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Toxicokinetics and bioavailability of bisphenol AF following oral administration in rodents: a dose, species, and sex comparison

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Abstract

We investigated the toxicokinetics and bioavailability of bisphenol AF (BPAF) in male and female Harlan Sprague Dawley rats and B6C3F1/N mice following a single gavage administration of 34, 110, or 340 mg/kg. A validated analytical method was used to quantitate free (unconjugated parent) and total (unconjugated and conjugated) BPAF in plasma. BPAF was rapidly absorbed in rats with the maximum plasma concentration, C_{max} , of free BPAF reached at 2.20 h. BPAF was cleared rapidly with a plasma elimination half-life of 3.35 h. C_{max} and the area under the concentration versus time curve, $AUC_{0-\infty}$, increased proportionally to the dose. Total BPAF C_{max} was reached 1.07 h in rats with both C_{max} (27-fold) and $AUC_{0-\infty}$ (52-fold) much higher than corresponding free values demonstrating rapid and extensive conjugation of BPAF following oral administration. Absorption of BPAF following a 34 mg/kg gavage dose in mice was more rapid than in rats with free BPAF C_{max} reached 0.455 h. Free BPAF was cleared rapidly in mice with an elimination half-life of 4.22 h. Similar to rats, total BPAF was much higher than corresponding free BPAF. There was no apparent sex-related effect in plasma toxicokinetic parameters of free or total BPAF in mice and rats. Bioavailability in rats was ~ 1% with no apparent dose-related effect. Bioavailability in mice was slightly higher than in rats (male ~ 6%, female 3%). These data demonstrate that BPAF was rapidly absorbed following gavage administration in rodents, rapidly and extensively conjugated with low bioavailability.

Keywords

bisphenol AF; bioavailability; toxicokinetics

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Declaration of Interest

The authors report no declarations of interest.

Introduction

Bisphenol AF (BPAF), a bisphenol analogue, is used as a crosslinking or curing agent for certain fluoroelastomers and as a monomer for synthesis of specialty polymers (Dupont, 2006; Halocarbon, 2008). BPAF-containing fluoroelastomers were approved by the U.S. Food and Drug Administration and these fluoroelastomers can contain up to 2% by weight BPAF (US FDA 2007 and 2008). The estimated annual production of BPAF in the United States ranges from 4500 kg to 227000 tons (US EPA, 2002), classifying it as a low to medium production chemical. The NIOSH National Occupational Exposure Survey (1981–1983) estimated that 4388 employees (1460 females) were potentially exposed to BPAF.

Eight bisphenol analogues, including BPAF, was investigated in indoor dust collected from several countries. Compared to the levels of bisphenol A (BPA) (range 0.63 to 3.3 ng/g), the frequency of detection and levels of BPAF was low (Liao et al., 2012; summarized in Chen et al., 2016). BPAF was detected in 76% of dust samples from Korea (0.091 µg/g) and 9% of dust samples from Japan (0.011 µg/g) although it was not detected in dust samples from China and the United States (Liao et al., 2012). BPAF has also been detected in water, sediment or sewage samples in China and Germany (Feng et al., 2012; Yang 2014). Liao et al. (2013) also compared the concentration of 8 bisphenol analogues in foodstuffs in the United States. Bisphenol analogues were found in 75% of the samples. The predominant analogues found were bisphenol A (BPA) (3 ng/g wet weight) and bisphenol F (0.929 ng/g wet weight), which accounted for 42 and 17%, respectively, of total bisphenol analogue concentration. However, the detection frequency (10.5%) and concentrations (0.012 ng/g wet weight) of BPAF was low. Although there are biomonitoring studies conducted to determine exposure to bisphenol analogues, BPAF has not been detected in such studies (Chen et al., 2016).

The majority of toxicity evaluations for BPAF have been in aquatic species. BPAF was toxic to aquatic organisms following both acute and chronic exposure (e.g., *Daphnia magna*, *Danio rerio* and *Desmodesmus subspicatus*) (Tisler, 2016). Disruption of thyroid function was observed in zebrafish larvae exposed to BPAF (5, 50, and 500 µg/L) for 7 d (Tang et al., 2015) and steroid hormonal balance was seen in larvae after 5 d of exposure and in 2-month old zebrafish after 28 d of exposure to 5, 25 and 125 µg/L (Shi et al., 2015; Yang et al., 2016). In rats, inhibition of testosterone production was noted in 7-week-old male animals exposed to BPAF (0, 2, 10, 50, and 200 mg/kg) for 14 d (Feng et al., 2012); however, exposure to BPAF during gestation days (GD) 3–19, during postnatal days (PND) 3–19, or exposure during both periods showed higher levels of BPAF in the testes and an increase in testosterone levels, and suggests that effects may depend when exposure occurs during the developmental period of the rat (Li et al., 2016). The Li et al. group also evaluated maternal and lactational transfer with 100 mg/kg BPAF and noted that pups exposed during the gestational period had measurable levels of BPAF in the testes and those exposed during the postnatal period had measurable BPAF levels in both serum and testes (Li et al., 2016) suggesting both gestational and lactational transfer may occur. The oral LD₅₀ of BPAF was reported as 3400 mg/kg in rat (sex and strain not specified) (Halocarbon, 2008).

Due to the lack of adequate toxicity data for BPAF, the National Toxicology Program (NTP) is evaluating the toxicity of BPAF in rodents following oral administration. Absorption, distribution metabolism, excretion (ADME) and toxicokinetics (TK) data are essential to provide broader context to toxicology data. Li et al. (2013) reported that following administration of 20 or 100 mg/kg BPAF in rats the maximum plasma BPAF and BPAF-glucuronide concentration was observed at 1 h and 30 min, respectively; BPAF-diglucuronide, BPAF-glucuronide, and BPAF-sulfate were found as the major metabolites. We have previously conducted a comprehensive investigation of ADME in rats and mice following gavage administration of [¹⁴C]BPAF (3.4, 34 or 340 mg/kg). Our data showed that BPAF was well absorbed in rodents, distributed in tissues, and excreted primarily via bile (Waidyanatha et al., 2015); as observed by Li et al. (2013), the major metabolites detected in rodent urine and bile were BPAF-glucuronide, BPAF-diglucuronide, mixed BPAF - glucuronide sulfate, and BPAF-sulfate. BPAF was cleared more slowly than BPA in hepatocytes from humans and rodents, in vitro. The clearance of BPAF followed the rank order rat > mouse > human (Waidyanatha et al., 2015). However, to the best of our knowledge, there is no comprehensive TK data for BPAF.

