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Abbreviations

AUClast:	Area under the plasma concentration-time curve from dosing time to the last sampling time
BPA:	Bisphenol A
BPAG :	Bisphenol A glucuronide
Cmax:	Maximum concentration
IV:	Intravenous
FAO:	Food and Agriculture Organization of the United Nations
LOQ:	Limit of Quantification
MRT:	Mean Residence Time
NOAEL:	No-Observed-Adverse-Effect-Level
TDI:	Tolerable Daily Intake
Tmax:	Time to maximum concentration
WHO:	World Health Organization

Abstract

Background: Bisphenol A (BPA) risk assessment is currently hindered by the rejection of reported higher than expected BPA plasma concentrations in humans after oral ingestion. These are deemed incompatible with the almost complete hepatic first-pass metabolism of BPA into its inactive glucurono-conjugated form, BPA glucuronide (BPAG).

Objectives: Using dogs as a valid model, plasma concentrations of BPA were compared over a 24-h period after intravenous, orogastric and sublingual administrations, in order to establish the absolute bioavailability of BPA administered sublingually and to compare it with oral bioavailability.

Methods: Six dogs were sublingually administered with BPA at 0.05 mg/kg and 5mg/kg. The time course of plasma BPA concentrations was compared with that obtained in the same dogs after intravenous administration of the same BPA doses and after a 20mg/kg BPA dose administrated by orogastric gavage.

Results: The data indicated that the systemic bioavailability of BPA deposited sublingually was high (70-90%) and that BPA transmucosal absorption from the oral cavity led to much higher BPA internal exposure than obtained for BPA absorption from the gastro-intestinal tract. The concentration ratio of BPAG to BPA in plasma was approximately 100-fold lower following sublingual administration than after oral dosing enabling the two pathways of absorption to be easily distinguished.

Conclusions: These findings demonstrate that BPA can be efficiently and very rapidly absorbed through the oral mucosa by the sublingual route. This efficient systemic entry route of BPA may lead to far higher BPA internal exposures than known for BPA absorption from the gastro-intestinal tract.

Introduction

Bisphenol A (BPA) is widely used in its monomeric form in the manufacture of polycarbonate plastics and epoxy resins (EFSA 2006). Vandenberg et al. (2007) have suggested that the release of BPA monomers from consumer products leads to the contamination of drinking water, food, dust and air thus providing considerable potential for human exposure to BPA. In support of this suggestion are data reported by Calafat et al. (2008) who found measurable levels of BPA metabolites in more than 90% of urine samples from a representative cohort of the US population. The principal source of BPA exposure is through the diet and, based on the measurement of urinary concentrations of BPA metabolites as a biomarker of aggregate human exposure levels, the median exposure has been estimated at only 0.01–0.12 μg/kg per day (FAO/WHO 2010). The current tolerable daily intake (TDI) is 0.05mg/kg/d (EFSA 2006).

Widespread human exposure to BPA raises concern among regulatory agencies because of its estrogenic properties *in vitro* (Wetherill et al. 2007) and *in vivo* (Richter et al. 2007). The risk assessment for BPA is controversial because the TDI which is based on guideline-driven toxicity studies (Ema et al. 2001; Tyl et al. 2002, 2008) is generally higher than doses that produce adverse effects on animals, especially if dosing occurs during the perinatal period (Cabaton et al. 2011; Vandenberg et al. 2008; Vom Saal and Hughes 2005).

It is generally assumed that the undesirable BPA effects are associated with plasma concentrations (internal dose) rather than to the administered BPA dose. Thus, some (Vandenberg et al. 2010) have questioned why reportedly high concentrations of unconjugated BPA in humans (in the ng/ml range) are not taken into account by regulatory agencies in the risk assessment process. Others (Mielke and Gundert-Remy 2009) have noted that the relatively low

estimated BPA daily intake and the observation of an extensive first-pass metabolism of oral BPA into its inactive glucurono-conjugated form, BPAG (Völkel et al. 2002), are not consistent with those high plasma levels of BPA observed in biomonitoring studies.

It has been suggested by Dekant and Völkel (2008) that the high plasma BPA levels reported in humans may be due to artifacts related to sample preparation, storage, overestimation by analytical techniques, or background contamination from labware or indoor dust. However, there is no little or no direct evidence for this assertion and there may be alternative explanations.

As most of the BPA exposure in humans occurs via the mouth, we hypothesized that BPA could be bioavailable sublingually, which could contribute to higher plasma concentrations. Sublingual refers to the route of administration by which a substance diffuses into the blood through the mucous membrane tissue under the tongue. As the sublingual mucosa is highly vascularised, a substance diffusing across this oral mucosal membrane has direct access to the systemic circulation via capillaries and venous drainage and will avoid first-pass hepatic metabolism (Patel et al. 2011).

