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Toxicokinetics of bisphenol A, bisphenol S, and bisphenol F in a pregnancy sheep model

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Abstract

Bisphenol A (BPA), S (BPS), and F (BPF) are among the most abundant bisphenols detected in humans, yet pregnancy toxicokinetics for BPS or BPF remain unknown. Because gestational BPS can disrupt placental function and result in reproductive and metabolic disorders in the progeny, the aim of the study was to investigate BPS and BPF toxicokinetics during pregnancy using an *in vivo* approach. Fetal catheterizations were conducted in pregnant sheep (n = 6) at mid-pregnancy and injected with either a single dose of BPS (n = 3, 0.5 mg/kg, s.c.), or a combination of BPS, BPF, and BPA (n = 3, 0.5 mg/kg for each chemical, s.c.). Maternal and fetal blood and urine and amniotic fluid were collected over 72h and analyzed for bisphenols by HPLC-MS/MS. We observed significant differences in half-life, maximum concentration, and total body clearance in maternal circulation among bisphenols. Longer half-lives were observed in fetal vs. maternal circulation for all bisphenols. Fetal toxicokinetics differed among bisphenols with BPS having the longest fetal half-life. All bisphenols reached basal levels at 48h in maternal plasma, but were still detectable in amniotic fluid, fetal urine, and fetal plasma at 72h. In this first pregnancy toxicokinetic study of BPS and BPF we have demonstrated maternal and fetal toxicokinetic differences among all three bisphenols. Higher BPS persistence in the fetal compartment warrants studies into progeny adverse outcomes following gestational exposure. Additionally, toxicokinetic

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differences among bisphenols call for a more careful approach when extrapolating kinetic information from one bisphenol chemical to another.

Keywords

bisphenols; toxicokinetics; pregnancy; fetal exposure

Introduction

Worldwide annual production of plastics has reached 8,300 million metric tons with ~80% accumulating in the environment (Geyer et al., 2017) and many of which are considered to contain endocrine disrupting chemicals (EDCs) (Gore et al., 2014). Bisphenols are EDCs used in the manufacturing of plastics, epoxy resins, and a variety of plastic and paper consumer products and food (Liao et al., 2012a; Liao and Kannan, 2013). Among the leading bisphenols present in humans are bisphenol A (BPA), bisphenol S (BPS), and bisphenol F (BPF) (Liao et al., 2012a; Philips et al., 2018). Depending on the cohort, BPA, BPS, and BPF can be detected in up to in 95.7, 89.4 and 66.5% of U.S. adults, respectively, of human urine samples (Liao et al., 2012b; Xue et al., 2015; Ye et al., 2015; Asimakopoulos et al., 2016; Lehmler et al., 2018; Philips et al., 2018; Rocha et al., 2018). BPS and BPF concentrations in humans are found in the same order of magnitude, but lower to that of BPA (Liao et al., 2012a; Ye et al., 2015). Despite the vast information available for BPA, much remains to be known regarding emerging BPA analogues (Chen et al., 2016; Wu et al., 2018). The detection of BPS, and to a lesser extent BPF, in human fetal cord blood (Kolatorova et al., 2018), the potential association between BPS exposure and gestational length (Wan et al., 2018a), and the fact that in mammals, fetal exposure to BPS can alter mammary gland development (Kolla et al., 2018), and that BPS and BPF can alter behavior (Catanese and Vandenberg, 2017; Ohtani et al., 2017), calls for the investigation of the materno-placental transfer of these bisphenols into the fetal compartment.

Recent studies have demonstrated that BPS can be acutely toxic in *D. magna* (Chen et al., 2002), induce neuronal dysfunction in zebrafish (Kinch et al., 2015) and *C. elegans* (Mersha et al., 2015) and impair fertility in aquatic species (Naderi et al., 2014; Qiu et al., 2015). In mammalian species, gestational or perinatal exposure to BPS induces obesity in mice offspring in a sex specific manner (Ivry Del Moral et al., 2016) and alters mammary gland development (Kolla et al., 2018). We have also recently demonstrated that gestational exposure to BPS reduces the endocrine secretory capacity of the placenta (Gingrich et al., 2018). Given the critical role of the placenta in pregnancy maintenance, oxygen and nutrient exchange with the developing fetus, and the detrimental effects that perturbations in placental homeostasis have on progeny outcomes (Gabory et al., 2013; Rosenfeld, 2015), understanding the toxicokinetics of BPS during pregnancy is much warranted. Although to a lesser extent, the effects of exposure to BPF have also been studied *in vivo* demonstrating that gestational BPF exposure alters neuronal behavior in mice (Ohtani et al., 2017).

Thus far, only one toxicokinetic study is available for BPS after oral administration in humans (Oh et al., 2018). However, this study was conducted in non-pregnant female and

male individuals. Physiologically based toxicokinetic (PBTK) mathematical models integrate chemical absorption, distribution, metabolism, and excretion, and help generate health risk assessment for chemical and pharmaceutical exposures due to their predictive capabilities (Zhuang and Lu, 2016; Ke et al., 2018). Recent work focusing on the development of a PBTK model for emerging bisphenols, such as BPS and BPF, have explicitly called for *in vivo* toxicokinetic data for BPS, BPF, and BPAF using biomonitoring or animal studies (Karrer et al., 2018). However, this novel PBTK model has only been developed in a non-pregnancy model. Given that toxicokinetics of BPA can be modulated by pregnancy (Corbel et al., 2013), we aimed to study the toxicokinetics of BPS and BPF during pregnancy. Our work will enable future development of precise pregnancy PBTK models for emerging bisphenols. Toxicokinetics during pregnancy are far more complex with the inclusion of the maternal, placental and fetal compartments (Ke et al., 2018). However, ethical constraints do not allow for these toxicokinetics studies to be conducted in pregnant women. The use of refined fetal surgery techniques in a sheep animal model represents a unique opportunity to monitor the maternal, amniotic, and fetal compartments, key elements of pregnancy PBTK models (Ke et al., 2018). Sheep are excellent models to study placental function (Fowden et al., 2015; Mourier et al., 2017) and have been used for the study of feto-maternal transfer of drugs (Krishna et al., 2002; Ngamprasertwong et al., 2016) and EDCs (Corbel et al., 2013; Corbel et al., 2015), as they allow for the simultaneous and longitudinal characterization of the pregnancy multi-compartment model in real time. The objective of this study was to comparatively determine the toxicokinetic profile of BPS, BPF and BPA in a pregnancy model by evaluating the maternal and the fetal compartments.

Materials and Methods

Chemicals

Bisphenols used included BPA (purity 99%, Cat#: 239658; Lot#: MKBQ5209V; Aldrich Chemical Co., Milwaukee, WI, USA), BPS (4,4'-sulfonyldiphenol, purity 99.7%, Cat#: 146915000, Lot#: A0337011, Acros Organics, Geel, Belgium), and BPF (purity 98%, Cat#: B47006; Lot#: 05712ME; Sigma-Aldrich, St. Louis, MO, USA) dissolved in corn oil. The internal standards $^{13}\text{C}_{12}$ -BPA (99%), $^{13}\text{C}_{12}$ -BPS (98%) and $^{13}\text{C}_{12}$ -BPF (99%) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA). β -glucuronidase (100,000 units/mL β -glucuronidase; 7,500 units/mL sulfatase) from *Helix pomatia* (Type HP-2) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of Michigan State University (MSU), are consistent with the National Research Council's Guide for the Care and Use of Laboratory Animals and meet the ARRIVE guidelines for reporting animal research (Kilkenny et al., 2010). The study was conducted at the MSU Sheep Teaching and Research Facility (East Lansing, MI; 42.7° N, 84.4° W) using animals selected from an in-house flock of Polypay x Dorset cross-bred sheep. Six female sheep at second or third parity were bred using a time mated pregnancy strategy using three vasectomized rams as previously described (Pu et al., 2017). After estrus detection by vasectomized rams, females were moved with a fertile ram previously tested using a

breeding soundness exam. Pregnancy and number of fetuses was confirmed at gestational day (GD) 45. Only singleton pregnancies were used in this study. Maternal and fetal characteristics are shown in Table 1. Animal diets have been previously described (Pu et al., 2017). In brief, starting 3 weeks preconception and continuing until 30 days after breeding, females were fed a total mixed ration of a diet providing an energy concentration of 3.2 Mcal/kg of digestible energy and crude protein concentration of 12%. From days 30 to 110 of gestation, energy concentration was reduced to 2.8 Mcal/kg of digestible energy. After GD110, females were fed a total mixed ration designed to maximize dry matter intake (40% neutral detergent fiber [NDF] and 70% NDF digestibility) ; 3.2 Mcal/kg digestible energy and 14% crude protein).

