

Supplementary Materials: Occurrence of Bisphenols and Benzophenone UV Filters in White-Tailed Eagles (*Haliaeetus albicilla*) from Smøla, Norway

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1. Physicochemical Properties of Studied BPs and BzPs

All the studied BPs and BzPs with their abbreviation, CAS number, molecular structure, molecular weight (g/mol), octanol-water partition coefficient ($\log K_{ow}$), solubility (25 °C; mg/L) and bioaccumulation factor are listed in **Table S1**.

2. UPLC-MS/MS Analysis

The chromatographic separation was carried out using an Acquity UPLC I-Class system (Waters, Milford, MA, USA) coupled to a triple quadrupole mass analyser (QQQ; Xevo TQ-S) with a ZSpray ESI ion source (Waters, Milford, MA, USA). The LC column used was a Kinetex C18 (50 × 2.1mm, 1.3 µm) connected to a Phenomenex C18 guard column (2.0 × 2.1mm). The column temperature was set at 30 °C. The mobile phase consisted of solvent (A) 0.1% v/v ammonium hydroxide in Milli-Q water and (B)methanol. The flowrate was 300 µLmin⁻¹ and the injection volume were 4 µL. The gradient elution initiated with 75% A, held for 10 s, decreased to 25% A within 3.4 min, then further decreased to 1% A, held for 30 s, and reverted to 75% A that was held for 20 s, for a total time run of 4 min. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Electrospray ionization was performed under negative ionization mode (ESI-). Optimal source settings were the following:source gas temperature 150 °C, capillary voltage -1500 V and nebulizer gas pressure 7.0 bar. Quantification of the target analytes was accomplished based on the internal standard method and with matrix-matched calibration standards [1], [2].

3. Method Performance

Correlation coefficients were assessed by running calibration curves prepared in methanol (fortified with target BPs and BzPs at 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 ng/mL and ISs at 20 ng/mL). The correlation coefficients in all cases were above 0.98 (**Table S3**). Precursor and product ions, retention times (RT) and relevant detection UPLC-MS/MS parameters of each target analyte and IS are presented in **Table S4**. The isomers, BPM and BPP, were quantified in samples as a single 1:1 mixture [3]. The method detection limits were ranging from 0.04 to 2.92 ng/g w.w. (**Table S3**). The precision of the UPLC-MS/MS method was evaluated in terms of repeatability expressed as relative standard deviation (RSD%) and for most target analytes was < 15% in the liver; overall ranged from 2.94 to 15.9% (**Table S3**). The instrumental repeatability was assessed by consecutive injections of standard solutions at an amount of 10 ng ($n = 5$, $k = 1$ day). The instrumental reproducibility was assessed by consecutive injections of standard solutions at 2.5 ($n = 5$) and at 20 ng ($n = 5$) in-between two days ($k = 2$) (**Table S3**). The matrix effects (%) for the target analytes are shown in **Table S3**. All target analytes demonstrated ionization suppression with the highest observed for BPAF and BPF, except for BzP-8 and BPM/BPP, which demonstrated slight signal enhancement.

Table S1. Studied BPs and BzPs with their abbreviation, CAS number, molecular structure, molecular weight (g/mol), octanol-water partition coefficient ($\log K_{ow}$), solubility (25 °C; mg/L) and bioaccumulation factor.

Target Analytes	Molecular Structure	Molecular Weight / g/mol	$\log K_{ow}$	Solubility (25 °C) / mg/L	Bioaccumulation Factor (log BAF) ^f
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Bisphenol S (BPS) 4,4'-sulfonyldiphenol CAS: 80-09-1		250.3	1.65 ^b	3518	0.548
Benzophenone-2 (BzP-2) 2,2',4,4'-tetrahydroxybenzophenone CAS: 131-55-5		246.2	3.16 ^c	398.5	0.614
4-hydroxybenzophenone (4-OH-BzP) CAS: 1137-42-4		198.2	3.07 ^d	405.8	1.619
Benzophenone-1 (BzP-1) 2,4-dihydroxybenzophenone CAS: 131-56-6		214.2	3.17 ^c	413.4	0.964
Bisphenol F (BPF) 4,4'-dihydroxydiphenylmethane CAS: 620-92-8		200.2	2.90 ^a	542.8	1.448
Benzophenone-8 (BzP-8) 2,2'-dihydroxy-4-methoxybenzophenone CAS: 131-53-3		244.2	3.93 ^c	52.73	1.673
Bisphenol AF (BPAF) 4,4'-(hexafluoroisopropylidene)diphenol CAS: 1478-61-1		336.2	5.50 ^a	4.302	2.808
Bisphenol A (BPA) 2,2-bis(4-hydroxyphenyl) propane CAS: 80-05-7		228.3	3.60 ^a	172.7	2.238
Bisphenol B (BPB) 2,2-bis(4-hydroxyphenyl) butane CAS: 77-40-7		242.3	4.20 ^a	29.23	2.231
Bisphenol M (BPM) 1,3-bis(2-(4-hydroxyphenyl)-2-propyl)benzene CAS: 13595-25-0		346.5	6.25 ^e	0.113	3.958
Bisphenol P (BPP) 4,4'-(1,4-phenylenediisopropylidene)bisphenol CAS: 2167-51-3		346.5	6.25 ^e	0.113	3.958

