Toxicokinetics of Bisphenol S in Male and Female Harlan Sprague Dawley Rats and B6C3F1/N Mice Following Oral Gavage Administration

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Abstract: 3115

ABSTRACT

Bisphenol S (BPS), a bisphenol analogue, is a component of polyether sulfones. As part of the toxicity testing of BPS, toxicokinetic studies were conducted following single gavage (34, 110, or 340 mg/kg) or intravenous (iv) administration of BPS to male and female Harlan Sprague Dawley rats and B6C3F1/N mice. Blood was collected at time points up to 72 h after dosing and plasma was analyzed for both total BPS (unconjugated and conjugated) and free BPS (unconjugated) using a validated liquid chromatograph-tandem mass spectrometry method. The concentration vs. time plots suggest enterohepatic recirculation which is more pronounced in mice than rats with a mono-exponential elimination in rats after oral administration but a bi-exponential elimination in mice (oral and iv) and after iv in rats. Individual plasma concentrations vs. time were analyzed using compartmental models. BPS was rapidly absorbed in rats with free BPS T_{max} ≤ 2 h in male, 5.9 h in female. Elimination half-lives of free BPS in rats increased from ~ 6 to ~12 h and $AUC_{0-\infty}$ increased disproportionally with dose, indicating a saturation of kinetics. Total BPS T_{max} was 1–3 h in rats with $AUC_{0-\infty}$ 15- to 32-fold higher than corresponding free BPAF $AUC_{0-\infty}$ indicating rapid and extensive conjugation of BPS in rats. A similar doubling of elimination half-life (8.5 to 17 h) was observed for total BPS with increasing dose, but AUC was linear with dose. In mice, highest concentrations were measured at first time point (15 min) following oral administration. In male mice, the elimination half-lives of free BPS and total BPS were shorter (ca. 2–4 h) than rats after 34 and 340 mg/kg oral doses. $AUC_{0-\infty}$ for total BPS and free BPS in mice increased non-linearly with increasing dose. $AUC_{0-\infty}$ for rats and mice was generally similar at each dose level. Similar to rats, extensive metabolism in mice is indicated by $AUC_{0-\infty}$ of total BPS ~12–15x that of free BPS. No sex differences were observed in either species. Oral bioavailability of free BPS in mice (11–19%) was similar to that of rats (9–21%). The data indicate that BPS is rapidly absorbed and rapidly and extensively metabolized to conjugates after oral administration in rats and mice.

Introduction

Bisphenol S (BPS) is a component of polyether sulfones which are used in a variety of consumer products, as a chemical intermediate, a monomer in plastics and resins, with applications in products such as baby bottles, microwave dishes, and artificial organs and joints (Liao et al., 2012a; Rochester and Bolden, 2015). BPS is also used in the preparation of developers for heat-sensitive paper, couplers for photography, fire retardants, and as modifiers for leather, fiber, and epoxy curing agents. (EFSA, 2015; Liao et al., 2012a; U.S.EPA, 2014)

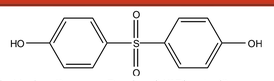
Due to lack of toxicity data for BPS, NTP has undertaken toxicity studies (*https://ntp.niehs.nih.gov/testing/status/agents/ts-m940150.html*) in Harlan Sprague Dawley (HSD) rats and B6C3F1/N mice. This work describes definitive single dose toxicokinetic studies conducted at doses of 34, 110, and 340 mg/kg. We have previously published ADME studies of [^{14}C]BPS following administration of 50, 150 and 500 mg/kg in rats and mice (Waidyanatha S et al., 2018)

Objectives

The objective of this study was to determine toxicokinetics of total BPS (conjugated and unconjugated) and free BPS following oral gavage or intravenous (iv) administration of BPS to male and female Harlan Sprague Dawley rats and B6C3F1/N mice. Dose response was evaluated in males, with limited studies in female of each species.

Methods

Bisphenol S (BPS)



Animals: Male and female Harlan Sprague Dawley (HSD) rats (9–10 weeks old at dosing) were obtained from Envigo (Indianapolis, IN) and B6C3F1/N mice (11–12 weeks old at dosing) were from Taconic Farms (Germantown, NY). Animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Animal procedures were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 2011) and were approved by the IACUC.

Doses and Routes: Oral gavage doses (34, 110, and 340 mg/kg in rats and mice) were formulated in 0.5% methylcellulose (5 and 10 mL/kg in rats and mice, respectively); 34 mg/kg iv doses in Kolliphor EL:95% Ethanol:DI water (20:10:70), (2 and 4 mL/kg in rats and mice, respectively).