The current studies were undertaken to generate TK data for free BPAF (unconjugated parent) and total BPAF (unconjugated and conjugated BPAF) following oral administration in Harlan Sprague Dawley (HSD) rats and B6C3F1/N mice, the two rodent strains used in the NTP toxicology studies. The study design is summarized in Table 1. The dose-response and sex difference in TK was investigated in male and female rats following a single oral administration of 34, 110, and 340 mg/kg BPAF. Species difference in TK was investigated in limited studies following a single oral administration of 34 mg/kg BPAF in male and female mice. To aid in the interpretation of oral data and to generate oral bioavailability, limited studies were also conducted following an intravenous (IV) dose of 34 mg/kg in male and female rats and mice. The doses were selected based on the reported oral LD50 in rodents, doses used in ADME studies, and proposed doses in toxicology studies.

Materials and methods

Chemicals and reagents:

BPAF (lot # 20100425) was obtained from 3B Pharmchem International (Wuhan) Co., Ltd (China). The chemical identity was confirmed by infrared spectroscopy, proton and carbon-13 nuclear magnetic resonance spectroscopy, mass spectrometry, and elemental analysis. The purity was determined to be > 99% based on high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) at 210 nm. The lot was also analyzed by differential scanning calorimetry (purity > 99.9%) and by Karl-Fischer titrimetry for water content (< 0.1%). 2,2-bis(3,5-Difluoro-4-hydroxyphenyl)-hexafluoropropane (DFBPA, lot # 20110525, purity 97%) to be used as internal standard (IS) was obtained from Pure Chemistry Scientific Inc. (Newton, MA). Control HSD rat matrices for analytical method validation and matrix calibration curves were obtained from Bioreclamation IVT Inc. (Westbury, NY). β -glucuronidase from *Helix pomatia* (100,000 units/mL and 7500 units/mL for β -glucuronidase and sulfatase activity, respectively) was

obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and reagents were procured from commercial sources.

Analytical method validation.

A method employing protein precipitation followed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used to quantitate free and total BPAF in plasma. A full validation was conducted in female HSD rat plasma for BPAF. The validation included an assessment of linearity, inter- and intra-day precision (estimated as relative standard deviation, RSD), inter- and intra-day accuracy (estimated as relative standard error, RE), absolute recovery, and experimental limits of quantitation (LOQ) and detection (LOD). Dilution verification was conducted to demonstrate that concentrations outside the validated range could be accurately quantitated after dilution with blank plasma into the validated range. The method was assessed for total BPAF (BPAF recovery following deconjugation) by preparing quality control (QC) samples in female HSD rat plasma (n = 6) under the enzyme deconjugation procedure (see below). The method was also assessed for male rat and male and female B6C3F1/N mouse plasma by preparing QC samples in respective matrix (n = 6 for each matrix).

Two stock solutions of BPAF were prepared in acetonitrile and further diluted in the same solvent to generate concentrations of standards in the working range. Stock solutions of DFBPA to be used as IS was prepared in acetonitrile and diluted in acetonitrile to generate working IS solutions. An eight-point solvent calibration curve (~ 3 to 100 ng/mL) in acetonitrile was prepared using alternate stock solutions. Eight-point matrix calibration curves (~ 3 to 100 ng/mL) were prepared in duplicate by adding BPAF in female HSD rat plasma, using alternate stock solutions. QC samples were prepared at three concentrations in female HSD rat plasma at 3 levels (~8, 30, and 90 ng/mL, n= 6 at each level per analysis day) using a procedure similar to that for the matrix standards, using an independent stock solution. Matrix blanks were prepared the same as matrix standards except the addition of the analyte.

For the determination of free BPAF, 50 μ L aliquots of plasma (matrix calibration standards, QC samples, or matrix blanks) were transferred to microcentrifuge tubes. For the determination of total BPAF, the samples were prepared as follows: to 50 μ L aliquots of plasma in microcentrifuge tubes, 10 μ L of 0.9% sodium chloride and 25 μ L of 190 mM sodium acetate buffer (pH 5) were added along with 10 μ L of β -glucuronidase from *Helix pomatia* and samples were incubated at ~ 37°C overnight. To all samples, acetonitrile (150 μ L for free and 250 μ L for total assessment) was added, and samples were vortexed for 30 sec and centrifuged for 5 min. To 125- μ L aliquot of the supernatant, 10 μ L of 750 ng/mL IS solution was added and analyzed by LC-MS/MS as described below.

Stability of BPAF in extracted samples from above was evaluated when stored at ambient and refrigerated temperatures. Stability of the BPAF in plasma was evaluated following three freeze-thaw cycles and when stored at -70°C for up to 9 months to cover the study sample storage conditions and duration.

All standards and samples were analyzed by LC-MS/MS using a Shimadzu (Columbus, MD) liquid chromatograph coupled to an Applied Biosystems API-4000 Q-Trap (Waltham, MA) mass spectrometer. Chromatography was performed using a Phenomenex Luna column (C18, 5 μ m, 50 $\times$ 2.0 mm; Santa Clara, CA). Mobile phases A (0.5% formic acid) and B (methanol with 0.5% formic acid) were run with a linear gradient from 30% B to 100% B over 4 min followed by a 4 min hold at a flow rate of 0.3 mL/min. The column temperature was maintained at 40°C. The turbospray ion source was operated in negative mode with a source temperature of 400°C and an ion spray voltage of –4500 V. Transitions monitored were 335.0 $\rightarrow$ 264.7 for BPAF, and 407.0 $\rightarrow$ 337.0 for DFBPA. The retention times were 6.3 min for BPAF and 6.7 min for DFBPA.

A linear regression with 1/X weighing was used to relate LC-MS/MS peak area response ratio of analyte to IS and concentration of BPAF in plasma. The concentration of free and total BPAF was calculated using response ratio, the regression equation, initial sample volume, and dilution when applicable.

Animal studies.

