Toxicokinetics of bisphenol S in rats for predicting human bisphenol S clearance from allometric scaling

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Abbreviations:
AUC_{last}: Area under the plasma concentration-time curve from dosing time to the last sampling time, $AUMC_{last}$: Area under the first moment plasma concentration-time curve from dosing time to the last sampling time, BPA: Bisphenol A, BPAG: Bisphenol A glucuronide, BPS: Bisphenol S, BPSG: Bisphenol S glucuronide, BW: Body Weight, Cl: Clearance, Cl_F:Apparent clearance, Cmax : Maximal concentration, IV: Intravenous, LOQ: Limit of Quantification, MRT: Mean Residence Time, Tmax: Time to maximal concentration, TK: Toxicokinetic, V_{SS}: Steady state volume of distribution

Abstract

Previous data obtained in piglets suggested that despite structural analogy with Bisphenol A (BPA), Bisphenol S (BPS) elimination may proceed more slowly, resulting in a much higher systemic exposure to unconjugated BPS than to BPA. Interspecies allometric scaling was applied to predict the toxicokinetic (TK) parameters of BPS, namely plasma clearance in humans from values obtained in animals, and thus contribute to assessment of the human internal exposure to BPS.

Allometric scaling was performed using mean BPS plasma clearance values measured in rats after intravenous administration of 5 mg BPS /kg body weight (BW) and those previously obtained in piglets and sheep using identical IV BPS dosing and analytical procedures.

The BPS plasma clearance, evaluated at 0.92 L/kg.h in rats, was proportional to species body weight, enabling the prediction of human BPS plasma clearance by extrapolating to a BW of 70 kg. The estimated BPS plasma clearance in humans was thus 0.92 L/min (0.79 L/kg.h), i.e. about two times lower than the previously estimated BPA clearance (1.79 L/min).

By increasing systemic exposure to the active moiety of an environmental estrogenic chemical, this less efficient clearance of BPS in humans, as compared with BPA, might worsen the harmful consequences of replacing BPA by BPS.

Keywords: Toxicokinetics; bisphenol S; allometric scaling, clearance

Introduction

Owing to great concern regarding the adverse effects of bisphenol A (BPA) on human health, restrictive measures have been applied that have led to its gradual replacement by structural analogues. Bisphenol S (BPS) is one of the main alternatives to BPA as a color developer in thermal papers and in the manufacture of plastics and epoxy resins (Wu et al. 2018).

The widespread and increasing use of BPS in consumer products is reflected by the high prevalence of BPS in urine from a cohort of US populations (Lehmler et al. 2018) and Asian countries (Liao et al. 2012). Despite insufficient data to assess the origin of such wide internal exposure, relevant pathways of human exposure to BPS could be the ingestion of BPS-contaminated food or dust and direct contact of BPS-contaminated hands with the mouth resulting from hand-to-mouth activity. A review of the literature suggests that substituting BPA with BPS may not lower the risk of endocrine disruption since BPS has been shown to display estrogen-like effects at concentrations similar to BPA (Pelch et al. 2019). Both BPS and BPA are largely conjugated to glucuronides (Le Fol et al. 2015; Skledar et al. 2016; Zhou et al. 2014), which are considered inactive due to their lack of estrogenicity (Matthews et al. 2001; Skledar et al. 2016). So, by increasing the internal exposure to the active form of BPA, a lower *in vivo* BPS plasma glucuronidation rate, as shown in piglets (Gayrard et al. 2019), may still aggravate the harmful consequences of BPA substitution by BPS. Indeed in piglets, the 3.5 times lower plasma clearance (Cl) of BPS as compared with BPA, combined with its 100 times lower first-pass glucuronidation following oral dosing, accounted for the 250 times higher systemic exposure to active unconjugated BPS (Gayrard et al. 2019). The relevance of this result for humans is supported by the higher serum concentrations of BPS, compared with BPA, that have been predicted for peroral exposure from a physiologically based toxicokinetic (TK) model (Karrer et al. 2018). However, the impossibility of directly measuring basic TK parameters in humans, such as plasma clearance, volume of distribution and mean residence time, limits our