Sample Collection: Blood samples were obtained from three animals per group per time point following dose administration. One or two interim blood samples (ca. 250 µL) were obtained from lateral tail vein of rats and terminal samples were obtained from rats and mice by terminal cardiac puncture after euthanasia with CO_2. Blood was immediately dispensed into a tube containing K_3EDTA, mixed by inversion and placed on ice. Plasma was prepared and frozen and stored at ca. -70 °C until analysis.

Sample Analysis: Plasma samples were analyzed for free and total BPS using a validated UPLC-MS/MS method. Samples (50 µL) were thawed and vortexed for analysis by addition of 10 µL of IS (100 µg/mL of BPS-*d8*), followed by 10 µL of methanol. Samples were extracted by adding 300 µL of acetonitrile, then transferred to autosampler vials with glass inserts for analysis.

Analysis of plasma extracts was conducted using Waters Acquity UPLC/Applied Biosystems 4000 QTRAP (Waters Corp., Milford, MA/ Applied Biosystems, Framingham, MA). Chromatography was conducted on Waters Acquity UPLC BEH C18 (2.1 x 50 mm, 1.7-µm) with Waters Acquity UPLC BEH C18 Guard (2.1 x 5 mm, 1.7-µm)(Milford, MA). Solvents were A: Water, B: Acetonitrile 0.3 mL/min. Initially 5% B for 1 min., ramp to 95% B in 5 min., hold at 95% B for 2 min., reverse to 5% B in 0.5 min., hold at 5% B for 1.5 min.; total run time = 10 min. The column temperature was 60 °C and autosampler temperature was 10 °C.

For each injection, the ratio of the area of the BPS peak to the area of the internal standard peak was calculated. The linear regression equation relating the peak area ratios of the matrix calibration standards to their BPS concentration was determined for data weighted by $1/x^2$.

Toxicokinetic Analysis: Toxicokinetic data was analyzed by 1- or 2-compartment modeling techniques using Phoenix WinNonlin Version 6.4 (Certara, Princeton, NJ). Model fit plots were examined visually to select the appropriate model and residual plots were examined to select an appropriate weighting scheme. Each selected model was further evaluated by examination of goodness of fit statistics including AIC, SBC, parameter CV%, residual run count, parameter count and condition number.

Results – Rat Toxicokinetics

GAVAGE ADMINISTRATION

Toxicokinetic parameters are presented in Table 1. The fitted concentration versus time plots are presented in Figure 1.

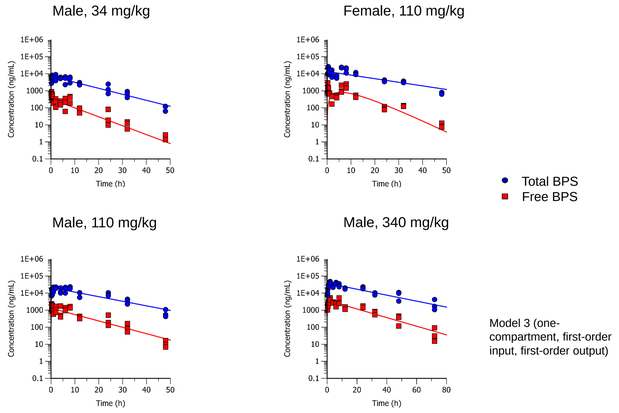
FREE BPS

- Free BPS concentrations in plasma peaked (T_{max}) between 0.350 and 5.88 h, indicating rapid absorption of BPS.
- Plasma half-lives (5.77–11.9 h) for free BPS increased with increasing dose for male rats.
- AUC increased nonlinearly with dose for male rats, suggesting saturation of elimination processes.
- Clearance of free BPS decreased with increasing dose.
- No sex difference was observed for any parameter.

TOTAL BPS

- Total BPS T_{max} values were 0.620–2.77 h across the dose groups, indicating rapid metabolism of BPS.
- Plasma half-lives for total BPS increased with increasing dose.
- AUC for total BPS was ~15- to 32-fold higher than AUC for free BPS, indicating extensive conjugation of BPS. AUC was proportional to dose across the 34–340 mg/kg dose range.
- Clearance of total BPS by male rats was consistent across all dose levels (range: 331–397 mL/h/kg).
- No sex difference was observed for any parameter.

