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Environment International 171 (2023) 107722

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

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ARTICLE INFO

Handling Editor: Marti Nadal

Keywords:
Bisphenols
Toxicokinetics
Oral exposure
Mixture
Pig model

ABSTRACT

Due to the restrictions of its use, Bisphenol A (BPA) has been replaced by many structurally related bisphenols (BPs) in consumer products. The endocrine disrupting potential similar to that of BPA has been described for several bisphenols, there is therefore an urgent need of toxicokinetic (TK) data for these emerging BPs in order to evaluate if their internal exposure could increase the risk of endocrine disruption. We investigated TK behaviors of eleven BPA substitutes (BPS, BPAF, BPB, BPF, BPM, BPZ, 3-3BPA, BP4-4, BPAP, BPP, and BPFL) by intravenous and oral administrations of mixtures of them to piglets and serial collection of blood over 72 h and urine over 24 h, to evaluate their disposition. Data were analyzed using nonlinear mixed-effects modeling and a comparison was made with TK predicted by the generic model HTTK package. The low urinary excretion of some BPs, in particular BPM, BPP and BPFL, is an important aspect to consider in predicting human exposure based on urine biomonitoring. Despite their structural similarities, for the same oral dose, all BPA analogues investigated showed a higher systemic exposure (area under the plasma concentration-time curve (AUC) of the unconjugated Bisphenol) than BPA (2 to 4 fold for 3-3BPA, BPAF, BPB and BPZ, 7–20 fold for BP4-4, BPAP, BPP, BPFL, BPF and BPM and 150 fold for BPS) due mainly to a considerable variation of oral bioavailability (proportion of BP administered by oral route that attains the systemic circulation unchanged). Given similarities in the digestive tract between pigs and humans, our TK data suggest that replacing BPA with some of its alternatives, particularly BPS, will likely lead to higher internal exposure to potential endocrine disruptive compounds. These findings are crucial for evaluating the risk of human exposure to these emerging BPs.

1. Introduction

Due to restrictions on the use of Bisphenol A (BPA) in food contact plastics, manufacturers have replaced BPA with structurally related substances. Some of these BPA substitutes, like BPS, BPF, BPAF, BPAP, BPB, BPP, and BPZ, have been detected in food (González et al., 2020; Karsauliya et al., 2021; Liao and Kannan, 2013, 2014a; Morgan and Clifton, 2021; Zoller et al., 2015), personal care products (Liao and Kannan, 2014b; Lu et al., 2018) and indoor dust (Liao et al., 2012; Wang et al., 2015; Zhang et al., 2020). Human exposure to bisphenols (BPs) is therefore ubiquitous. Recent biomonitoring studies have shown a change in the distribution of BPs detected in human urine between the late 2000s and the late 2010s, with a decrease in the BPA exposure in parallel with an increase in detection rates and urine concentrations of

Abbreviations: TK, Toxicokinetics; BPs, Bisphenols; BPF, Bisphenol F; BPA, Bisphenol A; BPB, Bisphenol B; BPAF, Bisphenol AF; 3-3BPA, 2,2-Bis(4-hydroxy-3-methylphenyl)propane; BPZ, Bisphenol Z; BPM, Bisphenol M; BPS, Bisphenol S; BP4-4, 4,4′-Dihydroxybiphenyl; BPAP, Bisphenol AP; BPFL, Bisphenol FL; BPP, Bisphenol P; DHDPE, Bis(4-hydroxyphenyl) Ether 4,4′-Oxydiphenol. LOQ, limit of quantification; QC, Quality Control; UHPLC, ultra-high performance liquid chromatography; NCA, non-compartmental approach; NLME, NonLinear Mixed Effect modeling; HTTK, High-Throughput Toxicokinetics. Vss, steady-state volume of distribution; AUC, area under the concentration-time curve; MRT, Mean Residence Time; Cl, Clearance; F, Bioavailability; Cl_F, apparent clearance; HL, terminal Half- life.

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https://doi.org/10.1016/j.envint.2022.107722
Received 27 October 2022; Received in revised form 20 December 2022; Accepted 24 December 2022
Available online 26 December 2022

BPS and BPF (Frederiksen et al., 2020; Gyllenhammar et al., 2017; Gys et al., 2021; Ye et al., 2015). Although BPA, BPS, and BPF remain currently the most frequently detected BPs in human urine, other emerging BPs such as BPAF, BPB, BPP, BPZ, and BPAP are increasingly being detected (Chen et al., 2018; Gys et al., 2021; Lucarini et al., 2020; Zhang et al., 2020).

Several BPs can cross the placental barrier (Gély et al., 2021b) and have endocrine disrupting potential similar to that of BPA (Pelch et al., 2019a; Rochester and Bolden, 2015; Rosenmai et al., 2014). Because developing foetuses are particularly vulnerable to endocrine disruption, pregnancy is considered as a critical period for BPs exposure. BPA, BPB, BPZ, and BPAP have been shown *in vitro* to exert a thyroid disrupting effect due to their binding affinity to the thyroid hormone receptor alpha and thyroxine-binding globulin (Beg and Sheikh, 2020). Moreover, BPA, BPAF, BPAP, BPB, BPE, BPF, BPS, BPP, and BPZ have estrogen receptor alpha agonist activity and, except for BPS, androgen receptor antagonist activity (Kojima et al., 2019). Furthermore, *in vivo* studies show that prenatal exposure to BPA, BPE, and BPS induces male and female reproductive dysfunctions (Shi et al., 2019, 2018). Altogether, these data suggest that these structurally related BPs could induce endocrine disrupting effects (Moreman et al., 2017; Pelch et al., 2019a; Rochester and Bolden, 2015; Rosenmai et al., 2014; Siracusa et al., 2018; Usman and Ahmad, 2016). Thus, the substitution of BPA with structurally related BPs may be of concern for human health.

Toxicokinetics (TK) provides critical information for integrating hazard toxicity and exposure assessments in order to determine potential risk of exposure to chemical compounds in human. Indeed, the amount of BP that can reach the target tissues and exert effects are dependent on plasma concentrations, these latter being related to the dose by a key TK parameter, namely the blood (plasma) clearance in addition to the bioavailability. Bioavailability, which corresponds to the amount of substance reaching the systemic circulation unchanged, is determined by both the extent of gastrointestinal absorption and of presystemic elimination by gut and hepatic first-pass effect when exposure occurs via the oral route. TK is needed to extrapolate toxicological data from *in vitro* or from animal models to human and to compare internal exposure of unconjugated (active) BP producing deleterious effects to those that can be expected from realistic human external exposure scenarios, i.e. the whole dose to which human is exposed. This information is also crucial for determining the dose and concentration range that should be used for *in vitro* testing. Previous BPA and BPS TK studies performed in human or in animal models show that the oral BPS systemic exposure (Area Under the plasma Concentration-time curve (AUC) of the unconjugated BPS) is on average several hundred times higher than for BPA for the same dose level due to its higher systemic availability and its lower plasma clearance (Collet et al., 2015; Gayrard et al., 2020, 2019; Khmiri et al., 2020). *In vitro* studies show that BPF, BPB, and BPAF, like BPA and BPS, are metabolized predominantly by the glucuronidation reaction (Gramec Skledar and Peterlin Mašič, 2016). In rodent, BPF is mostly metabolized in sulfate conjugate after oral administration (Cabaton et al., 2006) and BPAF, like BPA, is rapidly and extensively conjugated with a low oral bioavailability around 1 % (Waidyanatha et al., 2019, 2021).

Thousands of chemicals have been profiled by high-throughput screening programs in order to screen for their potential bioactivity. However, linking these hazard predictions to human exposure is required to determine potential chemical risk. In the context of reduction of *in vivo* animal toxicological testing, high-throughput TK models offer an alternative approach to predict the *in vivo* TK properties using *in vitro* measurements and chemical structure-based properties (Pearce et al., 2017). Among the software packages developed, the High-Throughput Toxicokinetic (HTTK) open source R package, a platform created by the U.S. EPA's National Center for Computational Toxicology is easy to use for a wider range of chemicals and readily available.

Due to the lack of adequate TK data for emerging BPs, the objective of our experimental study was to evaluate and compare the toxicokinetic properties determining the internal exposure of twelve unconjugated (active) BPs, i.e., BPA, BPS, BPAF, BPB, BPF, BPM, BPZ, 3-3BPA, BP4-4, BPAP, BPP, and BPFL after oral exposition. These twelve analogs were selected because of their industrial use (Caballero-Casero et al., 2016; "ECHA," n.d.; Liao and Kannan, 2013) and their detection in recent biomonitoring studies (Chen et al., 2018; Gys et al., 2021; Lucarini et al., 2020; Mok et al., 2022; Zhang et al., 2020). Two routes of administration were performed, intravenous administration to evaluate TK parameters such as plasma clearance and volume of distribution and oral route to determine the oral bioavailability of these 12 BPs. In the context of reducing *in vivo* animal toxicological testing, mixtures of these twelve BPs were administrated intravenously and by gavage to piglets, a species relevant to the human in terms of digestive function (Gonzalez et al., 2015; vom Saal and Welshons, 2006). Our mixture approach was validated for BPA and BPS by comparing the TK parameters obtained in this study with those previously determined when they were administered individually (Gayrard et al., 2019). We also used the HTTK package to predict TK parameters of these BPs and compare them with our *in vivo* experimental data.

2. Materials and methods

2.1. Animals

The experimental protocol was authorized by the French Ministry of Research under the number #22452_2019101609073159. The experiment was carried out on nine female piglets of Large White breed from a French farm (Tarn et Garonne, France). They were aged from 28 (weaning ages) to 51 days (study end) and were weighed between 8.75 kg and 21.3 kg, respectively with a mean (±SD), 11.2 ± 1.4, 13.7 ± 1.8 and 17.0 ± 2.2 kg in the first, second and third period. The piglets were fed with a flour-based growth food (PS2, Solieval, Villefranche de Rouergue, France) given ad libitum except the days of the administration for which piglets were fasted overnight and were give a standard meal 3 h post-dosing. Water was given ad libitum. The piglets were housed at room temperature (24 °C) with a 12 h light/dark cycle. During the first 24 h of sampling, the piglets were housed individually in stainless steel cages.

2.2. Experimental design

The experiment was designed as a cross-over in three periods separated by 72 h. At each period, three piglets received a mix of BPs containing BPA, BPAF, BPB, BPF, BPM, BPZ, and 3-3BPA each at a nominal dose of 6 μmol/kg bodyweight (BW) by intravenous route (IV), three other piglets received a mix of BPs containing BPS, BP4-4, BPAP, BPP, and BPFL each at a nominal dose of 6 μmol/kg BW by IV and the three other piglets received a mix of all 12 BPs each at a nominal dose 200 μmol/kg BW by oral route. For the IV route, only 5 to 7 BPs were administered in a mixture because of the poor solubility of BPs in DMSO and in pig blood. Another bisphenol, DHDPE [Bis(4-hydroxyphenyl) Ether 4,4′-Oxydiphenol; CAS number: 1965–09-9] was also administered in the mixture at the same corresponding molar dose but was not considered due to mass detection difficulties. The IV administrations were carried out via an indwelling catheter inserted into the auricular vein just before the administration (0.1 mL of solution by kg BW) and oral administrations were carried out by gastric intubation (2 mL of solution by kg BW). The IV and oral BPs doses were selected based on previous TK data (Gayrard et al., 2019) allowing to quantify plasma concentrations for a few hours, i.e., sufficiently long to observe the terminal phase slope and allow calculation of TK parameters considering the performance of the assay.

2.3. Samples collection

Serial jugular venous blood samples (2 mL) were taken before and at

2, 5, 15, 30, 60 min and at 2, 3, 4, 6, 8, 11, 24, 34, 48, and 72 h after BPs administration. They were collected in heparinized polypropylene tubes, immediately chilled in ice and centrifuged for 10 min at 3000 × g at 4 °C. Plasma was stored in polypropylene tubes at −20 °C until assay. Total urine was collected over 24 h with collection times at 0, 3, 6, 8, 11, and 24 h after BPs administration. Total urine was filtered through a nylon mesh (250 μm) and collected in glass containers and chilled in ice. The volume of each urine sample and the sampling time were recorded. Urine samples were immediately chilled on ice and centrifuged for 10 min at 3000 × g at 4 °C and stored at −20 °C until assay.