Studies were conducted at Mispro Biotech Services (RTP, NC) by RTI International (RTP, NC) and were approved by Institutional Animal Care and Use Committee. Animals were housed in facilities that are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Animal procedures were in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council 2011). Male and female Hsd:Sprague Dawley® SD® (HSD) rats were obtained from Harlan Laboratories (Indianapolis, IN). B6C3F1/N mice were obtained from Taconic Farms (Germantown, NY). The animals were quarantined for at least 7 d before they were randomized into dosing groups and used in a study. Mice and rats, had *ad libitum* access to certified, irradiated NTP 2000 feed (Ziegler Bros, Inc., Gardners, PA) and 5K96 (Purina Mills), respectively. All animals had access to city (Durham, NC) tap water. Feed was used within 180 days of milling. Environmental conditions included: room temperature 72 $\pm$ 3 °F (22 $\pm$ 2 °C), relative humidity 50% and a 12-h light/dark cycle. Enviro Dri packs (100% virgin kraft paper shreds (Sheperd Specialty Papers, Watertown, TN) were provided for environmental enrichment. Male (280–315 g) and female (190–237 g) rats were 9–10 weeks old and male (25.5–30.5 g) and female (18.9–25.2 g) mice were 10–11 weeks old at the time of dosing.

Oral dose formulations of BPAF (3.4 mg/mL for mice and 6.8, 22, and 68 mg/mL for rats) were prepared in corn oil and analyzed using a validated HPLC-UV method (linear range, 0.3 to 136 mg/mL; r 0.99; precision 5%; accuracy, $\pm$ 10%). IV dose formulations of BPAF (8.5 mg/mL for mice and 17 mg/mL for rats) were prepared in water:Cremophor:ethanol (67:23:10) and analyzed using a validated HPLC-UV method (linear range, 1 to 20 mg/mL r 0.99; precision 5%; accuracy, $\pm$ 10%). All oral and IV formulations were within 10% of the target concentration. Prior to study initiation, stability (10% of day 0) of both oral and IV formulations was confirmed for up to 42 d when stored in sealed clear bottles with Teflon-lined lids at ambient or refrigerated conditions.

Single oral doses were administered at 34, 110, or 340 mg/kg. Dose formulations were administered in a volume of 5 mL/kg for rat and 10 mL/kg for mouse by intragastric gavage using a syringe equipped with a ball-tipped gavage needle (16G for rats, 18G for mice). Single IV dose was administered at 34 mg/kg; dose was administered in a volume of 2 mL/kg for rats and 4 mL/kg for mice into a lateral tail vein using a syringe equipped with a 27G needle for rats and 30G needle for mice.

Following dose administration, blood samples were collected at target times of 0 (predose), 5, 15, 30 min, 1, 2, 4, 8, 12, 24, 32, and 48h for all dose groups. Blood samples were obtained from 3 animals per dose group per time point. (Note: Since the animal strains used are inbred the animal-to-animal variability in kinetics is minimal, the standard practice is to use 3 animals for timepoint.) Two blood samples were obtained from each rat: the first sample (~ 250 µL) was obtained from the tail vein using a 20–22G needle and the second sample (as much blood as possible) was obtained via cardiac puncture following euthanasia with CO₂. (Note: According to the guidelines of the National Center for the Replacement Refinement and Reduction of Animals in Research, the blood obtained from cardiac puncture is not considered solely as arterial blood (<https://www.nc3rs.org.uk/rat-cardiac-puncture-terminal>). Hence, the variability in concentration of BPAF between tail vein and cardiac blood is assumed to be minimal.) For animals administered BPAF via IV route, the tail vein used for dosing was marked, and the interim blood sample was collected from the other lateral vein. The blood sampling times were separated by at least 2 h for each rat. For non-terminal collections the total volume collected didn't exceed 2% of body weight. All blood samples from mice were obtained via cardiac puncture following euthanasia with CO₂. The actual times for blood collection was recorded at each time point. Immediately following collection, blood was dispensed into tubes containing K₃EDTA, mixed by inversion and placed on ice. Plasma was prepared within 1 h of collection by centrifugation of blood. All samples were stored at –70 °C within 2 h of collection.

A Debra™ data collection and reporting system (LabLogic Systems, Ltd., Sheffield, England) was used for collection of body weights, dose administered, dosing time, and blood sample collection times. The Debra system was used to calculate and report dose administered in each animal and the time between dosing and sample collection.

Study sample preparation and analysis.

Study samples were prepared and analyzed for free and total BPAF similar to matrix standards as described above, using the validated analytical method, with the exception that no standards were added. Study samples that exceeded the matrix calibration range were diluted into the validated range using respective control matrix. Each sample set was bracketed by method blanks, matrix calibration standards, and QC samples prepared at low and high ends of the calibration curve. Analyte quantitation was as similar to that described above. The concentration of analytes was expressed as ng/mL of plasma. All concentrations above LOD (0.82 ng/mL) of the assay were reported. Data from study samples were considered valid if: the matrix calibration curve was linear ($r = 0.99$); at least 75% of matrix standards were within 15% of nominal (except at the LOQ where it was 20%); at least 67% of the QC samples were within 15% of nominal values; all analyses met this criteria.

Toxicokinetic analysis.

Individual animal data were evaluated for aberrant concentrations and time points. The actual blood collection times were within 10% from nominal time and hence the actual collection time was used for TK analysis. All concentrations were evaluated to identify outliers by performing Q-tests. Based on these assessments, only one value was eliminated from TK analysis.

WinNonlin (Version 6.4, Certara, Princeton, NJ) was used for TK analysis. A variety of compartmental models were tested for BPAF concentration versus time data sets. For each compartmental model, data sets were analyzed with and without weighting. The model and the weighing factor that resulted in the best goodness of fit (evaluated using the Akaike Information Criterion (AIC) and Schwarz Bayesian Criterion (SBC)) was selected as the final model. Based on this, a two-compartment model with first order input, first order output and $1/y$ ($1/y^2$ weighting used for total BPAF 340 mg/kg group) weighting was used to calculate TK parameters following gavage administration (Model 11, equation 1) and a two-compartment model with bolus input, first order output and $1/y^2$ weighting was used to calculate TK parameters following IV administration (Model 7, equation 2).

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-k_{el}t} \quad (1)$$

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (2)$$

Results

Analytical method validation.