[a] from [4]; [b] from [5]; [c] from [6], [d] from [7]; [e] from [8]; and [f] from [9].

Table S2. List of samples with their code, year of sampling, sex and approximation of age of the individuals.

Sample Code	Year	Gender	Estimated Age
HA 07	2006	Female	Adult
HA 08	2006	Male	Adult
HA 11	2006	Female	Subadult
HA 14	2008	Male	Adult
HA 15A	2008	Male	Adult
HA 20	2008	Male	Adult
HA 21	2008	Male	Adult
HA 22A	2009	Male	Subadult
HA 25	2009	Female	Subadult
HA 26	2009	Male	Subadult
117862	2010	Male	Juvenile
HA 29	2010	Female	Adult
HA 30	2010	Male	Subadult
HA 31	2010	Female	Adult
HA 32	2010	Male	Subadult
HA 35	2010	Female	Subadult
HA 40	2011	Male	Adult
HA 41	2011	Female	Adult
HA 42	2011	Male	Adult
HA 45	2012	Male	Subadult
HA 46	2012	Male	Adult
172013	2013	Male	Nestling
HA 52	2013	Female	Subadult
23042014	2014	Female	Adult
HA 56	2014	Male	Subadult
HA 58	2014	Female	Subadult
HA 59	2014	Female	Adult
HA 60	2015	Male	Subadult
HA 61	2015	Male	Subadult
HA 62	2015	Female	Adult
HA 65	2016	Male	Subadult
HA 67	2016	Female	Adult
HA 68	2016	Female	Subadult
HA 72	2016	Female	Adult
HA 83	2017	Male	Adult
HA 81	2018	Male	Adult
HA 85	2018	Female	Adult
HA 88	2018	Female	Adult

Table S3. Bioanalytical method performance characteristics.

Target Analytes	Calibration Curves		Precision %	Repeatability %	Reproducibility		Detection Limits			Matrix Effects %
	R ²	Linearity (ng·mL ⁻¹)	10 ng (n = 3)	10 ng (n = 5)	2.5 ng (n = 5)	20 ng (n = 5)	IDL (ng/mL)	MDL (ng/g)	RSD %	
BPS	0.998	0.2–50	3.67	1.7	11	13	0.12	0.27	14	-39
BzP-2	0.9993	0.2–50	6.29	3.6	9.9	5.8	0.14	0.19	7.1	-54
4-OH-BzP	0.996	0.1–50	13.1	4.1	17	23	0.02	0.05	5.2	-20
BzP-1	0.998	0.1–50	8.04	5.6	24	19	0.01	0.04	15	-42
BPF	0.98	0.2–50	10.4	11	33	14	0.01	2.92	3.8	-1.9
BzP-8	0.9994	0.1–50	13.0	12	39	6.9	0.06	1.18	23	13
BPAF	0.9996	0.1–50	4.3	9.5	11	3.0	0.04	0.86	4.2	-0.8
BPA	0.9993	0.1–50	2.94	9.5	36	7.2	0.04	2.73	12	-20
BPB	0.9986	0.2–50	15.9	11	21	6.0	0.13	1.47	15	-75
BPM/BPP	0.9998	0.1–50	6.54	8.3	31	24	0.04	0.16	14	15

Table S4. Precursor ions and transitions of the target analytes and internal standards, their retention times, collision energies and cone voltage values for UPLC-MS/MS analysis.