2.4. Test materials and treatments

All materials for the preparation of solutions, including the materials used for sampling, processing and analysis, were made of glass or BPA and BPS-free plastic (polypropylene). Bisphenol S (BPS, purity ≥ 98 %), Bisphenol A (BPA, purity ≥ 99 %), 2,2-Bis(4-hydroxy-3-methylphenyl) propane (3-3BPA, purity ≥ 97 %), Bisphenol FL (BPFL, purity ≥ 97 %), Bisphenol Z (BPZ, purity ≥ 98 %), 4,4′-Dihydroxybiphenyl (BP4-4, purity ≥ 97 %), Bisphenol AP (BPAP, purity ≥ 99 %), Bisphenol AF (BPAF, purity ≥ 97 %), Bisphenol M (BPM, purity ≥ 99 %), and Bisphenol BP (BPBP, purity ≥ 98 %) were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Bisphenol B (BPB, purity ≥ 98 %), Bisphenol F (BPF, purity ≥ 96 %), Bisphenol P (BPP, purity ≥ 98 %) and five isotopes-labeled standards, namely, BPAF-d4 (purity ≥ 98 %), BPAP-d5 (purity ≥ 97.5 %), BPP-d16 (purity ≥ 98 %), BPS-d8 (purity ≥ 97 %), and BPA$^{13}C_{12}$ (purity ≥ 98 %), used as internal standards (IS), were purchased from Toronto Research Chemicals (Toronto, Canada) (Fig. 1).

Dimethyl sulfoxide (DMSO) and corn oil and β-glucuronidase type HP-2 were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Methanol was LC/MS quality and purchased from Fisher Scientific (Illkirch, France).

For IV administrations, BPs powders were dissolved in DMSO at a concentration of 60 mM. For administrations by gavage, BPs powders were dissolved in a DMSO-corn oil (50:50, v:v) at 100 mM and mixed before administration to obtain an emulsion. The volume administered to pigs were adjusted to the individual BW recorded during the day preceding BPs administrations. The concentrations of dosing solutions were verified by Acquity ultra performance liquid chromatography I Class coupled to PhotoDiode Array detector (UPLC-UV, Waters, Milford, MA, USA) and actual individually administrated doses for each BP were used in non-compartmental approach (NCA) and NonLinear Mixed Effect (NLME) modeling (Table S1).

2.5. Samples analysis

2.5.1. Quantification of urinary concentrations

For the twelve BPs, the unconjugated and total bisphenol were quantified in urine by ultra-high performance liquid chromatography coupled to UV detection (UHPLC-UV). Briefly, the unconjugated BPs were extracted from urine (400 μL of sample) and 100 μL of internal standard (BPBP at the concentration of 5 μg/mL) with Solid Phase Extraction on HR-XAW cartridge (Macherey Nagel, Düren, Germany) under vacuum with a protocol adapted from Gely et al. (Gély et al., 2021a). After elution with methanol, samples were evaporated to dryness, reconstituted with 200 μL of methanol:H_2O, 50:50 (v:v). The BPs were separated on a Acquity BEH Phenyl column (100 × 2.1 mm; 1.7 μm) at 0.3 mL/min and 40 °C using H_2O/Methanol gradient elution and detected by UV. The wavelengths for each compound were reported in Table S2. Chromatographic data were monitored by Empower® software (Water, Milford, MA, USA). The method was validated in urine according to the European Medicine Agency Guidelines (European Medecines Agency Science Medicines Health, 2011) with two calibration curves: high concentrations, from 0.5 to 20 μg/mL, and low concentrations from 0.01 μg/mL to 2 μg/mL were quantified at several wavelengths (Table S2). Blanks and quality control samples were used to monitor potential contamination during analysis and precision of the

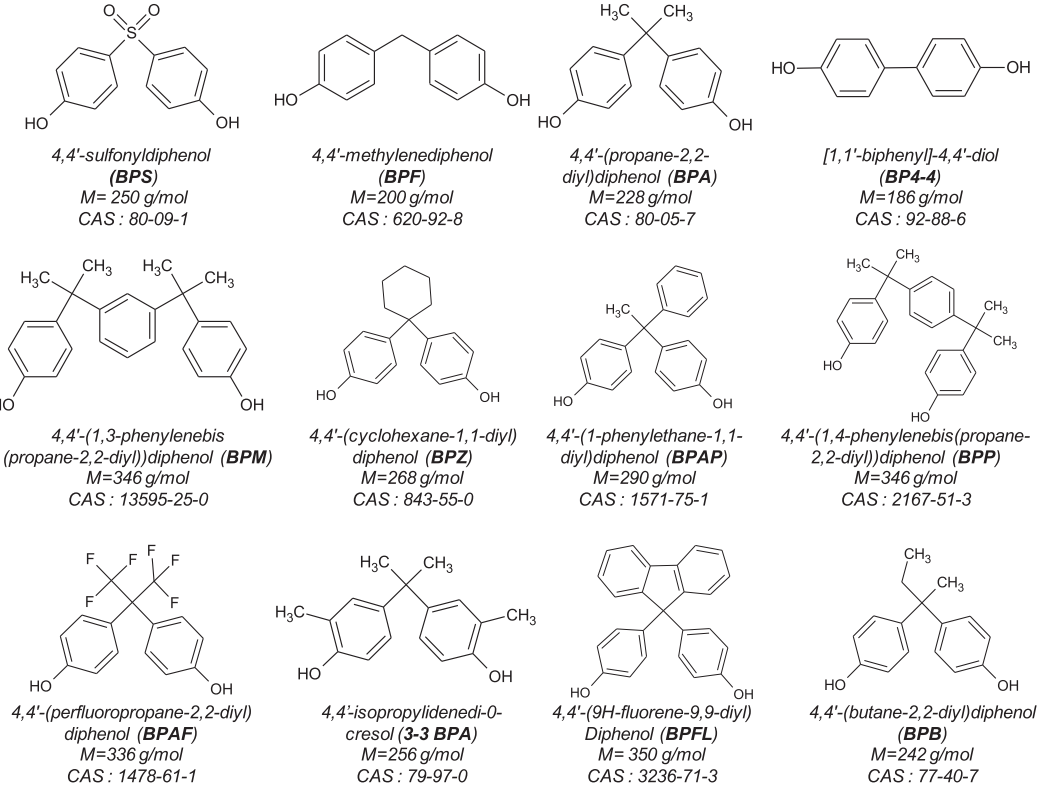

Fig. 1. Molecular structure of the twelve studied BPs.

method. The accuracy ranges of three QCs (0.03, 0.15 and 1.5 μg/mL, low calibration range) were 89–114 %, with mean intra- and inter-day coefficients of variation lower than 16 % for all BPs. The accuracy ranges of three QCs (1.5, 7.5 and 15 μg/mL, high calibration range) were 98–120 %, with mean intra- and inter-day coefficients of variation lower than 16 % for all BPs. For the LOQ (limit of quantification, 0.01–1 μg/mL, according to BPs), the accuracy ranges were 81–121 % with a CV % inter-day (n = 6) lower than 19 %. Performances of the assay (LOQ, model linearity, accuracy and inter and intra-day CV) for each BP were reported in Table S3a, b.

Total bisphenols were simultaneously quantified in urine by UHPLC-UV, after an enzymatic hydrolysis. Conjugated bisphenols were hydrolyzed by adding 100 μL of sodium acetate buffer containing β-glucuronidase (100,000 units/mL) and BPBP at 10 μg/mL as IS to 100 μL of sample. Samples were incubated during 2 h at 37 °C and 500 rpm. After the incubation, 200 μL methanol were added and shaken at 10 °C for 1 min at 1400 rpm and centrifuged at 20000 × g at 4 °C. Twenty μL of supernatant were eluted under the conditions previously described for unconjugated BPs. Blank samples were used to check the absence of contamination during assay. To validate our hydrolysis process, the linearity of the concentrations after serial dilutions of one urine sample and of BP-G solutions commercially available (BPA-G, BPF-G, BPS-G and BPAF-G) was checked. For a few urine samples with high levels of BPs, the efficiency of our hydrolysis process on the enzymatic deconjugation of urinary BPs was assessed by comparison with a hydrolysis using a 10-fold higher concentration of β-glucuronidase.

The assay was validated in pig urine according to the European Medicine Agency Guidelines from 0.2 to 100 μg/mL. The accuracy ranges of five QCs (0.6, 3, 7.5, 30 and 75 μg/mL) were 90–121 %, with intra-and inter-day precision lower than 20 % for all BPs. The accuracy ranges were 80–122 %, with intra-and inter-day precision lower than 12 % for all BPs at LOQ (between 0.2 and 1 μg/mL according to BPs). Performances of the method including LOQ, model linearity, accuracy and inter and intra-day CV results were reported in Table S4.

2.5.2. Quantification of plasma concentrations

Unconjugated bisphenols in plasma were simultaneously quantified after protein precipitation with ultra-performance liquid chromatography coupled to tandem mass spectrometry (Acquity-2D UPLC Xevo triple quadripole, Waters, Milford, MA, USA). Briefly, samples (100 μL) were purified by protein precipitation with 200 μL of methanol containing IS, $BPA_{13}C^{12}$, BPSd8, BPAFd4, BPPd16 and BPAPd5 (25 ng/mL). These internal standards were selected according to their commercial availability but also for their physicochemical properties, relevant to the 12 studied BPs, in terms of elution range and hydrophobicity, thus limiting the variability of the ionization due to both the matrix and the mobile phase. The mixture was shaken at 10 °C for 10 min at 1400 rpm and centrifuged at 20,000 g and 4 °C for 10 min. Then, the supernatant was eluted on BEH Phenyl column using H_2O/methanol gradient [0.3 mL/min, 40 °C] and detected in negative electrospray ionization using multiple reaction monitoring (MRM) mode. Chromatographic data were monitored by Targetlynx® software (Water, Milford, MA, USA). The MRM transitions of BPs and their respective cone voltages and collision energies were detailed in Supplemental Material Table S5. Two injection volumes were used, 20 μL for concentrations lower than 10 ng/mL and 10 μL for concentrations higher than 50 ng/mL. For the intermediate concentrations, a double injection was performed. Blank samples were used to check the absence of contamination during assays and the method was validated according to the European Medicine Agency Guidelines in piglet plasma over the calibration range between 2.5 and 5000 ng/mL. The upper-LOQ and lower-LOQ were evaluated for each BP and the performances of the method were reported in Table S6. The accuracy ranges of five QCs (2.5, 7.5, 25, 250 and 2500 ng/mL) for the twelve BPs were 72–122 %, with intra- and inter-day precisions lower than 25 % for all BPs, except for BPB, BPM and BPZ for which the accuracy was between 52 and 117 % and intra- and inter-day precisions were lower than 50 %. At LOQ (2.5–5 ng/mL, according to BPs), the accuracy ranges were 91–123 % with an inter-day CV (n = 6) lower than 26 %.

During each samples run, LOQs were adjusted to the performances of the assay and were between 2.5 and 25 ng/mL (Table S7).

2.6. Toxicokinetic analysis

Toxicokinetic analysis were performed using Phoenix WinNonlin® (version 8.3, Certara, L.P., Princeton, NJ USA). The cumulated molar amount of urinary excreted BPs was calculated by multiplying the molar concentration by the excreted urine volume at each sampling time.

2.6.1. Non-compartmental analysis

Plasma concentration–time profiles of the twelve BPs were first analyzed according to a NCA. The area under the concentration time curve ($AUC_{0\text{-tlast}}$) and the area under the first moment curve ($AUMC_{0\text{-tlast}}$) from dosing time (t = 0) to the time of the last measurable concentration were calculated using the linear trapezoidal rule after IV and oral dosing. The area under the plasma concentration time curve from dosing time to infinity ($AUC_{0\text{-inf}}$) was obtained by adding to $AUC_{0\text{-tlast}}$, the area extrapolated from the time of the last measurable concentration to infinity by dividing the last quantifiable plasma concentration by the slope of the terminal phase (λ_z), as estimated by linear regression using the best-fit option of Phoenix®. The clearance (Cl) and apparent clearance (Cl_F) were respectively estimated after IV and oral administrations by dividing the administered dose by the $AUC_{0\text{-inf}}$ calculated for each analyte. The plasma clearance (Cl), steady-state volume of distribution (Vss) and Mean Residence Time (MRT) were computed using classical equation with extrapolation to infinity (Rowland and Tozer, 1995). The oral bioavailability (F) was estimated by the ratio of $AUC_{0\text{-inf}}$ of the unconjugated bisphenols obtained after oral and IV dosing, normalized by the respective IV and oral dose.

