

Supplemental Material

Toxicity and bioconcentration of Bisphenol A alternatives in the freshwater pulmonate snail *Planorbella pilsbryi*

Ève AM Gilroy¹, Karyn Robichaud¹, Maria Villella¹, Kara Chan¹, David WG McNabney¹, Carmen Venier², Victor Pham-Ho¹, Émilie Montreuil Strub¹, Shelby A Ravary¹, Ryan S Prosser² and Stacey A Robinson³

¹ Aquatic Contaminants Research Division, Environment and Climate Change Canada, Burlington, ON

² School of Environmental Sciences, University of Guelph, Guelph, ON

³ Ecotoxicology and Wildlife Health Division, Environment and Climate Change Canada, Ottawa, ON

Table S1 Water quality parameters (mean $\pm$ standard deviation [*n*]) collected throughout the toxicity studies with adult freshwater snails *Planorbella pilsbryi*.

Test Duration	Compound	pH	Temperature (°C)	Dissolved Oxygen (mg/L)	Conductivity (μS/cm)	Ammonia (mg/L)
96 h	BPA	7.8 $\pm$ 0.44 (19)	23.0 $\pm$ 0.3 (19)	5.5 $\pm$ 3.15 (19)	401 $\pm$ 36 (19)	1.3 $\pm$ 2.24 (18)
	BPF	7.8 $\pm$ 0.23 (20)	21.4 $\pm$ 0.2 (20)	6.8 $\pm$ 1.92 (20)	358 $\pm$ 34 (20)	0.2 $\pm$ 0.90 (20)
	BPS	7.9 $\pm$ 0.17 (20)	21.9 $\pm$ 0.5 (20)	7.6 $\pm$ 1.01 (20)	369 $\pm$ 30 (20)	0.5 $\pm$ 2.06 (19)
28 d	BPAF	7.6 $\pm$ 0.23 (25)	23.1 $\pm$ 0.15 (25)	6.6 $\pm$ 2.33 (25)	346 $\pm$ 64 (20)	0.2 $\pm$ 0.41 (25)
	BPA	7.8 $\pm$ 0.18 (72)	23.0 $\pm$ 0.43 (72)	6.8 $\pm$ 1.55 (72)	388 $\pm$ 23 (72)	0.4 $\pm$ 1.28 (72)
	BPAF	8.0 $\pm$ 0.20 (64)	22.4 $\pm$ 0.43 (64)	8.1 $\pm$ 0.72 (64)	374 $\pm$ 20.7 (64)	1.0 $\pm$ 1.80 (64)

Table S2. Ion transitions and internal standards used for each analyte for chemical analysis of aqueous samples of Bisphenols A (BPA), F (BPF), S (BPS) and AF (BPAF) completed by the National Laboratories for Environmental Testing (Burlington, ON). Note: quantitation/qualifier ion ratios were used to determine if interferences are present in the quantitation ion signal. Analyses were completed in negative electrospray mode.

Instrument 1 (2019-2021)		Waters Xevo TQ-S UHPLC-MS/MS				
Source temperature (°C)		150				
Desolvation temperature (°C)		400				
Desolvation gas flow (L/hr)		800				
Cone gas flow (L/hr)		150				
Collision gas flow (mL/min)		0.07				
Capillary voltage (kV)		3				
Target Analyte	Quantitation & Qualifier Ion Pairs (m/z)	Internal Standard	Ratio Quantitation/Qualifier peak area (± limit*)	V _f (v)	Retention Time (min)	
BPA	227.1 – 212.0	¹³ C ₁₂ BPA	2.9	18	0.85	
	227.1 – 133.0			26		
¹³ C ₁₂ BPA	239.0 – 224.0	-	Internal standard	18		
BPF	199.1 – 105.1	¹³ C ₁₂ BPF	-	24	0.78	
¹³ C ₁₂ BPF	211.0 – 99.1	-	Internal standard	22		
BPS	249.0 – 108.0	¹³ C ₁₂ BPS	3.0	28	0.70	
	219.0 – 156.0			24		
¹³ C ₁₂ BPS	261.1 – 114.0	-	Internal standard	26		
BPAF	335.1- 265.1	¹³ C ₁₂ BPAF	-	24	0.95	
¹³ C ₁₂ BPAF	347.2- 277.1	-	Internal Standard	22		
Instrument 2 (2021-present)		Sciex 6500+ QTRAP UHPLC				
Curtain gas flow (PSI)		20.0				
Ion Source temperature (°C)		450				
Ion source gas 1 (PSI)		50.0				
Ion source gas 2 (PSI)		50.0				
Ion spray voltage (V)		-4,000				
Entrance potential (V)		-10				
Collision cell exit potential		-11				
Target Analyte	Quantitation* & Qualifier Ion** Pairs (m/z)	Internal Standard	Ratio Quantitation/Qualifier peak area (± limit*)	Retention Time (min)	Collision Energy (V)	Declustering Potential (V)
BPA	227.0 – 212.0*	¹³ C ₁₂ BPA	0.22	0.92	-26	-100
	227.0 – 133.0**					
¹³ C ₁₂ BPA	239.0 – 224.0	-	Internal standard			
PBF	199.0 – 93.0*	¹³ C ₁₂ BPF	4.09	0.84	-30	-97
	199.0- 105.0**					
¹³ C ₁₂ BPF	211.0- 99.0	-	Internal standard			
BPS	249.0 – 108.0*	¹³ C ₁₂ BPS	0.23	0.77	-33	-147
	249.0- 156.0**					
¹³ C ₁₂ BPS	261.0 – 114.0	-	Internal standard			
BPAF	335.0- 265.0*	¹³ C ₁₂ BPAF	0.00	1.03	-29	-133
	335.0- 197.0**					
¹³ C ₁₂ BPAF	347.0- 277.0	-	Internal standard			