An analytical method to quantitate free BPAF in female HSD rat plasma was developed and successfully validated. A summary of validation parameters investigated and corresponding results are shown in Table 2. The method was linear ($r = 0.99$), accurate (inter-day and intra-day %RE ± 13.6) and precise (inter-day and intra-day %RSD ≤ 7.1). Experimental LOQ was 2.8 ng/mL and LOD was 0.9 ng/mL. Standards as high as 100,000 ng/mL in plasma could successfully be diluted into the validated concentration range with observed %RE ± 15.3 and %RSD $\leq 2.7\%$. The method was acceptable to quantitate total BPAF in plasma with %RE ± 15.4 and %RSD $\leq 4.1\%$. The method was qualified to quantitate free BPAF in male HSD rat plasma (%RE, ± 10.4 ; %RSD, 5.8%) and male (%RE, ± 11.6 ; %RSD, 3.6) and female (%RE, ± 17.7 ; %RSD, 7.5) B6C3F1/N mouse plasma using spiked QC standards prepared at 25 ng/mL and analyzed with an 8-point standard curve prepared in female HSD rat plasma over the range of ~ 3 to 100 ng/mL (Table 2).

Stability of analytes in extracted samples were demonstrated when stored at ambient temperature or refrigerator at 3 and 90 ng/mL (% RE ± 18.5). Analyte stability in plasma was confirmed during 3 freeze-thaw cycles at 3, 30, and 90 ng/mL (%RE ± 11.5) or when stored $\sim -70^\circ\text{C}$ for at least 9 months at 5 and 70 ng/mL ($\geq 95.5\%$ of target). These data

confirm that the analytical method was suitable to quantitate free and total BPAF in rat and mouse plasma.

Free and total BPAF toxicokinetics in rat:

Free and total BPAF was detected at all timepoints in both male and female plasma following gavage administration of 34, 110, and 340 mg/kg BPAF. Plasma concentration versus time data were fitted using a two-compartment model with first order input, first order output and $1/y$ ($1/y^2$ for total BPAF in 340 mg/kg groups) weighting.

Model fits of free BPAF for male and female rats are shown in Figure 1 and TK parameters estimated are given in Table 3. BPAF was absorbed rapidly following gavage administration in male and female rats with T_{max} reached at 2.2 h; there was an apparent increase in T_{max} with the increasing dose (Table 3). In general, C_{max} increased proportionally to the dose. The volume of distribution was high and exceeded the reported body water volume in rats (688 mL/kg) (Davies and Morris, 1993) indicating distribution of free BPAF into the peripheral compartment. BPAF was cleared rapidly from plasma with an elimination half-life (K_{10} half-life) ranging from 1.68 to 3.35 h, depending on the dose administered. $AUC_{0-\infty}$ increased proportionally to the dose. There was no apparent sex-related difference in plasma toxicokinetic parameters of free BPAF in rats.

Model fits for total BPAF for male and female rats are shown in Figure 1 and TK parameters estimates are given in Table 4. Total BPAF C_{max} and AUC were 27- and 52-fold higher, respectively, than free BPAF following gavage administration of BPAF in rats (Tables 3 and 4). In addition, C_{max} was reached 1.07 h. These data demonstrate rapid and extensive conjugation of BPAF following gavage administration in rats. C_{max} increased less than proportionally to the dose at the highest dose of 340 mg/kg in both males and females. Total BPAF was cleared from plasma more slowly than free BPAF with an elimination half-life (K_{10} half-life) ranging from 2.60 to 4.61 h for all groups except 340 mg/kg female group where it was 20.2 h. $AUC_{0-\infty}$ increased proportionally to the dose in males but not in females where it was more than proportional to the dose.

Free and total BPAF was detected at all timepoints in both male and female plasma following IV administration of 34 mg/kg BPAF. Plasma concentration versus time data were fitted using a two compartment model with first order input, first order output and $1/y$ weighting and corresponding TK parameters are given in Table 5. Both free and total C_{max} and $AUC_{0-\infty}$ were similar between sexes. In male rats, plasma elimination half-lives (K_{10} half-life) of free and total BPAF were 0.412 h and 0.703 h and were similar between sexes.

Free and total BPAF toxicokinetics in mice:

Following gavage administration, free BPAF was below the LOD for a few animals at later timepoints. Total BPAF was detected above LOD at all timepoints for female mice but was above the LOD only through 32 h for male mice. Plasma concentration versus time data were fitted using a two compartment model with first order input and first order output and $1/y$ weighting.

Model fits for free and total BPAF for male and female mice are shown in Figure 2 and TK parameters estimated are given in Table 6. BPAF was absorbed rapidly following gavage administration in male and female mice with free BPAF $C_{\max}$ reached at 0.455 h and 0.342 h, respectively. $C_{\max}$ for males and females were 64.1 and 105 ng/mL, respectively. The volume of distribution was high and exceeded the reported a body water volume in mice (725 mL/kg) (Davies and Morris, 1993) indicating distribution of free BPAF into the peripheral compartment. BPAF was cleared rapidly from plasma with an elimination half-life (K10 half-life) of 4.22 and 1.33 h for males and females, respectively. $AUC_{0-\infty}$ was similar in males and females.

Total BPAF $C_{\max}$ and AUC were 30-fold and 12-fold higher, respectively, than free BPAF following gavage administration of 34 mg/kg BPAF in mice (Table 6). In addition, $C_{\max}$ was reached 0.298 h. These data demonstrate rapid and extensive conjugation of BPAF following gavage administration in mice. Total BPAF was cleared from plasma with an elimination half-life (K10 half-life) of 0.753 and 0.804 h for male and females, respectively.

Following IV administration of 34 mg/kg BPAF in male and female mice, free BPAF levels in plasma were above the LOD in all samples from 5 min through 12 h (female) or 24 h (male) and in some samples at 32 h; levels were below the LOD at 48 h post administration in both males and females. Total BPAF levels were above the LOD at all timepoints. Plasma concentration versus time data were fitted using a two-compartment model with first order input and first order output with $1/y^2$ weighting and corresponding TK parameters for free and total BPAF are given in Table 7. Free and total BPAF $C_{\max}$ were similar between male and female mice. However, free BPAF $AUC_{0-\infty}$ was 2-fold and total BPAF $AUC_{0-\infty}$ was 3-fold higher in female mice than male mice. In male mice, plasma elimination half-lives (K10 half-life) of free (0.698 h) and total (1.31 h) BPAF were higher than the corresponding free (0.119 h) and total (0.339 h) values in females.