Figure 1. Observed and Predicted Total and Free BPS Concentrations in Male and Female HSD Rat Plasma Following Gavage Administration of 34, 110, and 340 mg/kg BPS



Model 3 (one-compartment, first-order input, first-order output)

Table 1. Toxicokinetic Parameters for Free and Total BPS following Gavage Administration to Rats

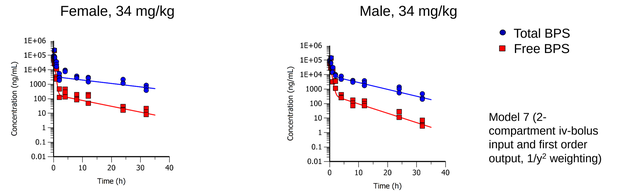
Variable	Free BPS	Free BPS	Free BPS	Free BPS	Total BPS	Total BPS	Total BPS	Total BPS
Dose Level (mg/kg)	34	110	110	340	34	110	110	340
Sex	M	F	M	M	M	F	M	M
Cmax (ng/mL)	313 (52.4)	804 (159)	1140 (145)	3240 (215)	6420 (432)	13000 (1400)	18500 (1550)	32800 (1840)
Tmax (h)	0.350 (1.02)	5.88 (1.71)	1.17 (0.544)	1.97 (0.311)	0.991 (0.224)	0.620 (0.494)	1.75 (0.365)	2.77 (0.339)
K01 (1/h)	13.6 (49.8)	0.171 (6.58)	3.16 (2.03)	1.80 (0.408)	4.02 (1.24)	8.36 (8.32)	2.06 (0.613)	1.29 (0.228)
K10 (1/h)	0.120 (0.0292)	0.169 (6.43)	0.086 (0.0185)	0.0582 (0.00663)	0.0812 (0.00941)	0.0485 (0.00976)	0.0619 (0.00908)	0.0403 (0.00425)
K01_HL (h)	0.0509 (0.186)	4.04 (155)	0.219 (0.141)	0.385 (0.0871)	0.172 (0.0531)	0.0829 (0.0825)	0.336 (0.0996)	0.537 (0.0947)
Elimination half-life K10_HL (h)	5.77 (1.40)	4.11 (157)	8.06 (1.73)	11.9 (1.35)	8.54 (0.989)	14.3 (2.88)	11.2 (1.64)	17.2 (1.81)
CL_F (mL/h/kg)	12500 (2390)	8550 (2030)	7480 (1260)	5450 (474)	397 (35.5)	398 (66.1)	331 (36.8)	373 (29.5)
V_F (mL/kg)	104000 (15200)	50800 (1940000)	87000 (13600)	93600 (7720)	4890 (394)	8200 (981)	5350 (555)	9260 (642)
AUC (h*ng/mL)	2710 (517)	12900 (3050)	14700 (2480)	62400 (5420)	85700 (7670)	277000 (45900)	333000 (37000)	911000 (71900)
AUC/D (h*ng/mL/mg/kg)	79.7	117	134	184	2520	2520	3030	2680

INTRAVENOUS ADMINISTRATION

The TK parameters are shown in Table 2. The concentration versus time plots are presented in Figure 2.

- The elimination half-life (beta half-life) for free and total BPS were slightly longer for female than for male rats
- The Cl for free BPS was ~4x that of total BPS for both sexes.
- No sex difference was found in AUC of free or total BPS.

Figure 2. Observed and Predicted Total and Free BPS Concentrations in Male and Female HSD Rat Plasma following Intravenous Administration of 34 mg/kg BPS



Model 7 (2-compartment *iv*-bolus input and first order output, $1/y^2$ weighting)

Table 2. Toxicokinetic Parameters for Free and Total BPS following Intravenous Administration to Rats

Variable	Free BPS	Free BPS	Total BPS	Total BPS
Dose Level (mg/kg)	34	34	34	34
Sex	M	F	M	F
Elimination half-life Beta_HL (h)	4.67 (0.426)	7.46 (1.1)	6.38 (0.46)	12.7 (2.91)
CL (mL/h/kg)	1140 (261)	1250 (240)	263 (19.1)	293 (33.5)
AUC (h*ng/mL)	29800 (6790)	27200 (5200)	129000 (9360)	116000 (13200)
AUC/D (h*ng/mL/mg/kg	876	800	3794	3412

Results – Mouse Toxicokinetics

GAVAGE ADMINISTRATION

Toxicokinetic parameters are presented in Table 3. The fitted concentration versus time plots are presented in Figure 3.