2.6.2. TK modeling. In a second step, we used a NLME modeling approach (also known as a population model) for each substance to analyze simultaneously all data obtained from the 9 pigs after both IV and oral administrations and to provide robust estimates of the typical TK parameters. For the oral route, visual inspection of the plasma concentration profiles suggested some discontinuous absorption with rebounds of plasma concentrations and the oral data were empirically modeled by sequentially including in the model different absorption rate constants (from 1 to 3). The typical TK parameters of the BPA, BPAF, BPB, BPF, BPM, BPZ, 3-3BPA, BP4-4, BPAP, BPP, BPFL were estimated using a two-compartment model and one (BP4-4, BPM, BPP), two (3-3BPA, BPA, BPAF, BPB, BPF, BPFL, BPS, BPZ) or three (BPAP) rates absorption constant. For all BPs, the inclusion of one or more rate constants of absorption and the two-compartment model depicted in Fig. 2 was selected based on the likelihood ratio test, the Akaike Information Criterion (AIC), and inspection of different diagnostic plots to better fit simultaneously the merged plasma concentrations obtained after both IV and oral administrations.

The primary parameters of the model, namely the volume of distribution of the central compartment (V_c), the first-order rate constants (K_{10}, K_{12}, K_{21} and K_{abs} or K_{a1}, K_{a2}, K_{a3}, according to the BP) and the bioavailability (F) were estimated by minimizing an objective function value (OFV) expressed as minus twice the log of the likelihood estimation (-2LL) using the Laplacian engine. Oral bioavailability was estimated by applying an ILogit transformation to preclude a value greater than 1.

The Between-Subject Variability (BSV) was evaluated using the exponential model using Equation (1):

$$\theta_{parameter\_i} = \theta_{tv\_parameter} \times Exp(\eta_i) \quad (1)$$

With $\theta_{parameter\_i}$ is the parameter estimated for the i^{th} animal,

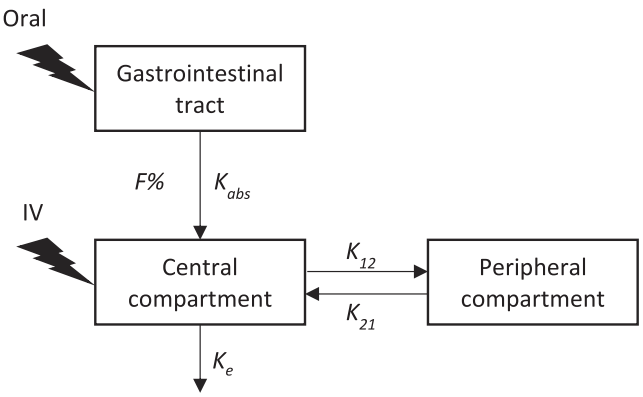

Fig. 2. Schematic representation of the two compartmental model. The model includes both a central and a peripheral compartment. For the oral route, BPs are administered in the absorption compartment (Gastrointestinal tract) and linked to the central compartment with a rate constant of transfer (K_{abs}). The exchanges between the central and the peripheral compartments are bilateral, with k_{12} and k_{21} being the distribution rate constants between the central and the peripheral compartments. Elimination of the BPs was modeled with a first-order rate constant designated K_e. The fraction of BPs that reaches the central compartment unchanged, i.e., the bioavailability, is designated by F%.

$\theta_{tv\_parameter}$ is the typical population value of this parameter and η_i is the deviation associated with the i^{th} animal from the corresponding value of the parameter at the population level. The distribution of η_i was assumed to be normal with a mean of 0 and a variance ω^2. BSV was reported as a coefficient of variation (CV) in the original scale (Equation (2)):

$$CV(\%) = 100 \times \sqrt{\exp(\omega^2) - 1} \quad (2)$$

The shrinkage of random effects toward the means was calculated for the ETAs (Savic and Karlsson, 2009) with equation (3):

$$shrinkage = 1 - \frac{SD(EBE_\eta)}{\omega} \quad (3)$$

Where ω is the estimated variability for the population and SD is the standard deviation of the individual values of the empirical Bayesian estimates (EBE) of η.

The residual variance was modeled using a combined additive and proportional error model, using equation (4):

$$Cobs_{ij} = Cpred_{ij}\left(1 + \varepsilon_{1ij}\right) + \varepsilon_{2ij} \quad (4)$$

where $Cobs_{ij}$ and $Cpred_{ij}$ are respectively the j^{th} observed and predicted concentrations for the i^{th} individual and ε_{1ij} and ε_{2ij} the multiplicative and additive residual errors.

The predictive ability of the model was checked graphically by plotting Visual Predictive Check (VPC) plots to compare the observed data with the prediction interval (20th-80th percentile) of data simulated using the model and obtained from 200 replicates (Figure S1-S3).

Secondary parameters were calculated from the population primary parameters.

The plasma clearance values (Cl) of BPs were estimated using obtained using equation (5):

$$Cl = V_c \times K_e \quad (5)$$

where V_c is the volume of the BPs central compartment, and K_e is the first-order BPs elimination rate constant from the central compartment.

V_{ss} and MRT were estimated with Equations (6) and (7):

$$V_{ss} = V_c \times \left(1 + \frac{K_{12}}{K_{21}}\right) \quad (6)$$

$$MRT = V_{SS}/Cl \quad (7)$$

Where V_{ss}, MRT, V_c as defined above.

The oral bioavailaibility (F) was estimated using equation (8):

$$F = \frac{e^f}{1 + e^f} \times 100 \quad (8)$$

Where f is the ilogit estimate.

The terminal half-life time ($t_{1/2}$) was estimated with the following equation (9):

$$t_{1/2} = \frac{\ln 2}{0.5 \times (K_e + K_{12} + K_{21}) - \sqrt{(K_e + K_{12} + K_{21})^2 - 4 \times K_{21} \times K_e}} \quad (9)$$

with K_e, K_{12}, K_{21} as defined above.

To validate the cocktail approach used to estimate the TK parameters of a mixture of the 12 BPs, the NLME modeling approach enabled the use of several historical information data sets for BPA and BPS to compare TK parameters of BPA and BPS administered in the present study as a mixture of 5–7 (IV) or 12 (oral route) BPs to those previously obtained in the same piglet model, with standard administration protocols, i.e. with the administration of only BPA/BPS (Gayrard et al., 2019). This previous dataset was described in Table S8. To assess the possible cocktail effect, we included in the NLME model a categorical covariate corresponding to the mode of administration (single versus mixture dosing), on two key TK parameters, i.e., Cl and F. The statistical significance was evaluated by considering the confidence interval of these estimate parameters as obtained using a bootstrap method.

2.7. High-throughput toxicokinetics

The R package HTTK (version 2.1.0, https://CRAN.R-project.org/package=httk) on R studio software (version 1.2.5001) was used to predict key TK parameters for each BP taken individually (Pearce et al., 2017). The toxicokinetic models within the R HTTK are parameterized using high-*throughput in vitro* data (plasma protein binding and hepatic clearance), as well as structure-derived physicochemical properties and species-specific physiological data. A one-compartment model is used to compare predictions with *in vivo* data. Total clearance is equal to hepatic metabolism, calculated with a well-stirred model using scaled *in vitro* intrinsic hepatic clearance, and passive glomerular filtration (non-metabolic renal clearance). The effective volume of distribution is calculated by summing the plasma volume and the products of each tissue volume and the *in vitro* ratio of unbound to total concentration in plasma and tissue, as given by the following equation:

$$Vss = V_P + \frac{fu}{fuT} \times V_T \quad (10)$$

Where V_P is the plasma volume, V_T is the tissue volume, and fu and fuT are the fraction of unbound drug in plasma and tissue respectively.

The elimination rate (Ke) is calculated by dividing the total clearance by the volume of distribution. Half-life is calculated by dividing the natural-log of 2 by the elimination rate from the one compartment model. For a 1-compartment model, MRT is calculated as the inverse of Ke.

3. Results

Unconjugated BPs were not detected or were below the limit of quantification in all 26 plasma blank samples except in three samples for BPS and one for BPM, these contaminations were attributed to analytical method carry over. Unconjugated BPs were not detected in the 22 urine blank samples, except for BP4-4, BPB and BPM detected in only one blank sample at a concentration close to LOQ (between 0.01 and 0.02 μg/mL according to BPs). Total BP4-4, BPA, BPAF, BPAP, BPFL, BPM, BPS, BPF and BPP were detected in only 1 to 3 urine blank samples at concentrations close to the LOQ (between 0.2 and 1 μg/mL according to BPs). These data suggested that little to no external contamination had occurred during sample collection, processing, and assay.

3.1. Validation of the mixture approach

The visual inspection of the concentration–time profiles of BPA and BPS obtained with the cocktail approach with those obtained in 27 pigs having received BPS or BPA in a single dose (Gayrard et al., 2019) did not identify any major difference between the two administration modalities (single vs mixture) for both BPS and BPA and for both IV and oral administrations (Figure S4). Plasma clearance and oral bioavailability estimated with the mixture approach were slightly but not significantly higher than those of a single BPA or BPS administration (28 and 11 % respectively for BPS and 29 and 26 % respectively for BPA), indicating that the mixture mode of administration did not affect the disposition of BPA and BPS.

3.2. Urinary excretion of BPs

The time courses of cumulative urinary excretion of all 12 total BPs at fixed intervals over 24 h are depicted in Fig. 3. By 24 h, the mean fractions (±SD) of the BP dose recovered in urine as total BP ranged from 3.0 ± 1.1 % (BPFL) to 76 ± 12 % (BPS) after IV dosing and from 0.9 ± 0.3 % (BPFL) to 59 ± 13 % (BPS) after oral dosing. For BPM, BPP and BPFL, the mean fractional urinary excretion recovered in urine 24 h after oral and IV dosing were both less than 10 % of the administrated dose. About half of the urinary BPs doses were excreted 3 h after IV dosing whereas 6 to 8 h were necessary to reach 50 % of the urinary excretion after oral dosing. By 24 h, unconjugated BPs in urine represented between 0.05 and 0.23 % of the corresponding IV BP dose except for BPS (0.68 ± 0.46 %) and BPF (0.81 ± 0.80 %).

3.3. Internal exposure to BPs after IV and oral dosing

Figs. 4 and 5 depict the time course of the plasma concentration of each of the 12 BPs after IV and oral dosing, respectively, to 8 piglets. After IV dosing, plasma concentrations of all 12 BPs decreased rapidly to below the LOQ of the assay (2.5 to 25 ng/mL depending on the BP) by as early as 30 min for BP4-4, 8 h for BPP, and about 2–4 h for the remaining 10 BPs.

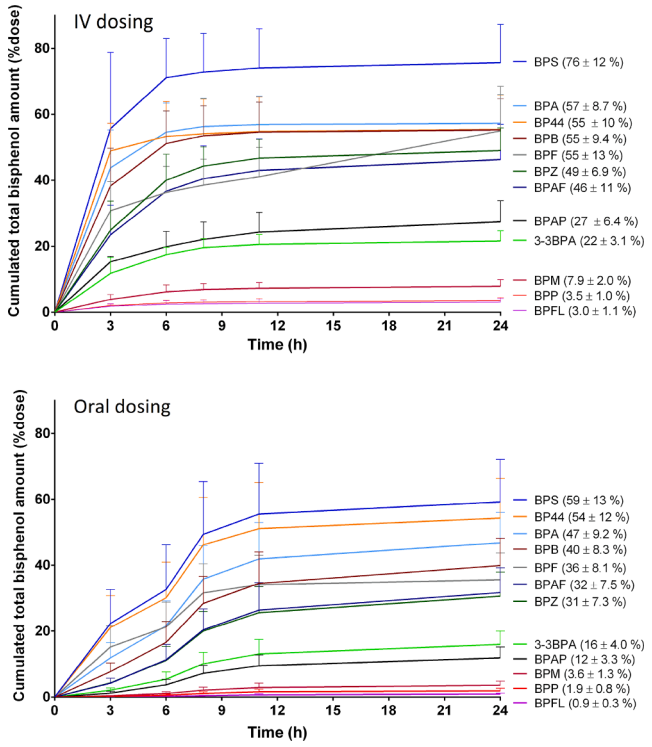

Fig. 3. Cumulated urinary amounts of total BPs in piglets.
The values indicated for each BP are the mean (±SD) fractions of the respective BP dose recovered in urine over 24 h period after a single IV administration at 6 μmol/kg (top) and oral administration at 200 μmol/kg (bottom). It should be noted that the 3 BPs with the lowest urinary excretion are the 3 BPs with the highest MW i.e., BPM, BPP and BPFL.

