kojima et al. (2019), BPS showed agonist activities for human estrogen receptors (ERα and ERß) in the same order of magnitude as BPA. In an *in vivo* study in female rats, Ahsan et al. (2018) also showed that BPS altered estrus cycle and ovarian function and development.

According to metabolism studies in animals, BPS is readily conjugated to glucuronides once absorbed in the body, similar its BPA analogue. Free BPS is considered the active moiety, while the detoxification product BPS-glucuronide (BPSG) has been documented to be the main compound excreted in urine (Gayrard et al., 2019a; Gayrard et al., 2019b; Grandin et al., 2018). In humans, there is a growing body of literature on exposure to BPS in the general population, as assessed from measurements of BPS and BPSG in accessible matrices (Frederiksen et al., 2020; Ghayda et al., 2019; Husøy et al., 2019; Lehmler et al., 2018; Liao et al., 2012; Liu et al., 2017; Ndaw et al., 2018; Philips et al., 2018; Wan et al., 2018; Ye et al., 2015). In particular, Liao et al. (2012) was the first study to report BPS levels in the general population; they showed that total BPS (the sum of free and glucurono-conjugated BPS) was detectable in 81% of spot urine samples (n = 315) collected in Americans and Asians (Chinese, Indians, Japanese, Korean, Kuwait, Malaysian and Vietnamese) aged 2 to 84 years old, with geometric mean concentrations of 0.168 µg/L (range of LOQ of 0.02 to 21 µg/L). Liu et al. (2017) also measured total BPS in 61 paired maternal and cord blood samples from the Chinese population. Although total BPS was detectable only in 4 maternal and 7 cord blood samples, with a range of < 0.03 to 0.07 µg/L, this study showed that BPS can cross the human placenta. More recently, Ndaw et al. (2018) measured total BPS in pre- and post-shift urine samples and first morning void of 17 French cashiers and 15 controls; they found that concentration values were significantly higher in cashiers compared to controls (geometric mean (range) of 2.48 (0.1–28.4 µg/L) versus 0.72 µg/L (< LOQ of 0.1–229 µg/L)). Lehmler et al. (2018) analyzed total BPS concentrations in spot urine samples of children (n = 868) and adults (n = 1808) participating in the National Health and Nutrition Examination Survey (NHANES) 2013–2014; total BPS was detected in 89.4% of samples with median concentration of 0.37 µg/L in adults (interquartile of 0.14–0.88 µg/L) and 0.29 (0.12–0.70) µg/L in children. In a prospective cohort of Netherland females enrolled during early pregnancy (n = 1396), Philips et al. (2018) reported a detection rate of total BPS of 67.8% in spot urine samples collected in 2004–2005 and median concentrations of 0.36 µg/L (interquartile of 0.17–1.08 µg/L), similar to results in NHANES participants. Ghayda et al. (2019) also documented semen and urinary concentrations of total BPS in 158 men enrolled in a prospective cohort in Massachusetts (USA) and seeking fertility treatment from 2011 to 2017, and reported a 76% detection rate (> 0.1 µg/L) from 338 urine samples, a geometric mean of 0.37 µg/L and interquartile of 0.2 to 0.9 µg/L.

To be able to interpret biomonitoring data, it is important to have information on the toxicokinetics of the biomarkers of exposure of interest. While the toxicokinetics of BPA has been largely documented in humans (Fisher et al., 2011; Teeguarden et al., 2015; Thayer et al., 2015), only a limited number of human toxicokinetic studies of BPS has been performed to date (Liu and Martin, 2019; Oh et al., 2018). Some animal studies have also been conducted (Gayrard et al., 2019a; Gayrard et al., 2019b; Gingrich et al., 2019; Gingrich et al., 2018; Grandin et al., 2018). The determination of the toxicokinetics of BPS and its glucurono-conjugate BPSG in humans is important given the potential animal-to-human differences in the kinetics. Oh et al. (2018) documented the time courses of BPS (free BPS and total BPS) in the plasma and urine of seven male and female Korean volunteers from Seoul orally exposed to 8.75 µg BPS-d4/kg body weight (bw). In this study, BPSG was not directly measured; it was rather derived from the difference between unconjugated BPS analyzed without enzymatic hydrolysis and the sum of unconjugated and glucurono-conjugated BPS (and possibly sulfo-conjugates) measured after enzymatic hydrolysis with β-glucuronidase solution with some arylsulfatase activity. Karrer et al. (2018) developed a physiologically-based pharmacokinetic model for BPA and extrapolated it to its analogs including BPS by (i) determining chemical-specific partition coefficients (tissue-to-serum) with a quantitative structure-activity relationship (QSAR) approach and (ii) metabolism parameters with an *in vitro* assessment of glucuronidation of BPS in human liver and intestinal microsomes. The model was then calibrated with the data of Oh et al. (2018). Some model adjustments had to be made to obtain a good fit to the available human kinetic data; according to these authors, the modeled higher plasma concentrations of unconjugated BPS after peroral exposure might be explained by a low glucuronidation rate.

The objective of the present work was to conduct a clinical study to compare, within a same framework, the kinetic profiles of unconjugated BPS and its specifically quantified BPSG conjugation product in female volunteers of childbearing age exposed orally and dermally to deuterated BPS (BPS-d8) in controlled conditions. This study aimed to further contribute to provide basic kinetic data that can serve to develop a human toxicokinetic model allowing reconstruction of absorbed doses from biomarkers measurements of specifically measured BPS and BPSG in vulnerable populations, such as pregnant women.

2. Materials and methods

2.1. Study design and Ethics approval

A clinical study was conducted to document the kinetics of BPS and its glucurono-conjugate BPSG specifically in plasma and urine of female subjects of childbearing age following a single oral and dermal exposure to BPS. The same volunteers (with the exception of three individuals) were exposed orally and then dermally one month later, to ensure complete elimination of the compound between exposures. The study protocol and consent form were approved by the Clinical Research Ethics Committee (CERES) of the University of Montreal prior to study onset (certificate number 17-153-CERES-P). Each participant gave its written consent to participate, was informed of the risks of participating and its right to withdraw from the study at any time. Each participant received a monetary compensation for its time. Volunteers spent the first sampling day at the University for blood and urine sampling and were then asked to return the next three mornings for a blood sampling and to return urine samples. Two accredited nurses were present during the whole study period, performed blood sampling and monitored signs and symptoms.

2.2. Volunteers and dosing

Eight adult female volunteers – in good health, aged between 21 and 30 years old and weighing between 55.5 and 88.9 kg (mean of 65.5 kg) – were recruited on a voluntary basis, seven of which participated in the oral exposure and six in the dermal application. Each of seven volunteers was administered a single oral dose of BPS-d8 (4,4′-sulfonylbisphenol-d8, purity > 99%, isotopic purity > 98%, MW of 258.32 g/mol, Toronto Research Chemicals, Toronto, Canada) of 0.1 mg/kg of body weight (bw) (corresponding to 7 mg for an individual of 70 kg or 0.39 μmol/kg bw). The dose administered orally to volunteers, in the deuterated form, is identical on a mass basis to that previously used in a kinetic study in volunteers exposed orally to BPA (0.1 mg/kg BPA-d6) and close on a molar basis (0.43 μmol/kg bw) (Thayer et al., 2015).

This dose is 100 times lower than the No-Observed Adverse Effect Level (NOAEL) for a subchronic exposure (USEPA, 2014). The NOAEL was established from a 45-day toxicity study in adult male rats orally exposed to BPS. For the parental toxicity, the NOAEL dose was established at 10 mg/kg bw and the Lowest-Observed-Adverse-Effect-Level (LOAEL) dose was established at 60 mg/kg bw/day. For the reproductive toxicity, NOAEL and LOAEL values of 60 and 300 mg/kg bw/day were established (USEPA, 2014). In the present study, the dose administered, which is 100 times lower than the NOAEL, considers an inter-species uncertainty factor (UF_A) of 10 and interindividual uncertainty factor (UF_H) of 10.