Target Analytes	Retention Time (min)	Precursor Ion (m/z)	Cone Voltage (V)	Quantification Transition (m/z)	Collision Energy (eV)	Confirmation Transition (m/z)	Collision Energy (eV)	Internal Standards Used
BPS	0.38	249 [M-H] ⁻	40	249 > 108	26	249 > 156	22	BPS (¹³ C)
BzP-2	0.39	245 [M-H] ⁻	40	245 > 109	16	245 > 135	14	BPS (¹³ C)
4-OH-BzP	0.48	197 [M-H] ⁻	60	197 > 92	28	197 > 120	22	BPS (¹³ C)
BzP-1	0.48	213 [M-H] ⁻	46	213 > 135	18	213 > 91	28	BPS (¹³ C)
BPF	1.81	199 [M-H] ⁻	46	199 > 93	20	199 > 105	20	BPF (¹³ C)
BzP-8	1.91	243 [M-H] ⁻	46	243 > 123	16	243 > 93	18	BPA (¹³ C)
BPAF	2.04	355 [M-H] ⁻	40	355 > 265	24	355 > 177	42	BPAF (¹³ C)
BPA	2.36	227 [M-H] ⁻	50	227 > 212	18	227 > 133	24	BPA (¹³ C)
BPB	2.71	241 [M-H] ⁻	20	241 > 212	18	--	--	BPB (¹³ C)
BPM/BPP	3.95	345 [M-H] ⁻	14	345 > 330	26	345 > 133	46	BPA (¹³ C)
BPS (¹³ C)	0.39	261 [M-H] ⁻	50	261 > 114	28	261 > 162	20	
BPF (¹³ C)	1.81	211 [M-H] ⁻	22	211 > 99	24	211 > 111	26	
BPAF (¹³ C)	2.04	347 [M-H] ⁻	20	347 > 277	24	347 > 208	36	
BPA (¹³ C)	2.36	239 [M-H] ⁻	30	239 > 224	20	--	--	
BPB (¹³ C)	2.71	253 [M-H] ⁻	16	253 > 224	18	--	--	

-- Not considered values.

Table S5. Recoveries (%R) of the target analytes (post-extraction fortified amounts: 2.5, 10, 20 and 50 ng; n = 2 replicates for each amount).

Target Analytes	Amount (ng)	Average (n = 2)	RSD % (n = 2)
BPS	2.5	not quantifiable	-
	10	100	5.0
	20	90	8.4
	50	84	5.5
BzP-2	2.5	62	5.3
	10	64	6.6
	20	65	2.7
	50	64	0.7
4-OH-BzP	2.5	120	23
	10	123	20
	20	117	9.4
	50	125	3.8
BzP-1	2.5	89	8.2
	10	86	9.9
	20	86	11
	50	89	17
BPF	2.5	53	10
	10	56	2.1
	20	54	6.6
	50	54	12
BzP-8	2.5	69	6.1
	10	72	8.0
	20	73	6.1
	50	70	3.9
BPAF	2.5	84	14.4
	10	95	2.1
	20	94	4.6

	50	91	6.3
BPA	2.5	46	13
	10	53	4.4
	20	53	3.0
	50	52	2.9
	2.5	49	23
BPB	10	55	15
	20	56	16
	50	53	17
BPM/BPP	2.5	70	5.7
	10	75	4.3
	20	75	3.7
	50	73	4.3

Table S6. Concentrations in ng/g w.w. of the detected target compounds in the livers from the white-tailed eagles (*Haliaeetus albicilla*) (N = 38); sorted by sex and estimated age.

	BzP-2	4-OH-BzP	BzP-1	BzP-8	BPAF	BPA
Females (n = 17)						
Detection rate	1/17	4/17	5/17	1/17	15/17	4/17
Median (ng/g w.w.)*	2.17	0.31	2.74	10.55	2.34	4.52
Mean (ng/g w.w.)*	2.17	0.35	3.69	10.55	2.92	6.86
SD*	n.c.	0.19	2.15	n.c.	1.61	4.78
RSD %*	n.c.	55.4	58.2	n.c.	55.0	69.7
Males (n = 21)						
Detection rate	0/21	4/21	5/21	1/21	17/21	4/21
Median (ng/g w.w.)*	n.c.	0.65	3.04	2.08	2.56	9.07
Mean (ng/g w.w.)*	n.c.	0.90	2.79	2.08	2.88	13.93
SD*	n.c.	0.73	0.45	0.00	1.02	11.74
RSD %*	n.c.	81.5	16.3	0.00	35.5	84.3
Adults (n = 21)						
Detection rate	1/21	5/21	7/21	0/21	19/21	3/21
Median (ng/g w.w.)*	2.17	0.22	3.14	n.c.	2.56	3.74
Mean (ng/g w.w.)*	2.17	0.37	3.68	n.c.	2.66	13.63
SD*	0.00	0.28	1.76	n.c.	0.92	14.26
RSD %*	0.00	74.7	47.9	n.c.	34.6	104.6
Sub-adults (n = 15)						
Detection rate	0/15	3/15	3/15	2/15	12/15	4/15
Median (ng/g w.w.)*	n.c.	0.64	2.14	6.32	2.28	9.07
Mean (ng/g w.w.)*	n.c.	1.04	2.23	6.32	3.15	9.62
SD*	n.c.	0.74	0.17	4.24	1.73	3.68
RSD %*	n.c.	71.8	7.8	67.1	54.9	38.2
Juvenile and nestling (n = 2)						
Detection rate	0/2	0/2	0/2	0/2	1/2	1/2
Median (ng/g w.w.)*	n.c.	n.c.	n.c.	n.c.	4.54	3.76
Mean (ng/g w.w.)*	n.c.	n.c.	n.c.	n.c.	4.54	3.76
SD*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
RSD %*	n.c.	n.c.	n.c.	n.c.	n.c.	n.c.