After oral administration of the mix of 12 BPs, the plasma concentrations increased to maximal values about 1–2 h for BPS, BP4-4, BPA, BPB, BPF, and BPAF and about 3–4 h for 3-3BPA, BPAP, BPP, BPFL, BPM and BPZ (Table 1). The average maximal plasma concentrations of BPS relative to the dose (171 ± 56 nmol/L per μmol/kg BW) were more than 100 times higher than the maximal values reached for 3-3BPA, BPA, BPAF, BPB and BPZ (ranging from 0.47 to 1.31 nmol/L per μmol/kg BW) and about 13 to 66 times higher than BP4-4, BPM, BPFL, BPP, BPAP and BPF (ranging from about 2.57–12.97 nmol/L per μmol/kg BW). Furthermore, the individual time-profiles of 3-3BPA, BP4-4, BPB, BPF, BPP and BPZ in plasma showed a rebound of plasma concentrations around 4–10 h, suggesting a possible enterohepatic recirculation.

Table S9 and 1 give the NCA estimates of TK parameters obtained after IV and oral dosing, respectively. The BPS systemic exposure after oral dosing ($AUC_{0\text{-last}}$ value relative to the dose) was on average between 5 and 30 times higher than that of BPP, BPFL, BPF, BP4-4, BPM and BPAP, between 40 and 100 times higher than that of BPAF, BPZ, BPB and 3-3BPA and 190 times higher than that of BPA (Table 1).

3.4. TK modeling

Figures S1-S3 show the results of the Visual Predictive Check of the model for the 12 BPs after IV and oral dosing. For each BP, the observed quantiles (20 %, 50 %, and 80 %) are reasonably well overlaid by the corresponding predictive check quantiles, indicating that the model was able to capture the general trend of the BP dispositions.

Table 2 shows the population parameters of the 12 BPs, determined from the estimated primary parameters of the model. Plasma clearance of bisphenols ranged from 1.28 to 4.95 L/kg/h, with BPS plasma clearance (1.28 L/kg/h) about 2 to 4 times lower than that of all other BPs. Steady-state volume of distribution (Vss) ranged between 0.67 (BPS) and 7.71 (BPZ) L/kg, with a Vss around 2 L/kg for 3-3BPA, BPAP, BPFL, BPA, BPAF, BPB and BPF and higher than 4 L/kg for BP4-4, BPP, BPM and BPZ. Mean BPS Vss (0.67 L/kg) was about 3 to 12 times lower than that of other BPs. The persistence of these 12 BPs, as reflected by the MRT values, were low and close to each other, ranging from 0.5 to 2 h. The elimination half-lives of the 12 BPs were of the same order of magnitude as the respective MRT values and ranged from 0.4 h (BPAF) to 3.55 h (BP4-4), indicating that these 12 bisphenols are rapidly eliminated either because of a relatively low Vss (BPS) or a rather high clearance (other BPs), see discussion.

The BPs showed a considerable range of oral bioavailability (the fraction of active BP that reaches the central compartment unchanged), with values below 7 % for 3-3BPA, BP4-4, BPA, BPAF, BPB, BPF, and BPZ and between 12 and 20 % for BPAP, BPP, BPFL and BPM. BPS showed a much higher oral bioavailability (59.5 %) in comparison with that of other BPs (Table 2). These key parameters determine the systemic exposure to the active bisphenol after an oral exposure (AUC_{VO} normalized by the dose, Table 2), which shows a wide range of value with BPA systemic exposure being 2 to 150-times lower than that of these emerging analogues.

3.5. TK prediction by HTTK package

The HTTK package enabled the prediction of TK parameters for only 5 BPs (BPS, BPFL, BPA, BPAF, and BPB, Table 3). The total clearance of BPS is about 20 to 30 times lower than that of BPA, BPAF and BPB, but is 10-times higher than that of BPFL. The steady state volume of distribution varied among these five BPs, with those of BPS and BPFL being 7–30 times lower than those of BPA, BPAF and BPB. These predicted TK parameters resulted in half-lives unexpectedly long for this class of

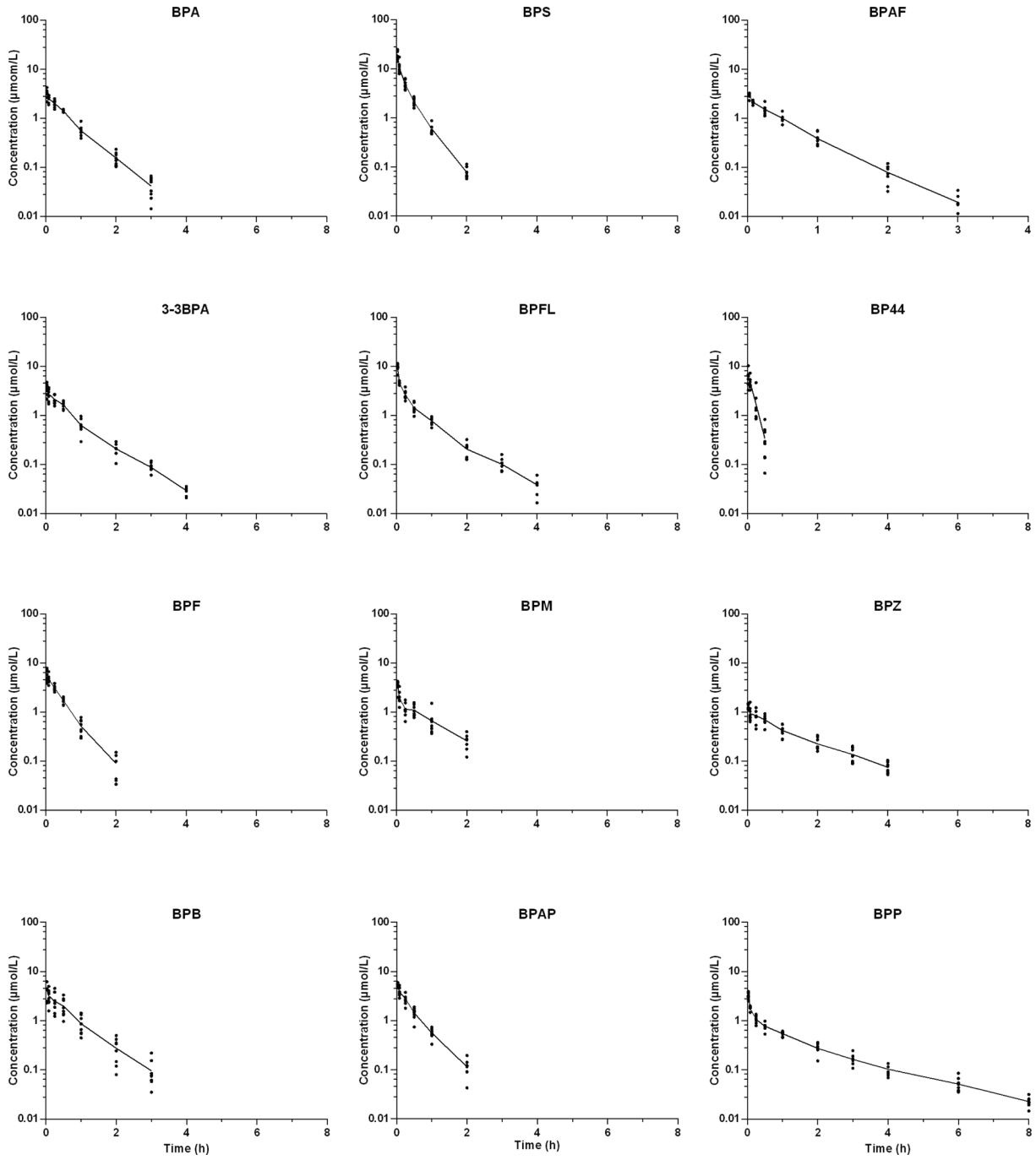

Fig. 4. Semi-logarithmic plots of individual and mean plasma concentrations versus time of 12 BPs after IV administration. Twelve BPs were administered either as a mixture of BPA, BPAF, BPB, BPF, BPM, BPZ and 3-3BPA or as a mixture of BPS, BP4-4, BPAP, BPP, and BPFL at a nominal dose of 6 µmol/kg BW in 8 piglets. Mean plasma concentration values (µmol/L) were calculated at the time periods (h) where at least six values were above the LOQ. BP, Bisphenol; IV, intravenous.

phenols, ranging from 6 to 215 h. These TK parameters predicted with the HTTK model were very different from those we estimated *in vivo* in piglets. This was especially true for plasma clearances, HTTK model predicted values were 25 (BPAF and BPB) to 3552 (BPFL) times lower than those we actually evaluated *in vivo* with the consequence of major differences in half-life times, with HTTK model predicted values being 10 (BPS) to 260 (BPFL) fold longer than those we evaluated *in vivo* (see Table 2).

4. Discussion

The purpose of our study was to evaluate the potential of internal exposure of 12 BPs by experimentally quantifying their key TK parameters, while respecting the welfare rule of 3R, and to compare them to those predicted *in silico* using the HTTK R package. This is the first study to evaluate the TK of 10 emerging BPs that have not been extensively investigated to date, by comparison with the well-documented BPA and BPS.

To respect a minimal usage of laboratory animals, a mixture approach involving the simultaneous administration of 5–7 BPs for IV dosing and of all BPs for oral dosing was used in piglets. Piglets are considered as a relevant species for translational research because of their important anatomical and physiological similarities with humans, with regard to gastrointestinal and renal function (Tang and Mayersohn,

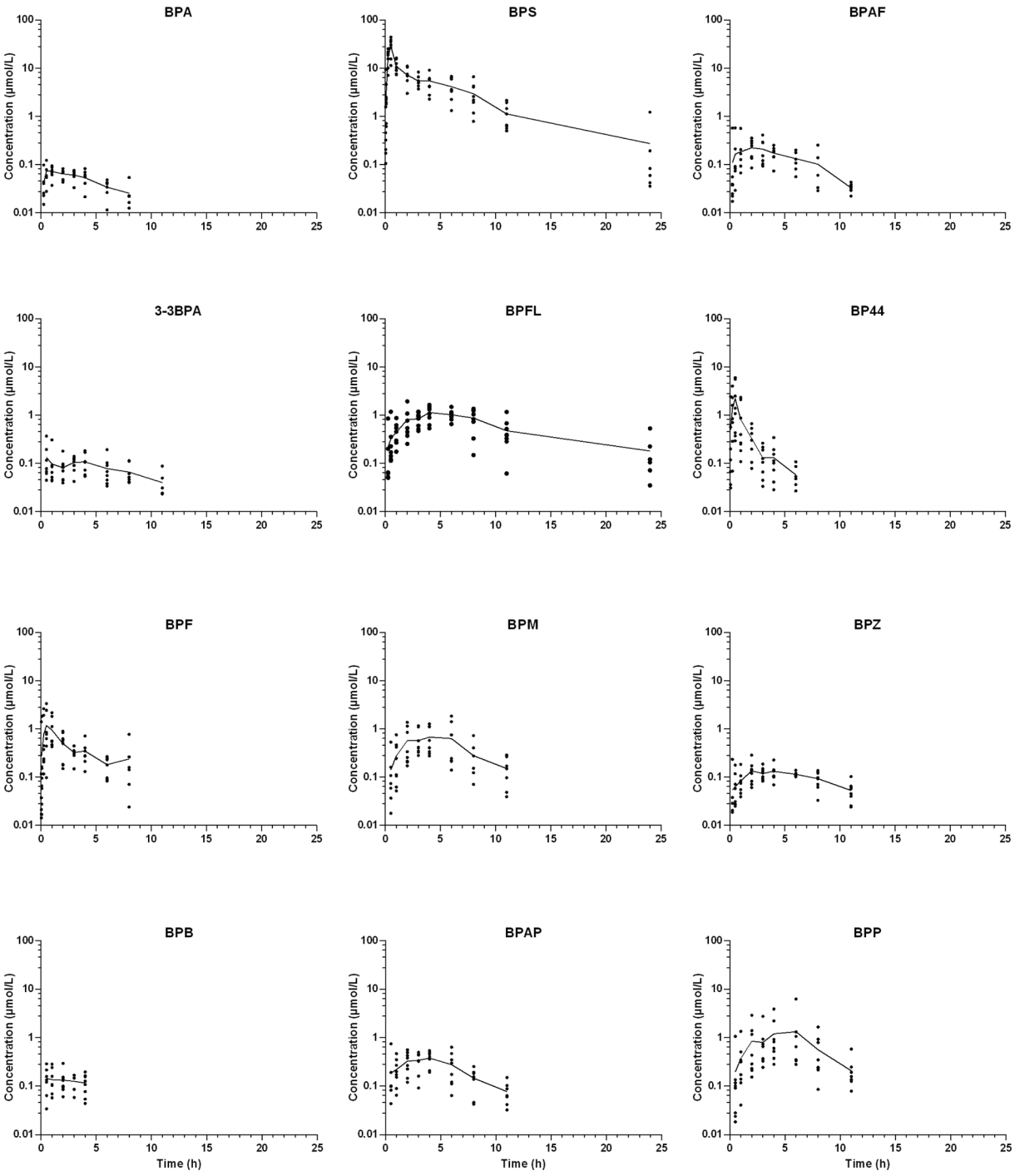

Fig. 5. Semi-logarithmic plots of individual and mean plasma concentrations versus time of 12 BPs after oral administration. The mix of 12 BPs at the nominal dose of 200 µmol/kg BW was administered to 8 piglets (except for BPA n = 7). Mean plasma concentration values (µmol/L) were calculated at the time periods (h) where at least six values were above the LOQ.