In the present study, dogs were used to evaluate the oral transmucosal passage of BPA. The permeability of the buccal membrane is very similar in dog and human and thus, dog is a reliable species to assess sublingual absorption of drugs for human use (Barsuhn et al. 1988). The objectives of the study were: (1) to determine the bioavailability of BPA administered sublingually, (2) to characterize the time course of the plasma BPA concentrations following sublingual BPA, and (3) to compare systemic plasma BPA concentrations as a measure of exposure after sublingual and conventional oral dosing routes.

Materials and methods

Animals

Animals used in this study were treated humanely and with regard for the alleviation of suffering. All animal procedures were carried out in accordance with the accepted standards of humane animal care under the agreement number 31-242 for animal experimentation from the French Ministry of Agriculture. The study was conducted in six dogs (2 male and 4 female) of the Beagle breed (Harlan, France). The dogs were aged 2-3 years old and their weights were within the range 15-19 kg. The dogs were housed in pairs in 12-m² rooms. The animals were fed a standard diet and had free access to drinking water. The animal rooms were illuminated by artificial light on a 12h light/dark cycle and the temperature was maintained at about 20°C. The dogs had access to outdoor exercise areas for about 4h per day.

Experimental design and dosing

The first experiment was divided into two periods separated by one week during which the dogs received intravenous (iv) and sublingual administrations of BPA at a dose of 5 mg/kg using a two-treatment, two-sequence, two-period crossover design. This dose was chosen based on the intravenous dose estimated to be required to achieve BPA plasma concentrations greater than the limit of quantification (LOQ, 1ng/ml) for about 8-10h, *i.e.* a duration sufficient to observe the terminal phase slope and allow calculation of BPA pharmacokinetic parameters. Two different sublingual modalities of BPA administration were used. A BPA solution in ethanol (approximately 1.3 mL, 5mg/kg) was deposited as a single bolus under the tongue of 3 dogs, briefly anesthetized by an iv injection of sodium thiopental (Nesdonal^{R}, Merial, Lyon, France, 11 mg/kg). The 3 other conscious dogs received the same volume and dose as 20 µl drops of an

aqueous solution containing 40% ethanol continuously delivered over a 10-min period towards the floor of the mouth.

In a second experiment, BPA was once administered at a dose equivalent to the TDI (0.05mg/kg/d) that was chosen to reflect better the maximal possible BPA human external exposure and to check the proportionality of BPA pharmacokinetics with dose. The experimental protocol of this experiment was divided into 3 periods separated by one week. During the two first periods, dogs received iv and sublingual administrations of BPA at a dose of 0.05 mg/kg using a two-treatment, two-sequence, two-period crossover design. BPA was sublingually delivered as repeated deposits of 20 µl drops of an aqueous solution containing 1% ethanol as described above. During the third period, BPA was orally administered by means of orogastric intubation at a dose of 20mg/kg. This dose was selected based on previous pharmacokinetic data to obtain unconjugated BPA plasma concentrations of the same order of those observed after sublingual administration of BPA at a TDI dose level.

For both experiments and for each dog, BPA was administered iv as a bolus via an indwelling catheter (22 G) into the cephalic vein under the same conditions of dose, volume and anesthesia as during the corresponding sublingual administrations. The animals were fasted overnight prior to the study day, had free access to drinking water and were given a standard meal 5 h post-dose. During sampling periods, the dogs were housed individually in stainless steel cages.

Test material and treatments

BPA and all chemicals were purchased from Sigma Aldrich (Saint-Quentin, Fallavier, France). For the first experiment, BPA solutions were extemporaneously prepared by dissolving BPA at a

concentration of 50 mg/ml in 1% ethanol/49% propylene glycol (iv dosing), ethanol (sublingual bolus) or 40% ethanol/60% water (v/v) (drop administration).

For the second experiment, BPA solutions were extemporaneously prepared by dissolving BPA at a concentration of 0.5 mg/ml in water containing 1% of ethanol (iv and sublingual administrations). For oral administrations, BPA was dissolved at 40 mg/ml in 1% ethanol/9% corn oil (v/v).

Blood sampling

Serial jugular venous blood samples were taken before, in the middle (5 min after commencement) and at the end of the sublingual drops administration and at 2, 4, 8, 15, 30, 60, 90, 120, 180, 240 min and every 2-h for 12 h (experiments 1 and 2) and at 24 h (experiment 1) after iv and sublingual BPA administrations. Serial blood samples were obtained at 15, 30, 60, 120, 180, 240 and every 2-h for 12 h and at 24 h after oral BPA administrations.

Blood samples were collected in heparinized polypropylene tubes, immediately chilled in ice and centrifuged for 10 min at 3000 g at 4°C, and the supernatant plasma stored in polypropylene tubes (Eppendorf®) at -20°C until assay.