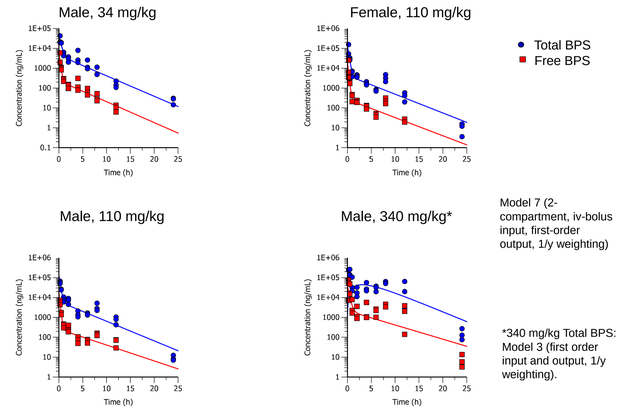
FREE BPS

- Tmax occurred before the first sampling time point (15 min).
- Elimination half-lives (beta half-life) for free BPS ranged from 2.86 to 4.21 h for male mice.
- AUC was proportional to dose for male mice at 34 and 340 mg/kg, but was disproportionately lower at 110 mg/kg for male and female mice.
- Clearance of free BPS was similar for 34 and 340 mg/kg in males (~13,000 mL/h/kg) but was higher at 110 mg/kg (21,700 mL/h/kg).
- No sex difference was observed for any parameter.

TOTAL BPS

- Tmax occurred before the first sampling time point (15 min).
- Elimination half-lives were about 3 h for all dose levels and sexes.
- Total BPS AUC was ~12- to 15-fold higher than that for free BPS for all dose groups.
- As for free BPS, AUC of total BPS increased proportionally with dose in male mice for 34 and 340 mg/kg groups, but disproportionately lower at 110 mg/kg for male and female mice.
- Clearance of total BPS by male mice was similar for 34 and 340 mg/kg dose levels (~900 mL/h/kg) but was higher (1770 mL/h/kg) for the 110 mg/kg group.
- No sex difference was observed for any parameter.

Figure 3. Observed and Predicted Total and Free BPS Concentrations in Male and Female B6C3F1/N Mouse Plasma following Gavage Administration of 34, 110, and 340 mg/kg BPS



Model 7 (2-compartment, iv-bolus input, first-order output, 1/y weighting)

*340 mg/kg Total BPS: Model 3 (first order input and output, 1/y weighting).

Table 3. Toxicokinetic Parameters for Free and Total BPS following Gavage Administration to Mice

Variable	Free BPS	Free BPS	Free BPS	Free BPS	Total BPS	Total BPS	Total BPS	Total BPS
Dose Level (mg/kg)	34.0	110	110	340	34.0	110	110	340[a]
Sex	M	F	M	M	M	F	M	M
Cmax (ng/mL)	7540 (2770)	21300 (13500)	19400 (3590)	39200 (22700)	47000 (10500)	142000 (48000)	140000 (23300)	43700 (24900)
K10 (1/h)	2.95 (0.853)	4 (1.85)	3.83 (0.566)	1.5 (0.803)	1.36 (0.283)	2.5 (0.704)	2.25 (0.325)	0.227 (0.389)
K12 (1/h)	1.5 (0.587)	1.12 (0.869)	1.31 (0.274)	1.2 (1.19)	1.1 (0.465)	1.13 (0.569)	1.44 (0.292)	NA[a]
K21 (1/h)	0.377 (0.185)	0.273 (0.379)	0.25 (0.11)	0.312 (0.283)	0.469 (0.197)	0.325 (0.209)	0.39 (0.101)	NA
Elimination half-life Beta_HL (h)	2.86 (1.41)	3.29 (4.78)	3.77 (1.78)	4.21 (3.17)	2.92 (0.958)	3.18 (1.99)	3.04 (0.763)	3.05[b] (5.23)
CL (mL/h/kg)	13300 (2100)	20600 (6130)	21700 (1950)	13000 (4670)	986 (109)	1940 (325)	1770 (131)	878 (619)
V1 (mL/kg)	4510 (1660)	5150 (3270)	5660 (1050)	8670 (5030)	724 (162)	775 (262)	788 (132)	3870 (7150)
AUC (h*ng/mL)	2550 (402)	5340 (1590)	5070 (455)	26100 (9350)	34500 (3790)	56700 (9500)	62100 (4600)	387000 (273000)
AUC/D (h*ng/mL/mg/kg)	75.0	48.5	46.1	76.8	1015	515	565	1138

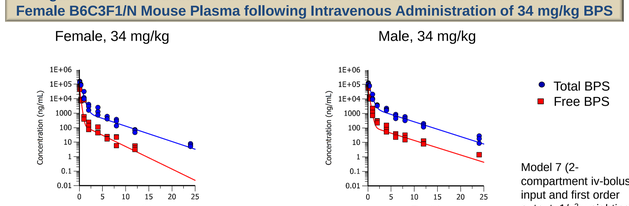
[a] A one compartment model was used for this group due to failure of fit to a 2-compartment model. Therefore, some parameters are not applicable.
[b] K10 Half-life

INTRAVENOUS ADMINISTRATION

The TK parameters are shown in Table 4. The concentration versus time plots are presented in Figure 4.