* Values > MDLs were used for the calculation.; n.c.: not calculated.

Table S7. Concentrations in ng/g w.w. of the detected target compounds in the livers from the white-tailed eagles (*Haliaeetus albicilla*) (N = 38); sorted by sampling year.

Sample Code	Year	Gender	Estimated Age	BzP-2	4-OH-BzP	BzP-1	BzP-8	BPAF	BPA
HA 07	2006	F	Adult	2.17	0.38	-	-	1.54	-
HA 08	2006	M	Adult	-	-	-	-	2.22	-

HA 11	2006	F	Subadult	-	0.64	-	-	-	-
Detection rate				1/3	2/3	0/3	0/3	2/3	0/3
Median (ng/g w.w.)*				2.17	0.51	n.c.	n.c.	1.88	n.c.
Mean (ng/g w.w.)*				2.17	0.51	n.c.	n.c.	1.88	n.c.
SD*				n.c.	0.13	n.c.	n.c.	0.34	n.c.
RSD %*				n.c.	25.5	n.c.	n.c.	18.1	n.c.
HA 14	2008	M	Adult	-	-	2.45	-	-	-
HA 15A	2008	M	Adult	-	-	3.14	-	2.21	-
HA 20	2008	M	Adult	-	-	-	-	5.19	33.8
HA 21	2008	M	Adult	-	0.21	-	-	2.90	-
Detection rate				0/4	1/4	2/4	0/4	3/4	1/4
Median (ng/g w.w.)*				n.c.	0.21	2.80	n.c.	2.90	33.8
Mean (ng/g w.w.)*				n.c.	0.21	2.80	n.c.	3.43	33.8
SD*				n.c.	n.c.	0.35	n.c.	1.27	n.c.
RSD %*				n.c.	n.c.	12.3	n.c.	37.1	n.c.
HA 22A	2009	M	Subadult	-	-	-	-	2.75	-
HA 25	2009	F	Subadult	-	-	2.14	10.5	6.39	-
HA 26	2009	M	Subadult	-	-	-	-	4.98	7.43
Detection rate				0/3	0/3	1/3	1/3	3/3	1/3
Median (ng/g w.w.)*				n.c.	n.c.	2.14	10.55	4.98	7.43
Mean (ng/g w.w.)*				n.c.	n.c.	2.14	10.55	4.71	7.43
SD*				n.c.	n.c.	n.c.	n.c.	1.50	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	31.8	n.c.
117862	2010	M	Juvenile	-	-	-	-	4.54	-
HA 29	2010	F	Adult	-	-	-	-	2.34	3.74
HA 30	2010	M	Subadult	-	2.08	-	-	-	-
HA 31	2010	F	Adult	-	0.14	-	-	2.57	3.36
HA 32	2010	M	Subadult	-	0.39	-	-	-	-
HA 35	2010	F	Subadult	-	-	-	-	6.68	15.0
Detection rate				0/6	3/6	0/6	0/6	4/6	3/6
Median (ng/g w.w.)*				n.c.	0.39	n.c.	n.c.	3.56	3.74
Mean (ng/g w.w.)*				n.c.	0.87	n.c.	n.c.	4.03	7.38
SD*				n.c.	0.86	n.c.	n.c.	1.75	5.42
RSD %*				n.c.	99.0	n.c.	n.c.	43.4	73.4
HA 40	2011	M	Adult	-	-	3.04	-	3.11	-
HA 41	2011	F	Adult	-	0.22	7.94	-	1.08	-
HA 42	2011	M	Adult	-	-	-	-	2.48	-
Detection rate				0/3	1/3	2/3	0/3	3/3	0/3
Median (ng/g w.w.)*				n.c.	0.22	5.49	n.c.	2.48	n.c.
Mean (ng/g w.w.)*				n.c.	0.22	5.49	n.c.	2.22	n.c.
SD*				n.c.	n.c.	2.45	n.c.	0.85	n.c.
RSD %*				n.c.	n.c.	44.6	n.c.	38.2	n.c.
HA 45	2012	M	Subadult	-	-	-	2.08	1.88	-
HA 46	2012	M	Adult	-	-	-	-	3.08	-
Detection rate				0/2	0/2	0/2	1/2	2/2	0/2
Median (ng/g w.w.)*				n.c.	n.c.	n.c.	2.08	2.48	n.c.
Mean (ng/g w.w.)*				n.c.	n.c.	n.c.	2.08	2.48	n.c.
SD*				n.c.	n.c.	n.c.	n.c.	0.60	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	24.1	n.c.