Experimental design

Experimental design is shown in Figure 1. Pregnant females underwent fetal catheterization surgery at $GD\ 114.8 \pm 0.8$ days. After a week of recovery, females ($n = 3$) were injected with a single s.c. dose of BPS (0.5 mg/kg) or a combination of BPA, BPS, and BPF ($n = 3$; 0.5 mg/kg for each chemical), at a dosing volume of 1.9 ± 0.1 ml. The dose and route of exposure used to calculate the pharmacokinetics of bisphenols stem from previous work in which a s.c. injection of 0.5 mg/kg BPA resulted in detectable BPA fetal concentrations (Veiga-Lopez et al., 2013) comparable to that of the median level of BPA measured in maternal circulation of U.S. women (Veiga-Lopez et al., 2015). The same doses of BPS and BPF were used to compare with the BPA dose used in the study. Although main exposure route of bisphenol exposures is oral (Liao and Kannan, 2013), the use of sheep as a model, a ruminant species, requires the use of an alternative exposure route to avoid confounding factors from ruminal metabolism.

Fetal catheterization surgery

All experimental animals underwent surgery to place a catheter in the fetal descending aorta and inferior vena cava as previously described (Ehrhardt et al., 2002). In brief, fasted animals were sedated using xylazine (0.2 mg/kg), anesthetically induced with ketamine (8 mg/kg), and inhaled isoflurane (2% vaporized) was used to maintain anesthesia throughout the procedure. A midline laparotomy was performed to isolate the uterine horn where the fetus was positioned. A hysterotomy was then performed in the uterine horn, through which the fetal limb was isolated and exposed past the tibio-tarsal joint. The cranial tibial vein was isolated with fine forceps and catheterized with a polyvinyl catheter (Saint-Gobain Performance Plastics, Beaverton, MI, USA; inner diameter: 0.86 mm, outer diameter: 1.37 mm). The catheter was threaded through the cranial tibial vein into the inferior vena cava (20 cm) and secured laterally onto the fetal hind limb using a non-absorbable suture (silk, USP 3–0). An additional catheter (Saint-Gobain Performance Plastics, Beaverton, MI, USA; inner diameter: 1.59 mm, outer diameter: 3.18 mm) was secured to the hind limb to provide access to amniotic fluid samples. The uterine incision was closed with an absorbable monofilament suture (polyglycolic acid, USP 0) using an inverted pattern finished with a single Cushing suture to avoid peri-uterine adhesion. Catheters were exteriorized through a small incision (~1 cm) in the flank of the animal nearest to the hysterotomy. The abdominal muscle wall and peritoneum were closed using an absorbable monofilament suture material (polyglycolic acid, USP 1) in an interrupted pattern. The midline skin incision was closed using non-

absorbable suture (silk, USP 0) in an interrupted mattress pattern. Catheters were flushed daily with sterile saline, followed by heparinized saline to maintain patency. Fetuses all received injections (i.m.) and amniotic infusions of gentamycin (2 ml at 100 mg/ml) at the time of surgery, and amniotic infusions every 2 days after surgery. Additionally, all ewes received penicillin G (6,000 IU/kg BW) and gentamycin sulfate (2 mg/kg BW) 24 h before surgery, at the time of the surgery, and every 12 h for 3 days after surgery. Maternal health, including body temperature, appetite, and vital signs were monitored twice daily for a week after surgery and daily thereafter. No abnormal signs were observed in any of the mothers through the termination of the experiment.

Sample collection

Except for fetal catheters and syringes (non-polycarbonate plastic) used to draw samples, all materials used for sample processing, storage, and analyses were glass. Blanks (double distilled water) for catheters and syringes were assayed for all three bisphenols to assess potential contamination from sampling collection materials. Serial maternal samples were collected before the chemical administration (Time 0 = t_0) and at 1, 3, 5, 9, 24, 36, and 48 h after the chemical administration. Serial fetal samples were collected at 0, 3, 9, 24, 36, 48, and 72 h after the chemical administration. To evaluate accumulation of bisphenols in the amniotic sac, amniotic fluid samples were collected at 0 and 72 h after the chemical administration. To evaluate the urinary excretion of bisphenol, maternal urine samples were collected before the chemical administration, and at 9 and 24 h after chemical administration. To evaluate if the bisphenols could be detected in fetal urine, samples were taken at 72 h after the chemical administration. All samples were kept frozen at -80°C until assayed.

HPLC-MS/MS analysis

Simultaneous extraction of total bisphenols (BPA, BPS and BPF) in the sheep biofluids was performed using a method reported earlier with slight modifications (Liao et al., 2012a; Gingrich et al., 2018). Because reversion of glucuronide and sulfate forms of BPA to the unconjugated form occurs through β -glucuronidase (Ginsberg and Rice, 2009) and arylsulfatase C (Stowell et al., 2006), which are highly expressed in human tissues, such as the placenta (Andra et al., 2016); in this study, we measured total BPA to provide with a combined measure for unconjugated and conjugated forms of bisphenols in this pregnancy model. Briefly, 250 μL of sheep plasma/urine/amniotic fluid was spiked with 20 ng of the internal standard (IS) mixture. To the spiked samples, an enzymatic digestion with β -glucuronidase followed by liquid-liquid extraction (LLE) with ethyl acetate was performed for the extraction of the target chemicals. Calibration curves were constructed for the target chemicals between the concentration ranges of 0.05 to 200 ng/ml with the regression coefficients > 0.998 . For every 20 samples, a procedural blank, a corresponding matrix blank, 2-level matrix spike experiments (20 ng and 40 ng) and sample duplicates were performed. There was no detectable concentration of BPF found in procedural blanks. However, trace levels of BPS (0.02 ng/ml) and BPA (0.05 ng/ml) were found in blanks and they were subtracted from concentrations found in sheep biofluids. The respective mean matrix spike recoveries (at 2-levels) were 99.1% and 90.7% (BPA), 87.9% and 95% (BPS), and 92.8% and 87.4% (BPF). Duplicate analysis of randomly selected samples yielded a

coefficient of variation of < 12% for the target chemicals. The instrumental level of quantification (LOQ) values for BPA, BPS and BPF were 0.33 ng/ml, 0.12 ng/ml and 0.50 ng/ml, respectively. An Agilent 1260 HPLC (Agilent Technologies Inc., Santa Clara, CA) system equipped with a Zorbax SB-Aq (150 mm x 2.1 mm, 3.5 μ m; Agilent Technologies Inc., Santa Clara, CA) column was used for the chromatographic separation of target chemicals under a gradient elution method using water (A) and methanol (B) as mobile phases. An API 4500TM electrospray QTRAP mass spectrometer (ESI-MS/MS; Applied Biosystems, AB Sciex, Framingham, MA, USA) was used for the selective identification (MRM transitions) and quantification (by isotopic dilution method) of the target chemicals.

Toxicokinetic analysis

All toxicokinetic parameters were calculated using a non-compartmental analysis based on plasma concentrations of total BPA, BPS, or BPF over time for each individual. The following parameters were calculated for each chemical for the maternal (_{mat}) and fetal (_{fet}) compartments: 1) maximum observed plasma concentration ($C_{\max\text{-mat}}$ and $C_{\max\text{-fet}}$), 2) the time at which the $C_{\max}$ was observed ($t_{\max\text{-mat}}$ and $t_{\max\text{-fet}}$), 3) area under the curve (AUC_{mat} and AUC_{fet}) from 0 to 48 h for maternal and from 0 to 72 h in fetal, and area under the first moment curve ($AUMC_{\text{mat}}$ and $AUMC_{\text{fet}}$) calculated using the logarithmic trapezoidal method (Purves, 1992), 4) mean residence time (MRT_{mat} and MRT_{fet}) calculated using the equation $MRT = AUMC/AUC$ (Karol, 1990), and 5) chemical half-life ($t_{1/2\text{mat}}$ and $t_{1/2\text{fet}}$). Total body clearance ($Cl_{\text{TB-mat}}$) was estimated for maternal samples and calculated using the equation $Cl_{\text{TB}} = \text{dose}/AUC$. All parameters were calculated using the software Kinetica with a non-compartmental analysis (NCA) assistant (Adept Scientific, version 5.0, 2007). Kinetica was also used to validate AUC, AUMC, and MRT calculations. To evaluate the bioaccumulation preference in mother vs. the fetus, the fetal to maternal (F/M) ratio was calculated by dividing the $C_{\max\text{-fet}}$ by the $C_{\max\text{-mat}}$.