2018). Despite the difficulty of resolving 12 BPs from plasma and urine matrices, UHPLC assay methods were successfully developed and validated for the simultaneous quantification of 12 BPs both in plasma and in urine for levels within the range of those required for this TK study.

The mixture approach cannot be considered as a limitation in the context of environmental toxicology and is an important subject of recent methodological development (Pletz et al., 2020). Indeed, the 12 BPs assessed simultaneously in pigs are currently used in replacement of BPA (Caballero-Casero et al., 2016; "ECHA," n.d.; Liao and Kannan, 2013). Moreover, by developing a NLME TK modeling that merged the data obtained here with a combination of 12 BPs and previous data sets corresponding to the plasma concentrations of BPA and BPS after IV and oral administrations obtained from the piglet model (Gayrard et al., 2019), we were able to validate our mixture approach by demonstrating that the combination of 12 BPs did not interfere with TK mechanisms, at least for BPA and BPS. However, considering the high doses of BPs used, a saturation of some metabolic pathway cannot be fully ruled out.

The levels of unconjugated forms of BPs recovered in urine are very low and close to that observed in previous studies for BPA and BPS (Gayrard et al., 2019), indicating that the renal clearances of BPs are negligible in comparison with hepatic clearance. By 24 h, the mean fractions of the dose of BPA or BPS recovered in urine after IV dosing, as

Table 1
Toxicokinetic parameters estimated by noncompartmental analysis after oral dosing. The toxicokinetic parameters (mean ± SD) of 3-3BPA, BPS, BP4-4, BPAP, BPP, BPFL, BPA, BPAF, BPB, BPF, BPM and BPZ were determined after simultaneous oral administration of 12 BPs (200 μmol/kg BW) to 8 piglets.

	3-3BPA	BPS	BP4-4	BPAP	BPP	BPFL	BPA	BPAF	BPB	BPF	BPM	BPZ
Cmax (nmol/L per μmol/kg BW)	0.87 ± 0.25	170.77 ± 56.02	12.97 ± 13.22	2.57 ± 0.83	5.57 ± 3.16	7.83 ± 2.23	0.47 ± 0.19	1.31 ± 0.59	0.97 ± 0.23	7.90 ± 6.26	4.17 ± 2.18	0.85 ± 0.19
Tmax (h)	2.89 ± 2.80	0.47 ± 0.11	0.64 ± 0.61	3.85 ± 1.22	4.42 ± 1.99	4.71 ± 1.26	1.17 ± 0.79	2.71 ± 0.76	2.32 ± 1.64	0.74 ± 0.59	4.14 ± 1.35	4.14 ± 1.46
$AUC_{0\text{-last}}$ (μmol × h/L) per μmol/kg BW)	0.0042 ± 0.0008	0.4003 ± 0.1805	0.0122 ± 0.0115	0.0174 ± 0.0125	0.0317 ± 0.0138	0.0719 ± 0.0447	0.0021 ± 0.0009	0.0091 ± 0.0053	0.0034 ± 0.0014	0.0175 ± 0.0086	0.0259 ± 0.0131	0.0067 ± 0.0035
CL_F (L/kg/h)	185.91 ± 44.97	2.86 ± 1.06	130.00 ± 96.45	78.83 ± 45.78	32.41 ± 11.49	18.02 ± 10.19	465.80 ± 234.86	130.43 ± 63.31	230.52 ± 114.56	65.21 ± 28.55	38.47 ± 23.56	133.65 ± 57.27
Half-life (h)	4.54 ± 2.94	3.43 ± 1.39	2.25 ± 1.19	5.53 ± 5.89	3.71 ± 1.74	5.74 ± 3.26	3.51 ± 1.37	5.42 ± 3.21	3.18 ± 1.79	2.22 ± 0.37	3.92 ± 2.34	6.82 ± 4.34
MRT (h)	7.96 ± 3.04	4.77 ± 2.09	2.59 ± 1.52	8.41 ± 5.29	7.16 ± 2.20	10.18 ± 4.20	5.44 ± 1.60	8.44 ± 3.64	5.73 ± 2.87	3.56 ± 0.78	7.65 ± 3.17	10.79 ± 5.31
F (%)	1.91 ± 0.75	49.26 ± 22.54	5.86 ± 4.19	6.05 ± 4.57	10.42 ± 3.64	17.43 ± 10.08	0.91 ± 0.45	4.05 ± 1.94	1.40 ± 0.82	5.23 ± 3.08	9.09 ± 5.07	3.82 ± 1.13

Cmax: Maximal plasma concentration after oral administration; Tmax: time of maximal concentration; CL_F: apparent plasma clearance MRT: Mean residence time; F: Bioavailability by the oral route; $AUC_{0\text{-last}}$/Dose: Dose scaled area under the plasma concentration-time curve from dosing time to the time of the last measurable plasma concentration after administration.
BPF: Bisphenol F; BPA: Bisphenol A; BPB: Bisphenol B; BPAF: Bisphenol AF; 3-3BPA: 3-3 Bisphenol A; BPZ: Bisphenol Z; BPM: Bisphenol M; BPS: Bisphenol S; BP4-4: Bisphenol 4-4; BPAP: Bisphenol AP; BPFL: Bisphenol FL; BPP: Bisphenol P.

Table 2
Population parameters as obtained with a two-compartment model.

	3-3BPA	BPS	BP4-4	BPAP	BPP	BPFL	BPA	BPAF	BPB	BPF	BPM	BPZ
Bioavailability (%)	3.35	59.50	6.88	12.66	16.96	19.70	0.89	4.30	1.62	5.73	12.90	4.46
Clearance (L/kg/h)	3.30	1.28	4.52	3.25	3.33	2.49	3.60	4.53	2.64	2.86	3.56	4.95
Vss (L/kg)	2.65	0.67	4.73	1.78	6.13	1.97	2.38	2.60	2.07	2.49	4.79	7.71
AUC_{VO} (μmol × h/L per μmol/kg BW)	0.0041	0.309	0.017	0.014	0.035	0.041	0.0020	0.0069	0.0045	0.020	0.021	0.0049
Half-life (h)	0.73	2.43	3.55	0.56	1.42	0.83	0.53	0.40	0.68	2.86	1.02	1.14
MRT (h)	0.80	0.52	1.05	0.55	1.84	0.79	0.66	0.57	0.79	0.87	1.34	1.56

Vss: steady-state volume of distribution. AUC_{VO}: Dose scaled area under the plasma concentration-time curve from dosing time to infinity after oral dosing. MRT: Mean residence time. BPF: Bisphenol F; BPA: Bisphenol A; BPB: Bisphenol B; BPAF: Bisphenol AF; 3-3BPA: 3-3 Bisphenol A; BPZ: Bisphenol Z; BPM: Bisphenol M; BPS: Bisphenol S; BP4-4: Bisphenol 4-4; BPAP: Bisphenol AP; BPFL: Bisphenol FL; BPP: Bisphenol P.

Table 3
Predicted Human toxicokinetic parameters of BPS, BPFL, BPA, BPAF and BPB calculated using the HTTK R package.

	BPS	BPFL	BPA	BPAF	BPB
Clearance (L/kg/h)	0.0062	0.0007	0.111	0.180	0.103
Vss (L/kg)	0.211	0.212	6.34	1.58	1.86
HL_lambda (h)	23.7	215.5	39.6	6.1	12.6
MRT (h)	34.2	311.0	57.1	8.8	18.1

BPS: Bisphenol S; BPFL: Bisphenol FL; BPA: Bisphenol A; BPB: Bisphenol B. The TK parameters are calculated for a one compartment model for plasma. The total clearance is assumed to be entirely due to hepatic metabolism and passive glomerular filtration (kidney). The effective volume of distribution is calculated by summing the plasma volume and the product of each tissues volume to its partition coefficient relative to plasma. Half-life is calculated by dividing the natural-log of 2 by the elimination rate (ratio of the total clearance by the volume of distribution) from the one compartment model. MRT is calculated as the inverse of the elimination rate constant.

total BP (quantified after enzymatic hydrolysis of phase II conjugates), are of the same order of magnitude as the corresponding BPA glucuronide and BPS glucuronide fractions determined in a previous study (Gayrard et al., 2019), suggesting that these urinary BPs are mainly as glucuronidated form and that the conditions of the hydrolysis of these conjugated BPs in mixture can be considered as suitable for biomonitoring. In our study, somewhat lower BPA excretion values after oral dosing could be attributed in part to the loss of urine at some collection points. Moreover, the urinary excretion of BPA in pig is slightly lower than that described in human (84–109 % over 24 h after administration (Thayer et al., 2015; Völkel et al., 2002) whereas it is of the same order of magnitude for BPS (56–70 % over 72 h period post-dosing (Khmiri et al., 2020; Oh et al., 2018)).

The BP urinary excretion varied considerably among the 12 BPs, the total percentage of the oral dose recovered in urine over 24 h being lower than 16 % after oral dosing for BPAP, 3-3BPA, BPP, BPFL, and BPM. This low urinary recovery rate cannot be explained by the duration of the urine collection, the urinary excretion being near complete at 24 h for most of studied BPs. Phase II metabolic reactions are identified as the main detoxification pathway both *in vivo* and *in vitro* for a few investigated BPs (BPA, BPS, BPF and BPAF) (Gramec Skledar and Peterlin Mašič, 2016; Li et al., 2013; Skledar et al., 2016; Waidyanatha et al., 2021, 2018), but metabolism pathways remain largely unidentified for most emerging BP analogues. Thus, the contribution from metabolites other than glucuronides, such as hydroxylated compounds (Cabaton et al., 2008; Gramec Skledar and Peterlin Mašič, 2016; Skledar et al., 2016), could lead to an underestimation of total BPs urinary excretion.

Moreover, some BPs could be mainly excreted in feces as observed in rodent. The molecular weight thresholds for biliary excretion are different between rats (about 200–300 g/mol) and humans (about 500–600 g/mol) (Clark et al., 1969; Hughes et al., 1973; Roberts et al., 2002). Hence, in rodent model, some orally administered investigated BPs were extensively excreted in feces, such as BPA (around 80 %, Kurebayashi et al., 2003) and BPAF (65–80 %, Waidyanatha et al., 2015) whereas urine is the predominant route of excretion for BPS (40–50 %, Waidyanatha et al., 2018) and BPF (around 50 %, Cabaton et al., 2006).

Therefore, due to the higher molecular weight of the conjugated form of some BPs (around or above 500 g/mol for BPFL, BPP, BPM, BPAF and BPAP) favoring biliary excretion, a significant part may be eliminated by feces. This is most certainly the case for the three BPs with the least elimination by the urine (BPM, BPP and BPFL), which are also the three BPs with the highest MW (346, 346 and 350, respectively), suggesting that their glucuronides have MW higher than the MW biliary threshold in pigs. Most importantly, our data highlight the need to take into account the differences in urinary excretion between BPs to evaluate external exposures based on urinary biomarker levels. Further studies are needed to evaluate the feces elimination, at least for BPs with a low urinary elimination.