BPA and BPA-G assays

BPA and BPA-G in plasma samples were simultaneously quantified with an Acquity ultra performance liquid chromatograph coupled to a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA), according to the method previously described (Lacroix et al. 2011).

Briefly, samples (100 µL) were purified by protein precipitation, diluted with 150 µL of acetonitrile and 50 µL of internal standards BPA-d_{16} and BPA-G $^{13}C_{12}$ separated on a C18 column with a water/acetonitrile gradient elution. The multiple reaction monitoring transitions used to detect BPA, BPA-d_{16}, BPA-G and BPA-G $^{13}C_{12}$ were 227 > 212, 241 > 142, 403>227 and 415>239 with collision energies of 28, 20 and 30 eV, respectively. Chromatographic data were monitored by Targetlynx® software (Water, Milford, MA, USA). Blanks and quality control samples were used to monitor potential contamination during analysis and the accuracy and precision of the method.

The mean intra- and inter-day coefficients of variation for three concentration levels and for BPA and BPA-G were lower than 15% respectively and the limits of quantifications (LOQ) were validated at 1 ng/ml and 5 ng/ml, respectively.

Pharmacokinetic analysis

Plasma concentration-time profiles of BPA and BPAG were analyzed according to a non-compartmental approach using WinNonlin® (WinNonlin® professional 5.3, Pharsight Corporation, Cary, NC, USA). From the plasma BPA and BPAG concentration-time data in individual dogs, the maximum concentration (Cmax), and the time to maximum concentration (Tmax) were derived. The areas under the BPA and BPA-G plasma concentration-time curves (AUClast) and the areas under the first moment curves (AUMClast) were calculated using the linear trapezoidal rule from dosing time to the last sampling time. The mean residence time (MRT) was calculated as the ratio of AUMC to the AUC values.

The first 8 min following the end of sublingual administrations were not taken into account to derive the BPA pharmacokinetic parameters (AUClast, AUMClast, Cmax, Tmax, MRT) because

high plasma BPA levels encountered during this lag time may reflect the immediate input of BPA drained from the tongue into the jugular vein (that was also the site of blood sampling) before its rapid mixing in the general circulation. The AUClast values were normalized by the corresponding BPA dose.

For each dog, the absolute bioavailability of BPA administered sublingually at 5mg/kg was measured as the ratio of the normalized BPA AUClast for the sublingual route to the equivalent AUClast for the iv route. For the two BPA doses (0.05 and 5mg/kg), the extent of BPA sublingual absorption was defined as the ratio of the BPAG AUClast values obtained for the sublingual route to the equivalent BPAG AUClast for the iv route. For the lower BPA sublingual dosing, this latter value was also considered as an appropriate measure of the absolute bioavailability of BPA based on the assumption that BPAG is not formed at the site of administration or by a first pass effect. For oral bioavailability and absorption rate computation, only the iv dose of 5mg/kg was considered.

Statistical analyses

All results are presented as mean ± SD. The Student t test and SYSTAT12® software (Systat Software Inc., CA, USA) were used to analyze differences in mean BPA and BPAG pharmacokinetic parameters (AUClast, Cmax, Tmax, MRT) according to the route of administration.

Results

BPA was not detected in any of the control samples obtained before the administrations. Tables 1 and 2 give the values for pharmacokinetic parameters of BPA and BPAG following BPA

administrations at the different doses and according to the different routes (iv, sublingual and oral) of BPA administration.

Experiment 1: IV and sublingual BPA dosing at 5mg/kg

Due to the fact that the two sublingual administration methods (bolus *vs* 10 minutes drops) gave comparable results, they have been combined into one data set. The time course of mean plasma BPA concentrations after BPA sublingual administrations was very close to that obtained in the same dogs after iv administration (Figure 1A); the plasma concentrations during the first minutes following sublingual application were even higher than obtained after intravenous administration.

The mean time at which BPA reached Cmax after the 8 min following the end of sublingual administration of BPA at 5 mg/kg was 13 ± 9 min (Figure 1A, Table 1). The mean Cmax after the sublingual administrations of BPA at 5mg/kg was not significantly different from the corresponding value obtained after iv administration (7296 ± 1615 ng/ml *vs* 6443 ± 3910 ng/ml, iv *vs* sublingual administration, P=0.6, Table 1). The MRT of BPA was not different according to the iv *vs* sublingual routes of administration (69 ± 13 *vs* 73 ± 33 min, P=0.7, Table 1).

For BPAG, the mean Cmax did not significantly differ according to the route of administration (15657 ± 6426 *vs* 11808 ± 10419 ng/ml, iv *vs* sublingual administration, P=0.2, Table 2). However, the time at which BPAG reached Cmax after sublingual administration of BPA was delayed (Figure 1C, Table 2) as compared with the iv route, reaching 16 ± 7 *vs* 35 ± 13 min for iv *vs* sublingual administrations (P=0.04).