understanding of the TK mechanisms underlying the different fates of bisphenol analogues, thus requiring assumptions when predicting BPS plasma kinetic profiles for external environmentally-relevant exposures. Clearance represents the most important TK parameter because it is the only parameter controlling the internal exposure following intravenous (IV) administration, as measured by the Area Under the plasma concentration vs time Curve (AUC). For an oral route of exposure, AUC is entirely controlled by both Cl and bioavailability (noted F). The steady-state volume of distribution (noted Vss) not only reflects the extent of distribution of a substance in the body but, in conjunction with Cl, also controls its persistence as measured by the Mean Residence Time (MRT). MRT is defined as the average time that a single molecule of the substance of interest remains in the body. Direct calculations of Cl, Vss and MRT require an IV injection that is impossible to carry out in man and an alternative approach consists of estimating these TK parameters by applying an indirect approach such as allometry. So-called simple allometric scaling consists of relating body size to a parameter of interest using a power equation (Chappell and Mordenti 1991). This has proved to be a useful tool for predicting human clearance of BPA from animal data (Collet et al. 2015). In the present study, this approach was used to estimate the Cl, Vss and MRT of BPS in humans from plasma BPS kinetic profiles obtained in rats following IV BPS dosing and TK data previously obtained using identical IV BPS dosing and analytical procedures in piglets (Gayrard et al. 2019) and in pregnant and non-pregnant ewes (Grandin et al. 2017, 2018).

Materials and methods

Animal handling procedures and sample collection

The experiment was declared to the regional Ethical Committee “Toxcomethique” (C2EA n°86) and to the French Ministry of Research. It was authorized by the French Ministry of Research under the number #5215_2016042815224143). The experiment was carried out with

15 female Wistar rats (Envigo, Gannat, France) weighing 266±26 g. They were fed *ad libitum* a commercial rat chow (2016 Teklad global 16% protein rodent diets, Envigo) and were housed individually in metabolism cages during the sampling periods.

The rats, briefly anesthetized using volatile anesthesia (2 % isoflurane, AErrane®, Baxter SA, Maurepas, France, in 0.7 L/min of O_2), were administered BPS into the lateral tail vein at a dose of 5 mg/kg. Serial blood samples (0.25 ml) were collected in propylene tubes before and at each of the designated time points post-administration: 10 min (n=8), 20 min (n=6), 30 min (n=8), 40 min (n=6), 60 min (n=8), 1.5h (n=6), 2h (n=8), 4h (n=5), 8h (n=8), 12h (n=6), 24h (n=8) and 36h (n=4), through a catheter surgically inserted in the left femoral vein under volatile anesthesia at least two days before BPS administration. After each blood sampling, physiological saline was administered at a volume equivalent to the withdrawn blood volume. The catheter was then filled with 200 µL of heparinized saline (50 IU/mL). Total urine was collected in propylene tubes at 3, 6, 9, 12, and 24 h after BPS administration in 9 rats. The volume of each urine sample and the sampling time were recorded. Blood and urine samples were immediately chilled on ice and centrifuged for 10 min at 3000 g at 4°C and the supernatant was stored in propylene tubes at -20°C until assayed.

Test material and treatments

All materials for the preparation of solutions, including the materials used for sampling, processing and analysis, were made of glass or polypropylene. BPS (purity >99%) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). BPS dosing solutions were extemporaneously prepared by dissolving BPS in ethanol-saline (1:4, vol:vol) at 5 mg/ml.

BPS assay

Analytes were quantified by Acquity ultra performance liquid chromatography coupled to a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA). BPS and BPSG in

plasma and urine samples were measured simultaneously, without resorting to a hydrolysis step, using the method described previously (Grandin et al. 2017).