For the oral administration, BPS-d8 was dissolved in ethanol (100 mg/mL equivalent to 10 mg/100 μL) and the solution was applied on a cookie (70 μL of solution on a cookie for a 70-kg individual). The ethanol deposited on the cookie was left to evaporate for 10 min prior to ingestion by each volunteer. Each participant then drank 100 mL of water.

One month after the oral exposure, volunteers were exposed dermally to an acute dose of BPS of 1 mg/kg bw. It is important to note that there is currently no recommended reference dose for dermal exposure. Studies on the toxicokinetics of bisphenol A (BPA) in animals and humans estimated that the absorption fraction was 8.6 ± 2.1% in human skin explants (n = 7) (Demierre et al., 2012). To establish the dermally applied dose, it was considered that dermal absorption was < 10%, based on the study by Demierre et al. (2012) and therefore it was set 10 times higher than the oral dose. About 48 h before dermal application of BPS, participants were recommended to remove hair on their forearm while taking care not to irritate the skin. The solution was then applied to an area of 40 cm^2 of the forearm delimited by an indelible marker. BPS was suspended in a 0.1 M phosphate buffer (pH 7.4) containing 1% sodium carboxymethylcellulose of medium viscosity (100 mg/mL or 100 μg/μL) (a synthetic gel used against dry eye and as a food additive) and applied as drops (700 μL for an individual of 70 kg). The treated area was left uncovered and unwashed for a period of 6 h. After 6 h of dermal contact, a wipe was used (Hines et al 2017) to clean the entire application area. The application site was then washed with soap and water. The duration of application of 6 h was chosen to represent the normal duration of exposure of an employee handling receipts and to take into account the constraints related to protecting the application area. Furthermore, it was decided not to occlude the application area to prevent the transfer of the product on occlusion material.

2.3. Blood and urine sampling

The time-courses of the parent product BPS-d8 and its glucurono-conjugate BPSG-d8 in plasma were established by performing serial blood sampling by venipuncture on the arm 30 min before exposure (corresponding to a control sample) and at fixed time periods over 48 h following exposure, *i.e.* at 15 min, 30 min, 45 min, 1 h, 1 h 15, 1 h 30, 1 h 45, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 24 h and 48 h post-dosing (n = 17 samples per individual). In order to facilitate blood sampling, a catheter was placed during the first sampling day. Blood was collected in heparin tubes.

For the cutaneous application, the catheter was installed at the level of the ulnar vein of the arm contralateral to that on which the BPS-d8 was applied. Samples were taken by repeated venous punctures at previously fixed times. A volume of 10 mL was taken at each sampling time for a total of 170 mL, which is less than a donation of blood.

In parallel, in order to document the urinary excretion time courses of BPS-d8 and BPSG-d8, complete urine voids were collected in separate and clearly identified Nalgene® bottles (group, individual, time) at fixed periods, namely a nocturnal collection before exposure (-10 h-0 h) and 0–2 h, 2–4 h, 4–6 h, 6–8 h, 8–10 h, 10–12 h, 12–14 h, 14–24 h, 24–48 h and 48–72 h post-administration (n = 11 collections per individual and all urine voided during an established period was combined to the same bottle). Participants were asked to drink plenty of water on the day of exposure (1.5 L per day) to allow frequent urine collections. A 1.5 L bottle of drinking water was given to each participant on the first sampling day.

2.4. Treatment of samples and analysis

Immediately after collection, blood samples were stored at 4 °C in the refrigerator. Within the hour following blood withdrawal, plasma was isolated by centrifuging samples for 10 min at 1500 g at 4 °C. Four aliquots of 1 mL were prepared in polypropylene tubes with Fischer screw cap of 2 mL and then samples frozen at −20 °C. Two aliquots were shipped one dry ice at INRA in Toulouse for analysis of BPS-d8 and BPSG-d8.

Urine samples were stored in the refrigerator at 4 °C immediately after collection the first day of sampling or kept in coolers with ice packs afterwards by participants until they were brought to the University. Urine volumes were measured the day of collection. For each sample, 3 aliquots of 3 mL were prepared in 5 mL polypropylene tubes and 1 aliquot was prepared of 90–100 mL in a 120 mL Sarstedt container. Samples were subsequently frozen at −20 °C until analysis. Aliquots were shipped on dry ice for analysis at INRA in Toulouse.

Plasma and urine samples were assayed with an on-line solid phase extraction (SPE) ultra-performance liquid chromatography coupled to tandem mass spectrometry (Acquity-2D UPLC® Xevo® TQ, Waters, Milford, MA, USA). BPS-d8, BPSG-d8, BPSG (used as internal standard) were purchased from Toronto Research Chemicals and BPS was obtained from Merck KGaA, Darmstadt, Germany. All these standard purities were higher than 97% and isotopic purities were higher than 98%. Urine samples collected after oral administration were simultaneously quantified according to the method previously validated and published (Grandin et al., 2017; Grandin et al., 2018). Briefly, 100 μL of urine samples were diluted with 200 μL of acetonitrile/zinc sulfate containing BPS and BPSG as internal standard (100 ng/mL and 1000 ng/mL). The centrifuged mixture was loaded onto the on-line C8 cartridge for clean-up, separated on a CSH C18 column (Acquity 100 × 2.1, 1.7 μm, Waters) and detected in negative electrospray ionization (ESI-) using multiple reaction monitoring (MRM) mode. The urine samples collected after dermal administration and all the plasma samples were assayed as described in a previous study with minor modifications (Rancière et al., 2019). Briefly, samples were extracted on ion exchange SPE cartridges (HR-XAW, Macherey Nagel, Hoerdt, France) and labelled with dansyl chloride. The dansylated BPS-d8 and BPSG-d8 were loaded onto the on-line C8 SPE cartridge and separated on a Phenyl Hexyl column (Acquity 100 × 2.1 mm; 1.7 μm, Waters) with a water/acetonitrile gradient (both eluents containing 0.1% formic acid) [0.3 mL/min, 40 °C]. Analytes were detected by mass spectrometry after electrospray ionization in positive mode. The MRM transitions used for the quantification of dansylated BPS-d8 and BPSG-d8 were 725 > 171 and 668 > 171, respectively (see Table S1 for structures and fragmentations). The method was validated in plasma according to the European Medicine Agency Guidelines from 0.05 to 10 ng/mL (*i.e.* 0.2 to 39 nM) for BPS-d8