Statistical analysis

All data are presented as mean $\pm$ SEM. Appropriate logarithmic transformations were applied, as needed, to account for normality of data. First, maternal and fetal toxicokinetic parameters for BPS comparing females exposed to only BPS (n = 3) vs. the mixture (BPA, BPS, and BPF) (n = 3) was conducted using an independent T-test to evaluate if the co-administration of BPA and BPF may have affected BPS kinetics. No significant differences were observed and thus all 6 females exposed to BPS were combined and analyzed as a single group. Except for $t_{\max\text{-mat}}$, comparisons of maternal and fetal toxicokinetic parameters among the three treatment groups were analyzed by a mixed model or ANOVA with Tukey posthoc tests with fetal sex as a covariate. For $t_{\max\text{-mat}}$, comparisons among the three treatment groups were analyzed using a Kruskal-Wallis followed by a Dunn-Bonferroni posthoc test. In addition, to test the effect of fetal sex and interaction with treatment a general linear model was used. For comparisons within treatment group between 2 time points (i.e.: amniotic fluid) an independent T-test was used. Statistical software used was PASW Statistics for Windows release 18.0.1. Differences were considered significant at $P < 0.05$.

Results

Maternal non-compartmental toxicokinetic analysis

Maternal bisphenol concentrations after a single s.c. dose of BPA, BPS, or BPF are shown on Figure 2 and corresponding estimated toxicokinetic parameters using the above-referenced non-compartmental analysis are displayed on Table 2 (*left panel*). As described in the statistics` section, no significant differences were observed in maternal BPS toxicokinetics when comparing females exposed to only BPS ($n = 3$) vs. the mixture (BPA, BPS, and BPF) ($n = 3$) and thus all 6 females exposed to BPS were combined for comparison with BPA and BPF toxicokinetics. Mean $C_{\max\text{-mat}}$ was statistically different among treatments with $C_{\max\text{-mat}}$ for BPS (643 ± 29.9 ng/ml; $P < 0.001$) being an order of magnitude higher than for BPA and BPF (66.7 ± 1.7 and 48.8 ± 0.2 ng/ml, respectively; $P < 0.005$). $t_{\max\text{-mat}}$ was achieved at the same time after BPA and BPS exposure (3.0 ± 0.0 h) and earlier (1.7 ± 0.7 h) for BPF ($P < 0.05$). Both AUC_{mat} and $AUMC_{\text{mat}}$ reported after BPS exposure were significantly higher than those of BPA and BPF ($P < 0.02$; see Table 2). MRT_{mat} varied among bisphenols with BPF having the longest (10.3 ± 2.5 h) and BPS having the shortest (6.7 ± 0.3 h) MRT_{mat} , although no statistical difference was observed. Mean $Cl_{\text{TB-mat}}$ was statistically different among all treatments with BPF having the fastest $Cl_{\text{TB-mat}}$ (103.8 ± 22.7 ml/h) compared to BPA and BPS (45.4 ± 5.5 and 5.2 ± 0.5 ml/h, respectively; $P < 0.02$).

Urinary bisphenols concentrations are shown in Table 3. At t_9 after bisphenol administration, maternal urine peak concentrations for BPA and BPS were $1,300 \pm 210$ and $3,870 \pm 888$ ng/ml, respectively and were reduced by one order of magnitude at t_{24} . In contrast, BPF in maternal urine was detected at much lower concentrations and with means increasing over time but with a high variability. Urinary bisphenol concentrations in maternal samples were only found to be significantly different between BPF and either BPA or BPS ($P < 0.05$; see Table 3), but not between BPA and BPS.

Fetal non-compartmental toxicokinetic analysis

Fetal bisphenol concentrations after a single s.c. dose of BPA, BPS, or BPF are shown in Figure 2 and corresponding estimated toxicokinetic parameters using the above-referenced non-compartmental analysis are displayed on Table 2 (*right panel*). There were no statistical differences in fetal toxicokinetics between female or male fetuses in the BPS group (data not shown) and thus data for both fetal sexes were combined. The number was too small to test this in BPA or BPF groups. Additionally, as described in the statistical section, no significant differences were observed in fetal BPS toxicokinetics when comparing females exposed to only BPS ($n = 3$) vs. the mixture (BPA, BPS, and BPF) ($n = 3$) (data not shown) and thus all 6 females exposed to BPS were combined for comparison with BPA and BPF toxicokinetics. Mean $C_{\max\text{-fet}}$ after BPA administration (87.6 ± 6.9) was significantly higher than either BPS or BPF (18.5 ± 1.1 and 20.9 ± 2.0 ng/ml, respectively; $P < 0.001$), but within the same order of magnitude. $T_{\max\text{-fet}}$ observed was bisphenol dependent with BPF being the earliest and statistically different (3.0 ± 0.0 h; $P < 0.05$), from either BPA (14.0 ± 5.0 h) or BPS (21.0 ± 4.2 h). Both, AUC_{fet} and $AUMC_{\text{fet}}$ differed significantly among bisphenols, with BPA being the highest for both variables (3.9 ± 0.2 mg*h/ml and 120 ± 17 mg*h²/ml

respectively), followed by BPS (0.99 ± 0.05 mg*h/ml and 34 ± 1.5 mg*h²/ml respectively), and lastly BPF (0.21 ± 0.05 mg*h/ml and 1.7 ± 0.4 mg*h²/ml respectively) ($P < 0.001$, see Table 2). MRT_{fet} after BPA and BPS administration was within the same order of magnitude (31.3 ± 3.6 and 34.1 ± 1.7 h, respectively), and were both significantly higher to that observed after BPF administration (8.5 ± 0.2 h) ($P < 0.001$).

Prior to maternal administration of bisphenols, BPA, BPS, and BPF concentrations measured in fetal amniotic fluid (Table 4) were $< LOQ$ ($< 0.33 \pm 0.0$), 0.3 ± 0.2 , and $< LOQ$ ($< 0.5 \pm 0.0$) ng/ml, respectively. At t_{72} , all bisphenols were detectable in amniotic fluid, but concentrations were not different among bisphenols. At t_{72} , fetal urine total BPA was detected at 144.3 ± 46.7 ng/ml, an order of magnitude higher to that of BPS or BPF (6.6 ± 1.5 and 13.8 ± 3.4 ng/ml, respectively), however no statistical differences were detected. The F/M ratio was 1.31, 0.03, and 0.42 in BPA, BPS, and BPF, respectively.

Discussion

Fifteen different bisphenols exist, at least 8 are detectable in human urine, and concomitantly, BPA, BPS and BPF are detected in 95.7, 89.4 and 66.5% of U.S. adults, respectively (Lehmle et al., 2018). We have recently demonstrated that gestational exposure to BPS can result in placental dysfunction (Gingrich et al., 2018), and can also result in reproductive and metabolic disorders in the progeny (Ivry Del Moral et al., 2016; Kolla et al., 2018). Pregnancy toxicokinetics of less prevalent bisphenols, such as BPS and BPF, remain unknown. The current study provides with novel insights into the toxicokinetics of these two bisphenols in a pregnancy model. We demonstrate that BPS reaches maternal circulation at a concentration one order of magnitude above that of the other bisphenols (s.c. administration) and has the shortest half-life. In contrast, in the fetal compartment, BPS has the longest half-life, persisting at a low concentration. We have also evaluated the pregnancy toxicokinetics of BPF, the first study of its kind in any model system and demonstrated that BPF has a comparatively long half-life in maternal circulation, but clears faster from fetal circulation compared to BPA or BPS. The persistence of BPS in the fetal compartment, suggestive of a higher availability compared to other bisphenols, highlights the need to conduct additional studies to understand the effects of chronic BPS exposure on the developing fetus.

In this study, sheep was used as the animal model to help understand the kinetics of three commonly found bisphenols in a pregnancy model. Although there are essential differences in placentation between sheep and humans (Hyttel, 2010; Benirschke et al., 2012), sheep are considered a valid and useful model in the understanding of human placental function (Bird, 2017). Importantly, the sheep allows for fetal catheterization during mid-pregnancy and the evaluation of the chemical transfer from the maternal into the fetal and amniotic fluid compartment (Gingrich et al., 2018). Fetal catheterization to monitor dynamic toxicokinetics is only feasible in large mammalian species, such as monkeys and sheep. However, the use of the sheep represents a challenge, as it is a ruminant species, with different digestive physiology. Thus, to avoid differences in pre-absorptive metabolism in the ruminant digestive system, s.c. was selected as the route of choice in this study. In addition to the oral

route (Liao and Kannan, 2014), transdermal is also a major route of exposure to bisphenols (Bernier and Vandenberg, 2017).