Toxicokinetic analyses

The plasma mass concentrations were converted into molar concentrations before analysis. The cumulated molar quantities of excreted BPS (BPSG) in urine were calculated by multiplying the molar concentrations of BPS (BPSG) by the volume of urine at each sampling time.

Plasma concentration-time profiles of BPS were analyzed using Phoenix (version 8.0 Pharsight Corporation) according to a non-compartmental approach (NCA) involving a sparse data option. Sparse data methodology consists of calculating TK parameters based on the mean profile of all the subjects in the dataset and is appropriate when no rich data series can be obtained, as in the present experiment. The AUC and the Area under the first moment curve from the time of dosing to the last measurable concentration, AUC_{last} and $AUMC_{last}$ were calculated using the linear trapezoidal rule. The mean plasma concentration-time profile of BPSG was used to derive the maximum concentration (Cmax), the time to maximum concentration (Tmax), and the AUC_{last} and $AUMC_{last}$ values. The Cl of BPS and apparent clearance of BPSG (Cl_F) were estimated after IV administration of BPS by dividing the dose by the AUC_{last} calculated for each analyte as previously described. V_{ss} and MRT were computed using the usual pharmacokinetic equations and the moment statistical approach (Gibaldi and Perrier 1983).

Allometric approach

Allometric scaling was based on the mean clearance and body weight data obtained in rats and those previously obtained using identical 5mg/kg IV BPS dosing and analytical procedures in female piglets (Gayrard et al. 2019), and in pregnant and non-pregnant female sheep (Grandin et al. 2017, 2018). The data used for allometric scaling are given in Table 1.

The mean clearance values (Cl) obtained in each animal species were plotted against the mean animal BW using the following standard power equations:

$C_l = a \times BW^b$ Eq1

where a and b are the allometric coefficient and the exponent of the allometric equation, respectively.

The Cl and BW were transformed logarithmically and fitted to Equation 2 by linear regression analysis using Systat^R (Version 11, Systat Software, Inc., San Jose California USA).

$logC_l = loga + b \times logBW$ Eq2

where *log a* is the y-intercept, and b is the slope.

Based on the animal data, the predicted value of Cl in humans was obtained by solving the allometric equation for a standard BW of 70 kg. Vss and MRT were scaled using the same approach as for Cl. Since the classical allometric relationship for Vss produced a very low and unexpected computed exponent (0.526) and predicted an unrealistically low Vss (0.267L/kg, see discussion), this approach was no longer considered to estimate Vss in man. The alternative approach proposed by Bachmann et al. (1996), based exclusively on rat Vss, was implemented to predict V_{SS} in man using a power model with slope and intercept values of 0.91 and 0.705 respectively. MRT was deduced from Cl and V_{SS} estimates according to Equation 3.

$MRT = V_{SS}/Cl$ Eq3

Results

BPS was not detected in any of the control samples obtained before the administrations, suggesting that little-to-no sample contamination had occurred during sample collection, processing and assay. The time course of plasma concentrations of BPS and BPSG in rats after IV administration is shown in Figure 1. Table 2 gives the values estimated by NCA for TK parameters of BPS and BPSG after IV BPS administration. The plasma concentration-time