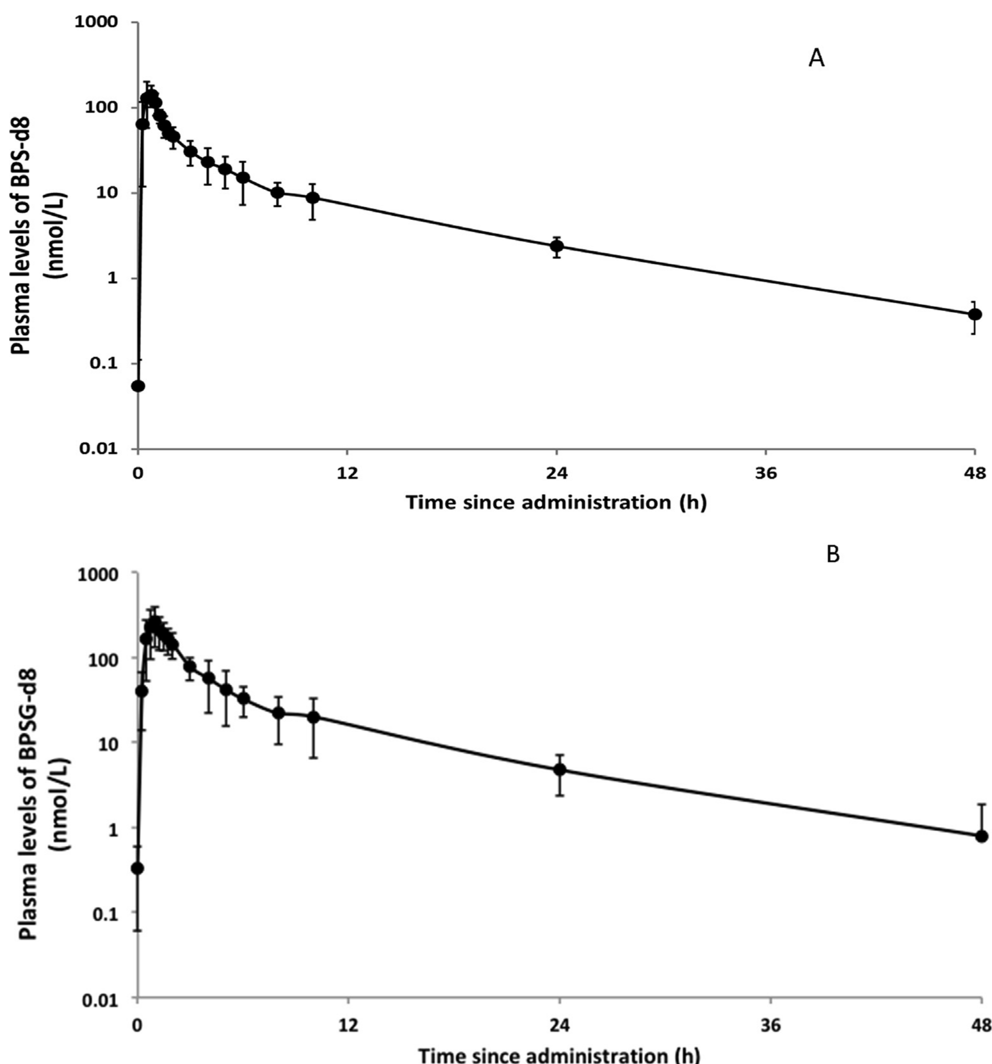

Fig. 1. Concentration-time courses of BPS-d8 (A) and BPSG-d8 (B) in the plasma of volunteers (nmol/L) following a single oral administration of 0.1 mg/kg bw (0.39 µmol/kg bw) of BPS-d8 (mean ± SD) (n = 6). Symbols represent mean values and vertical bars are standard deviations.

using a linear model weighted by 1/X (X = concentration) and from 0.5 to 100 ng/mL (*i.e.* 1.15 to 230 nM) for BPSG-d8 using a linear model weighted by $1/X^2$. Blank samples were used to check the absence of contamination during assays. The accuracy and the intra- and inter-day precisions (assessed by the coefficients of variation, CV%) of the method were evaluated from quality control samples at three concentration levels (0.08, 0.8 and 8 ng/mL). The CV% were below 17% for BPS-d8 and 9% for BPSG-d8 with accuracy ranging from 80% to 96% for both molecules. The lower limits of quantification (LLOQ) were estimated in plasma at 0.05 and 0.5 ng/mL (*i.e* 0.2 and 1.15 nM) and in urine at 0.5 and 5 ng/mL (*i.e.* 2 and 11.5 nM) for BPS-d8 and BPSG-d8, respectively. The LLOQ values were determined with 5 replicates at the lowest concentration that can be quantified with an accuracy ranging from 80% to 120% and an intraday precision lower than 20%. The limits of detection were estimated at 0.02 and 0.14 ng/mL (*i.e.* 0.08 nM and 0.32 nM) in plasma for BPS-d8 and BPSG-d8, respectively.

2.5. Toxicokinetic analysis

Following ingestion, the plasma concentration-time courses of BPS-d8 and BPSG-d8 showed three phases, a phase of appearance in plasma followed by a biexponential decrease in concentrations. The different rate constants for each of the three phases were determined by least-square fit adjustments of the following general function (Eq. (1)) to observed individual plasma concentration-time profiles of BPS-d8 and BPSG-d8 following oral administration of BPS-d8, by using "curve fitting" tool in Matlab. Apparent appearance and biphasic elimination phases were represented by: $C(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$ (Eq. (1)), where *i*) C (t) is the plasma concentration as a function of time; *ii*) A, B and C are the preexponential coefficients; *iii*) α, β and γ are the hybrid rate coefficients for the three phases. Apparent half-life ($t_{1/2}$) values were calculated using the equation $t_{1/2} = 0.693/k$ where k = α, β or γ (Hayes, 2007). For the cutaneous exposure, there were too many undetectable values to allow such determinations.

From plasma concentration (C) - time profiles after oral exposure, other calculated parameters include maximal concentration (C_{max}), time-to-peak levels (T_{max}), the discrete version of the area under the concentration-time curve (AUC), the area under the first moment of concentration-time curve (AUMC), the mean residence time (MRT), the apparent oral clearance (Cl_{oral}), estimated bioavailability (F) and the systemic plasma clearance (Cl_{plasma}) (Gibaldi and Perrier, 1982; Hayes, 2007; Weiss, 1990). Equations used to calculate these parameters are:

$$AUC_{oral} = \frac{1}{2}\sum_{\forall i}(t_i - t_{i-1})[C(t_i) + C(t_{i-1})]$$

$$AUMC_{oral} = \frac{1}{2}\sum_{\forall i}(t_i - t_{i-1})[t_iC(t_i) + t_{i-1}C(t_{i-1})]$$

$$MRT_{oral} = \frac{AUMC_{oral}}{AUC_{oral}}$$

$$Cl_{oral} = \frac{Dose}{AUC_{oral}}$$

where Dose is the administered dose.

Bioavailability (fraction of BPS-d8 reaching the systemic circulation unchanged) was indirectly estimated from Cl_{oral} using the following equation (Gibaldi and Perrier, 1982):

$$F = \frac{Q}{Q + Cl_{oral}}$$

where Q is the hepatic blood flow rate (considered here at 25 mL/kg bw/min).

This equation assumes that first pass metabolism only occurs in the liver (*i.e.* no metabolism in the gastrointestinal tract) and that BPS-d8 is completely absorbed from the gut lumen after oral dose (*i.e.* that the oral absorption fraction f_{abs} is 1; not to be confused with the bioavailability), given that by default F = 1 – ER, where ER is the extraction ratio due to first-pass metabolism.

The systemic plasma clearance was therefore deduced from BPS-d8 bioavailability and according to Gibaldi and Perrier (1982) and Weiss (1990) as follows:

$$Cl_{plasma} = \frac{F \times Dose_{oral}}{AUC_{oral}} = F \times Cl_{oral} = \frac{Q}{\frac{Q}{Cl_{oral}} + 1} = \frac{Q}{\frac{AUC_{oral} \times Q}{Dose_{oral}} + 1}$$

With an absorption fraction < 1, lower Cl_{plasma} values would be obtained.

3. Results

3.1. Time courses of BPS-d8 and BPSG-d8 in plasma after oral administration

The concentration-time courses of BPS-d8 and BPSG-d8 in plasma over a 48-h period following oral administration of 0.1 mg/kg bw of BPS-d8 in volunteers are presented in Fig. 1 (and Fig. S1). Calculated toxicokinetic parameters determined from the plasma concentration-time courses are compiled in Tables 1 and 2. While BPSG-d8 was found in higher concentrations than BPS-d8 (roughly 2-fold based on peak levels), it is readily apparent that the plasma profiles of BPS-d8 and BPSG-d8 over 48 h evolved in parallel, and showed rapid appearance and elimination phases. Apparent appearance rates (mean ± SD) calculated from the time courses of BPS-d8 and BPSG-d8 were 2.3 ± 2.3 and 1.4 ± 2.3 h^{-1} (translating into $t_{1/2\alpha}$ of 0.3 ± 0.3 and 0.5 ± 0.3 h), respectively. Average peak values (± SD) were reached at about the same time for both compounds, that is at 0.7 ± 0.1 and 1.1 ± 0.4 h postdosing, respectively. From the terminal phase of BPS-d8 and BPSG-d8 in plasma, average (± SD) apparent elimination half-lives ($t_{1/2\gamma}$) of 7.9 ± 1.1 and 9.3 ± 7.0 h were calculated, respectively. Similarities in the time courses of BPS-d8 and BPSG-d8 in plasma (appearance and elimination rates) are also reflected by the same calculated mean residence time (MRT) value of both compounds (as calculated from $\frac{AUMC}{AUC}$). The fraction of BPS reaching the systemic circulation unchanged (*i.e.* bioavailability) was further calculated at 62 ± 5% on average (± SD) (with an Cl_{oral} of 0.94 ± 0.19 L/kg bw/h) and the systemic plasma clearance was estimated at 0.57 ± 0.07 L/kg bw/h. Furthermore, from the individual time-courses of BPS-d8 and BPSG-d8 in plasma, the curve showing small plasma peak concentrations around 4–10 h is compatible with an enterohepatic recirculation (see supplementary Fig. S1).