Table S3. Analytes, ions and quantification references for chemical analysis of tissue samples of Bisphenols A (BPA), B (BPB), E (BPE), F (BPF), S (BPS) and AF (BPAF), completed by SGS Axys (Sydney, BC).

Target Analyte	Quantitation* & Qualifier Ion** Pairs (m/z)	Typical Retention Time (Minutes)	Internal Standard
Analytes			
BPA	227.211 – 133.041* 227.211 – 212.051**	5.92	¹³ C ₁₂ -BPA
BPB	241.29 – 212.028* 241.29 – 226.207**	6.29	¹³ C ₁₂ -BPB
BPE	213.259 – 197.967* 213.259 – 118.971**	5.72	¹³ C ₁₂ -BPF
BPF	199.18 – 92.942* 199.18 – 104.984**	5.54	¹³ C ₁₂ -BPF
BPS	249.19 – 107.917* 249.19 – 91.963**	4.71	¹³ C ₁₂ -BPS
BPAF	335.09 – 265.09* 335.09 – 196.988**	5.95 5.89	¹³ C ₁₂ -BPAF
Surrogate Standards			
¹³ C ₁₂ -BPF	211.092	5.54	D ₆ -BPA
¹³ C ₁₂ -BPA	239.187	5.92	D ₆ -BPA
¹³ C ₁₂ -BPAF	347.258	5.95	D ₆ -BPA
¹³ C ₁₂ -BPB	253.203	6.3	D ₆ -BPA
Recovery standard			
D ₆ -Bisphenol A	233.312	5.90	External

* Quantitation ions were used for quantification.

**Qualifier ions represent confirmation product ions and may be used in instances of interference. For compounds having two MRMs, ion ratios were monitored and compared with the ion ratio generated from the opening calibration run. An interim ion ratio criterion of 50% was used for concentrations that produce S:N ratios greater than 10 in both MRMs.

Table S4. Survival and frequency of activity (mean [standard deviation]) of the freshwater snail *Planorbella pilsbryi* exposed to Bisphenol A, F, S and AF during aqueous 96-h static tests. Asterisks denote significant differences from pooled controls.

Chemical Tested	Treatment/Measured Concentration (µg/L)	Survival (%)	Activity (%)
Bisphenol A	Control	90 [11.0]	90 [3.3]
	Solvent Control	93 [10.3]	95 [5.5]
	0.072	87 [16.3]	89 [6.9]
	0.086	80 [17.9]	87 [13.5]
	0.65	87 [16.3]	91 [6.0]
	3.8	87 [24.2]	86 [8.3]
	67	83 [19.7]	89 [5.9]
	692	97 [8.2]	89 [9.4]
	2,843	30 [21.0]*	3 [8.2]*
Bisphenol F	Control	100 [0]	97 [8.2]
	Solvent Control	100 [0]	99 [2.0]
	0.012	93 [10.3]	88 [8.2]
	0.16	93 [16.3]	93 [8.2]
	0.41	83 [23.4]	81 [24.2]
	4.4	97 [8.2]	96 [8.0]
	46	100 [0]	97 [4.1]
	476	100 [0]	93 [6.9]
	1,930	97 [8.2]	70 [12.2]
	BPA (9,285)	0 [0]*	0 [0]*
Bisphenol S	Control	100 [0]	100 [0]
	Solvent Control	100 [0]	99 [1.6]
	0.12	100 [0]	100 [0]
	0.37	100 [0]	99 [1.6]
	0.88	100 [0]	99 [2.1]
	8.3	100 [0]	98 [2.2]
	89	100 [0]	99 [2.1]
	843	100 [0]	99 [1.6]
	8,590	100 [0]	97 [4.1]
	BPA (7,035)	33 [51.6]*	0 [0]*
Bisphenol AF	Control	100 [0]	100 [0]
	Solvent Control	100 [0]	100 [0]
	0.48	100 [0]	100 [0]
	0.23	100 [0]	100 [0]
	2	100 [0]	100 [0]
	5.2	100 [0]	98 [4.1]
	37	100 [0]	100 [0]
	486	100 [0]	58 [26.4]
	1,930	0 [0]*	0 [0]*
	BPA (7,190)	0 [0]*	2 [4.1]*

Table S5. Survival and frequency of activity (mean [standard deviation]) of adult freshwater snail *Planorbella pilsbryi* exposed to Bisphenol A (BPA) and Bisphenol AF (BPAF) during aqueous 28-d static renewal tests. Values followed by an asterisk denote significant difference from pooled controls. [§] denotes a significant difference from the control, but not the solvent control or pooled controls.