Bioavailability of BPAF in rats and mice:

Absolute bioavailability of BPAF following gavage administration was estimated in male and female rats and mice using $AUC_{0-\infty}$ of free BPAF following gavage and IV administration, adjusted for dose administered. In general, absolute bioavailability was very low in both species and sexes (Table 8). In rats, absolute bioavailability was ~ 1% with no dose-related effect. In mice, following administration of 34 mg/kg, absolute bioavailability was slightly higher with ~ 6 and 3%, in males and females, respectively.

Discussion

BPAF has gained attention in recent years due to its structural similarity to BPA. However, there is paucity of safety data for BPAF. To address the data gaps, the NTP has undertaken toxicity testing of BPAF using rodent models. Disposition data are essential to explain toxicological data and provide broader context. Previously, we have reported the ADME data for BPAF in rodents following a single gavage administration of [^{14}C]BPAF (Waidyanatha et al., 2015). In the current investigation, we report the plasma TK data of free and total BPAF following a single gavage administration of BPAF in male and female rats

and mice. An analytical method was validated to measure free and total BPAF in rats and mice with good inter- and intra-day precision and accuracy. Free and total BPAF data from rats and mice were modeled using two-compartment models. While the model fit appears reasonable, the high SE values for the absorption phase and the distribution phase reflect the rapid absorption and metabolism, with few time points available to define these parts of the curves.

Overall, these data suggest linear kinetics of free BPAF over the dose range examined and absence of sex difference in kinetics in rats. BPAF was rapidly absorbed in male and female rats following a gavage dose of 34, 110, and 340 mg/kg with $T_{\max}$ 2.20 h; $T_{\max}$ of free BPAF increased with the dose and the increase was more prominent in males (0.812 to 2.3 h) than in females (0.767 to 1.34 h). Li et al. (2013) also reported rapid absorption of BPAF in rats following a single gavage administration of 20 and 100 mg/kg BPAF with an estimated free BPAF $T_{\max}$ of 1 h. Systemic exposure parameters, $C_{\max}$ and $AUC_{0-\infty}$, of free BPAF, in rats increased proportionally to the dose. In rats, BPAF was eliminated with a half-life 3.35 h. In general, the disposition of free BPAF in mice and rats administered 34 mg/kg gavage dose was similar except that the maximum plasma concentration was reached slightly faster in mice (0.342–0.455 h) than in rats (0.767–0.812 h).

In both rats and mice of both sexes, BPAF was rapidly and extensively conjugated following oral administration. In rats, total BPAF $T_{\max}$ increased with the dose with 0.41 and 3.97 h, respectively, for 34 and 340 mg/kg. In mice, total BPAF $T_{\max}$ (0.298 h) was shorter than in rats for the comparable dose of 34 mg/kg. More importantly, in both rats and mice, the conjugation was extensive with total BPAF $C_{\max}$ and $AUC_{0-\infty}$ much higher than the free BPAF. For example, in male and female rats, respectively, total $C_{\max}$ was 19-fold and 27-fold higher than free $C_{\max}$ and total $AUC_{0-\infty}$ was 52-fold and 77-fold higher than free $AUC_{0-\infty}$. Similarly, in male and female mice given a 34 mg/kg dose, total $C_{\max}$ was 30- and 38-fold higher than free $C_{\max}$ and total $AUC_{0-\infty}$ was 12- and 23-fold higher than free $AUC_{0-\infty}$, respectively. This is likely due to extensive first pass conjugation in the intestine and the liver following oral exposure of BPAF in rodents. Subsequently, the absolute bioavailability of BPAF in both species was low but mice have slightly higher bioavailability (3 – 6%) than rats (~ 1%).

A comprehensive review of the disposition of BPA in rodents and primates were provided by Willhite et al. (2008). We have compared our data for free BPAF in female rats and mice following a single gavage administration of BPAF to two more recent publications (Doerge et al. (2010); Taylor et al. (2011) on TK of BPA in female rodents and non-human primates (Table 9). This comparison reveals that the pattern of disposition of BPAF is similar to well-researched BPA. Both BPAF and BPA were absorbed rapidly with $T_{\max}$ reached within an hour. Free BPAF and BPA were eliminated rapidly from plasma (or serum) with estimated terminal half-lives (beta half-life) of ~ 9 h for BPAF (at 34 mg/kg) and 3–9 h for BPA. Dose-normalized systemic exposure parameters from the current study for BPAF were compared, after converting to molar units, to those from Doerge et al. (2010) and Taylor et al. (2011) for BPA in rodents. Dose-normalized $C_{\max}$ ($C_{\max}$ for unit exposure, (nmol/mL)/(mmol/kg)) for BPAF was 1–3 and for BPA was ~ 1–10 in rodents. Dose-normalized $AUC_{0-\infty}$ ((nmol.h/mL)/(mmol/kg)) for BPAF was ~ 13–15 and for BPA was 6–30. This

suggests that systemic exposure to BPA and BPAF is similar in rodents. Dose-normalized $C_{\max}$ and $AUC_{0-\infty}$ of BPA in monkeys were ~ 10 (nmol/mL)/(mmol/kg) and ~ 34 (nmol.h/mL)/(mmol/kg) suggesting similarities in systemic exposure of BPA between rodents and non-human primates and subsequently of BPAF.