Maternal toxicokinetics of bisphenols

In this study, total BPS reached the highest $C_{\text{max-mat}}$ in plasma but had the shortest $t_{1/2\text{-mat}}$ and MRT_{mat} demonstrating that despite reaching the highest circulating concentrations, BPS persisted less time in maternal circulation compared to BPA or BPF. To our knowledge, only a single study has evaluated BPS toxicokinetics after a single oral dose (8.7 $\mu\text{g/kg BW}$ of d⁴-BPS) (Oh et al., 2018). For total BPS, this human study reported a higher MRT (9.8 ± 1.03 h) and longer $t_{1/2}$ (6.81 ± 0.72 h) (Oh et al., 2018) compared to the current study (MRT_{mat} : 6.7 ± 0.3 h and $t_{1/2\text{-mat}}$: 3.7 ± 0.1 h). Although within the same magnitude, differences in route of exposure between studies (oral vs. s.c.) and lack of first pass metabolism in s.c. exposure may be accounting for a shorter $t_{1/2}$ in the present study. However, the human BPS toxicokinetic study (Oh et al., 2018) has been challenged for assumptions regarding BPS bioavailability and exclusion of contributions from first-pass metabolism (Grandin et al., 2018). Importantly, exclusion of first pass metabolism following an oral dose could result in the overestimation of MRT_{mat} and $t_{1/2\text{-mat}}$.

To our knowledge this is the first BPF toxicokinetic study in a pregnancy model. We have demonstrated that BPF kinetics during pregnancy differ to that of BPA and BPS, with BPF reaching the lowest $C_{\text{max-mat}}$ (48.8 ± 0.2 ng/ml), but having the fastest clearance rate (103.8 ± 22.7 ml/h) compared to BPS and BPA. To date, the only information on the kinetics of BPF is limited to an *in vivo* study that compared BPF excretion between pregnant and non-pregnant female rats following a high oral dose (7 or 100 mg/kg H³-BPF) (Cabaton et al., 2006). As in rats (Cabaton et al., 2006) and humans (Lehmle et al., 2018), we were able to confirm that BPF is excreted through maternal urine (Table 3), although we are unable to account for BPF excretion through other routes, such as biliary, or the urinary clearance of the main BPF conjugates, glucuronide or sulfate (Cabaton et al., 2008). To note, BPF was detected in one out of three pre-exposure urine samples. As BPF has been detected in grain products (Liao and Kannan, 2013) and because animals were housed outdoors until a week prior to surgery, it is possible the mothers were environmentally exposed to BPF prior to the start of the kinetic experiment.

The current BPA toxicokinetic results add to previous pregnancy reports in sheep and rhesus monkeys (Corbel et al., 2013; Patterson et al., 2013). Our observed $t_{1/2\text{-mat}}$ of 5.3 ± 0.4 h was similar to $t_{1/2}$ reported following oral administration in humans (6.4 ± 2.0 h (Thayer et al., 2015); 5.4 h (Volkel et al., 2002)), s.c. administration in neonatal rats ($3.4 - 4.3$ h (Doerge et al., 2010)), i.v. administration in pregnant female rhesus monkeys (2.8 ± 1.1 h (Patterson et al., 2013)), and i.v. administration in adult female rats (8.1 ± 6.1 h (Doerge et al., 2010)). The similar $t_{1/2}$ observed in human studies compared to our study despite the different species and route of exposure highlight the usefulness of using sheep to understand bisphenol toxicokinetics. For unconjugated BPA, a lower $t_{1/2}$ (0.76 ± 0.66 h) was reported following an i.v. administration (2 mg/kg/day) in a pregnant Lacaune sheep model when compared with the current study, despite similar MRT (9.56 ± 0.25 h) (Corbel et al., 2013) to that of our study (MRT_{mat} : 8.3 ± 0.6 h). Differences in $t_{1/2}$ likely relate to form of BPA

measured and the fact that Corbel *et al.* (Corbel et al., 2013) did not account for fetal number, which could blunt the $t_{1/2}$, as observed with betamethasone pharmacokinetics (Ballabh et al., 2002). Furthermore, BPA administration (s.c.) to non-pregnant female rats resulted in a $t_{\max}$ (1 – 4 h) similar to this study (3.0 h), (Pottenger et al., 2000), which may be attributed to the higher dose used (10 mg/kg) or the reproductive state of the female (non-pregnant). In humans, a single oral dose of d⁶-BPA resulted in a similar but faster $t_{\max}$ (1.1 ± 0.5 h), and a $C_{\max}$ a magnitude higher ($1,711 \pm 496$ nM) than our reported $C_{\max\text{-mat}}$ value (Thayer et al., 2015). The difference in $t_{\max}$ and $C_{\max}$ between the current study and the study following an oral dose of BPA in humans may be due to differences in route of exposure, species, and/or dose administered. Unlike other studies that have reported inter-individual differences in BPA toxicokinetics (Thayer et al., 2015), in our study, all maternal and fetal BPA kinetics showed very small variability among animals. To note, BPA $t_{1/2\text{-mat}}$ was similar to a human oral administration study (Thayer et al., 2015), while BPS $t_{1/2\text{-mat}}$ was shorter to that of another human oral exposure study (Oh et al., 2018). In addition to the pitfalls noted for the BPS human oral study, such as exclusion of first-pass metabolism in their modeling, this discrepancy may be partly explained by the MRT_{mat} calculated for each bisphenol. The faster MRT observed for BPS over BPA indicates a faster turnover BPS rate, and a larger probability that BPS toxicokinetics are altered depending on the route of exposure compared with BPA, which has a slower MRT . Additional factors that may contribute to the $t_{1/2}$ differences between our study and the two human studies may relate to pregnancy status and exposure dose, both of which differ from the current study.

Our comparative evaluation of the three bisphenols toxicokinetics demonstrated that the largest differences were evident between BPS and BPF, with BPS having the highest $C_{\max\text{-mat}}$ and AUC_{mat} , and BPF the lowest, while BPF had the highest $t_{1/2\text{-mat}}$, MRT_{mat} , and $Cl_{\text{TB-mat}}$, while BPS had the lowest. With the shortest $t_{1/2\text{-mat}}$ and despite a slower clearance, BPS clears from maternal plasma by 24 h, while BPA cleared by 48 h, and BPF persisted at low concentrations and was still detected (< 9.2 ng/ml) at 48 h post-injection. Toxicokinetic differences are the result of variation in absorption, distribution, metabolism, and excretion that are directly attributed to differences in the chemical properties between BPS and BPF, including solubility, acidity constant, plasma protein binding, and molecular weight (see Table 5; (Cao et al., 2011; Pan et al., 2014; Pivnenko et al., 2015; Luo et al., 2016)). Chemical transport and metabolism, and competition for transport and metabolism highly depend on these properties (Griffiths and Campbell, 2015). In addition, absorption from the subcutaneous compartment into the blood circulation is not yet fully understood and factors like depth of subcutaneous adipose tissue may alter drug kinetics (Richter et al., 2012). Thus, lower water solubility and higher $\log K_{\text{o/w}}$ of BPA (300 mg/l and 3.32, respectively or BPF (5.4 and 2.91, respectively compared to BPS's (1,100 mg/l and 1.65, respectively (see Table 5; (PubChem; Pivnenko et al., 2015))) could have contributed to larger retention of the BPA and BPF dose in the subcutaneous adipose tissue. Further work is required to validate this hypothesis. Overall, differences in this pregnancy toxicokinetic model highlight the need to exert caution when extrapolating kinetic information from BPA, from which much is known, to other bisphenol chemicals.

In this study, half of the females received only BPS, while the other half received a mixture of BPA, BPS, and BPF, similar to a co-exposure of bisphenols that humans are exposed to (>

50% of people may be exposed to the three bisphenols simultaneously in the U.S. (Lehmle et al., 2018)). Maternal or fetal BPS toxicokinetic parameters did not differ in animals exposed to only BPS *vs.* the combination of bisphenols suggesting that the co-exposure to BPA or BPF did not interfere with absorption, distribution, metabolism, and excretion of BPS. Our experimental design did not allow to address this for BPA or BPF. However, the similar BPA toxicokinetic results obtained in this study compared to previous work (Volkel et al., 2002; Doerge et al., 2010; Gayraud et al., 2013; Patterson et al., 2013; Thayer et al., 2015) suggest that co-exposure to other bisphenols did not interfere with BPF or BPA kinetics. Although, a recent study has demonstrated that the co-administration of BPA (oral, 50 µg/kg C¹⁴-BPA) with BPS (s.c. injections, 1, 3, 6, and 9 mg) results in elevated circulating levels of total BPA in mice at the 9 mg BPS dose (Pollock et al., 2018) and hypothesized that the BPA kinetics may be altered due to competition for metabolic enzymes, such as sulfotransferase (SULT) or UDP-glucuronosyltransferase (UGT) (Pollock et al., 2018).