The mean area under the BPA plasma concentration-time curve (AUClast) (normalised for administered dose) after sublingual administration of BPA at 5mg/kg was lower than that

obtained after iv administration (P=0.04, Table 1, Figure 2A) while the corresponding mean BPAG AUClast values did not significantly differ. The mean BPA sublingual bioavailability for the high dose was 70 ± 31%. This high systemic bioavailability was confirmed by the mean ratio of BPAG AUC values (81 ± 18%) that is also an estimate of the systemic bioavailability provided that the BPAG is not formed at the site of administration or by a first-pass effect, which seems to be a reasonable assumption for a direct buccal absorption (see discussion).

Experiment 2: IV and sublingual BPA dosing at 0.05mg/kg

BPA was no longer detected about 2h after iv and sublingual BPA administrations at 0.05mg/kg while BPAG plasma levels remained above the LOQ for 8-10h after BPA administrations in 3 dogs.

Following BPA sublingual applications at 0.05mg/kg, the BPA plasma levels were more variable and higher than obtained in the same dogs after BPA intravenous administration at the same dose (Figure 1B)

The mean time value at which BPA reached Cmax (Tmax) after the 8 min following the end of sublingual administration of BPA at 0.05 mg/kg was 10 ± 4 min (Figure 1 B, Table 1). The mean Cmax after the sublingual administrations of BPA at 0.05mg/kg was more variable than the corresponding value obtained after iv administration (64 ± 36 ng/ml *vs* 249 ± 331 ng/ml, iv *vs* sublingual administration, Table 1). For BPAG, the mean Cmax following sublingual BPA applications was lower than obtained after iv administration (78 ± 38 *vs* 46 ± 20 ng/ml, P=0.03, Table 2). However, the time at which BPAG reached Cmax after sublingual administration of BPA was delayed (Figure 1D, Table 2) as compared with the iv route, reaching 12 ± 4 *vs* 35 ± 20 min for iv *vs.* sublingual administrations (P=0.06).

The BPA systemic exposure resulting from BPA sublingual dosing at 0.05mg/kg as reflected by the mean BPA AUClast was more variable and higher than that obtained after iv administration and was not considered for bioavailability computation (see discussion).

The mean BPA sublingual bioavailability for this low dose was 90 ± 26%, as computed by the ratio of the BPAG AUClast values obtained for the sublingual route to the equivalent BPAG AUClast for the iv route.

Oral BPA dosing at 20mg/kg

The mean Cmax and Tmax values of plasma BPA observed after BPA oral administration at 20mg/kg were 47 ± 20 ng/ml and 20 ± 8 min, respectively. The mean BPA oral bioavailability was 0.72 ± 0.28%. This value was lower than the mean ratio of BPAG AUC values (54 ± 19%) showing that BPA was rather well absorbed by the gastro-intestinal tract but that most absorbed BPA is metabolized by a first-pass effect at the hepatic level. A major difference between the two modalities of oral administration (orogastric *vs* sublingual dosing) was the BPAG:BPA plasma molar concentration ratio. During the first 120 min following sublingual BPA administrations at 5mg/kg, the mean BPAG: BPA ratio ranged from 1:1 to 13:1. This ratio ranged between 1:1 to 6:1 during the 120 min that followed BPA sublingual dosing at 0.05mg/kg. This was almost 100 times lower than that obtained after BPA absorption from the gastro-intestinal tract after oral dosing, which ranged from 237:1 to 634:1 over the same time period (Figure 2B).

Discussion

Much of the concern regarding BPA safety in humans has centered on the adverse effects of BPA in experimental animal studies, when blood concentrations were close to values of unconjugated BPA concentrations, in the ng/ml range, that have been reported from numerous human biomonitoring surveys (Vandenberg et al. 2010). However, these high BPA concentrations are considered to be erroneous and are discounted for risk assessment purposes, because of: (1) their deemed incompatibility with the low BPA estimated daily intake, which is mainly through the diet (FAO/WHO 2010); and (2) the tenet based on oral pharmacokinetic data in humans, which indicates extensive hepatic first pass glucuronidation of BPA leading to inactivation of almost all ingested BPA (Völkel et al. 2002).

To our knowledge, the present study is the first to demonstrate that BPA delivered sublingually is almost totally bioavailable. Indeed, this pathway of BPA absorption allows hepatic first-pass glucuronidation to be bypassed, leading to much higher BPA internal exposures than those obtained after conventional oral administration.