3.2. Time courses of BPS-d8 and BPSG-d8 in urine after oral administration

The time courses of BPS-d8 and BPSG-d8 excretion rate in the urine of volunteers over the 72-h period following ingestion of 0.1 mg/kg bw of BPS-d8 are depicted in Fig. 2. Urinary excretion rate profiles appeared to evolve in parallel for both substances and were similar to plasma profiles, as expected.

The time courses of BPS-d8 and BPSG-d8 cumulative excretion over the 72-h period post-administration were also derived (Fig. 3 and Fig. S2). Although the urinary excretion time courses of BPS-d8 and BPSG-d8 were similar, BPSG-d8 was present in ≈30-times higher amounts

Table 1
Mean (± SD) time to peak levels, first-order apparent appearance and elimination half-lives and excretion fraction derived from individual time courses of BPS-d8 and BPSG-d8 in the plasma and urine of volunteers following ingestion of 0.1 mg/kg bw of BPS-d8 (n = 6 in plasma and n = 7 in urine).

	Mean ± SD[a] (n = 6 in plasma and n = 7 in urine)			
	BPS		BPSG	
	Plasma	Urine	Plasma	Urine
Time-to-peak levels T_{max} (h)[b]	0.7 ± 0.1		1.1 ± 0.4	
Maximal concentration C_{max} (nmol/L)[b]	158 ± 47		273 ± 127	
Apparent appearance $t_{1/2\alpha}$ (h)	0.3 ± 0.3		0.5 ± 0.3	
Apparent elimination $t_{1/2\beta}$ (h)	1.0 ± 0.6		1.0 ± 0.8	
Apparent terminal elimination $t_{1/2\gamma}$ (h)	7.9 ± 1.1		9.3 ± 7.0	
Excreted fraction (molar % of total dose)[c]		1.7 ± 1.3[d]		54 ± 10[d]

[a] Represents mean ± SD values of kinetic parameters derived from individual time course data.
[b] Mean time-to-peak levels and maximal concentration (± SD) were calculated from peak values observed for each individual.
[c] Represents the molar percentage of total administered dose of BPS-d8 recovered in urine as BPS-d8 and BPSG-d8.
[d] Range of values of 0.6–4.4% for BPS-d8 and 37–72% for BPSG-d8.

Table 2
Toxicokinetic parameters calculated from plasma concentration-time profiles of BPS-d8 and BPSG-d8 (on a molar basis) in volunteers following ingestion of 0.1 mg/kg bw of BPS-d8 (n = 6).

Toxicokinetic parameters	Mean ± SD (n = 6)	
	BPS-d8	BPSG-d8
$AUC_{0\to\infty\ oral}$ (nmol/L × h)	432 ± 89	946 ± 345
$AUMC_{oral}$ (nmol/L × h^2)	3082 ± 886	6774 ± 3852
MRT (h)	7.1 ± 1.0	6.9 ± 2.6
Cl_{oral} (L/kg bw/h)	0.94 ± 0.19	0.45 ± 0.13
Bioavailability (F) (%)[a]	62 ± 5	
Cl_{plasma} (L/kg bw/h)[b]	0.57 ± 0.07	

[a] Bioavailability (F) corresponds to the fraction of BPS-d8 reaching the systemic circulation unchanged. This calculation (to be valid) assumes that the oral absorption fraction (f_{abs}) is 1 given that by default F = 1 – ER, where ER is the extraction ratio due to first-pass metabolism that is considered to occur only in the liver.
[b] Cl_{plasma} was computed from Cl_{oral} and the estimated bioavailability (F).

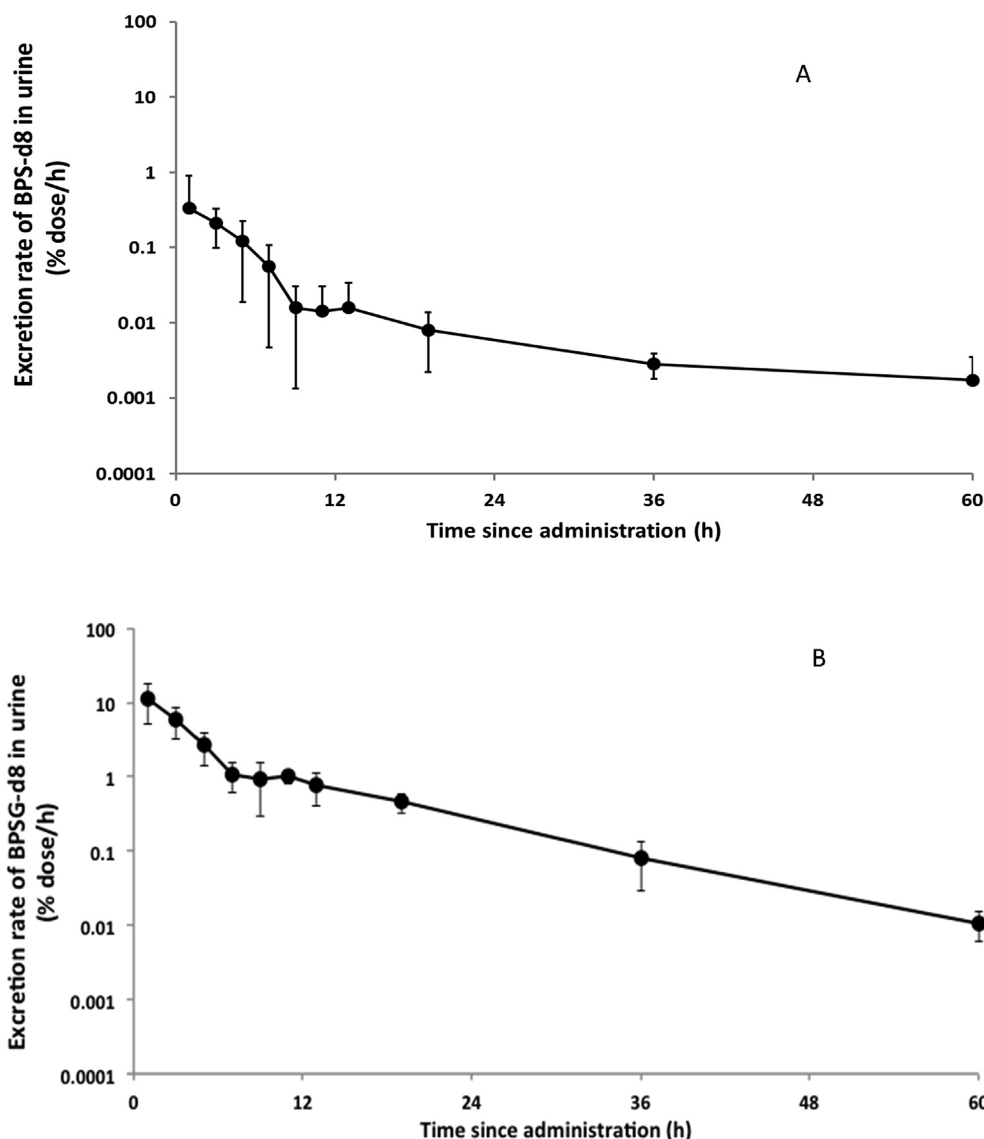

Fig. 2. Time courses of BPS-d8 (A) and BPSG-d8 (B) excretion rate in the urine (% dose/h) of volunteers following a single oral administration of 0.1 mg/kg bw of BPS-d8 (mean ± SD) (n = 7) (B). Symbols represent mean values and vertical bars are standard deviations.

than BPS-d8. The average percent (± SD) of the administered dose recovered in urine as BPS-d8 and BPSG-d8 over the 0–72 h period postdosing was 1.72 ± 1.3 and 54 ± 10%, respectively (with a range of 0.6–4.4 and 37–72%, respectively; Table 1). Urinary excretion appears near complete after 72 h (asymptote being reached at 72 h).