Our TK analysis was based on using NLME modeling, allowing to merge IV and oral data sets, thereby overcome the difficulty of estimating the terminal slope for analytical reasons for some BPs, such as BP4-4 after IV dosing, and a robust estimation of bioavailability. The much higher oral bioavailability of BPS (60 %) in comparison with that of BPA (0.89 %) estimated in this study reinforces data previously obtained in piglet (Gayrard et al., 2019) and humans (Khmiri et al., 2020; Thayer et al., 2015). For other emerging BPA analogues, the systemic bioavailability is 2 to 14 times higher than that of BPA. For BPAF, the oral bioavailability determined in piglet (4.3 %) is close to that evaluated in male and female mice (respectively 5.64 % and 3.13 %) (Waidyanatha et al., 2019). Oral bioavailability is the product of fraction absorbed, fraction escaping first-pass gut-wall elimination, and fraction escaping first-pass hepatic elimination. For the BPs mainly eliminated by urine (BPS, BPA, BP4-4, BPB, BPF, BPZ), the urinary excretion of total BPs over 24 h are relatively close for the two routes of administration, indicating a rather high absorption (not to be confused with bioavailability) following gavage as already observed for BPA and BPS (Gayrard et al., 2019). The great extent of absorption of these BPs may be explained partly by their relatively low molecular size (less than400 g/mol) and their rather high lipophilicity (Varma et al., 2010). Using a database of 309 drugs in humans, Varma et al. (2010) show that although lipophilicity favors absorption, the fractions of drugs escaping gut-wall and hepatic presystemic elimination decreases with increasing lipophilicity, with compounds having logD values greater than 3 demonstrating higher gut and hepatic first-pass extraction. This single physicochemical descriptor is, however, insufficient to explain the highly different extents of first-pass extractions of BPs, in particular the high bioavailability of BPS (Log P value of 2.15) in comparison with those of other investigated BPs (Fig. 6). We have previously shown (Gayrard et al., 2019) that BPS, unlike BPA, is unlikely to be subjected to an intestinal first-pass effect. According to the available metabolism data, phase II metabolic reactions is the main detoxification pathway both *in vivo* and *in vitro* for BPA, BPS, BPAF and BPF. However, the great extent of their first-pass elimination could be attributed to changes in molecular properties that affect their interactions with metabolic enzymes and the metabolic reactions (Gramec Skledar and Peterlin Mašič, 2016; Ramírez et al., 2021).

BPS plasma clearance, as determined in piglets (1.28 L/kg per h), is about 2 to 4 times lower than that of all BPs, indicating that BPS is eliminated less efficiently than the other 11 studied BPs. This result confirms and extends previous data comparing plasma clearances of BPS and BPA in several species (Collet et al., 2015; Gayrard et al., 2020, 2019; Grandin et al., 2017). For BPA, BPF, BPAF and BPS predominantly metabolized by the glucuronidation reaction (Gramec Skledar and Peterlin Mašič, 2016), the difference in clearance values is consistent with the relative liver intrinsic clearance values of BPA, BPF and BPAF vs. BPS (respectively 11, 11 and 77 fold differences) determined *in vitro* from hepatic glucuronidation in humans (Karrer et al., 2018) or with the relative total clearances predicted by HTTK package for BPA, BPAF and BPB vs. BPS (respectively 18, 29 and 17 fold differences). By contrast, the relative clearance of BPFL vs BPS predicted by HTTK package is disproportionately low by comparison with the plasma clearance experimentally determined. Surprisingly, the clearances computed in HTTK package (0.0007 L/kg per h for BPFL, 0.0062 L/kg per h for BPS and between 0.1 and 0.18 L/kg per h for BPAF, BPA and BPB) are far below the plasma clearances measured here in pig (*in vivo*/prediction ratio ranging from 25 to 3552) and those estimated in human for BPA and BPS [respectively, 1.54 L/kg per h (Collet et al., 2015) and 0.57 L/kg per h (Khmiri et al., 2020)]. A great difference has already been reported for BPA, with a clearance estimated from the *in vivo* data obtained in a rat model approximately 100 fold higher than the predicted clearance using HTTK data (Wambaugh et al., 2018). This discrepancy between HTTK predictions and *in vivo* data is primarily due to the underprediction of the hepatic intrinsic clearance determined *in vitro*, in particular for high intrinsic clearance values (Wood et al., 2017). Moreover, intrinsic clearance is only predicted from the hepatic intrinsic clearance and kidney glomerular excretion ignoring other pathways of elimination such as extra-hepatic metabolism or active transport in the kidneys (Wambaugh et al., 2018).

Assuming that the plasma and blood BPs concentrations are equal, plasma clearances determined experimentally are high and of the same order of magnitude as the hepatic blood flow [estimated at 2.6 L/kg per h in adult pig from allometric scaling (Björkman and Redke, 2010;

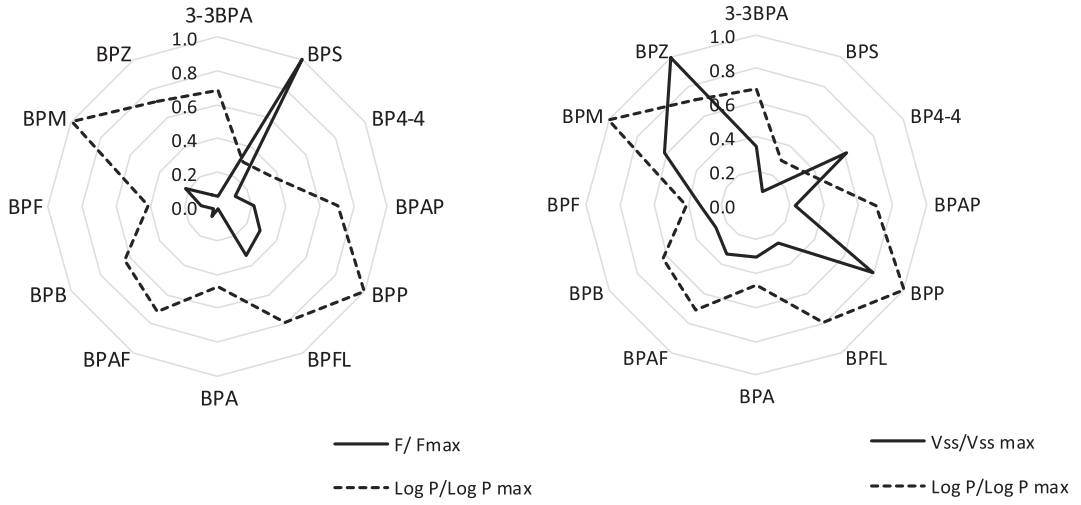

Fig. 6. Kiviat plots describing for the 12 bisphenols the oral bioavailability (F, left) and the steady-state volume of distribution (Vss, right) determined in piglets and their potential relationship with the Log P. The Log P was determined with ChemDraw17.1 (ChemPropPro)). Each value is normalized by the corresponding maximal value. No relationship between Log P and bioavailability and between Log P and Vss was evidenced.

Boxenbaum, 1980)] for all the BPs investigated, except for BPS (estimated hepatic extraction ratio of 0.49). The plasma clearances of BP4-4 and BPAF exceed the hepatic blood flow, indicating substantial extrahepatic clearance. The resulting fraction of BPs administered by oral route that escapes presystemic metabolism can be as high as 50.8 % for BPS and low or very low for other studied BPs. The agreement of these values with the corresponding oral bioavailability measured in pigs (59.5 % for BPS and between 1 and 17 % for the others investigated BPs) confirms the predominant role of liver and its first-pass effect for BPs clearance and BPs plasma exposure.

The plasma clearance of BPs represents the key parameter to predict the average plasma BPs concentrations while the steady-state volume of distribution only influences the persistency of BPs in the organism. In piglet, the BPs Vss are moderate to high and varied between 1.8 for BPAP to 7.7 L/kg for BPZ, except for BPS (0.67 L/kg). The BPA and BPS steady state volumes of distribution values are consistent with those previously evaluated in pig (Collet et al., 2015; Gayrard et al., 2020) and are close to those estimated in human by an allometric approach (1.00 and 2.26 L/kg, respectively). For BPAF, the steady state volume of distribution is close to those observed in rodent species (2.6 L/kg in our pig model, 0.7 L/kg in rat and 1.4 and 8.3 L/kg, respectively, in female and male mice). The volumes of distribution calculated using the HTTK package for BPS, BPFL, BPA, BPAF, and BPB (between 0.21 and 6.34 L/kg) are of the same order of magnitude as those determined in piglet (ratio measured/predicted from 0.4 to 9.3).

The extent of distribution of a substance in the body is dependent on the tissue partitioning determined by the relative fraction unbound in plasma and outside plasma and tissue affinity (see equation (10)). The determinants of the overall unbound in a given tissue can be both nonspecific (e.g., partitioning into tissue phospholipid) and specific (binding to proteins such albumin). The extent of membrane/tissue binding is also dependent on the lipophilicity of the molecule (Log P) (Smith et al., 2015). However, for the investigated BPs, the visual inspection of the Fig. 6 does not reveal any relationship between Log P and Vss. The percent unbound to albumin of several BPs are determined both by *in vitro* biomimetic LC and *in silico* predictions (BPM: 0.98 %; BPAF: 1.10 %; BPB: 3.21 %; BPA: 4.09 % and BPF: 7.58 % and BPS: 13.23 %) and are shown to be largely non-specific and mainly lipophilicity-driven (Grumetto et al., 2019). Here again, differences in Vss among BPs cannot be clearly explained by differences of their predicted unbound fractions in serum, in particular for BPS.

The persistence of BPM, BPZ, BPP and BP4-4, as reflected by the MRT, is 2 to 3 times higher (1–2 h) than those of other investigated BPs, resulted mainly from their 2–12 times higher Vss (4.7 to 7.7 L/kg) and not of a lower clearance. The lower MRT for BPS (0.52 h) is due to its 3–12 times lower Vss (0.67 L/kg), despite a lower clearance.

Terminal Half-life (HL), corresponding to the rate of elimination during the terminal phase, is a hybrid parameter reflecting both the plasma clearance and the volume associated with this terminal phase (Varea). HL, as directly measured in piglets, range between 0.4 and 3.6 h and are close to those of MRT, confirming that the body distribution of this class of phenols is rather moderate. The high BPs HL predicted by HTTK package (between 6 and 216 h) results from the gross underestimation of the plasma clearance, in particular for BPFL, BPS and BPA.

In the pig model, for the same amount of BP ingested, BPA results in the lowest systemic exposure to unconjugated, active bisphenol in comparison to 11 structural analogs (2 to 4 times for 3-3BPA, BPAF, BPB and BPZ, 7–20 times for BP4-4, BPAP, BPP, BPFL, BPF, BPM and much lower, 150 times for BPS). For BPA and BPS, our results are in good agreement with the kinetics observed in humans (Khmiri et al., 2020; Thayer et al., 2015). Assuming equal oral exposure, a physiologically based pharmacokinetic (PBPK) model predicts that human BPS exposure leads to the highest internal concentration of unconjugated BP in serum by comparison to those of BPA, BPF and BPAF (Karrer et al., 2018). The predicted profiles of internal exposure are rather close for BPF, BPA and BPAF. However, the authors point out the need of TK animal studies to calibrate PBPK for BPF and BPAF.

The TK parameters estimated by HTTK model can in turn be used to calculate relevant steady-state concentration for human exposure scenarios that can be compared to those identified as bioactive *in vitro*. While HTTK predicts BPs Vss rather well, as previously shown for BPA (Wambaugh et al., 2018), the underestimation of total clearance of BPs by the HTTK method could lead to a large overestimation of internal exposure. Hence, the BPA time-concentration profile for a daily dose of 1 mg/kg computed using HTTK model shows an accumulation over 50 days of exposure (Pearce et al., 2017) and is then completely inconsistent with concentrations profiles of unconjugated BPA observed *in vivo* or modeled with a calibrated and validated PBPK model, considering that metabolism is linear (Karrer et al., 2018). Moving forward, these TK considerations show the importance of experimental data that provide input parameters to refine or to challenge the parameterization of these TK models, in particular for bioavailability for which HTTK predictions are not effective.

Most of the BPA analogues investigated in our study (BPAF, BPB, BPZ, BPA, BPAP and BPS) are shown to be agonist of estrogen receptors *in vitro* (Pelch et al., 2019a, 2019b) and may have similar effects on endocrine system as BPA. Our TK data indicate that for equal external exposures, the endocrine disrupting effect of all these investigated BPA analogues could be exacerbated because of their higher or even much higher internal exposure in comparison to BPA and this applies particularly for BPS.

5. Conclusion

This TK assessment of this mixture of environmental BPs in pigs, while complying with the strategy to replace, reduce, and refine the use of animals, provides a better understanding of TK mechanisms determining the differences of patterns of internal exposure of these structurally analogues of BPA that underlie their potential adverse effects. Thus, for an equivalent oral exposure, all BPA analogues investigated showed a higher internal exposure to unconjugated, active BP compared to BPA (2 to 4 fold for 3-3BPA, BPAF, BPB and BPZ, 7–20 fold for BP4-4, BPAP, BPP, BPFL, BPF and BPM and 150 fold for BPS) due mainly to a considerable variation of oral bioavailability. Given similarities in the digestive tract between pigs and humans, our TK data suggest that replacing BPA with some of its alternatives will likely lead to higher amount of BPA analogues, particularly BPS, that can reach the target tissues and exert effects.