172013	2013	M	Nestling	-	-	-	-	-	3.76
HA 52	2013	F	Subadult	-	-	-	-	2.23	-
Detection rate				0/2	0/2	0/2	0/2	1/2	1/2
Median				n.c.	n.c.	n.c.	n.c.	2.23	3.76
(ng/g w.w.)*									
Mean				n.c.	n.c.	n.c.	n.c.	2.23	3.76
(ng/g w.w.)*									
SD*				n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
23042014	2014	F	Adult	-	-	-	-	1.12	-
HA 56	2014	M	Subadult	-	-	-	-	2.15	10.7
HA 58	2014	F	Subadult	-	-	-	-	1.79	-
HA 59	2014	F	Adult	-	-	3.18	-	3.38	-
Detection rate				0/4	0/4	1/4	0/4	4/4	0.25
Median				n.c.	n.c.	3.18	n.c.	1.97	10.7
(ng/g w.w.)*									
Mean				n.c.	n.c.	3.18	n.c.	2.11	10.7
(ng/g w.w.)*									
SD*				n.c.	n.c.	n.c.	n.c.	0.82	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	38.9	n.c.
HA 60	2015	M	Subadult	-	-	-	-	1.70	-
HA 61	2015	M	Subadult	-	-	-	-	1.98	-
HA 62	2015	F	Adult	-	-	2.74	-	3.12	-
Detection rate				0/3	0/3	1/3	0/3	3/3	0/3
Median				n.c.	n.c.	2.74	n.c.	1.98	n.c.
(ng/g w.w.)*									
Mean				n.c.	n.c.	2.74	n.c.	2.27	n.c.
(ng/g w.w.)*									
SD*				n.c.	n.c.	n.c.	n.c.	0.61	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	27.1	n.c.
HA 65	2016	M	Subadult	-	-	2.07	-	2.99	-
HA 67	2016	F	Adult	-	-	-	-	3.73	-
HA 68	2016	F	Subadult	-	-	2.47	-	2.33	5.30
HA 72	2016	F	Adult	-	-	-	-	3.28	-
Detection rate				0/4	0/4	2/4	0/4	4/4	1/4
Median				n.c.	n.c.	2.27	n.c.	3.14	5.30
(ng/g w.w.)*									
Mean				n.c.	n.c.	2.27	n.c.	3.08	5.30
(ng/g w.w.)*									
SD*				n.c.	n.c.	0.20	n.c.	0.51	n.c.
RSD %*				n.c.	n.c.	8.81	n.c.	16.4	n.c.
HA 83	2017	M	Adult	-	-	-	-	2.56	-
Detection rate				0/1	0/1	0/1	0/1	1/1	0/1
Median				n.c.	n.c.	n.c.	n.c.	2.56	n.c.
(ng/g w.w.)*									
Mean				n.c.	n.c.	n.c.	n.c.	2.56	n.c.
(ng/g w.w.)*									
SD*				n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	n.c.	n.c.
HA 81	2018	M	Adult	-	0.90	3.25	-	2.32	-
HA 85	2018	F	Adult	-	-	-	-	-	-
HA 88	2018	F	Adult	-	-	-	-	2.25	-
Detection rate				0/3	1/3	1/3	0/3	2/3	0/3
Median				n.c.	0.90	3.25	n.c.	2.29	n.c.
(ng/g w.w.)*									
Mean				n.c.	0.90	3.25	n.c.	2.29	n.c.
(ng/g w.w.)*									
SD*				n.c.	n.c.	n.c.	n.c.	0.03	n.c.
RSD %*				n.c.	n.c.	n.c.	n.c.	1.53	n.c.

*Values < MDLs.; *Values > MDLs were used for the calculation.; n.c.: not calculated.

References

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