Chemical Tested	Treatment/Measured Concentration (µg/L)	Survival (%)	Activity (%)	Reproductive Output (egg masses/snail/week)	Length (mm)	Weight (g)	Number of juveniles	Day to first hatch (d post-exposure)
BPA	Control	90 [11.0]	95 [5.4]	4.5 [0.95]	16.3 [0.29]	1.18 [0.054]	362 [106.1]	8.0 [0.00]
	Solvent Control	93 [10.3]	93 [3.3]	4.7 [1.26]	16.3 [0.23]	1.18 [0.047]	234 [135.0]	8.2 [0.41]
	0.014	100 [0]	92 [2.9]	4.7 [0.40]	16.0 [0.30]	1.15 [0.085]	195 [48.1]	9.0 [0.63]
	0.13	100 [0]	94 [4.1]	4.1 [1.00]	15.8 [0.66]	1.11 [0.088]	107 [114.5] [§]	12.7 [5.24]
	0.45	97 [8.2]	94 [2.7]	4.8 [0.79]	16.2 [0.29]	1.17 [0.058]	241 [176.2]	11.7 [5.28]
	3.7	93 [10.3]	91 [5.9]	4.3 [0.30]	16.4 [0.55]	1.20 [0.115]	202 [166.6]	9.7 [3.2]
	42	97 [8.2]	94 [5.8]	4.4 [1.09]	16.4 [0.30]	1.20 [0.052]	78 [85.1]*	11.2 [4.26]
	479	100 [0]	92 [3.1]	4.1 [1.01]	16.1 [0.33]	1.13 [0.064]	36 [54.9]*	16.0 [5.73]*
BPAF	Control	93 [10.3]	100 [0]	8.1 [1.48]	16.5 [0.75]	0.98 [0.127]	263 [175.0]	9.2 [1.17]
	Solvent Control	97 [8.2]	100 [0]	8.0 [0.82]	16.7 [0.86]	1.01 [0.143]	189 [151.9]	7.7 [0.82]
	0.27	93 [10.3]	99 [2.3]	8.3 [0.75]	16.6 [0.88]	1.00 [0.166]	176 [181.9]	9.5 [2.59]
	0.07	97 [8.2]	100 [0]	8.2 [1.13]	16.4 [0.84]	0.98 [0.155]	163 [101.4]	8.3 [1.37]
	0.56	93 [10.3]	99 [1.0]	8.6 [1.47]	16.4 [0.91]	0.95 [0.141]	182 [147.5]	8.2 [0.98]
	5	100 [0]	100 [0]	8.0 [0.91]	16.4 [0.97]	0.98 [0.156]	152 [112.8]	9.8 [2.93]
	49	93 [10.3]	99 [1.0]	8.4 [0.71]	16.7 [0.89]	0.97 [0.160]	221 [165.8]	9.2 [2.93]
	547	97 [8.2]	100 [0]	8.0 [1.10]	16.8 [1.16]	1.05 [0.178]	98 [104.9]	9.8 [4.02]

Table S6. Toxicity of Bisphenols A, F, S and AF to *Planorbella pilsbryi* embryos in aqueous 13-d static renewal tests (embryos), expressed as ECx values for hatching. Concentrations are reported as µg/L (**measured**). All calculations were completed using the *drc* statistical package v.1.1.456 (Ritz et al., 2015) in R v .4.1.0 (R Core Team, 2021). The best-fitting among 3- or 4-parameter log-logistic and Weibull models was selected.

Compound	Effect Measure	Estimate [SE]	95% Confidence Interval
Bisphenol A	EC10	402 [76.2]	244.3-560.4
	EC25	709 [66.4]	571.4-846.8
	EC50	1,165 [121.1]	913.4-1,415.7
Bisphenol F	EC10	907 [225.9]	438.7-1375.7
	EC25	1,198 [217.9]	746.3-1650.2
	EC50	1,529 [428.9]	639.2-2418.3
Bisphenol S	EC10	4,460 [1,390.1]	1,577.0-7,342.8
	EC25	6,450 [1,193.1]	3,975.8-8,924.6
	EC50	8,910 [713.0]	7,431.1-10,388.4
Bisphenol AF	EC10	192 [12.1]	167.2-217.4
	EC25	214 [44.0]	122.5-305.2
	EC50	238 [84.3]	63.0-412.6

References

- R Core Team, 2021. R: A Language and Environment for Statistical Computing. Vienna, Austria.
<https://www.R-project.org/>
- Ritz, C., Baty, F., Streibig, J.C., Gerhard, D. 2015. Dose-Response Analysis using R. PLoS ONE
10:e0146021. <https://doi.org/10.1371/journal.pone.0146021>