Moreover, the great variation in the urinary excretion of BPs, particularly low for BPM, BPP or BPFL, is an important aspect to consider for predicting human exposure from urine biomonitoring. It means that the amount of BPM, BPP and BPFL excreted in urine cannot reflect the external exposure level.

Our TK data determined in animal model are crucial to parameterize and calibrate the PBPK model for a better interpretation of human exposure to these emerging BPs. These TK parameters are also indispensable to develop quantitative Toxicodynamic/TK approaches to translate *in vitro* potencies to equivalent *in vivo* doses in order to evaluate the risk of human exposure to these emerging BPs.

Funding

This work was supported by the French Ministry of Agriculture [BQR2020].

CRediT authorship contribution statement

Clémence A. Gély: Conceptualization, Methodology, Investigation, Writing – original draft. **Marlène Z. Lacroix:** Conceptualization, Methodology, Investigation, Funding acquisition. **Béatrice B. Roques:** Investigation. **Pierre Louis Toutain:** Conceptualization. **Véronique Gayrard:** Conceptualization, Investigation, Funding acquisition. **Nicole**

Picard-Hagen: Conceptualization, Methodology, Investigation, Writing – original draft, Funding acquisition.

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.

Data availability

Data will be made available on request.

Acknowledgments

The authors thank Cedric Lacassagne, Mathieu Gaillard, Noémie Deschamps and Théo Fargues for their assistance and involvement in animal care. This manuscript was edited at Life Science Editors.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107722.

References

Beg, M.A., Sheikh, I.A., 2020. Endocrine disruption: Molecular interactions of environmental bisphenol contaminants with thyroid hormone receptor and thyroxine-binding globulin. Toxicol. Ind. Health 36, 322–335. https://doi.org/10.1177/0748233720928165.

Björkman, S., Redke, F., 2010. Clearance of Fentanyl, Alfentanil, Methohexitone, Thiopentone and Ketamine in Relation to Estimated Hepatic Blood Flow in Several Animal Species: Application to Prediction of Clearance in Man. J. Pharm. Pharmacol. 52, 1065–1074. https://doi.org/10.1211/0022357001774985.

Boxenbaum, H., 1980. Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin. J. Pharmacokinet. Biopharm. 8, 165–176. https://doi.org/10.1007/BF01065191.

Caballero-Casero, N., Lunar, L., Rubio, S., 2016. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal. Chim. Acta 908, 22–53. https://doi.org/10.1016/j.aca.2015.12.034.

Cabaton, N., Chagnon, M.-C., Lhuguenot, J.-C., Cravedi, J.-P., Zalko, D., 2006. Disposition and Metabolic Profiling of Bisphenol F in Pregnant and Nonpregnant Rats. J. Agric. Food Chem. 54, 10307–10314. https://doi.org/10.1021/jf062250q.

Cabaton, N., Zalko, D., Rathahao, E., Canlet, C., Delous, G., Chagnon, M.-C., Cravedi, J.-P., Perdu, E., 2008. Biotransformation of bisphenol F by human and rat liver subcellular fractions. Toxicol. In Vitro 22, 1697–1704. https://doi.org/10.1016/j.tiv.2008.07.004.

Chen, Y., Fang, J., Ren, L., Fan, R., Zhang, J., Liu, G., Zhou, L., Chen, D., Yu, Y., Lu, S., 2018. Urinary bisphenol analogues and triclosan in children from south China and implications for human exposure. Environ. Pollut. 238, 299–305. https://doi.org/10.1016/j.envpol.2018.03.031.

Clark, A.G., Hirom, P.C., Millburn, P., Smith, R.L., Williams, R.T., 1969. Reabsorption from the biliary system as a factor influencing the biliary excretion of organic anions. Biochem. J. 115, 62P–P. https://doi.org/10.1042/bj1150062Pa.

Collet, S.H., Picard-Hagen, N., Lacroix, M.Z., Puel, S., Viguié, C., Bousquet-Melou, A., Toutain, P.-L., Gayrard, V., 2015. Allometric scaling for predicting human clearance of bisphenol A. Toxicol. Appl. Pharmacol. 284, 323–329. https://doi.org/10.1016/j.taap.2015.02.024.

ECHA [WWW Document], n.d. URL https://echa.europa.eu/fr/home (accessed 10.13.20).

European Medecines Agency Science Medicines Health, 2011. European Medecines Agency Science Medicines Health. Guideline on bioanalytical method validation.

Frederiksen, H., Nielsen, O., Koch, H.M., Skakkebaek, N.E., Juul, A., Jørgensen, N., Andersson, A.-M., 2020. Changes in urinary excretion of phthalates, phthalate substitutes, bisphenols and other polychlorinated and phenolic substances in young Danish men; 2009–2017. Int. J. Hyg. Environ. Health 223, 93–105. https://doi.org/10.1016/j.ijheh.2019.10.002.

Gayrard, V., Lacroix, M.Z., Gély, C.A., Grandin, F.C., Léandri, R., Bouchard, M., Roques, B., Toutain, P.-L., Picard-Hagen, N., 2020. Toxicokinetics of bisphenol S in rats for predicting human bisphenol S clearance from allometric scaling. Toxicol. Appl. Pharmacol. 386, 114845 https://doi.org/10.1016/j.taap.2019.114845.

Gayrard, V., Lacroix, M.Z., Grandin, F.C., Collet, S.H., Mila, H., Viguié, C., Gély, C.A., Rabozzi, B., Bouchard, M., Léandri, R., Toutain, P.-L., Picard-Hagen, N., 2019. Oral Systemic Bioavailability of Bisphenol A and Bisphenol S in Pigs. Environ. Health Perspect. 127, 077005 https://doi.org/10.1289/EHP4599.

Gély, C.A., Huesca, A., Picard-Hagen, N., Toutain, P.L., Berrebi, A., Gauderat, G., Gayrard, V., Lacroix, M.Z., 2021a. A new LC/MS method for specific determination of human systemic exposure to bisphenol A, F and S through their metabolites: Application to cord blood samples. Environ. Int. 151, 106429 https://doi.org/10.1016/j.envint.2021.106429.

Gély, C.A., Lacroix, M.Z., Morin, M., Vayssière, C., Gayrard, V., Picard-Hagen, N., 2021b. Comparison of the materno-fetal transfer of fifteen structurally related bisphenol analogues using an ex vivo human placental perfusion model. Chemosphere 276, 130213. https://doi.org/10.1016/j.chemosphere.2021.130213.

Gonzalez, L.M., Moeser, A.J., Blikslager, A.T., 2015. Porcine models of digestive disease: the future of large animal translational research. Transl. Res. 166, 12–27. https://doi.org/10.1016/j.trsl.2015.01.004.

González, N., Cunha, S.C., Ferreira, R., Fernandes, J.O., Marquès, M., Nadal, M., Domingo, J.L., 2020. Concentrations of nine bisphenol analogues in food purchased from Catalonia (Spain): Comparison of canned and non-canned foodstuffs. Food Chem. Toxicol. 136, 110992 https://doi.org/10.1016/j.fct.2019.110992.

Gramec Skledar, D., Peterlin Mašič, L., 2016. Bisphenol A and its analogs: Do their metabolites have endocrine activity? Environ. Toxicol. Pharmacol. 47, 182–199. https://doi.org/10.1016/j.etap.2016.09.014.

Grandin, F., Picard-Hagen, N., Gayrard, V., Puel, S., Viguié, C., Toutain, P.-L., Debrauwer, L., Lacroix, M.Z., 2017. Development of an on-line solid phase extraction ultra-high-performance liquid chromatography technique coupled to tandem mass spectrometry for quantification of bisphenol S and bisphenol S glucuronide: Applicability to toxicokinetic investigations. J. Chromatogr. A 1526, 39–46. https://doi.org/10.1016/j.chroma.2017.10.020.

Grumetto, L., Barbato, F., Russo, G., 2019. Scrutinizing the interactions between bisphenol analogues and plasma proteins: Insights from biomimetic liquid chromatography, molecular docking simulations and in silico predictions. Environ. Toxicol. Pharmacol. 68, 148–154. https://doi.org/10.1016/j.etap.2019.02.008.

Gyllenhammar, I., Glynn, A., Jönsson, B.A.G., Lindh, C.H., Darnerud, P.O., Svensson, K., Lignell, S., 2017. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ. Res. 153, 48–54. https://doi.org/10.1016/j.envres.2016.11.012.

Gys, C., Bastiaensen, M., Bruckers, L., Colles, A., Govarts, E., Martin, L.R., Verheyen, V., Koppen, G., Morrens, B., Den Hond, E., De Decker, A., Schoeters, G., Covaci, A., 2021. Determinants of exposure levels of bisphenols in flemish adolescents. Environ. Res. 193, 110567 https://doi.org/10.1016/j.envres.2020.110567.

Hughes, R.D., Millburn, P., Williams, R.T., 1973. Molecular weight as a factor in the excretion of monoquaternary ammonium cations in the bile of the rat, rabbit and guinea pig. Biochem. J. 136, 967–978. https://doi.org/10.1042/bj1360967.

Karrer, C., Roiss, T., von Goetz, N., Gramec Skledar, D., Peterlin Mašič, L., Hungerbühler, K., 2018. Physiologically Based Pharmacokinetic (PBPK) Modeling of the Bisphenols BPA, BPS, BPF, and BPAF with New Experimental Metabolic Parameters: Comparing the Pharmacokinetic Behavior of BPA with Its Substitutes. Environ. Health Perspect. 126, 077002 https://doi.org/10.1289/EHP2739.

Karsauliya, K., Bhateria, M., Sonker, A., Singh, S.P., 2021. Determination of Bisphenol Analogues in Infant Formula Products from India and Evaluating the Health Risk in Infants Asssociated with Their Exposure. J. Agric. Food Chem. 69, 3932–3941. https://doi.org/10.1021/acs.jafc.1c00129.

Khmiri, I., Côté, J., Mantha, M., Khemiri, R., Lacroix, M., Gely, C., Toutain, P.-L., Picard-Hagen, N., Gayrard, V., Bouchard, M., 2020. Toxicokinetics of bisphenol-S and its glucuronide in plasma and urine following oral and dermal exposure in volunteers for the interpretation of biomonitoring data. Environ. Int. 138, 105644 https://doi.org/10.1016/j.envint.2020.105644.

Kojima, H., Takeuchi, S., Sanoh, S., Okuda, K., Kitamura, S., Uramaru, N., Sugihara, K., Yoshinari, K., 2019. Profiling of bisphenol A and eight its analogues on transcriptional activity T via human nuclear receptors. Toxicology 413, 48–55. https://doi.org/10.1016/j.tox.2018.12.001.

Kurebayashi, H., Betsui, H., Ohno, Y., 2003. Disposition of a Low Dose of 14C-Bisphenol A in Male Rats and Its Main Biliary Excretion as BPA Glucuronide. Toxicol. Sci. 73, 17–25. https://doi.org/10.1093/toxsci/kfg040.

Li, M., Yang, Y., Yang, Y.i., Yin, J., Zhang, J., Feng, Y., Shao, B., 2013. Biotransformation of Bisphenol AF to Its Major Glucuronide Metabolite Reduces Estrogenic Activity. PLoS ONE 8, e83170.

Liao, C., Kannan, K., 2014a. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China 31, 319–329. https://doi.org/10.1080/19440049.2013.868611.

Liao, C., Kannan, K., 2014b. A Survey of Alkylphenols, Bisphenols, and Triclosan in Personal Care Products from China and the United States. Arch. Environ. Contam. Toxicol. 67, 50–59. https://doi.org/10.1007/s00244-014-0016-8.

Liao, C., Kannan, K., 2013. Concentrations and Profiles of Bisphenol A and Other Bisphenol Analogues in Foodstuffs from the United States and Their Implications for Human Exposure. J. Agric. Food Chem. 61, 4655–4662. https://doi.org/10.1021/jf400445n.

Liao, C., Liu, F., Guo, Y., Moon, H.-B., Nakata, H., Wu, Q., Kannan, K., 2012. Occurrence of Eight Bisphenol Analogues in Indoor Dust from the United States and Several Asian Countries: Implications for Human Exposure. Environ. Sci. Technol. 46, 9138–9145. https://doi.org/10.1021/es302004w.