Our investigation used an *in vivo* dog model to establish the systemic uptake of buccal administered BPA. The relevance of this model is supported by similarity of the mechanisms of drug transport and of histology of the dog buccal mucosa compared with human oral mucosa (Barsuhn et al. 1988), which is not the case for rats where the buccal epithelium is keratinised (Shojaei 1998). In the present trials, we selected the jugular vein as the site of blood sampling. The advantage of the jugular vein is to collect blood from the venous drainage of the tongue. Thus, the fact that after BPA sublingual application, the jugular blood BPA concentrations were transiently higher than obtained after the iv administration of the same dose, is the direct proof of

a rapid and efficient passage of BPA by the transmucosal oral route. The disadvantage of this blood collection site is that the corresponding plasma BPA concentrations do not properly reflect the BPA systemic exposure during the buccal absorption phase (Sohlberg et al. 2013), *i.e.* before mixing of the jugular blood with systemic blood. It is for this reason that to evaluate the systemic exposure to BPA, and to derive the BPA AUC values calculated for the BPA sublingual dosing, the kinetic analysis discounted the BPA plasma concentrations measured in the jugular blood during the BPA administration itself and during the 8 min following the end of BPA sublingual applications. This delay was considered as sufficient to not bias the bioavailability estimation because the BPA MRT values did not differ between the iv *vs* sublingual routes indicating a very short buccal absorption phase of about a few minutes. In a supplementary experiment performed on two of the dogs previously used, we have taken blood samples in parallel from the jugular and the cephalic veins after BPA sublingual dosing at 2 doses, 0.05 and 0.5mg/kg. We have observed that after BPA sublingual dosing at 0.05 and 0.5mg/kg, BPA plasma concentrations in the jugular vein were higher and more variable than corresponding concentrations in the cephalic vein during the first 60 and 15 minutes post BPA sublingual dosing, respectively (see Supplemental Material, Figure S1). The systemic BPA exposures estimated from blood samples taken from the cephalic vein represented about 57 and 94% of that estimated from jugular blood samples obtained from 8 min after the completion of the BPA sublingual dosing at 0.05 and 0.5mg/kg, respectively (see Supplemental Material, Table S1), indicating that for the high BPA dose, the BPA bioavailability (70%) was properly calculated from jugular blood BPA concentrations obtained during and up to 8 min after the completion of BPA sublingual application. This view was comforted by the high extent of BPA bioavailability when computed using the systemic exposure to BPAG (81%). Indeed, the bioavailability can be

also determined by the AUC ratio of the metabolite provided that the metabolite is not formed at the site of administration or by a first-pass effect (Cutler 1981; Weiss 1990); that seems to be a reasonable assumption for a direct buccal absorption.

The mean absolute BPA bioavailability resulting from sublingual administration, (70%) as computed using BPA plasma concentrations after the high BPA administration, showed high bioavailability. For this high dose experiment, we used an alcoholic vehicle (40-100% ethanol) and a highly concentrated dosing solution to carry out the BPA sublingual administrations and a vehicle effect facilitating the sublingual absorption cannot be ruled out. In order to check the relevance of our findings with a high BPA dose, in a second experiment, we have administered BPA in an aqueous solution containing 1% of ethanol at a 100 times lower dosage, corresponding to the TDI (0.05mg/kg). In this experiment, the fact that BPA was no longer detected about 2h after the iv and the sublingual BPA administrations prevented accurate evaluation of the terminal slope and of BPA pharmacokinetic parameters (AUClast, bioavailability) that were more accurately evaluated after the administration of the highest dose. However, the BPA systemic exposure observed after BPA sublingual dosing at 0.05mg/kg, when compared to that obtained after iv administration of the same BPA dose, clearly indicates that the findings obtained for the high BPA dose are consistent with those obtained with a lower dose level. In addition when considering BPAG, the bioavailability of BPA after administration of a low BPA dose can be properly computed and was 90%

The physico-chemical properties of BPA, namely its moderate water solubility (LogP of 3.3), and its relative low molecular weight (228) are likely to explain its penetration across the sublingual membrane and may explain the high extent of BPA absorption.

The use of this *in vivo* canine model showed that the extensive uptake of BPA following sublingual applications, by by-passing the hepatic first-pass glucuronidation mechanism, may lead to a BPA internal exposure about 100-fold higher than would be obtained after oral administration of the same external BPA dose. The markedly increased BPA internal exposure, resulting from transmucosal absorption, highlights the possible limitations of those investigations in which BPA was administered as a single oral bolus (Doerge et al. 2010a, 2010b). These limitations were discussed by Sieli et al. (2011), who have reported some differences in BPA internal exposures in mice following exposure through the diet versus a single oral bolus exposure. The results of the present study suggest that the presence of BPA in food may increase the internal exposure to bioactive BPA of animals and humans when compared with a single bolus oral administration, although in rodents the totally keratinized oral mucosal lining (Shojaei 1998) may limit transmucosal BPA absorption. Currently, the results of Teeguarden et al. (2011) do not support a high contribution of sublingual absorption of the only dietary source of BPA to a much higher than expected human internal exposure. The conditions controlling absorption after sublingual dosing in our experimental design may be different from those prevailing during oral exposure to BPA contained in food or dust. The potential contribution of sublingual absorption of BPA entering the mouth to high blood unconjugated concentrations (in the ng/ml range) must be evaluated through biomonitoring surveys designed to integrate both dietary and non-dietary sources of BPA, including the potential non-dietary ingestion route associated to hand-to-mouth activity. Indeed, a meta-analysis addressing the question of mouthing behaviours in children have shown that the frequency of hand-to-mouth activity, that is up to 28 contacts per hour, is an important variable for exposure assessments (Xue et al. 2007). Considering the potential non-food sources of BPA, it is also important to note that a significant amount of BPA