We also compared the conjugation of BPA and BPAF following oral exposure. In both rodents and monkeys, $T_{\max}$ of total or conjugated BPA was short demonstrating rapid conjugation. In adult female rhesus monkeys, following a single 400 $\mu\text{g/kg}$ oral dose, total $C_{\max}$ and $AUC_{0-\infty}$, respectively, were ~ 38 - and ~ 91 -fold higher, respectively, than the corresponding free values pointing to extensive conjugation (Taylor et al., 2011). Similar observations were noted in adult female CD-1 mice following a single oral dose of 100 mg/kg BPA, where total $C_{\max}$ and $AUC_{0-\infty}$, respectively, were ~ 120 - and ~ 124 -fold higher than the corresponding free values (Taylor et al., 2011). In studies by Doerge et al. (2010), in adult female Sprague-Dawley rats administered 100 $\mu\text{g/kg}$ BPA orally, total $C_{\max}$ and $AUC_{0-\infty}$, respectively, were ~ 187 - and ~ 261 -fold higher than the corresponding free values. These observations are similar to our investigation where total $C_{\max}$ and $AUC_{0-\infty}$ were much higher than the for free BPAF. Doerge et al. (2010) reported an absolute bioavailability of free BPA in adult mice to be 0.028 (2.8%) which was similar to our observation in mice following an oral dose of 34 mg/kg (3–6%). They also compared TK parameters of BPA in Sprague-Dawley rat pups at varying ages following a 100 $\mu\text{g/kg}$ of an oral BPA dose; the study clearly demonstrated that the development of metabolic capabilities with increasing age attenuate internal exposure to free BPA. Overall, these comparisons strengthen our confidence in the high degree of similarity in the toxicokinetics and bioavailability of BPA and BPAF following oral administration in rodents. In a recent investigation by Karrer et al. (2018), physiologically based pharmacokinetic modeling of the bisphenol analogues concluded that BPAF behaves similar to BPA. However, the greater estrogenic activity of BPAF both in vitro and in vivo suggests a greater response for a similar exposure (Conley et al., 2016). In a comparison of estrogenic activity in vitro (the T47D-KBluc estrogen receptor transcriptional activation (ERTA) assay), BPAF had a substantially lower EC_{50} than BPA (2.06×10^{-8} M vs 1.27×10^{-7} M), and was active at lower doses administered orally in the rat uterotrophic assay (ED_{50} values of 209.1 and 1118.0 mg/kg/day for BPAF and BPA, respectively).

Conclusion

Our data demonstrate that BPAF is rapidly absorbed in male and female rats and mice following gavage administration and extensively conjugated leading to very low bioavailability ($\sim 6\%$). BPAF was rapidly eliminated in rats and mice with half-lives ~ 4.22 h. There were minimal dose, species- or sex-related effects on the plasma toxicokinetics of free BPAF in rats and mice.

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Figure 1.

Plasma concentration versus time profiles of free BPAF (open circle) and total BPAF (filled square) following a single gavage administration of 34, 110, and 340 mg/kg BPAF in male and female Harlan Sprague Dawley rats. Data presented are for individual animals. Two-compartmental model with first order input, first order output and $1/y$ ($1/y^2$ for total BPAF in 340 mg/kg groups) was used to fit the data.

Figure 2.

Plasma concentration versus time profiles of free BPAF (open circle) and total BPAF (filled square) following a single gavage administration of 34 mg/kg BPAF in male and female B6C3F1/N mice. Data presented are for individual animals. Two-compartmental model with first order input, first order output, lag time and 1/y weighting was used to fit the data.

Table 1.Study design^a

Species, Sex	Route	Dose (mg/kg)
Rat, Male	Oral	34, 110, 340
Rat, Female	Oral	34, 110, 340
Rat, Male	Intravenous	34
Rat, Female	Intravenous	34
Mouse, Male	Oral	34
Mouse, Female	Oral	34
Mouse, Male	Intravenous	34
Mouse, Female	Intravenous	34

^aTarget times for blood collection following the dose administration were 0 (predose), 5, 15, 30 min, 1,2,4,8,12,24,32, 48h for all dose groups

Table 2.Analytical method validation and stability data for BPAF in rat and mouse plasma^a

Matrix	Parameter	Data
Female rat plasma (free BPAF)	Concentration range (ng/mL)	2.8 to 105
	Linearity (r) (1/x weighting)	0.99
	LOQ ^b (ng/mL)	2.80
	LOD ^c (ng/mL)	0.89
	Accuracy (%RE) (7.8, 30, 90 ng/mL) ^d Intraday Inter-day	±13.6 ± 13.6
	Precision (%RSD) (7.8, 30, 90 ng/mL) ^d Intraday Inter-day	5.8 7.1
	Absolute recovery (%)	83.8 to 108.5
	Dilution Verification (% RE, %RSD) 1,100 ng/mL 100,000 ng/mL	±6.7, 2.7 ±15.3, 2.5
	Extracted sample stability (%RE) Ambient temperature, refrigerator (~5 days) (3 and 90 ng/mL)	±18.5, ± 17.3
	Matrix freeze-thaw stability ^d (3 cycles) (%RE, 3, 30, and 90 ng/mL)	±11.5
	Matrix storage stability (–70°C, up to 9 months) (%target, %RE) (5 and 70 ng/mL) ^e	95.5, 22.3
Female rat plasma (total BPAF)	Accuracy, precision (% RE, %RSD) (30 ng/mL)	±15.4, 4.1
Male rat plasma (free BPAF)	Accuracy, precision (% RE, %RSD) (25 ng/mL)	±10.4, 5.8
Male mouse plasma (free BPAF)	Accuracy, precision (% RE, %RSD) (25 ng/mL)	±11.6, 3.6
Female mouse plasma (free BPAF)	Accuracy, precision (% RE, %RSD) (25 ng/mL)	±17.7, 7.5

^a A full validation was performed in female rat plasma for free BPAF with assessments for total BPAF in female and matrix evaluations in male rat and male and female mice.

^b LOQ, limit of quantitation; LOD, limit of detection, RE, relative error; RSD, relative standard deviation.

^c Estimated as 3 times the standard deviation of the LOQ (n=6 replicates).

^d Values given are based on the 3 concentrations.

^e Values given are based on the 2 concentrations.

Table 3.

Plasma toxicokinetic parameters of free BPAF following a single gavage administration in male and female Harlan Sprague Dawley rats^a