3.3. Time courses of BPS-d8 and BPSG-d8 in plasma after cutaneous application

The individual time courses of BPS-d8 and BPSG-d8 in plasma over a 48-h period following the onset of a 6-h cutaneous application of 1 mg/kg bw of BPS-d8 in volunteers are displayed in Table S2. Values were under the lower limit of quantification (LLOQ) for most time points such that average time course were not reported (Hecht et al., 2018). With such low levels, it was not possible to determine toxicokinetic parameters, as was done for oral exposure. Nevertheless, an increase in plasma levels of both compounds was detectable from individual profiles and peak values were reached between 5 and 8 h depending on individuals, which is close to the time of cleaning of the treated area. BPSG-d8 was also found in somewhat higher molar concentrations than BPS-d8 (roughly 2- to 9-fold at peak levels).

3.4. Time courses of BPS-d8 and BPSG-d8 in urine after cutaneous application

In line with blood profiles, limited amounts of BPS-d8 and its conjugate were recovered in urine after cutaneous application and peak excretion rates were reached between 5 and 11 h postdosing depending on the volunteer while mean value shows a peak at 1 h (Fig. 4). BPSG-d8 appears to be excreted somewhat more slowly than BPS-d8 over the 72-h collection period, but again values were close to the LLOQ, such that this should be interpreted with caution. The cumulative excretion time courses of BPS-d8 and BPSG-d8 show that BPSG-d8 is excreted in about 20-times higher amounts than BPS-d8, and that excretion is not totally complete after 72 h (lack of asymptote) at least for part of the volunteers (Fig. 5 and Fig. S3). The average percent (± SD) of the administered dose recovered in urine as BPS-d8 and BPSG-d8 was about 0.004 ± 0.003 and 0.09 ± 0.07%, respectively. This suggests a rather low relative bioavailability by the cutaneous route when compared to the oral route.

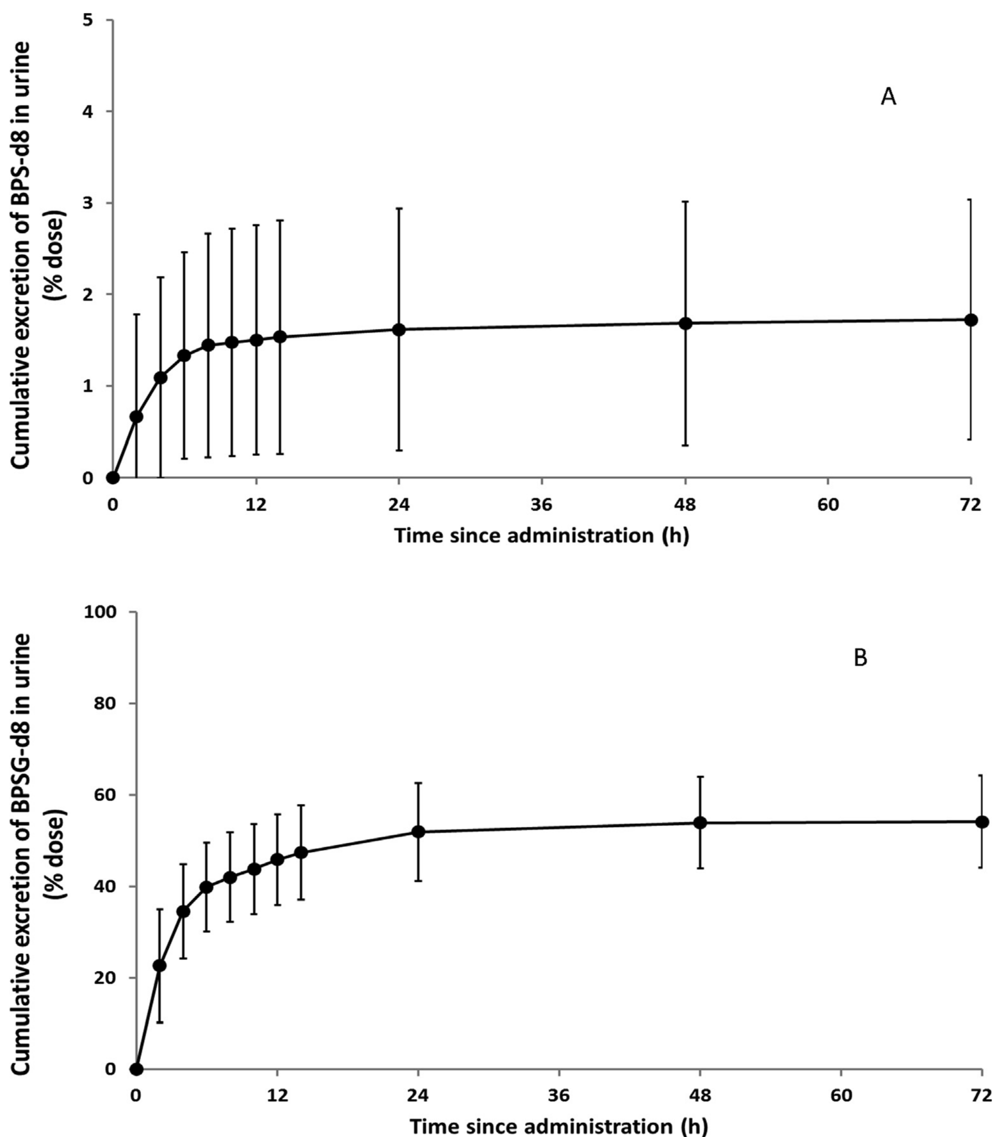

Fig. 3. Time courses of BPS-d8 (A) and BPSG-d8 (B) cumulative excretion (as a molar % of administered dose) in the urine of volunteers following a single oral administration of 0.1 mg/kg bw of BPS-d8 (mean ± SD) (n = 7). Symbols represent mean values and vertical bars are standard deviations.

4. Discussion

4.1. Toxicokinetics of BPS in humans after oral exposure

There are limited data on the toxicokinetics of BPS to date. This is the first study to document the toxicokinetics of BPS and BPSG specifically following dermal and oral exposure in volunteers within a same experimental framework, and complements the available oral kinetic data of Oh et al. (2018) and dermal data of Liu and Martin (2019). Female volunteers of childbearing age were chosen for our study, given that the acquired data aimed to serve, in a next step, for the development of a human toxicokinetic model allowing reconstruction of absorbed doses from measurements of BPS and BPSG in the plasma and/or urine of vulnerable populations, such as pregnant women. Deuterated compound was administered (BPS-d8) to ensure that measured levels in plasma and urine were not partially due to a body burden resulting from environmental exposure to BPS (Andra et al., 2016; Thayer et al., 2015). Oh et al. (2018) recently documented the plasma and urinary time courses of unconjugated and total BPS; BPSG levels were estimated by the difference between total BPS and unconjugated BPS levels. In the current work, BPS-d8 and BPSG-d8 were quantified specifically by UHPLC-MS-MS.

Plasma profile and urinary rate time course obtained in our study after oral exposure to a single low-dose show the rapid appearance of BPS in plasma and elimination from the body of volunteers (elimination $t_{1/2}$ from the terminal phase in plasma of ≈7.9–9.3 h on average), mainly in the conjugated form (see Table 1 and Figs. 1 and 2). The similar apparent initial rate of BPS-d8 and BPSG-d8 after oral exposure suggests that both forms reach the systemic bloodstream at about the same time period, indicative of a rapid conjugation of BPS-d8 in intestines/enterocytes or liver prior to reaching the systemic circulation (first-pass effect). This has also been suggested from toxicokinetic studies of BPS in pigs (Gayrard et al., 2019a; Gayrard et al., 2019b), but also by Karrer et al. (2018) who developed a physiologically-based pharmacokinetic (PBPK) model for BPA and adapted the model to other analogues, such as BPS. These authors suggest that first-pass glucuronidation occurs mainly in the liver rather than in enterocytes.