Fetal toxicokinetics of bisphenols

We have demonstrated that, like BPA (Corbel et al., 2013; Patterson et al., 2013), both BPS and BPF cross the placenta reaching the fetal compartment and entering the amnion. Maternal *vs.* fetal toxicokinetic parameters were different with lower concentrations detected in the fetal compartment for BPS and BPF. This finding points to an incomplete maternal to fetal transfer, supportive of an active transport, rather than passive diffusion, across the placental barrier as the transfer mechanism (Pacifici and Nottoli, 1995; Yoshikawa et al., 2002). Similar to the maternal compartment, the non-compartmental analysis also revealed marked toxicokinetic differences among all three bisphenols within the fetal compartment.

Despite BPS being the bisphenol with the highest concentration in maternal circulation, BPA had the highest fetal C_{max}-fet compared to BPS and BPF. This suggests that active placental transport is more permissive for BPA compared to BPS and BPF, which may relate to differences in lipophilicity, solubility, plasma protein binding, and/or molecular weight (Griffiths and Campbell, 2015). In this study, we observed that only BPA had an F/M ratio > 1. Because an F/M ratio > 1 reflects that the chemical has a higher affinity to bioaccumulate in the fetus compared to the mother, we would predict that only BPA would accumulate in the fetal compartment. In fact, the higher logK_{o/w} for BPA and BPF (3.32 and 2.91) points to a higher risk of bioaccumulation in tissues compared to BPS (1.65). However, BPS's long t_{1/2}-fet (402.2 ± 102.6 h) demonstrates that despite a lower placental transfer, the clearance from the fetal compartment occurs at a much slower rate to that of BPA (t_{1/2}-fet: 52.0 ± 20.3 h), resulting in BPS's long persistence in the fetal compartment. Previous studies have also demonstrated that conjugated BPA can remain trapped in the fetal compartment (Nishikawa et al., 2010; Corbel et al., 2013). This phenomenon has been hypothesized to occur due to the inability of water-soluble conjugated BPA to cross the placenta, and is therefore excreted in fetal urine and trapped in the amniotic fluid (Corbel et al., 2013). In this study, we have not evaluated the conjugated forms of bisphenols and thus cannot confirm this for BPS. It has also been hypothesized that once present in the amniotic fluid, BPA may be swallowed and recirculated into the fetus (Corbel et al., 2013). The accumulation of BPA in amniotic fluid has been previously reported in rhesus monkeys (Patterson et al., 2013) and rats

(Doerge et al., 2011). In support of the idea that the amniotic fluid may serve as a reservoir that helps recirculate bisphenols into the fetal compartments, we demonstrate that BPS and BPF can also accumulate in the amniotic fluid up to 72 h after administration (Table 4). Given the predicted $t_{1/2\text{-fet}}$, BPS may be detected in the fetal compartment up to a month after administration.

Chemical accumulation in the fetal compartment is not unique to bisphenols and has been reported with other chemicals (Mitro et al., 2015). Previous studies have shown that a decrease in the efflux transporter placental-glycoprotein (P-gp), also known as MDR1 or ATP-binding cassette transporter B1 (ABCB1), is associated with an accumulation of P-gp substrates (Bloise et al., 2017), such as BPA (Yoshikawa et al., 2002), in the fetal compartment. It is possible that the accumulation of both BPA and BPS, and the comparatively high levels of BPA reached in fetal circulation could potentially be the result of competition between bisphenols for efflux through transporters, such as P-gp. Additional complexity arises from the fact that BPA can also modify ABC transporters in placental cells (Jin and Audus, 2005).

Our previous work has shown that gestational BPS, but not BPA, leads to a placental disruption (Gingrich et al., 2018). Despite a longer BPS half-life in the fetal compartment, the fact that both chemicals are detectable in the amniotic fluid and fetal compartment after 72 h suggests that the placenta was exposed to either bisphenol to a similar degree. Whether effects on the placenta may relate to different placental bioaccumulation of BPA and BPS or a different exposure in bioactive unconjugated bisphenols, remains unknown. It is also important to note that the current results are specific to mid-to late gestation. Numerous studies have demonstrated how gestational age modifies toxicokinetics, which largely depend on changes in placental transport receptor, cytochrome P450 enzymes expression in liver and placenta, or fetal to maternal albumin ratio (Ewing et al., 2015). These factors should be considered when extrapolating current results to other gestational ages.

In addition to amniotic fluid, this study is the first to detect bisphenols in fetal urine after maternal exposure. In addition to previous studies reporting BPA excretion through meconium (Arbuckle et al., 2015), our study demonstrates that the fetus, like the mother, is also able to excrete bisphenols through urine. Urinary BPA concentrations are similar in the mother compared to the fetus after 72 h of exposure. A similar relationship has been reported for other chemicals, such as triclosan (Arbuckle et al., 2015). Although it has been demonstrated *in vitro* that there are differences in glucuronidation efficiency between bisphenols (Karrer et al., 2018), these glucuronidation rates were determined in adult human liver and intestinal microsomes, whose metabolic profiles are developmental stage dependent (Chiba et al., 1997). However, because we did not evaluate conjugated forms of bisphenols it is not possible to address such conjugation rates in our fetal urine samples. On the other hand, we detected BPS above the limit of quantification in both amniotic fluid and fetal samples prior to exposure (t_0) in over half of the samples analyzed. As observed for BPF, and because animals were housed outdoors until a week prior to surgery, we hypothesize that these higher baseline BPS concentrations in the fetal compartment are the combination of the longer half-life for BPS in the fetal compartment (402.2 ± 102.6 h or

~16.5 days) and potential environmental exposures (i.e. water source (Wan et al., 2018b)) prior to the surgical intervention.

Conclusion

To our knowledge, this is the first report on the toxicokinetics of either BPS or BPF in a pregnancy model. This study has not only demonstrated differences in toxicokinetic parameters among three of the most prevalent bisphenols, but also between differences in maternal and fetal toxicokinetics. Toxicokinetic differences among bisphenols call for a more careful approach when extrapolating kinetic information from one bisphenol chemical to another.

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Abbreviations

ABCB1	ATP-binding cassette transporter B1
AUC_{fet}	fetal area under the curve
AUC_{mat}	maternal area under the curve
AUMC_{fet}	fetal area under the first moment curve
AUMC_{mat}	maternal area under the first moment curve
BPA	Bisphenol A
BPS	Bisphenol S
BPF	Bisphenol F
Cl_{TB-mat}	maternal total body clearance
C_{max-fet}	maximum observed fetal plasma concentration
C_{max-mat}	maximum observed maternal plasma concentration
EDC	endocrine disrupting chemicals
F/M	fetal to maternal
GD	gestational day

LLE	liquid-liquid extraction
LOQ	level of quantification
MRT_{fet}	fetal mean residence time
MRT_{mat}	maternal mean residence time
NCA	non-compartmental analysis
NDF	neutral detergent fiber
P-gp	placental-glycoprotein
PBTK	Physiologically based toxicokinetic
SULT	sulfotransferase
t_{1/2-fet}	fetal chemical half-life
t_{1/2-mat}	maternal chemical half-life
UGT	UDP-glucuronosyltransferase