168 profile of BPS was characterized by a phase of rapid decay of concentrations during the first
169 four hours followed by a slow elimination phase. Plasma BPSG concentrations increased
170 rapidly after IV administration of BPS, and the maximum BPSG concentration (C_{max}) was
171 attained within the first 10 minutes. After a rapid decline, an increase of BPSG plasma
172 concentrations was observed about 4 h after BPS injection, after which the concentrations then
173 decreased slowly from 12 h post dosing. The BPS Cl and BPSG Cl_F values were 0.92 L/kg.h
174 and 0.18 L/kg.h, respectively. The respective values of BPS Vss and MRT were 3.64 L/kg and
175 3.96 h. By 24h, the mean fraction of the BPS dose (± SD) recovered in urine was 37.5 ± 12.3%
176 (n=9) mostly as BPSG (93.5 ± 7.1%), unconjugated BPS in urine representing 1.9 ± 2.1 % of
177 the BPS dose.
178 The allometric relationship between BW and Cl is shown in Figure 2. Data were fitted using
179 equation 1 and the resulting allometric equation 4 was used to predict Cl according to BW.
180 $Cl = 0.0151 \times \mathrm{BW}^{0.968}$ Eq4
181 with Cl in L/min, and BW in kg
182 The 95 % confidence interval for the slope of the allometric relationship between BW and Cl
183 (b value) was 0.8437 to 1.100 meaning that the slope was not significantly different from 1 with
184 P > 0.05. On applying Equation 4, the estimated BPS Cl for a 70-kg human was 0.92 L/min
185 (0.79 L/kg.h). Using the empirical model proposed by Bachmann et al. (1996), the estimated
186 BPS Vss was 2.26 L/kg in man, based on the rat Vss value of 3.6L/kg, and the deduced value
187 of MRT was 2.86 h.
188
189 **Discussion**
190 Our estimates of BPS Cl, Vss and MRT in humans, based on scaling from animal data, provide
191 some additional TK data for BPS. While the TK properties of BPA have been the subject of a
192 large number of studies, to our knowledge this is the first study to describe the TK parameters

of BPS and BPSG after BPS IV dosing in rats, a species commonly used in toxicological studies.

The BPS clearance in rats (0.92 L/kg.h) was shown to be equivalent to that previously estimated in larger species such as sheep (Grandin et al. 2017, 2018) and piglets (Gayrard et al. 2019) while the persistency of BPS as reflected by the MRT was 5 to 13 times higher (4 h) due to its 5 to 21 times higher V_{SS} (3.6 L/kg). The rebound in BPSG plasma concentrations, that peaked 12 h post BPS IV dosing, may reflect the BPS enterohepatic recirculation previously suggested by the rather high fecal excretion of BPS and its metabolites following IV administration in rats (32.5% of the dose, Waidyanatha et al. 2018). In the present experiment, the mean fraction of BPS dose recovered in urine over the 0-24 h post-dosing period was 37.5%, mostly in the form of BPSG, indicating that the renal clearance of unconjugated BPS was minimal. This result is consistent with those of other authors who reported that 42% of the radioactivity was eliminated in urine following injection of radiolabeled BPS in rats (Waidyanatha et al. 2018). This suggests that the clearance of BPS is driven by its biotransformation to more hydrophilic secondary metabolites (mainly BPSG) with subsequent elimination either in urine or bile. Enterohepatic recycling of BPA has been shown to occur in rats (Kurebayashi et al. 2003). An enterohepatic recirculation pathway has also been incorporated in the PBTK model of BPS for peroral exposure (Karrer et al. 2018) to improve the fitting of model predictions for BPS data biomonitoring (Oh et al. 2018) and to account for the longer persistence of BPS compared with BPA. The relevance of this modality of BPS elimination in humans is however questionable since BPSG has been shown to be cleared mostly by renal clearance in humans (Oh et al. 2018).

The main result of our study is that BPS Cl can be estimated in man, with a good degree of confidence, by applying a simple allometric approach and selecting three species with BWs spanning a large range (from 0.2 to 77Kg BW). We were thus able to estimate Cl by interpolation and thereby reduce the uncertainties associated with extrapolation. In addition, the