The elimination time course of BPSG-d8 in plasma after oral exposure was also similar to that of BPS-d8 (see Fig. 1 and Tables 1 and 2). The observed plasma kinetic features further suggest that the most important contribution to the overall elimination rate of BPS from blood is phase II conjugation to form more polar moieties, given the

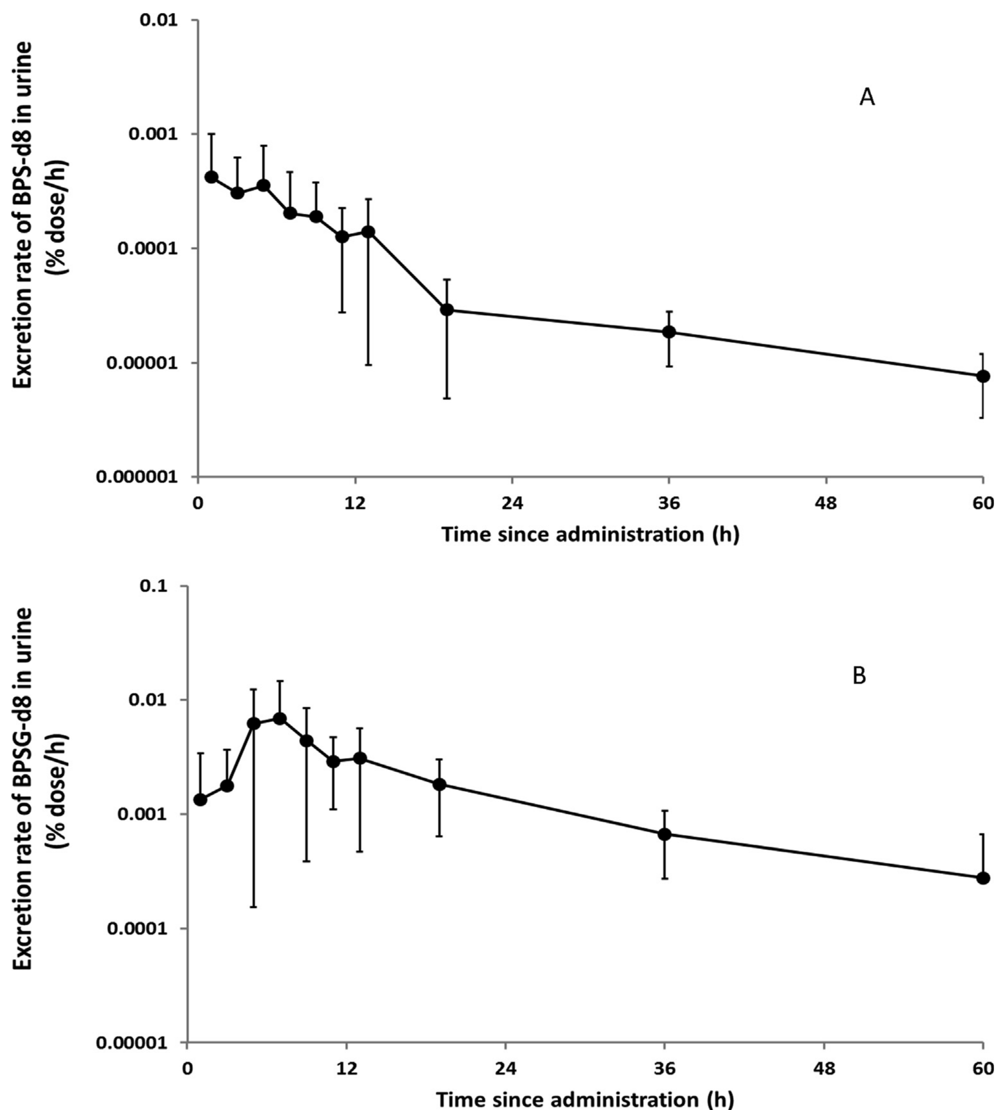

Fig. 4. Time courses of BPS-d8 (A) and BPSG-d8 (B) excretion rate in the urine (% dose/h) of volunteers following a cutaneous application of 1 mg/kg bw of BPS-d8 during 6 h (n = 6). Symbols represent mean values and vertical bars are standard deviations.

very small levels of unconjugated compound in urine. On the other hand, elimination of BPSG through excretion contributes mainly to BPSG overall clearance from blood. Furthermore, the observed time profiles of BPS-d8 and BPSG-d8 in plasma (behaviour at 6–10 h in particular) is indicative of a significant entero-hepatic recycling as was reported in rats (Gayrard et al., 2019b) and from the modeling of BPA in humans (Karrer et al., 2018). Although bisphenol conjugates are rapidly formed, the high levels of β-glucuronidase and arylsulfatase in tissues such as the liver, kidneys and placenta should allow deconjugation to the active unconjugated form, and in the case of intestines bacterial enzymes favors reabsorption from the gut lumen (Andra et al., 2016; Beaud et al., 2005; Danovitch and Laster, 1969; Parkinson et al., 2012).

From the plasma-concentration time course in volunteers of our study, a systemic plasma clearance (Cl_{plasma}) of BPS of 0.57 ± 0.07 L/kg bw/h and an oral bioavailability of 62 ± 5% were estimated (see Table 2), provided that the following two assumptions are met: *i*) oral absorption of BPS was total and *ii*) BPS is only eliminated by hepatic metabolism, which is consistent with a very low renal clearance of BPS. The plasma clearance as obtained in the current study is rather close to the human plasma clearance value of 0.79 L/kg bw/h inferred by allometric extrapolation from toxicokinetic data in rats, ewes and piglets (Gayrard et al., 2019a), thus confirming these results.

In our study, the total percentage of the administered molar dose recovered in urine as unconjugated BPS-d8 following oral exposure was similar to that observed in the study of Oh et al. (2018), with a range of 0.6 to 4.4% in our study compared to 0.9 to 3.3% in female volunteers of the study of Oh et al. (2018), indicating that the renal clearance of unconjugated BPS-d8 was low. However, with regard to cumulative excretion of BPSG-d8 in urine, somewhat lower excretion values were found in volunteers of our study. More specifically, our individual results show that 37 to 72% of the administered oral dose were recovered in urine as BPSG-d8 over the 0–72 h collection period postdosing, depending on the volunteers; when excluding the volunteer with the lowest cumulative excretion in urine, the observed total urinary excretion ranged between 51 and 72%, suggesting that clearance of BPS-d8 is mainly driven by its glucuronidation. In the study of Oh et al. (2018), the reported total percentage of the oral dose recovered in urine as BPSG (total BPS minus unconjugated BPS) varied between 59 and 77% in the female participants (n = 3), with a mean value of 70%, *i.e.* in the same order of magnitude as that observed in our study. In the male participants of that study, corresponding range of values was 67–104%, with a mean of 92% (n = 4). Differences between our results and those of Oh et al. (2018) may partly be explained by the fact that