Lu, S., Yu, Y., Ren, L., Zhang, X., Liu, G., Yu, Y., 2018. Estimation of intake and uptake of bisphenols and triclosan from personal care products by dermal contact. Sci. Total Environ. 621, 1389–1396. https://doi.org/10.1016/j.scitotenv.2017.10.088.

Lucarini, F., Krasniqi, T., Bailat Rosset, G., Roth, N., Hopf, N.B., Broillet, M.-C., Staedler, D., 2020. Exposure to New Emerging Bisphenols Among Young Children in Switzerland. Int. J. Environ. Res. Public. Health 17, 4793. https://doi.org/10.3390/ijerph17134793.

Mok, S., Lim, J.-E., Lee, A., Kim, S., Kim, S., Lee, I., Kho, Y., Park, J., Kim, S., Choi, K., Moon, H.-B., 2022. Within- and between-person variability of urinary phthalate metabolites and bisphenol analogues over seven days: Considerations of biomonitoring study design. Environ. Res. 209, 112885 https://doi.org/10.1016/j.envres.2022.112885.

Moreman, J., Lee, O., Trznadel, M., David, A., Kudoh, T., Tyler, C.R., 2017. Acute Toxicity, Teratogenic, and Estrogenic Effects of Bisphenol A and Its Alternative Replacements Bisphenol S, Bisphenol F, and Bisphenol AF in Zebrafish Embryo-Larvae. Environ. Sci. Technol. 51, 12796–12805. https://doi.org/10.1021/acs.est.7b03283.

Morgan, M.K., Clifton, M.S., 2021. Exposure to Triclosan and Bisphenol Analogues B, F, P, S and Z in Repeated Duplicate-Diet Solid Food Samples of Adults. Toxics 9, 47. https://doi.org/10.3390/toxics9030047.

Oh, J., Choi, J.W., Ahn, Y.-A., Kim, S., 2018. Pharmacokinetics of bisphenol S in humans after single oral administration. Environ. Int. 112, 127–133. https://doi.org/10.1016/j.envint.2017.11.020.

Pearce, R.G., Setzer, R.W., Strope, C.L., Sipes, N.S., Wambaugh, J.F., 2017. httk R Package for High-Throughput Toxicokinetics. J. Stat. Softw. 79. https://doi.org/10.18637/jss.v079.i04.

Pelch, K., Wignall, J.A., Goldstone, A.E., Ross, P.K., Blain, R.B., Shapiro, A.J., Holmgren, S.D., Hsieh, J.-H., Svoboda, D., Auerbach, S.S., Parham, F.M., Masten, S. A., Walker, V., Rooney, A., Thayer, K.A., 2019a. A scoping review of the health and toxicological activity of bisphenol A (BPA) structural analogues and functional alternatives. Toxicology 424, 152235. https://doi.org/10.1016/j.tox.2019.06.006.

Pelch, K.E., Li, Y., Perera, L., Thayer, K.A., Korach, K.S., 2019b. Characterization of Estrogenic and Androgenic Activities for Bisphenol A-like Chemicals (BPs). In Vitro Estrogen and Androgen Receptors Transcriptional Activation, Gene Regulation, and Binding Profiles. Toxicol. Sci. 172, 23–37. https://doi.org/10.1093/toxsci/kfz173.

Pletz, J., Blakeman, S., Paini, A., Parissis, N., Worth, A., Andersson, A.-M., Frederiksen, H., Sakhi, A.K., Thomsen, C., Bopp, S.K., 2020. Physiologically based kinetic (PBK) modelling and human biomonitoring data for mixture risk assessment. Environ. Int. 143, 105978 https://doi.org/10.1016/j.envint.2020.105978.

Ramírez, V., Gálvez-Ontiveros, Y., Porras-Quesada, P., Martinez-Gonzalez, L.J., Rivas, A., Álvarez-Cubero, M.J., 2021. Metabolic pathways, alterations in miRNAs expression and effects of genetic polymorphisms of bisphenol a analogues: A systematic review. Environ. Res. 197, 111062 https://doi.org/10.1016/j.envres.2021.111062.

Roberts, M.S., Magnusson, B.M., Burczynski, F.J., Weiss, M., 2002. Enterohepatic Circulation: Physiological, Pharmacokinetic and Clinical Implications. Clin. Pharmacokinet. 41, 751–790. https://doi.org/10.2165/00003088-200241100-00005.

Rochester, J.R., Bolden, A.L., 2015. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. Environ. Health Perspect. https://doi.org/10.1289/ehp.1408989.

Rosenmai, A.K., Dybdahl, M., Pedersen, M., Alice van Vugt-Lussenburg, B.M., Wedebye, E.B., Taxvig, C., Vinggaard, A.M., 2014. Are Structural Analogues to Bisphenol A Safe Alternatives? Toxicol. Sci. 139, 35–47. https://doi.org/10.1093/toxsci/kfu030.

Rowland, M., Tozer, T.N., 1995. Clinical Pharmacokinetics Concepts and Application, 3rd ed. Williams & wilkins.

Savic, R.M., Karlsson, M.O., 2009. Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. AAPS J. 11, 558–569. https://doi.org/10.1208/s12248-009-9133-0.

Shi, M., Sekulovski, N., MacLean, J.A., Hayashi, K., 2018. Prenatal Exposure to Bisphenol A Analogues on Male Reproductive Functions in Mice. Toxicol. Sci. 163, 620–631. https://doi.org/10.1093/toxsci/kfy061.

Shi, M., Sekulovski, N., MacLean, J.A., Whorton, A., Hayashi, K., 2019. Prenatal Exposure to Bisphenol A Analogues on Female Reproductive Functions in Mice. Toxicol. Sci. 168, 561–571. https://doi.org/10.1093/toxsci/kfz014.

Siracusa, J.S., Yin, L., Measel, E., Liang, S., Yu, X., 2018. Effects of bisphenol A and its analogs on reproductive health: A mini review. Reprod. Toxicol. 79, 96–123. https://doi.org/10.1016/j.reprotox.2018.06.005.

Skledar, D.G., Schmidt, J., Fic, A., Klopčič, I., Trontelj, J., Dolenc, M.S., Finel, M., Mašič, L.P., 2016. Influence of metabolism on endocrine activities of bisphenol S. Chemosphere 157, 152–159. https://doi.org/10.1016/j.chemosphere.2016.05.027.

Smith, D.A., Beaumont, K., Maurer, T.S., Di, L., 2015. Volume of Distribution in Drug Design: Miniperspective. J. Med. Chem. 58, 5691–5698. https://doi.org/10.1021/acs.jmedchem.5b00201.

Tang, H., Mayersohn, M., 2018. Porcine Prediction of Pharmacokinetic Parameters in People: A Pig in a Poke? Drug Metab. Dispos. 46, 1712–1724. https://doi.org/10.1124/dmd.118.083311.

Thayer, K.A., Doerge, D.R., Hunt, D., Schurman, S.H., Twaddle, N.C., Churchwell, M.I., Garantziotis, S., Kissling, G.E., Easterling, M.R., Bucher, J.R., Birnbaum, L.S., 2015. Pharmacokinetics of bisphenol A in humans following a single oral administration. Environ. Int. 83, 107–115. https://doi.org/10.1016/j.envint.2015.06.008.

Usman, A., Ahmad, M., 2016. From BPA to its analogues: Is it a safe journey? Chemosphere 158, 131–142. https://doi.org/10.1016/j.chemosphere.2016.05.070.

Varma, M.V.S., Obach, R.S., Rotter, C., Miller, H.R., Chang, G., Steyn, S.J., El-Kattan, A., Troutman, M.D., 2010. Physicochemical Space for Optimum Oral Bioavailability: Contribution of Human Intestinal Absorption and First-Pass Elimination. J. Med. Chem. 53, 1098–1108. https://doi.org/10.1021/jm901371v.

Völkel, W., Colnot, T., Csanády, G.A., Filser, J.G., Dekant, W., 2002. Metabolism and Kinetics of Bisphenol A in Humans at Low Doses Following Oral Administration. Chem. Res. Toxicol. 15, 1281–1287. https://doi.org/10.1021/tx025548t.

vom Saal, F.S., Welshons, W.V., 2006. Large effects from small exposures. II. The importance of positive controls in low-dose research on bisphenol A. Environ. Res. 100, 50–76. https://doi.org/10.1016/j.envres.2005.09.001.

Waidyanatha, S., Black, S.R., Aillon, K., Collins, B., Patel, P.R., Riordan, F., Sutherland, V., Robinson, V.G., Fernando, R., Fennell, T.R., 2019. Toxicokinetics and bioavailability of bisphenol AF following oral administration in rodents: A dose, species, and sex comparison. Toxicol. Appl. Pharmacol. 373, 39–47. https://doi.org/10.1016/j.taap.2019.04.015.

Waidyanatha, S., Black, S.R., Croutch, C.R., Collins, B.J., Silinski, M.A.R., Kerns, S., Sutherland, V., Robinson, V.G., Aillon, K., Fernando, R.A., Mutlu, E., Fennell, T.R., 2021. Comparative toxicokinetics of bisphenol S and bisphenol AF in male rats and mice following repeated exposure via feed. Xenobiotica 51, 210–221. https://doi.org/10.1080/00498254.2020.1829171.

Waidyanatha, S., Black, S.R., Snyder, R.W., Yueh, Y.L., Sutherland, V., Patel, P.R., Watson, S.L., Fennell, T.R., 2018. Disposition and metabolism of the bisphenol analogue, bisphenol S, in Harlan Sprague Dawley rats and B6C3F1/N mice and in vitro in hepatocytes from rats, mice, and humans. Toxicol. Appl. Pharmacol. 351, 32–45. https://doi.org/10.1016/j.taap.2018.05.008.

Waidyanatha, S., Mathews, J.M., Patel, P.R., Black, S.R., Snyder, R.W., Fennell, T.R., 2015. Disposition of bisphenol AF, a bisphenol A analogue, in hepatocytes *in vitro* and in male and female Harlan Sprague-Dawley rats and B6C3F1/N mice following oral and intravenous administration. Xenobiotica 45, 811–819. https://doi.org/10.3109/00498254.2015.1021732.

Wambaugh, J.F., Hughes, M.F., Ring, C.L., MacMillan, D.K., Ford, J., Fennell, T.R., Black, S.R., Snyder, R.W., Sipes, N.S., Wetmore, B.A., Westerhout, J., Setzer, R.W., Pearce, R.G., Simmons, J.E., Thomas, R.S., 2018. Evaluating In Vitro-In Vivo Extrapolation of Toxicokinetics. Toxicol. Sci. 163, 152–169. https://doi.org/10.1093/toxsci/kfy020.

Wang, W., Abualnaja, K.O., Asimakopoulos, A.G., Covaci, A., Gevao, B., Johnson-Restrepo, B., Kumosani, T.A., Malarvannan, G., Minh, T.B., Moon, H.-B., Nakata, H., Sinha, R.K., Kannan, K., 2015. A comparative assessment of human exposure to tetrabromobisphenol A and eight bisphenols including bisphenol A via indoor dust ingestion in twelve countries. Environ. Int. 83, 183–191. https://doi.org/10.1016/j.envint.2015.06.015.

Wood, F.L., Houston, J.B., Hallifax, D., 2017. Clearance Prediction Methodology Needs Fundamental Improvement: Trends Common to Rat and Human Hepatocytes/Microsomes and Implications for Experimental Methodology. Drug Metab. Dispos. 45, 1178–1188. https://doi.org/10.1124/dmd.117.077040.

Ye, X., Wong, L.-Y., Kramer, J., Zhou, X., Jia, T., Calafat, A.M., 2015. Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000–2014. Environ. Sci. Technol. 49, 11834–11839. https://doi.org/10.1021/acs.est.5b02135.

Zhang, H., Quan, Q., Zhang, M., Zhang, N., Zhang, W., Zhan, M., Xu, W., Lu, L., Fan, J., Wang, Q., 2020. Occurrence of bisphenol A and its alternatives in paired urine and indoor dust from Chinese university students: Implications for human exposure. Chemosphere 247, 125987. https://doi.org/10.1016/j.chemosphere.2020.125987.

Zoller, O., Brüschweiler, B.J., Magnin, R., Reinhard, H., Rhyn, P., Rupp, H., Zeltner, S., Felleisen, R., 2015. Natural occurrence of bisphenol F in mustard. Food Addit. Contam. Part A 1–10. https://doi.org/10.1080/19440049.2015.1110623.