can be released from resin-based dental materials, estimated at 13µg and 30mg of BPA respectively, in the average and the worst case scenario after one full crown restoration of a molar (Geens et al. 2012; Van Landuyt et al. 2011) and that BPA present in thermal papers may be taken in orally through direct contact of unwashed hands with the mouth (Geens et al. 2012).

Another major finding of the present experiments is that the two pathways of BPA systemic availability (*i.e.* with or without an hepatic first pass effect) can be easily distinguished taking into account the plasma BPAG:BPA molar concentration ratio. Following BPA entry into the systemic circulation by the sublingual route, this ratio was about 100 times lower than that obtained after BPA orogastric ingestion. The latter ratio obtained after oral dosing in dogs is consistent with the data on oral pharmacokinetics in humans and non-human primates showing that the peak serum concentrations of unconjugated BPA following oral administration are approximately 0.2-1% (Völkel et al. 2002) and 0.1-3% of the total (unconjugated plus conjugated) BPA (Doerge et al. 2010b; Taylor et al. 2011; Tominaga et al. 2006). The remarkably lower BPAG:BPA ratio obtained after sublingual administration justifies the claim of differences relating to systemic absorption bypassing hepatic metabolising enzymes. These data suggest that unconjugated BPA concentrations in human serum giving a BPAG:BPA plasma concentration ratio less than 10 are achievable. It follows that such data do not have to be attributed to sample contamination. Therefore, recent data, indicating that BPAG is not abundant in human serum relative to total BPA levels (Kosarac et al. 2012) should be re-evaluated in the light of the present results demonstrating a possible direct systemic entry of BPA from sublingual absorption.

Conclusions

The finding that BPA can be efficiently and very rapidly absorbed by the sublingual route suggests that that sublingual absorption of BPA entering the mouth from both dietary and non-dietary sources may contribute to much higher internal exposure to unconjugated form of BPA than expected following the passage through the gastro-intestinal tract. The study further shows that the ratio of BPAG to BPA plasma concentrations clearly differentiates the routes of BPA entry to the systemic circulation bypassing or not the liver. This finding is likely to have major implications for the interpretation of human biomonitoring data; such interpretation should take into account that BPA blood concentrations cannot directly be extrapolated from the BPAG levels by assuming a systematic extensive hepatic first-pass effect under all circumstances.

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Table 1: Mean values (±SD) for pharmacokinetic parameters of BPA following intravenous, sublingual and oral BPA dosing.

	Intravenous		Sublingual		Oral
Pharmacokinetic parameter[a]	0.05 mg/kg	5 mg/kg	0.05 mg/kg	5 mg/kg	20 mg/kg
Cmax (ng/ml)	64 ± 36	7296 ± 1615	249 ± 331	6443 ± 3910	47 ± 20
Tmax (min)	2 ± 0	3 ± 1	10 ± 4	13 ± 9	20 ± 8
AUClast (x 10^3 ng.min/ml)	1 ± 0	221 ± 54	2 ± 1	145 ± 44*	6 ± 2
MRT (min)	NC	69 ± 13	NC	73 ± 33	112 ± 37
BPA bioavailability (%)	NA	NA	NC	70 ± 31	0.72 ± 0.28

NA: not applicable

NC: not calculated

[a]The first 8 min following the end of sublingual administrations were not taken into account to derive the BPA pharmacokinetic parameters

* Significantly different from mean values obtained after the intravenous administration of BPA at the same dose (P value for acceptance set at 0.05, Student t test)

Table 2: Mean values (±SD) for pharmacokinetic parameters of BPAG following intravenous, sublingual and oral BPA dosing

Pharmacokinetic parameter	Intravenous		Sublingual		Oral
	0.05 mg/kg	5 mg/kg	0.05 mg/kg	5 mg/kg	20 mg/kg
Cmax (ng/ml)	78 ± 38	15 657 ± 6426	46 ± 20*	11 808 ± 10 419	30 777 ± 13 604
Tmax (min)	12 ± 4	16 ± 7	35 ± 20	35 ± 13*	38 ± 18
AUClast (x 10^3 ng.min/ml)	8 ± 5	2884 ± 776	7 ± 5	2355 ± 893	6081 ± 1935
MRT (min)	NC	417 ± 65	NC	562 ± 164	501 ± 200
BPA absorption and/or bioavailability (%)	NA	NA	90 ± 26	81 ± 18	54 ± 19

NA: not applicable

NC: not calculated

* Significantly different from mean values obtained after the intravenous administration of BPA at the same dose (P value for acceptance set at 0.05, Student t test)

Figure legends

Figure 1. Semi-logarithmic plots of mean (± SD) plasma concentrations (ng/mL) of BPA and BPAG *versus* time (min) after a single intravenous (open symbols, n=6) or sublingual (closed symbols, n=6) administration of BPA at 5mg/kg (A, C) and 0.05 mg/kg (B, D), respectively. Time 0 represents the end of the administrations.