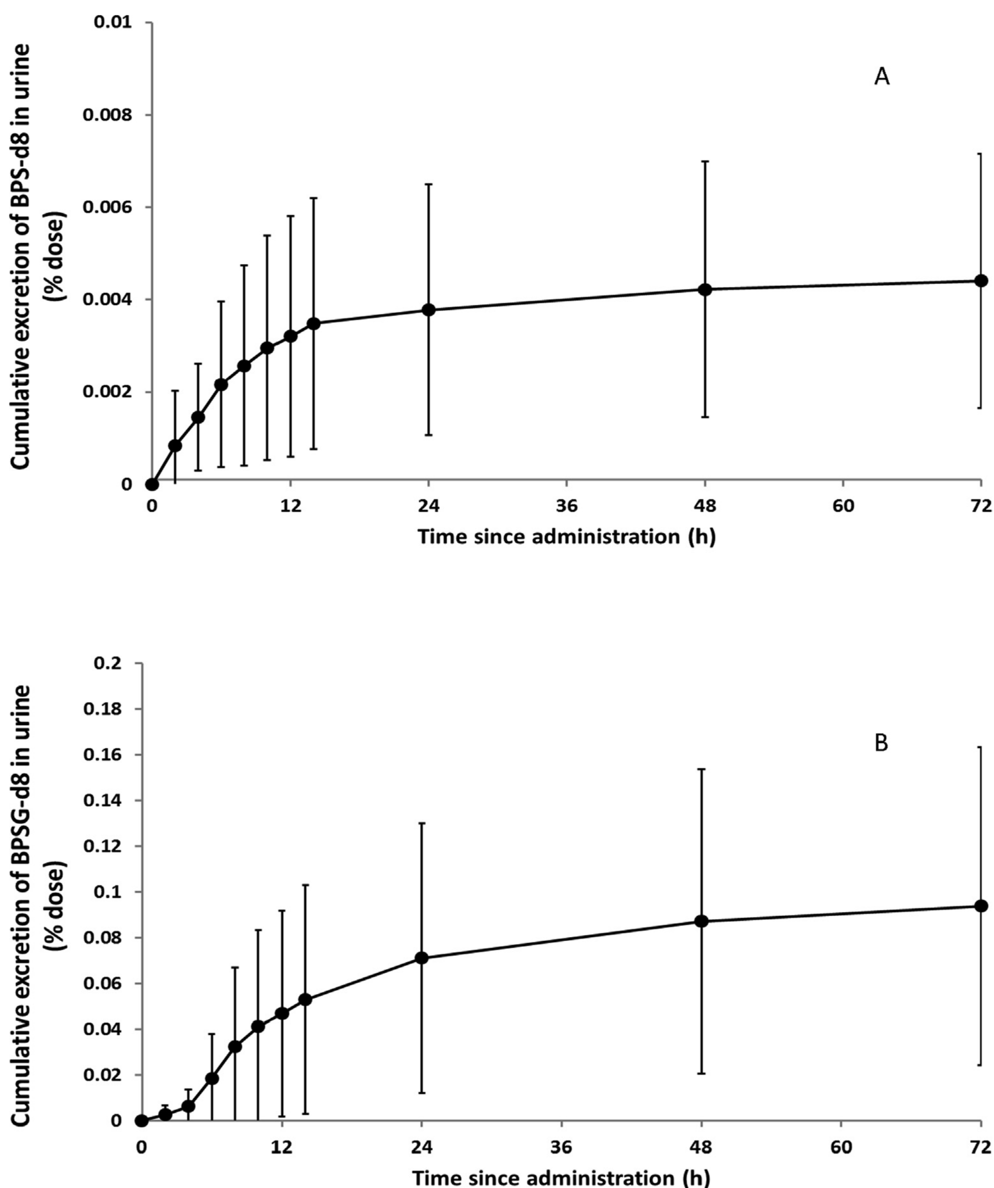

Fig. 5. Time courses of BPS-d8 (A) and BPSG-d8 (B) cumulative excretion (as a molar % of administered dose) in the urine of volunteers following a cutaneous application of 1 mg/kg bw of BPS-d8 during 6 h (n = 6). Symbols represent mean values and vertical bars are standard deviations.

the latter account for BPS conjugated to sulfates (even if reported sulfatase enzyme activity was low), given that conjugated BPS was estimated by the difference between total BPS and unconjugated BPS. In our work, the glucurono-conjugate was rather measured specifically. Other studies in the general population confirmed the presence of sulfate conjugates of bisphenol analogues in urine (Andra et al., 2016; Gerona et al., 2016; Liao and Kannan, 2012; Liu and Martin, 2019), and there is a known polymorphism between men and women in uridine-diphosphate (UDP)-glucuronosyltransferase (UGTs), in addition to ethnical variations (Gallagher et al., 2010; Iwai et al., 2004; Kojima and Degawa, 2014; Lampe et al., 2000; Mazur et al., 2010; Mehboob et al., 2017; Sparks et al., 2004). In particular, Gerona et al. (2016) reported that 15% of BPA forms recovered in the urine of the monitored individuals from the general population (pregnant women of diverse ethnic background) were sulfate conjugates. Karrer et al. (2018) considered that bisphenols are conjugated to sulfates in their model, but to a lesser extent than glucurono-conjugates. In an *in vitro* study in human HepaRG hepatic cell line, Le Fol et al. (2015) found that 85.8% of BPS was conjugated to glucuronides and 10.5% to sulfates. In the present study, the volunteers were of different origins (4 North American Caucasians, 3 of North African Arabic origin). Furthermore, in both our study and the study of Oh et al. (2018), total recovery of the different BPS forms in urine was less than 100%, suggesting the remaining is either i) excreted in feces as observed in animals, although the threshold molecular weight for biliary excretion is higher in humans than animals (325 ± 50 g/mol for rats and 500 ± 50 g/mol for humans) (Waidyanatha et al., 2018; Yang et al., 2009) or ii) forms other metabolites, such as hydroxylated BPS in the ortho- and *meta*-positions of the phenol rings (Skledar and Mašič, 2016; Skledar et al., 2016). The possible polymorphisms and variability in the urinary excretion of BPS forms in urine is an important aspect to consider for the biomonitoring of exposure to BPS and interpretation of biomonitoring data.

4.2. Comparison of BPS and BPA toxicokinetics in humans after oral exposure

The rates of appearance and elimination calculated from the time courses of BPS-d8 and BPSG-d8 in plasma (Table 1) were similar to those reported by Thayer et al. (2015) based on serum profiles of unconjugated BPA-d6 and total BPA-d6 in volunteers orally exposed to

BPA-d6 (mean initial $t_{1/2}$ (± SD) of 0.52 ± 0.28 and 0.26 ± 0.11 h, respectively; corresponding elimination $t_{1/2}$ from the terminal phase of 5.6 ± 1.2 h and 6.4 ± 2.0, respectively). However, concentrations of unconjugated BPS-d8 at peak levels (C_{max} of 158 ± 57 nmol/L) were found in much higher proportion compared to those of BPA-d6 (C_{max} of 6.5 ± 3.2 nmol/L) and $AUC_{0\to\infty}$ of unconjugated BPS-d8 was on average 19 times higher than that of BPA (23 ± 6.2 nmol/L × h) for a similar administered molar dose (0.39 µmol BPS-d8/kg bw versus 0.43 µmol BPA-d6/kg bw) (see Table 2 for BPS-d8 values in our study). Furthermore, on average, molar percent of the administered dose excreted in total as BPS-d8 and BPSG-d8 in the urine of volunteers was 1.72 ± 1.3 and 54 ± 10%, respectively, while values of 0.11 ± 0.19 and 87% ± 6.9% were observed for BPA-d6 and BPAG-d6 after oral dosing with BPA-d6 in volunteers (Thayer et al., 2015). This suggests that the active unconjugated form of BPS is more prevalent than that of BPA in humans.

Although there are known animal-to-human differences in the glucuronidation, Gayrard et al. (2019b) showed that a significant fraction of BPS dose in orally exposed piglets reached the systemic circulation in the unconjugated form (57.4% on average). On the other hand, BPA underwent a significant first-pass phase II metabolism to BPAG after oral exposure such that, on average, only 0.5% of the administered dose was found unconjugated in plasma. These authors also found that plasma clearance of BPS was 3.5 times lower than that of BPA. From the piglet data, a toxicokinetic model was also developed by these authors and predicted that virtually all (99%) of BPS oral dose was absorbed as compared to about 77% on average for BPA. Their model also suggested that BPS conjugation did not occur in enterocytes but rather in the liver (44% of absorbed fraction) contrary to BPA for which an extensive first-pass glucuronidation in the gastrointestinal (GI) tract (44%) and liver (99% of the BPA that did not undergo conjugation in enterocytes) was simulated. In turn, systemic bioavailability of active unchanged BPS was estimated to be 57.4% versus 0.5% for BPA in piglets. Our human data on BPS compared to those of Thayer et al. (2015) also suggest a difference in the kinetics of BPS and BPA (in particular in the glucuronoconjugation).