coefficient of determination (R^2) associated with the linear regression was very high (0.99) meaning that 99 % of the variability in BPS Cl between species was captured by the interspecies variation of BW. For the three species investigated, the allometric model predicted that the plasma Cl of BPS was directly related to BW and that the allometric exponent was not significantly different from 1. It has been argued that if the exponent of a simple allometric equation lies between 0.71 and 1, the product of Maximum Life-span potential (MLP) and clearance will predict Cl better than simple allometry (Mahmood and Balian 1996). We explored this so-called exponent rule to estimate the Cl in humans but did not find that such a correction was justified. The plasma Cl in man was thus estimated at a typical value of 0.92 L/min for a BW of 70 kg (0.79 L/kg.h). Assuming that the plasma and blood BPS concentrations are equal, as observed in sheep (unpublished observations), it can be concluded that the blood BPS Cl in humans is equal to 18% of the cardiac output (about 5 L/min) and 60% of the hepatic blood flow rate (1.24 L/kg.h, i.e. 1.45L/min for a 70-kg BW, Davies and Morris 1993). The mechanism of BPS Cl in man is unknown but assuming that all BPS is solely cleared by the liver (better case scenario for an oral exposure to an endocrine disruptor), the estimated hepatic extraction ratio (ERh) would therefore be 60%. The resulting fraction of BPS administered by oral route that escapes a hepatic first-pass effect can be as high as 40% (i.e. 1-ERh). This percentage will be even higher if a significant fraction of BPS is actually metabolized by some extra-hepatic mechanism such as renal clearance. This result is consistent with the bioavailability of BPS (57.4 %) that we directly measured in pigs (Gayrard et al. 2019). From our allometric estimate of BPS Cl in humans, it can therefore be tentatively concluded that if the liver is the only clearing organ of BPS in man (i.e. only an hepatic first-pass effect and no intestinal first-pass effect as we recently predicted in pigs, Gayrard et al. 2019), the oral bioavailability of BPS in man could be 50% or more. The relevance of a high BPS oral bioavailability in humans is strengthened by the similar values obtained for human BPS plasma

clearance estimated by allometry (0.79 L/kg.h) and the apparent clearance values computed from observed time courses in volunteers reported by Oh et al. (2018), using the area under the curve of unconjugated BPS (61 L/h *i.e.* 0.88L/kg.h). The predicted BPS oral bioavailability in man is much higher than the systemic oral BPA bioavailability that has been evaluated at less than 1% in adult monkeys (Doerge et al. 2010). It should be understood that when the hepatic clearance approaches a value close to the hepatic blood flow (1.5 L/min in man), the resulting increased hepatic first-pass effect leads to a much higher than proportional decrease of the oral bioavailability. This is likely the main explanation for the much higher BPS oral bioavailability compared with BPA, as shown in piglets (57.4% vs 0.50%, Gayrard et al. 2019). Indeed, the clearance of BPA in man (1.5 L/kg.h, Collet et al. 2015), mainly hepatic (Trdan Lušin et al. 2012) is close to the human hepatic blood flow (1.24 L/kg.h, Davies and Morris 1993) thereby allowing a near total first-pass effect. On the other hand, BPS clearance (0.79L/kg.h), although only two times lower than that of BPA, is not enough to ensure a near total hepatic first-pass effect, and results in a much higher than proportional increase of BPS bioavailability compared with BPA. In humans, the lower estimated BPS clearance compared with that of BPA (0.79 *vs* 1.5 L/kg.h, Collet et al. 2015) is consistent with the lower intrinsic hepatic clearance (0.4 vs 4.2 L/kg/h) predicted from the BPS and BPA glucuronidation kinetic parameters in human liver microsomes (Karrer et al. 2018).

The classical allometric relationship for Vss led to an unexpectedly low computed exponent of 0.526 whereas for most drugs this exponent is around 1 (Mahmood 2010), and therefore predicted an unrealistically low Vss in man. This is why we implemented the approach proposed by Bachmann et al. (1996) who showed that the human volume of distribution for 100 xenobiotics could be well predicted solely from the rat Vss. Using this empirical model, the BPS Vss estimated from the rat V_{SS} value (3.6 L/kg) was 2.26 L/kg in man. This prediction is

close to the reported human Vss/F that can be approximated at 5.15 L/kg by computing Vss/F from the human oral TK data published by Oh et al. (2018) and using equation 5.

$$V_{SS} = Cl \times MRT \qquad \text{Eq 5}$$

where Cl represents the apparent clearance of BPS (0.88 L/kg.h) and MRT is equal to 5.85 h