- The elimination half-lives (beta half-life) for free BPS (~2.5 h) and for total BPS (~2.9 h) show no marked sex difference.
- Cl of free BPS was 5–7x higher than for total BPS for male and female mice.
- The AUCs for free BPS (~12600 h*ng/mL) and total BPS (~70600 h*ng/mL) were similar across sexes.

Figure 4. Observed and Predicted Total and Free BPS Concentrations in Male and Female B6C3F1/N Mouse Plasma following Intravenous Administration of 34 mg/kg BPS



Model 7 (2-compartment iv-bolus input and first order output, $1/y^2$ weighting)

Table 4. Toxicokinetic Parameters for Free and Total BPS following Intravenous Administration to Mice

Variable	Free BPS	Free BPS	Total BPS	Total BPS
Dose_Level	34.0	34.0	34.0	34.0
Sex	M	F	M	F
Elimination half-life Beta_HL (h)	3.00 (0.464)	1.93 (0.262)	2.95 (0.196)	2.83 (0.249)
CL (mL/h/kg)	2450 (424)	3010 (649)	506 (53.3)	459 (62.5)
AUC (h*ng/mL)	13900 (2390)	11300 (2430)	67100 (7060)	74100 (10100)
AUC/D (h*ng/mL/mg/kg)	409	332	1974	2180

Bioavailability

Bioavailability is shown in Table 5.

- Calculated bioavailability of free BPS increased with increasing dose (range: 9.1–20.9%) in male rats.
- Calculated bioavailability of free BPS was similar at 34 and 340 mg/kg (~18%) for male mice, but was lower (11–15%) at 110 mg/kg for male and female mice.

Table 5. Bioavailability (%) of Free and Total BPS in Rats and Mice

Variable	Free BPS	Free BPS	Free BPS	Free BPS	Total BPS	Total BPS	Total BPS	Total BPS
Dose Level (mg/kg)	34	110	110	340	34	110	110	340
Sex	M	F	M	M	M	F	M	M
Rat	9.1	14.7	15.2	20.9	66.5	73.8	79.9	70.7
Mouse	18.3	14.6	11.2	18.7	51.5	23.6	28.7	57.8

Conclusions

- Bisphenol S (BPS) is rapidly absorbed and metabolized to conjugates following gavage administration (34 to 340 mg/kg) to rats and mice.
- The toxicokinetics of BPS administered orally to rat conformed to a one-compartment model, while in mice orally administered BPS conformed to a two-compartment model with no discernable absorption phase.
- Tmax for free BPS in male rat was less than 2 h and for male mouse occurred before the first sampling time point (15 min), demonstrating a species difference.
- AUC for total BPS was many times higher than that of free BPS in both rats (15- to 32-fold) and mice (12- to 15-fold). No sex differences in AUC were noted in either species.
- Elimination half-lives for total BPS in rats administered 34–340 mg/kg were 8.54–17.2 h while those for total BPS in mice were 2.92–3.18 h. Mice eliminated total BPS ~3–6 times faster than in rat.
- Elimination half-lives for free BPS increased with increasing dose in male rat (5.77 and 11.9 h for 34 and 340 mg/kg), indicating a saturation of elimination processes. Half-lives for free BPS in mice were faster (2.86–4.21 h).
- Clearance of total BPS was faster in mice (range: 878–1940 mL/h/kg) than in rat (range: 331–398 mL/h/kg).
- Clearance of free BPS decreased with increasing dose in male rats from 12,500 to 5450 mL/h/kg at 34 and 340 mg/kg doses. In mice, clearance of free BPS was ~13,000 mL/h/kg for both the low and high doses, but increased to 21,700 mL/h/kg for the 110 mg/kg dose.
- TK parameters were similar for male and female of each species following administration of 110 mg/kg BPS.
- The calculated absolute bioavailability of free BPS in rats and mice was moderate (~9–21% for rat and 11–19% for mice), and the data show rapid absorption and metabolism of BPS with most circulating equivalents associated with conjugates.

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