4.3. Toxicokinetics of BPS after dermal exposure and comparison with BPA

Dermal time course values were too close to the limit of detection or quantification to provide a direct comparison with the oral kinetic data. Nonetheless, as observed following oral exposure, our results also show that BPS is very rapidly absorbed in the systemic circulation and eliminated from the body following dermal application of a low dose; however, elimination did not appear to be complete after 72 h, suggesting a somewhat longer residence time after dermal than oral exposure. Similarly, Liu and Martin (2017) reported that BPA was eliminated more slowly from the body after dermal contact with thermal receipts containing BPA compared to dietary exposure; they also reported that the proportion of unconjugated BPA in the systemic circulation was higher after dermal exposure than ingestion. In animals, Gingrich et al. (2019) determined the time courses of total BPS (conjugated and non conjugated) in the plasma of pregnant sheep following subcutaneous exposure of 0.5 mg/kg of BPS and the calculated MRT (6.7 ± 0.3 h) was very close to that calculated in the current study for BPS following oral exposure.

Results of our study further highlight that the dermal absorption fraction is very low, that is < 0.1% based on BPS and BPSG profiles, which is smaller than that reported for BPA by Demierre et al. (2012) from human skin explants. In the latter study, on average (± SD), 56.9 ± 4.9% of the applied ^{14}C-BPA remained on the skin surface 24 h after application on skin explants and 8.6 ± 2.1% were found in the receptor fluid at 24 h post-application (n = 7 skin membranes prepared from two skin explants). With an experimental protocol similar to that of Demierre et al. (2012) but using fresh metabolically-active human skin explants and different ^{14}C-BPA exposure levels, Toner et al. (2018) obtained lower recoveries of ^{14}C-BPA in the receptor fluid - translating in a dermal absorption of 2–4% - and found a certain metabolism in the skin. More recently, Liu and Martin (2019) compared the percutaneous absorption and biotransformation of BPS and BPA *in vitro* using human epidermal cells (EpiDerm™ EPI-212 tissue constructs, a three-dimensional tissue model consisting of normal human epidermal keratinocytes on tissue culture inserts). They found that the permeability coefficient of BPS was lower than that of BPA. At both doses, < 10% of total BPS had migrated into the receiver solutions, whereas 43–46% of total BPA were recovered in receiver solutions (0.003–0.009 cm/h versus 0.033–0.036 cm/h after application of 1.5 or 7.7 µg/cm^2). They also reported a limited metabolism in the skin, given that more than 70% of total BPS and total BPA were found in the unconjugated form in skin tissue and in receiver solutions. These authors further compared the dermal penetration of BPS (not reported to be deuterated) and BPA-d6 from cumulative urinary excretion in volunteers simulating handling of thermal receipts and found that free BPS levels in 48-h urine collections, expressed as a proportion of total urine bisphenol, were higher than free BPA-d6 (6.9 ± 2.8% versus 2.7 ± 1.9%). In line with these results, the average molar ratio of BPS-d8/(BPS-d8 + BPSG-d8) in the urine of volunteers of our study can be calculated at 4.3%.

It is to be noted that BPS was applied on the forearm on our study. Ideally, it would have been better to apply BPS on a hand, which is the main exposure site, but this is hardly feasible. During biomathematical modeling, the permeability constant estimated from the data obtained can be adjusted to take into account the greater permeability of the skin of the forearm compared to that of the hands. Furthermore, the choice of the application area (40 cm^2) was to allow applying from 10 to 20 µL/cm^2 for a body weight between 50 and 70 kg, in accordance with the OECD (2004) recommendations of 10 µL/cm^2. This suspension applied to an area of 40 cm^2 translates into an applied dose of 1.25 to 1.75 mg/cm^2 (body weight between 50 and 70 kg), which is in the same order of magnitude as the maximum dose recommended by the USEPA (1998) of 1 mg/cm^2 to ensure the absence of saturation of the absorption process. It is also in the same order of magnitude as the maximum exposure dose of skin estimated for BPA when handling cash receipts and determined from the maximal transfer coefficient of BPA (21522 ng/s) and the skin surface area of hands in contact with tickets (23.5 cm^2, *i.e.* 0.6 mg/cm^2) (Bernier and Vandenberg, 2017).

In addition, BPS was applied on the skin in a solution of phosphate buffer (0.1 M) containing 1% sodium carboxymethylcellulose of medium viscosity (100 mg/mL) in our study, this medium being recommended as it does not modify the permeability of the skin (Vlaia et al., 2016). Amounts remaining on the skin at 6 h postdosing, hence at the time of washing, were not quantified. There could be differences in the absorption rate depending on the dissolution/suspension vehicle. In particular, dermal absorption rate of BPS through manipulation of thermal papers (Björnsdotter et al., 2017; Hines et al., 2017; Liu and Martin, 2017; 2019; Ndaw et al., 2018; Russo et al., 2017) may be somewhat different from that observed in the current work. In particular in a study in volunteers manipulating thermal receipts, Hormann et al. (2014) showed that dermal absorption - as assessed from serum and urinary levels of BPA, BPAG and sulfo-conjugate of BPA (BPAS) - was enhanced in individuals who had used hand sanitizers immediately prior to holding receipts for 45 s compared to when handling receipts with dry hands. Interestingly, at least one volunteer of that study showed relatively high BPAS levels.

4.4. Interest of the toxicokinetic data for the purpose of biomonitoring

From a biomonitoring perspective, the data collected in this study will be used to develop a toxicokinetic model specific to BPS, which will allow dose reconstruction of BPS from plasma or urinary BPS and BPSG measurements in exposed individuals. Similar to BPA, the glucuronoconjugate of BPS is confirmed to be the main form of BPS excreted in urine and exhibited kinetics similar to the active unconjugated BPS

moiety in humans with a short half-life. The rather short half-life of BPS-d8 and BPSG-d8 observed in our study following oral exposure suggests that steady-state equilibrium should be rapidly reached in individuals repeatedly exposed by ingestion, but there could be significant fluctuations in plasma and urinary concentrations for punctual BPS exposure through ingestion of contaminated food and drinks. Furthermore, the dermal data show a limited skin absorption such that dermal dose must be very high to contribute significantly to the absorbed dose by multiple routes, that is following combined oral, dermal and respiratory exposure. However, the apparent longer residence time of BPS in the body after dermal exposure suggests that a possible accumulation may be more important after dermal exposure than oral exposure upon repeated daily exposure.

4.5. Conclusions

Overall, the current data allowed to further document the toxicokinetics of BPS in humans. It confirmed the rapid appearance and elimination of the compound and its conjugate in the systemic circulation following oral exposure, with a majority of the dose being rapidly converted to the BPSG form. The present data also evidenced major differences between BPA and BPS kinetics with much higher systemic levels of active BPS than BPA, an observation explained by a higher oral bioavailability of BPS than BPA. These data indicate that the replacement of BPA by BPS could lead to increased exposure to a hormonally active substance. This work also confirmed the limited dermal absorption of BPS compared to oral absorption, but with a potentially longer residence time observed from plasma and urinary rate time courses after dermal exposure. These data should be useful for the development of a toxicokinetic model for a better interpretation of biomonitoring data. The current kinetic data is limited to a few volunteers for feasibility and cost reasons; as highlighted by Andra et al. (2016), future studies are needed to document the variability in phase II conjugation between individuals (male, female, pregnancy status), which may affect interpretation of biomonitoring data.

CRediT authorship contribution statement

Imèn Khmiri: Investigation, Visualization, Writing - original draft. **Jonathan Côté:** Methodology, Formal analysis, Visualization, Investigation, Writing - review & editing. **Marc Mantha:** Methodology, Investigation, Formal analysis. **Rania Khemiri:** Methodology, Investigation. **Marlène Lacroix:** Methodology, Investigation, Writing - review & editing, Funding acquisition. **Clémence Gely:** Validation. **Pierre-Louis Toutain:** Formal analysis, Writing - review & editing. **Nicole Picard- Hagen:** Methodology, Writing - review & editing, Funding acquisition. **Véronique Gayrard:** Methodology, Writing - review & editing, Funding acquisition. **Michèle Bouchard:** Conceptualization, Methodology, Investigation, Formal analysis, Resources, Supervision, Writing - original draft, Writing - review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105644.

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