References

- Andra SS, Austin C, Yang J, Patel D, Arora M, 2016 Recent advances in simultaneous analysis of bisphenol A and its conjugates in human matrices: Exposure biomarker perspectives. *Science of the Total Environment* 572, 770–781. [PubMed: 27586167]
- Arbuckle TE, Weiss L, Fisher M, Hauser R, Dumas P, Berube R, Neisa A, LeBlanc A, Lang C, Ayotte P, Walker M, Feeley M, Koniecki D, Tawagi G, 2015 Maternal and infant exposure to environmental phenols as measured in multiple biological matrices. *Science of the Total Environment* 508, 575–584. [PubMed: 25483107]
- Asimakopoulos AG, Xue J, De Carvalho BP, Iyer A, Abualnaja KO, Yaghmoor SS, Kumosani TA, Kannan K, 2016 Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia. *Environmental research* 150, 573–581. [PubMed: 26654562]
- Ballabh P, Lo ES, Kumari J, Cooper TB, Zervoudakis I, Auld PA, Krauss AN, 2002 Pharmacokinetics of betamethasone in twin and singleton pregnancy. *Clin Pharmacol Ther* 71, 39–45. [PubMed: 11823756]
- Benirschke K, Burton G, Baergen RN, 2012 *Pathology of the human placenta*, 6th ed. Springer, Berlin ; London.
- Bernier MR, Vandenberg LN, 2017 Handling of thermal paper: Implications for dermal exposure to bisphenol A and its alternatives. *PLoS One* 12, e0178449. [PubMed: 28570582]
- Bird SD, 2017 Artificial placenta: Analysis of recent progress. *Eur J Obstet Gynecol Reprod Biol* 208, 61–70. [PubMed: 27894031]
- Bloise E, Petropoulos S, Iqbal M, Kostaki A, Ortiga-Carvalho TM, Gibb W, Matthews SG, 2017 Acute Effects of Viral Exposure on P-Glycoprotein Function in the Mouse Fetal Blood-Brain Barrier. *Cell Physiol Biochem* 41, 1044–1050. [PubMed: 28222448]
- Cabaton N, Chagnon MC, Lhuguenot JC, Cravedi JP, Zalko D, 2006 Disposition and metabolic profiling of bisphenol F in pregnant and nonpregnant rats. *Journal of agricultural and food chemistry* 54, 10307–10314. [PubMed: 17177575]
- Cabaton N, Zalko D, Rathahao E, Canlet C, Delous G, Chagnon MC, Cravedi JP, Perdu E, 2008 Biotransformation of bisphenol F by human and rat liver subcellular fractions. *Toxicology in*

vitro : an international journal published in association with BIBRA 22, 1697–1704. [PubMed: 18672047]

- Cao J, Guo LH, Wan B, Wei Y, 2011 In vitro fluorescence displacement investigation of thyroxine transport disruption by bisphenol A. *J Environ Sci (China)* 23, 315–321. [PubMed: 21517007]
- Catanese MC, Vandenberg LN, 2017 Bisphenol S (BPS) Alters Maternal Behavior and Brain in Mice Exposed During Pregnancy/Lactation and Their Daughters. *Endocrinology* 158, 516–530. [PubMed: 28005399]
- Chen D, Kannan K, Tan HL, Zheng ZG, Feng YL, Wu Y, Widelka M, 2016 Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity-A Review. *Environmental science & technology* 50, 5438–5453. [PubMed: 27143250]
- Chen MY, Ike M, Fujita M, 2002 Acute toxicity, mutagenicity, and estrogenicity of bisphenol-A and other bisphenols. *Environmental toxicology* 17, 80–86. [PubMed: 11847978]
- Chiba M, Nshime JA, Lin JH, 1997 In vitro metabolism of indinavir in the human fetal liver microsomes. *Drug metabolism and disposition: the biological fate of chemicals* 25, 1219–1222. [PubMed: 9321528]
- Corbel T, Gayrard V, Viguie C, Puel S, Lacroix MZ, Toutain PL, Picard-Hagen N, 2013 Bisphenol A disposition in the sheep maternal-placental-fetal unit: mechanisms determining fetal internal exposure. *Biol Reprod* 89, 11. [PubMed: 23699389]
- Corbel T, Perdu E, Gayrard V, Puel S, Lacroix MZ, Viguie C, Toutain PL, Zalko D, Picard-Hagen N, 2015 Conjugation and deconjugation reactions within the fetoplacental compartment in a sheep model: a key factor determining bisphenol A fetal exposure. *Drug metabolism and disposition: the biological fate of chemicals* 43, 467–476. [PubMed: 25576162]
- Doerge DR, Twaddle NC, Vanlandingham M, Brown RP, Fisher JW, 2011 Distribution of bisphenol A into tissues of adult, neonatal, and fetal Sprague-Dawley rats. *Toxicol Appl Pharmacol* 255, 261–270. [PubMed: 21820460]
- Doerge DR, Twaddle NC, Vanlandingham M, Fisher JW, 2010 Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley rats. *Toxicol Appl Pharmacol* 247, 158–165. [PubMed: 20600215]
- Ehrhardt RA, Bell AW, Boisclair YR, 2002 Spatial and developmental regulation of leptin in fetal sheep. *Am J Physiol Regul Integr Comp Physiol* 282, R1628–1635. [PubMed: 12010744]
- Ewing G, Tatarchuk Y, Appleby D, Schwartz N, Kim D, 2015 Placental transfer of antidepressant medications: implications for postnatal adaptation syndrome. *Clin Pharmacokinet* 54, 359–370. [PubMed: 25711391]
- Fowden AL, Forhead AJ, Sferruzzi-Perri AN, Burton GJ, Vaughan OR, 2015 Review: Endocrine regulation of placental phenotype. *Placenta* 36 Suppl 1, S50–59. [PubMed: 25524059]
- Gabory A, Roseboom TJ, Moore T, Moore LG, Junien C, 2013 Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. *Biol Sex Differ* 4, 5. [PubMed: 23514128]
- Gayrard V, Lacroix MZ, Collet SH, Viguie C, Bousquet-Melou A, Toutain PL, Picard-Hagen N, 2013 High bioavailability of bisphenol A from sublingual exposure. *Environ Health Perspect* 121, 951–956. [PubMed: 23761051]
- Geyer R, Jambeck JR, Law KL, 2017 Production, use, and fate of all plastics ever made. *Sci Adv* 3.
- Gingrich J, Pu Y, Roberts J, Karthikraj R, Kannan K, Ehrhardt R, Veiga-Lopez A, 2018 Gestational bisphenol S impairs placental endocrine function and the fusogenic trophoblast signaling pathway. *Arch Toxicol* 92, 1861–1876. [PubMed: 29550860]
- Ginsberg G, Rice DC, 2009 Does Rapid Metabolism Ensure Negligible Risk from Bisphenol A? *Environ Health Persp* 117, 1639–1643.
- Gore AC, Crews D, Doan LL, La Merrill M, Patisaul HB, Zota A, 2014 Introduction to endocrine disrupting chemicals (EDCs) a guide for public interest organizations and policy-makers. *Endocrine Society*, pp. 1–69.
- Grandin FC, Gayrard V, Lacroix MZ, Picard-Hagen N, Mila H, Toutain PL, 2018 Comment on ‘Pharmacokinetics of bisphenol S in humans after a single oral administration’. *Environ Int* 116, 29. [PubMed: 29635094]
- Griffiths SK, Campbell JP, 2015 Placental structure, function and drug transfer. *Bja Educ* 15, 84–89.