Figure 2. Mean (± SD) BPA and BPAG area under plasma concentration-time (AUC) normalised for the actual administered dose (A) and semi-logarithmic plot of the mean ratio of BPAG to BPA molar concentrations *versus* time (min, B). A 5-mg/kg dose of BPA was given to dogs by iv (white bars, n=6) or sublingual routes (black bars, n=6). A 20-mg/kg dose of BPA was given to dogs by oral route (hatched bars, n=6). The numbers above the bars represent the mean value of the ratio of BPAG to BPA molar concentrations.

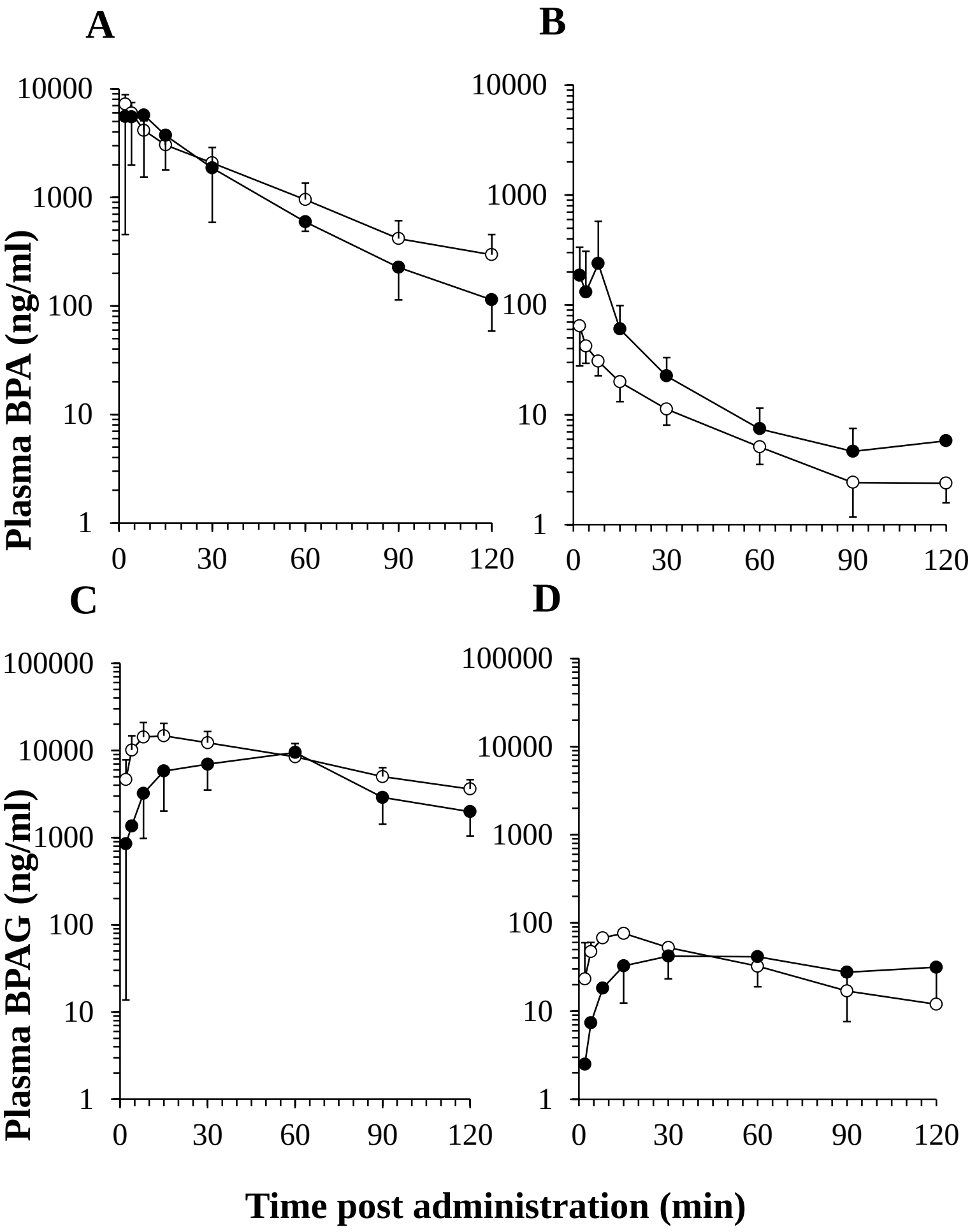
A
B
C
D
Plasma BPA (ng/ml)
Plasma BPAG (ng/ml)
10000
1000
100
10
1
100000
0
30
60
90
120
Time post administration (min)