Parameter ^b	Dose (mg/kg)		
<i>Male</i>	34	110	340
C _{max} (ng/mL)	60.7 (8.39)	142 (31.6)	552 (138)
T _{max} (h)	0.812 (0.352)	1.43 (0.339)	2.20 (0.718)
K01 Half-Life (h)	0.491 (286)	0.910 (123)	1.44 (381)
K12 (1/h)	0.849 (604)	0.402 (62.7)	0.156 (49.5)
K21 (1/h)	0.257 (0.137)	0.0972 (0.207)	0.0814 (0.223)
Alpha Half-life (h)	0.491 (285)	0.918 (125)	1.43 (379)
Beta Half-life (h)	10.2 (4.5)	18.4 (35.5)	13.9 (28.1)
K10 Half-Life (h)	1.85 (1080)	2.36 (320)	2.33 (619)
Cl1_F (L/h/kg)	85.2 (20.0)	89.1 (52.4)	70.3 (20.8)
Cl2_F (L/h /kg)	193 (24800)	122 (2520)	36.9 (1910)
V1_F (L/kg)	227 (132000)	304 (41100)	237 (62900)
V2_F (L/kg)	752 (966600)	1260 (23900)	454 (24400)
AUC _{0-∞} (h*ng/mL)	399 (93.5)	1230 (726)	4830 (1430)
<i>Female</i>			
C _{max} (ng/mL)	47.4 (8.39)	245 (29.3)	555 (99.5)
T _{max} (h)	0.767 (0.201)	0.658 (0.1)	1.34 (0.24)
K01 Half-Life (h)	0.350 (6.52)	0.405 (6.32)	0.892 (30.4)
K12 (1/h)	0.981 (31.1)	1.07 (19.3)	0.433 (15.4)
K21 (1/h)	0.698 (1.29)	0.215 (0.115)	0.0391 (0.0929)
Alpha Half-life (h)	0.384 (7.67)	0.422 (6.63)	0.912 (31.2)
Beta Half-life (h)	8.68 (2.6)	12.8 (4.83)	44.4 (152)
K10 Half-Life (h)	3.35 (61.8)	1.68 (26)	2.29 (79.2)
Cl1_F (L/h/kg)	73.8 (13.3)	74.7 (13.4)	70.7 (94.0)
Cl2_F (L/h /kg)	350 (4650)	194 (503)	101 (180)
V1_F (L/kg)	357 (6600)	181 (2810)	33 (7920)
V2_F (L/kg)	502 (5820)	899 (2050)	2580 (6150)
AUC _{0-∞} (h*ng/mL)	461 (82.7)	1470 (264)	4810 (6390)

^aBased on two-compartment model with first order input, first order output and 1/y weighting.

^bValues given are mean (standard error).

Table 4.

Plasma toxicokinetic parameters for total BPAF following a single gavage administration in male and female Harlan Sprague Dawley rats^a

Parameter ^b	Dose (mg/kg)		
<i>Male</i>	34	110	340
C _{max} (ng/mL)	2750 (525)	7970 (1920)	10500 (2400)
T _{max} (h)	0.734 (0.180)	1.07 (0.386)	3.97 (0.974)
K01 Half-Life (h)	0.447 (12)	0.561 (171)	2.43 (129)
K12 (1/h)	1.13 (35.9)	0.731 (321)	0.0931 (6.73)
K21 (1/h)	0.276 (0.308)	0.380 (0.568)	0.0682 (0.177)
Alpha Half-life (h)	0.427 (11.2)	0.558 (170)	2.53 (136)
Beta Half-life (h)	15.3 (8.95)	11.9 (6.14)	18.5 (29.7)
K10 Half-Life (h)	2.60 (70.1)	3.65 (1110)	4.61 (244)
Cl1_F (L/h/kg)	1.32 (0.382)	1.17 (0.314)	1.35 (0.303)
Cl2_F (L/h /kg)	5.58 (26.9)	4.51 (603)	0.836 (16.1)
V1_F (L/kg)	4.95 (134)	6.18 (1890)	8.98 (476)
V2_F (L/kg)	20.2 (115)	11.9 (1600)	12.3 (208)
AUC _{0-∞} (h*ng/mL)	25700 (7450)	93700 (25100)	252000 (56400)
<i>Female</i>			
C _{max} (ng/mL)	3760 (695)	10500 (2400)	15200 (5530)
T _{max} (h)	0.410 (0.126)	0.564 (0.166)	2.19 (0.64)
K01 Half-Life (h)	0.204 (38.1)	0.352 (17.2)	1.35 (35)
K12 (1/h)	2.19 (595)	1.64 (91.5)	0.411 (11.7)
K21 (1/h)	1.06 (0.809)	0.240 (0.216)	0.0498 (0.0765)
Alpha Half-life (h)	0.203 (37.9)	0.344 (16.8)	1.41 (36.5)
Beta Half-life (h)	9.74 (2.08)	38.7 (46.9)	200 (1380)
K10 Half-Life (h)	3.03 (566)	4.59 (225)	20.2 (586)
Cl1_F (L/h/kg)	0.954 (0.172)	0.623 (0.49)	0.309 (1.78)
Cl2_F (L/h /kg)	9.14 (766)	6.78 (43.2)	3.70 (10.3)
V1_F (L/kg)	4.17 (780)	4.13 (204)	9.01 (232)
V2_F (L/kg)	8.61 (725)	28.3 (195)	74.4 (185)
AUC _{0-∞} (h*ng/mL)	35600 (6420)	177000 (139000)	1100000 (6340000)

^aBased on two-compartment model with first order input, first order output and 1/y (1/y² for 340 mg/kg groups) weighting.

^bValues given are mean (standard error).

Table 5.

Plasma toxicokinetic parameters for free and total BPAF following a single intravenous administration of 34 mg/kg BPAF in male and female Harlan Sprague Dawley rats^a

Parameter ^b	Free		Total	
	Male	Female	Male	Female
C _{max} (ng/mL)	75900 (13700)	71500 (17300)	96700 (20000)	130000 (29000)
K ₁₂ (1/h)	0.0503 (0.0151)	0.0656 (0.0398)	0.333 (0.149)	0.340 (0.125)
K ₂₁ (1/h)	0.0892 (0.0147)	0.141 (0.0339)	0.128 (0.0235)	0.151 (0.0299)
Alpha Half-life (h)	0.400 (0.039)	0.420 (0.0749)	0.512 (0.113)	0.463 (0.0933)
Beta Half-life (h)	8.01 (1.28)	5.15 (1.15)	7.46 (0.829)	6.13 (0.883)
K ₁₀ Half-Life (h)	0.412 (0.0394)	0.439 (0.0724)	0.703 (0.12)	0.620 (0.109)
Cl ₁ (L/h/kg)	0.753 (0.102)	0.751 (0.118)	0.347 0.0429	0.292 0.0322
Cl ₂ (L/h /kg)	0.0225 (0.00797)	0.0312 (0.0177)	0.117 (0.0498)	0.0887 (0.0294)
V ₁ (L/kg)	0.448 (0.0811)	0.476 (0.115)	0.352 (0.0729)	0.261 (0.0581)
V ₂ (L/kg)	0.253 (0.0785)	0.222 (0.0922)	0.917 (0.277)	0.587 (0.143)
V _{ss}	0.700 (0.146)	0.697 (0.166)	1.27 (0.305)	0.848 (0.173)
AUC _{0-∞} (h*ng/mL)	45200 (6110)	45300 (7110)	98000 (12100)	117000 (12800)

^aBased on two-compartment model with first order input, first order output and 1/y weighting.