- Hyttel P, 2010 Essentials of domestic animal embryology Saunders/Elsevier, Edinburgh ; New York.
- Ivry Del Moral L, Le Corre L, Poirier H, Niot I, Truntzer T, Merlin JF, Rouimi P, Besnard P, Rahmani R, Chagnon MC, 2016 Obesogen effects after perinatal exposure of 4,4'-sulfonyldiphenol (Bisphenol S) in C57BL/6 mice. *Toxicology* 357–358, 11–20.
- Jin H, Audus KL, 2005 Effect of bisphenol A on drug efflux in BeWo, a human trophoblast-like cell line. *Placenta* 26 Suppl A, S96–S103. [PubMed: 15837075]
- Karol MD, 1990 Mean residence time and the meaning of AUMC/AUC. *Biopharm Drug Dispos* 11, 179–181. [PubMed: 2328302]
- Karrer C, Roiss T, von Goetz N, Gramec Skledar D, Peterlin Masic 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. [PubMed: 29995627]
- Ke AB, Greupink R, Abduljalil K, 2018 Drug Dosing in Pregnant Women: Challenges and Opportunities in Using Physiologically Based Pharmacokinetic Modeling and Simulations. *CPT Pharmacometrics Syst Pharmacol* 7, 103–110. [PubMed: 29349870]
- Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG, 2010 Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research. *PLoS biology* 8, e1000412. [PubMed: 20613859]
- Kinch CD, Ibahazehiebo K, Jeong JH, Habibi HR, Kurrasch DM, 2015 Low-dose exposure to bisphenol A and replacement bisphenol S induces precocious hypothalamic neurogenesis in embryonic zebrafish. *Proc Natl Acad Sci U S A* 112, 1475–1480. [PubMed: 25583509]
- Kolatorova L, Vitku J, Hampl R, Adamcova K, Skodova T, Simkova M, Parizek A, Starka L, Duskova M, 2018 Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. *Environmental research* 163, 115–122. [PubMed: 29433019]
- Kolla S, Morcos M, Martin B, Vandenberg LN, 2018 Low dose bisphenol S or ethinyl estradiol exposures during the perinatal period alter female mouse mammary gland development. *Reproductive toxicology* 78, 50–59. [PubMed: 29526645]
- Krishna R, Riggs KW, Kwan E, Wong H, Szeitz A, Walker MP, Rurak DW, 2002 Clearance and disposition of indometacin in chronically instrumented fetal lambs following a 3-day continuous intravenous infusion. *J Pharm Pharmacol* 54, 801–808. [PubMed: 12078996]
- Lehmler HJ, Liu B, Gadogbe M, Bao W, 2018 Exposure to Bisphenol A, Bisphenol F, and Bisphenol S in U.S. Adults and Children: The National Health and Nutrition Examination Survey 2013–2014. *ACS Omega* 3, 6523–6532. [PubMed: 29978145]
- 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. *Journal of agricultural and food chemistry* 61, 4655–4662. [PubMed: 23614805]
- Liao C, Kannan K, 2014 A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. *Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment* 31, 319–329.
- Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon HB, Nakata H, Kannan K, 2012a Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. *Environmental science & technology* 46, 6860–6866. [PubMed: 22620267]
- Liao C, Liu F, Guo Y, Moon HB, Nakata H, Wu Q, Kannan K, 2012b Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. *Environmental science & technology* 46, 9138–9145. [PubMed: 22784190]
- Luo H, Li C, Fang H, Wang X, 2016 Comparative study of the interactions between bisphenol analogues and serum albumins by electrospray mass spectrometry and fluorescence spectroscopy. *Rapid Commun Mass Spectrom* 30 Suppl 1, 162–167. [PubMed: 27539432]
- Mersha MD, Patel BM, Patel D, Richardson BN, Dhillon HS, 2015 Effects of BPA and BPS exposure limited to early embryogenesis persist to impair non-associative learning in adults. *Behav Brain Funct* 11, 27. [PubMed: 26376977]
- Mitro SD, Johnson T, Zota AR, 2015 Cumulative Chemical Exposures During Pregnancy and Early Development. *Curr Environ Health Rep* 2, 367–378. [PubMed: 26341623]

- Mourier E, Tarrade A, Duan J, Richard C, Bertholdt C, Beaumont M, Morel O, Chavatte-Palmer P, 2017 Non-invasive evaluation of placental blood flow: lessons from animal models. *Reproduction* 153, R85–R96. [PubMed: 27845691]
- Naderi M, Wong MY, Gholami F, 2014 Developmental exposure of zebrafish (*Danio rerio*) to bisphenol-S impairs subsequent reproduction potential and hormonal balance in adults. *Aquatic toxicology* 148, 195–203. [PubMed: 24508763]
- Ngamprasertwong P, Dong M, Niu J, Venkatasubramanian R, Vinks AA, Sadhasivam S, 2016 Propofol Pharmacokinetics and Estimation of Fetal Propofol Exposure during Mid-Gestational Fetal Surgery: A Maternal-Fetal Sheep Model. *PLoS One* 11, e0146563. [PubMed: 26752560]
- Nishikawa M, Iwano H, Yanagisawa R, Koike N, Inoue H, Yokota H, 2010 Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. *Environ Health Perspect* 118, 1196–1203. [PubMed: 20382578]
- Oh J, Choi JW, Ahn YA, Kim S, 2018 Pharmacokinetics of bisphenol S in humans after single oral administration. *Environ Int* 112, 127–133. [PubMed: 29272776]
- Ohtani N, Iwano H, Suda K, Tsuji E, Tanemura K, Inoue H, Yokota H, 2017 Adverse effects of maternal exposure to bisphenol F on the anxiety- and depression-like behavior of offspring. *J Vet Med Sci* 79, 432–439. [PubMed: 28025458]
- Pacifici GM, Nottoli R, 1995 Placental transfer of drugs administered to the mother. *Clin Pharmacokinet* 28, 235–269. [PubMed: 7758253]
- Pan F, Xu T, Yang L, Jiang X, Zhang L, 2014 Probing the binding of an endocrine disrupting compound-Bisphenol F to human serum albumin: insights into the interactions of harmful chemicals with functional biomacromolecules. *Spectrochim Acta A Mol Biomol Spectrosc* 132, 795–802. [PubMed: 24973668]
- Patterson TA, Twaddle NC, Roegge CS, Callicott RJ, Fisher JW, Doerge DR, 2013 Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. *Toxicol Appl Pharmacol* 267, 41–48. [PubMed: 23261975]
- Philips EM, Jaddoe VWV, Asimakopoulos AG, Kannan K, Steegers EAP, Santos S, Trasande L, 2018 Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004–5. *Environmental research* 161, 562–572. [PubMed: 29245124]
- Pivnenko K, Pedersen GA, Eriksson E, Astrup TF, 2015 Bisphenol A and its structural analogues in household waste paper. *Waste Manag* 44, 39–47. [PubMed: 26194879]
- Pollock T, Greville LJ, Weaver RE, Radenovic M, deCatanaro D, 2018 Bisphenol S modulates concentrations of bisphenol A and oestradiol in female and male mice. *Xenobiotica*, 1–9.
- Pottenger LH, Domoradzki JY, Markham DA, Hansen SC, Cagen SZ, Waechter JM, Jr., 2000 The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol Sci* 54, 3–18. [PubMed: 10746927]
- Pu Y, Gingrich J, Steibel JP, Veiga-Lopez A, 2017 Sex-specific modulation of fetal adipogenesis by gestational bisphenol A and bisphenol S exposure. *Endocrinology*. PubChem, PubChem, Open Chemistry database.
- Purves RD, 1992 Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-moment-curve (AUMC). *J Pharmacokinet Biopharm* 20, 211–226. [PubMed: 1522479]
- Qiu W, Zhao Y, Yang M, Farajzadeh M, Pan C, Wayne NL, 2015 Actions of Bisphenol A and Bisphenol S on the Reproductive Neuroendocrine System During Early Development in Zebrafish. *Endocrinology*, en20151785.
- Richter WF, Bhansali SG, Morris ME, 2012 Mechanistic determinants of biotherapeutics absorption following SC administration. *AAPS J* 14, 559–570. [PubMed: 22619041]
- Rocha BA, Asimakopoulos AG, Honda M, da Costa NL, Barbosa RM, Barbosa F, Jr., Kannan K, 2018 Advanced data mining approaches in the assessment of urinary concentrations of bisphenols, chlorophenols, parabens and benzophenones in Brazilian children and their association to DNA damage. *Environ Int* 116, 269–277. [PubMed: 29704805]
- Rosenfeld CS, 2015 Sex-Specific Placental Responses in Fetal Development. *Endocrinology* 156, 3422–3434. [PubMed: 26241064]

- Stowell CL, Barvian KK, Young PCM, Bigsby RM, Verdugo DE, Bertozzi CR, Widlanski TS, 2006 A role for sulfation-desulfation in the uptake of bisphenol A into breast tumor cells. *Chem Biol* 13, 891–897. [PubMed: 16931338]
- Thayer KA, Doerge DR, Hunt D, Schurman SH, Twaddle NC, Churchwell MI, Garantziotis S, Kissling GE, Easterling MR, Bucher JR, Birnbaum LS, 2015 Pharmacokinetics of bisphenol A in humans following a single oral administration. *Environ Int* 83, 107–115. [PubMed: 26115537]
- Veiga-Lopez A, Kannan K, Liao C, Ye W, Domino SE, Padmanabhan V, 2015 Gender-Specific Effects on Gestational Length and Birth Weight by Early Pregnancy BPA Exposure. *The Journal of clinical endocrinology and metabolism* 100, E1394–1403. [PubMed: 26406292]
- Veiga-Lopez A, Luense LJ, Christenson LK, Padmanabhan V, 2013 Developmental programming: gestational bisphenol-A treatment alters trajectory of fetal ovarian gene expression. *Endocrinology* 154, 1873–1884. [PubMed: 23525218]
- Volkel W, Colnot T, Csanady GA, Filser JG, Dekant W, 2002 Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol* 15, 1281–1287. [PubMed: 12387626]
- Wan Y, Huo W, Xu S, Zheng T, Zhang B, Li Y, Zhou A, Zhang Y, Hu J, Zhu Y, Chen Z, Lu S, Wu C, Jiang M, Jiang Y, Liu H, Yang X, Xia W, 2018a Relationship between maternal exposure to bisphenol S and pregnancy duration. *Environ Pollut* 238, 717–724. [PubMed: 29621731]
- Wan Y, Xia W, Yang S, Pan X, He Z, Kannan K, 2018b Spatial distribution of bisphenol S in surface water and human serum from Yangtze River watershed, China: Implications for exposure through drinking water. *Chemosphere* 199, 595–602. [PubMed: 29459349]
- Wu LH, Zhang XM, Wang F, Gao CJ, Chen D, Palumbo JR, Guo Y, Zeng EY, 2018 Occurrence of bisphenol S in the environment and implications for human exposure: A short review. *Science of the Total Environment* 615, 87–98. [PubMed: 28963899]
- Xue J, Wu Q, Sakthivel S, Pavithran PV, Vasukutty JR, Kannan K, 2015 Urinary levels of endocrine-disrupting chemicals, including bisphenols, bisphenol A diglycidyl ethers, benzophenones, parabens, and triclosan in obese and non-obese Indian children. *Environmental research* 137, 120–128. [PubMed: 25531816]
- Ye X, Wong LY, Kramer J, Zhou X, Jia T, Calafat AM, 2015 Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000–2014. *Environmental science & technology* 49, 11834–11839. [PubMed: 26360019]
- Yoshikawa Y, Hayashi A, Inai M, Matsushita A, Shibata N, Takada K, 2002 Permeability characteristics of endocrine-disrupting chemicals using an in vitro cell culture model, Caco-2 cells. *Curr Drug Metab* 3, 551–557. [PubMed: 12369897]
- Zhuang X, Lu C, 2016 PBPK modeling and simulation in drug research and development. *Acta Pharm Sin B* 6, 430–440. [PubMed: 27909650]