Figure 1

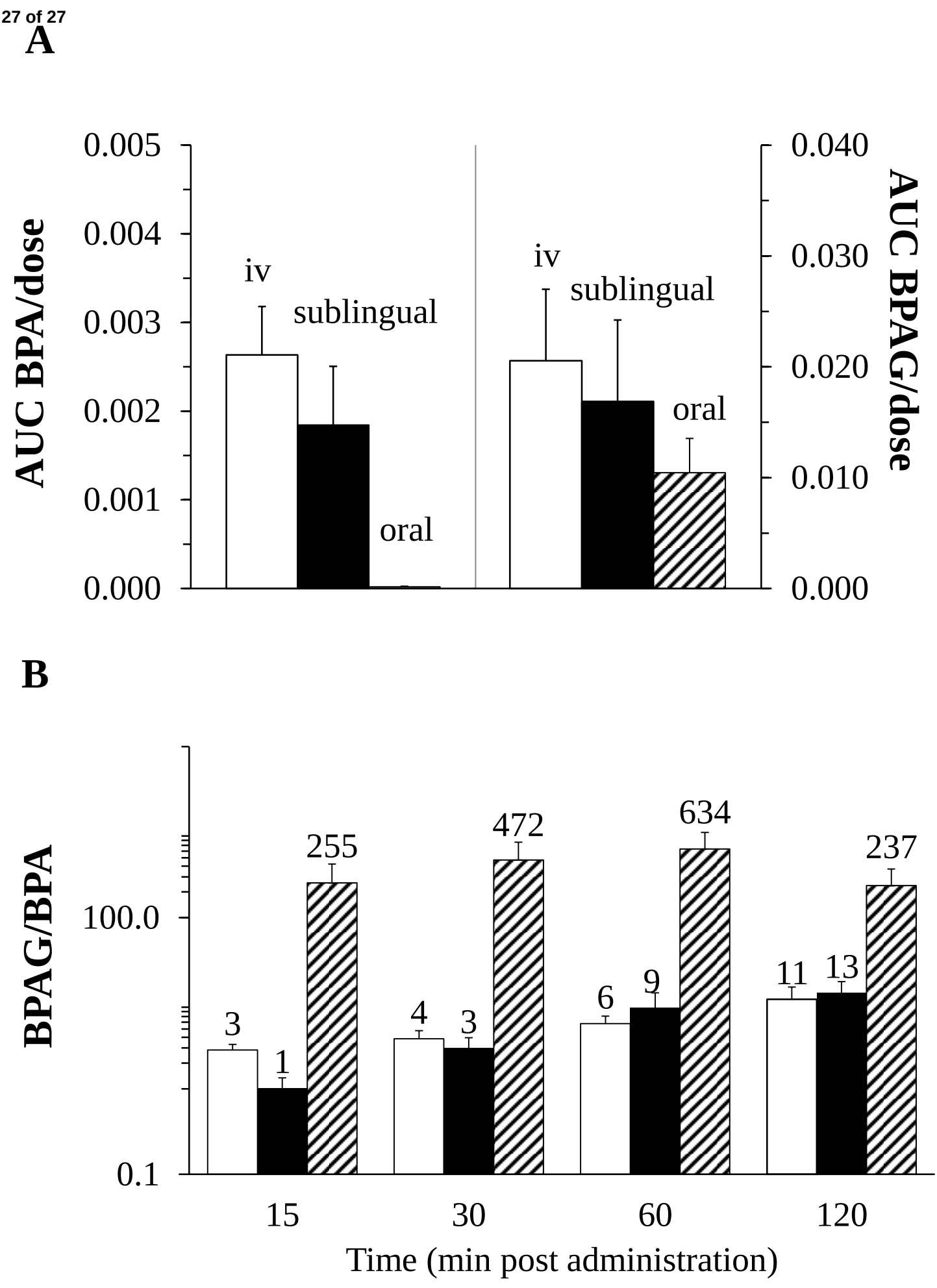
A
AUC BPA/dose
AUC BPAG/dose
0.005
0.004
0.003
0.002
0.001
0.000
0.040
0.030
0.020
0.010
0.000
iv
sublingual
oral
iv
sublingual
oral
B
BPAG/BPA
100.0
0.1
3
1
255
4
3
472
6
9
634
11
13
237
15
30
60
120
Time (min post administration)

Figure 2