An additional step for understanding the BPS disposition consists of considering the minimal model for Vss, as given by equation 6:

$$V_{SS} = V_P + \frac{fu}{fuT} \times V_T \quad \text{Eq 6}$$

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 unbound fraction of BPS in plasma has been shown to be slightly different in rats (0.15) and pregnant women (0.10, Gayrard, unpublished observation). Solving equation 6 with fu values for humans and rats and physiological values for human and rat tissues and plasma volumes (De Buck et al. 2007) predicts that fuT (a measure of BPS tissue affinity) would be very similar in rats (0.038) and man (0.053); this means that the difference in Vss between men and rats may simply reflect differences in the extent of BPS binding to plasma proteins. The lower V_{SS} of BPS in humans compared with rats resulted in a lower MRT (2.86 h).

Conclusion

Based on our allometric approach, we conclude that BPS is less efficiently metabolized than BPA in humans, as reflected by its at least two-times lower plasma clearance. Since BPA substitution by BPS should lead to equivalent external exposures in terms of doses and routes, this lower BPS plasma clearance might lead to much higher oral serum concentrations of unconjugated BPS, as compared with BPA, due to the direct effect of a lower plasma clearance of BPS and indirectly by a much lower hepatic first-pass effect. Considering that BPA and BPS have comparable estrogen-like effects, our results suggest that oral BPS exposure might be associated with higher risks for human health than BPA.

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386 Table 1: Mean species body weight and toxicokinetic parameters used for allometric scaling

387

Species	n	Body weight (kg)	Clearance (L/kg.h)	V_{SS} (L/kg)	MRT (h)	Reference
Female rats	15	0.27 ± 26.2	0.92	3.64	3.96	Present study
Female piglets	16	11.2 ± 1.42	0.95 ± 0.24	0.75 ± 0.38	0.75 ± 0.53	(Gayrard et al. 2019)
Female sheep (not pregnant)	4	59.6 ± 13.5	0.57 ± 0.13	0.17 ± 0.04	0.30 ± 0.04	(Grandin et al. 2017)
Sheep (pregnant)	6	77.4 ±12.3	0.99	0.38	0.38	(Grandin et al. 2018)

388 V_{SS}: Steady state volume of distribution
389 MRT: Mean Residence Time

390

391 Table 2: Toxicokinetic parameters of BPS and BPSG estimated by NCA analysis after BPS

392 intravenous dosing.

393

Toxicokinetic parameters	BPS	BPSG
Cmax (µM)	27.3	17.0
Tmax (h)	NA	0.17
AUC_{last} (µM.h)	21.8	106.1
Cl (L/kg.h)	0.92	NA
Cl_F (L/kg.h)	NA	0.18
V_{SS} (L/kg)	3.64	NA
MRT (h)	3.96	14.2

394 The toxicokinetic parameters of BPS and BPSG were estimated after IV administration of BPS
395 at 20 µmoles/kg (5mg/kg).
396 AUClast: Area under the plasma concentration-time curve from dosing time to the time of the
397 last measurable plasma concentration
398 BPS: Bisphenol S
399 BPSG: Bisphenol S glucuronide
400 Cl: Clearance
401 Cl_F: Apparent clearance
402 V_{SS}: Steady state volume of distribution
403 MRT: Mean Residence Time
404 NA: not applicable

405 **Figure captions**

406

407 Figure 1: Semi-logarithmic plots of the plasma concentrations (μM) of BPS and BPSG versus
408 time (h) in rats (n=15) following IV administration of BPS at 5 mg/kg.

409

410 Figure 2: Allometric scaling plot for BPS plasma clearance (Cl). Data were obtained in rats
411 (open circle), piglets (closed circle), non-pregnant sheep (open triangle), and pregnant sheep
412 (closed triangle). The continuous line represents linear regression analysis of all the data points.
413 The allometric equation is provided and the open square indicates where a 70-kg BW adult
414 human falls with the corresponding equation.