^bValues given are mean (standard error).

Table 6.

Plasma toxicokinetic parameters for free and total BPAF following a single gavage administration of 34 mg/kg BPAF in male and female B6C3F1/N mice^a

Parameter ^b	Free		Total	
	Male	Female	Male	Female
C _{max} (ng/mL)	64.1 (18.9)	105 (20.3)	1930 (449)	3970 (1060)
T _{max} (h)	0.455 (0.214)	0.342 (0.0703)	0.298 (0.071)	0.275 (0.101)
K01 Half-Life (h)	0.0807 (0.0747)	0.208 (66.9)	0.186 (42.4)	0.170 (13.9)
K12 (1/h)	0.0271 (0.476)	2.38 (899)	2.42 (635)	2.74 (260)
K21 (1/h)	0.369 (5.1)	0.487 (0.497)	0.487 (0.542)	0.536 (0.557)
Alpha Half-life (h)	1.67 (21.9)	0.209 (67.2)	0.187 (42.7)	0.172 (14.2)
Beta Half-life (h)	4.73 (5.8)	9.02 (4.74)	5.73 (3.16)	6.03 (3.27)
K10 Half-Life (h)	4.22 (2.34)	1.33 (426)	0.753 (171)	0.804 (65.8)
Cl1_F (L/h/kg)	80.0 (22.5)	68.7 (22.5)	6.41 (1.94)	2.95 (1.08)
Cl2_F (L/h /kg)	13.2 (226)	313 (17400)	16.8 (586)	9.38 (122)
V1_F (L/kg)	487 (238)	131 (42200)	6.97 (1590)	3.43 (281)
V2_F (L/kg)	35.8 (285)	642 (35300)	34.6 (1170)	17.5 (216)
AUC _{0-∞} (h*ng/mL)	425 (119)	495 (162)	5300 (1600)	11500 (4210)

^aBased on two-compartment model with first order input, first order output and 1/y weighting.

^bValues given are mean (standard error).

Table 7.

Plasma toxicokinetic parameters for free and total BPAF following a single intravenous administration of 34 mg/kg BPAF in male and female B6C3F1/N mice^a

Parameter ^b	Free		Total	
	Male	Female	Male	Female
C _{max} (ng/mL)	7490 (1750)	92300 (32300)	11700 (2540)	140000 (29800)
K ₁₂ (1/h)	0.189 (0.0802)	0.696 (0.190)	0.126 (0.0997)	0.572 (0.148)
K ₂₁ (1/h)	0.227 (0.0304)	0.257 (0.0225)	0.213 (0.0622)	0.263 (0.0277)
Alpha half-life (h)	0.566 (0.121)	0.106 (0.0193)	0.976 (0.308)	0.259 (0.0366)
Beta half-life (h)	3.76 (0.316)	3.03 (0.218)	4.37 (0.594)	3.45 (0.209)
K ₁₀ Half-Life (h)	0.698 (0.135)	0.119 (0.0225)	1.31 (0.269)	0.339 (0.0478)
Cl ₁ (L/h/kg)	4.51 (0.547)	2.15 (0.426)	1.54 (0.223)	0.496 (0.058)
Cl ₂ (L/h /kg)	0.860 (0.324)	0.256 (0.108)	0.367 (0.267)	0.139 (0.0422)
V ₁ (L/kg)	4.54 (1.06)	0.368 (0.129)	2.91 (0.634)	0.242 (0.0517)
V ₂ (L/kg)	3.78 (1.10)	0.995 (0.388)	1.73 (0.841)	0.528 (0.125)
V _{ss}	8.32 (1.64)	1.36 (0.499)	4.64 0.994	0.770 0.163
AUC _{0-∞} (h*ng/mL)	7540 (912)	15800 (3130)	22100 (3190)	68500 (8000)

^aBased on two-compartment model with first order input, first order output and 1/y² weighting.

^bValues given are mean (standard error).

Table 8.

Bioavailability of free BPAF following a single gavage administration in Harlan Sprague Dawley rats and B6C3F1/N mice

Dose (mg/kg)	Bioavailability (%) ^a			
	Male Rats	Female Rats	Male Mice	Female Mice
34	0.88	1.02	5.64	3.13
110	0.84	1.00	-	-
340	1.07	1.06	-	-

^a Bioavailability (%F) was calculated as AUC/Dose (oral) ÷ AUC/Dose (IV) x 100.

Table 9.

Comparison of free BPAF and free BPA TK parameters following gavage administration

	BPAF ^a		BPA		
	HSD Rats ^a	B6C3F1/N Mice ^a	SD Rats ^b	CD-1 mice ^c	Rhesus Monkeys ^c
Dose (mg/kg)	34	34	0.1	100	0.4
Dose (mmol/kg) ^d	0.101	0.101	0.00044	0.439	0.00175
T _{max} (h)	0.767	0.342	-	1	1
Half-life (h) ^e	8.68	9.02	3.0	4.90	8.88
C _{max} (nmol/mL)	0.142 ^f	0.313 ^f	0.00039 ^g	4.16 ^h	0.0173 ^h
Dose-normalized C _{max} (nmol/mL)/(mmol/kg)	1.406	3.099	0.886	9.47	9.88
AUC _{0-∞} (nmol.h/mL)	1.37 ^f	1.47 ^f	0.0026 ^g	13.1 ⁱ	0.0589 ⁱ
Dose-normalized AUC _{0-∞} (nmol.h/mL)/(mmol/kg)	13.6	14.6	5.91	29.8	33.7

^aData from current study;^bData from Dorege et al., (2010);^cData from Taylor et al., (2015);^dMolar masses of 228 and 336 g/mol were used for conversion of BPA and BPAF, respectively;^eTerminal half-lives ((beta half-lives) are shown;^fC_{max} (ng/mL) values were 47.4 and 105, for rats and mice, respectively; AUC_{0-∞} (ng.h/mL) values were 461 and 495 for rats and mice, respectively;^gC_{max} and AUC_{0-∞} values reported were 0.39 nM and 2.6 nmol.h/L, respectively;^hC_{max} values reported were 949 and 3.95 ng/mL for mice and monkeys, respectively;ⁱAUC_{0-∞} values reported were 2991 and 13.44 ng.h/mL, respectively;