Highlights

- Maternal and fetal pharmacokinetics were different among all three bisphenols
- Half-lives were longer in fetal vs. maternal circulation for all three bisphenols
- Bisphenol S had the longest fetal half-life compared to bisphenol A or bisphenol F

Figure 1. Experimental design and sample collection scheme for blood (*circles*), urine (*diamonds*), and amniotic fluid (*triangles*) over 72 h after bisphenol administration. Mat: maternal

Figure 2.

Toxicokinetic profiles for BPA, BPS, and BPF in maternal and fetal compartments. Mean ($\pm$ SEM) maternal (A-C) and fetal (D-F) plasma concentrations of BPA (A and D; closed squares; $n = 3$), BPS (B and E; gray circles; $n = 6$), or BPF (C and F; open triangles; $n = 3$) after a single 0.5 mg/kg s.c. dose of BPS or the combination of BPA, BPS, and BPF. Half ($n = 3$) of the females were only exposed to BPS while the other half ($n = 3$) were co-exposed simultaneously to BPA, BPS, and BPF.

Table 1.

Maternal and fetal characteristics at time of euthanasia.

	Mean $\pm$ SEM
Maternal weight (kg)	76.3 $\pm$ 3.6
Gestational age (days)	121.8 $\pm$ 0.7
Fetal weight (g)	4,389.7 $\pm$ 112.3
Fetal biparietal diameter (cm)	70.1 $\pm$ 0.8
Fetal Sex (n = Female:Male)	3:3

Note: F:M ratio for BPS only group = 2:1 and for mixture group = 1:2.

Table 2.

Toxicokinetic parameters for bisphenols in maternal and fetal plasma samples (mean $\pm$ SEM) after a single s.c. injection (0.5 mg/kg).

	Maternal			Fetal		
	BPA (n = 3)	BPS (n = 6)	BPF (n = 3)	BPA (n = 3)	BPS (n = 6)	BPF (n = 3)
C _{max} (ng/ml)	66.7 $\pm$ 1.7 ^a	643.1 $\pm$ 29.9 ^b	48.8 $\pm$ 0.2 ^c	87.6 $\pm$ 6.9 ^a	18.5 $\pm$ 1.1 ^b	20.9 $\pm$ 2.0 ^b
t _{max} (h)	3.0 $\pm$ 0.0 ^{a,b}	3.0 $\pm$ 0.0 ^a	1.7 $\pm$ 0.7 ^b	14.0 $\pm$ 5.0 ^a	21.0 $\pm$ 4.2 ^a	3.0 $\pm$ 0.0 ^b
t _{1/2} (h)	5.3 $\pm$ 0.4 ^{a,b}	3.7 $\pm$ 0.1 ^a	7.7 $\pm$ 2.2 ^b	52.0 $\pm$ 20.3 ^{a,b}	402.2 $\pm$ 102.6 ^a	14.2 $\pm$ 1.8 ^b
AUC (mg*h/ml)	0.81 $\pm$.07 ^a	7.5 $\pm$ 0.6 ^b	0.40 $\pm$ 0.1 ^c	3.9 $\pm$ 0.2 ^a	0.99 $\pm$.05 ^b	0.21 $\pm$.05 ^c
AUMC (mg*h ² /ml)	6.7 $\pm$ 0.4 ^a	51.0 $\pm$ 5.2 ^b	4.5 $\pm$ 2.3 ^a	120.0 $\pm$ 17.0 ^a	34.0 $\pm$ 1.5 ^b	1.7 $\pm$ 0.4 ^c
MRT (h)	8.3 $\pm$ 0.6	6.7 $\pm$ 0.3	10.3 $\pm$ 2.5	31.3 $\pm$ 3.6 ^a	34.1 $\pm$ 1.7 ^a	8.5 $\pm$ 0.2 ^b
Cl _{TB} (ml/h)	45.4 $\pm$ 5.5 ^a	5.2 $\pm$ 0.5 ^b	103.8 $\pm$ 22.7 ^c			

AUC: area under the curve

AUMC: area under the first moment curve

C_{max}: maximum plasma concentration observed

Cl_{TB}: total body clearance

MRT: mean residence time

t_{1/2}: chemical half-life

t_{max}: time at which the C_{max} is observed

Different superscripted letters denote statistical differences within maternal and fetal

compartments after ANOVA or Kruskal-Wallis posthoc test (P < 0.05).

Table 3.

Concentration of bisphenols (mean $\pm$ SEM) in maternal urine before (t_0), at 9 (t_9), and at 24 (t_{24}) h after a single s.c. injection (0.5 mg/kg).

	BPA (ng/ml) (n = 3)	BPS (ng/ml) (n = 6)	BPF (ng/ml) (n = 3)
t_0	< LOQ ^{a,b}	< LOQ ^a	1.5 $\pm$ 1.0 ^b
t_9	1,300 $\pm$ 210.0 ^a	3,870 $\pm$ 888 ^a	3.9 $\pm$ 3.4 ^b
t_{24}	298 $\pm$ 104.4 ^a	344 $\pm$ 50.1 ^a	18.7 $\pm$ 18.2 ^b

LOQ for BPA, BPS, and BPF are 0.33, 0.12, and 0.50 ng/ml, respectively.

Different superscripted letters denote statistical differences at each time point ($P < 0.05$).

Table 4.

Concentration of bisphenols (mean $\pm$ SEM) in amniotic fluid and fetal urine before (t_0) and 72 h (t_{72}) after a single s.c. injection (0.5 mg/kg).

		BPA (ng/ml) (n = 3)	BPS (ng/ml) (n = 6)	BPF (ng/ml) (n = 3)
Amniotic fluid	t_0	< LOQ	0.3 ± 0.2	< LOQ
	t_{72}	119 ± 137.0	5.6 ± 3.0	16.4 ± 22.6
Fetal urine	t_{72}	144 ± 46.7	6.6 ± 1.5	13.8 ± 3.4

LOQ for BPA, BPS, and BPF are 0.33, 0.12, and 0.50 ng/ml, respectively.

Note: No significance was observed among groups or time points within groups.

Table 5.

Chemical properties of bisphenols.

	BPA	BPS	BPF
Molecular weight (g/mol)	228.29 ⁵	250.27 ⁵	200.24 ⁵
Plasma protein binding (10 ⁴ l/mol)	2.90 – 3.14 ¹	3.14 – 5.14 ²	0.36 – 3.52 ⁴
pKa	9.6 ⁵	8.2 ⁵	7.55 ⁵
Solubility in water (mg/l)	300 ⁵	1,100 ³	5,43 ³
log K _{ow}	3.32 ⁵	1.65 ⁵	2.91 ⁵

Plasma protein binding constants are for human serum albumin (HSA). K_{ow}: octanol/water partition coefficient, pKa: acidity constant.

Superscripted numbers represent the following references:

¹⁾ Cao et al., 2011;

²⁾ Luo et al., 2016;

³⁾ Pivenko et al., 2015;

⁴⁾ Pan et al., 2014;

⁵⁾ NIH PubChem compound summaries.