Factors influencing the bioaccumulation of persistent organic pollutants in food webs of the Scheldt estuary

Evy Van Ael, Adrian Covaci, Krishna Das, Gilles Lepoint, Ronny Blust and Lieven Bervoets

Summary

POP analyses

The individual PCB standards were obtained from Dr. Ehrenstorfer Laboratories (Augsburg, Germany), standards of OCPs were obtained from Accustandard (New Haven, CT, USA) and PBDE standard mixtures were obtained from Wellington Laboratories (Guelph, Ontario, Canada). CB 143, BDE 77 and ε-HCH were used for the quantification of the analytes. Hexane, acetone, dichloromethane, *iso*-octane (all pesticide grade) and concentrated sulfuric acid (analytical grade) were obtained from Merck (Darmstadt, Germany). Silica gel 60 (63-230 mesh) and anhydrous Na_2SO_4 (Merck) were heated at 150 °C for 24 h. All solvents used for the analysis (acetone, dichloromethane, *iso*-octane, *n*-hexane, methanol) were of SupraSolv grade (Merck, Darmstadt, Germany). Sodium sulphate, concentrated sulfuric acid (analytical grade) and silica gel (0.063-0.200 mm), all from Merck, were pre-washed with *n*-hexane and heated overnight at 150 °C before use. Extraction thimbles (25 x 100 mm, Whatman®, England) were pre-extracted for 1 h with hexane/acetone (3/1; *v/v*) and dried at 100 °C for 12 h. Empty polypropylene columns for clean-up (25 mL) were purchased from Alltech (Lokeren, Belgium).

From the biota samples, an adequate amount (between 1 and 5 g) was ground with anhydrous Na_2SO_4, spiked with internal standards (CB 143, BDE 77, and ε-HCH) and extracted for 2 h by hot Soxhlet with 80 ml hexane/acetone (3/1; v/v). After lipid determination on an aliquot of the extract, the rest of the extract was cleaned-up on 8 g acid silica and analytes were eluted with 20 ml hexane and 15 ml dichloromethane. The eluate was concentrated and reconstituted in 100 µl *iso*-octane.

For sediment samples, the same procedure was repeated, but 5 g of activated copper powder was mixed with the sample (3 g) and an extra internal standard (^{13}C-BDE 209) was added before Soxhlet extraction. During the clean-up step, copper powder (2 g) was also added on top of the acid silica. The rest of the clean-up procedure was similar to the biota protocol, except for lipid determination.

PBDEs, chlordanes and HCHs were measured with an Agilent 6890-5973 gas chromatograph coupled with a mass spectrometer system (GC-MS). The GC was equipped with a 30 m x 0.25 mm x 0.25 µm DB-5ms capillary column (J&W Scientific, Folsom, CA, USA) and the MS was operated in electron capture negative ionisation (ECNI) mode. Methane was used as reagent gas and the ion source, quadrupole and interface temperatures were set at 170, 150 and 300 °C, respectively. The MS was used in the selected ion-monitoring (SIM) mode with ions *m/z* = 79 and 81 (for PBDEs and MeO-PBDEs acquired

during the whole run and with 2 most intense characteristic ions for each pesticide. Dwell times were set at 30 ms. One µl of the cleaned extract was injected in solvent vent mode (injector temperature: 90 °C, held for 0.05 min, then with 700 °C/min to 300 °C and kept for 25 min; vent flow was set at 75 ml/min and the purge vent opened at 1.5 min). Helium was used as carrier gas at constant flow (1.0 ml/min). The temperature of the DB-5ms column was kept at 90 °C for 1.50 min, then increased to 300 °C at a rate of 10 °C/min, kept for 20 min.

For the measurements of PCBs, DDXs, and HCB, we have used an Agilent 6890 GC – 5973 MS system operated in electron impact ionisation (EI) mode equipped with a 25 m x 0.22 mm x 0.25 µm HT-8 capillary column (SGE, Zulte, Belgium). The ion source, quadrupole and interface temperatures were set at 230, 150 and 300 °C, respectively. One µl of the cleaned extract was injected in cold pulsed splitless mode (injector temperature 90 °C (0.03 min) rising to 300 °C with 700 °C/min), pressure pulse 25 psi and pulse time 1.50 min. The splitless time was 1.50 min. Helium was used as the carrier gas at constant flow (1.0 ml/min). The temperature of the HT-8 column was kept at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min (kept for 2.0 min), further increased to 280 °C at a rate of 5 °C/min and finally raised to 300 °C at a rate of 40 °C/min, and kept for 20 min. The MS was used in the selected ion-monitoring (SIM) mode with 2 ions monitored for each PCB homologue group or individual OCP. Dwell times were set to 30 ms.

Quality assurance/quality control

Retention times, ion chromatograms and relative abundance of the monitored ions were used as identification criteria. A deviation of ion abundance ratios within 15% of the mean values for calibration standards was considered acceptable. Quantification was based on five-point calibration curves in which either the sum of *m/z* = 79 and 81 for PBDEs or the most intense ion from the molecular cluster for PCBs were monitored.

The peaks were positively identified as target compounds if: (1) the retention time matched that of the standard compound within ± 0.1 min and (2) the signal-to-noise ratio (S/N) was higher than 3:1. Procedural blanks were analyzed simultaneously with every batch of seven samples to check for interferences or contamination from solvent and glassware. Procedural blanks were consistent (RSD < 30%) and therefore the mean value was calculated for each compounds and subtracted from the values in the samples. After blank subtraction,

the *limit of quantification (LOQ)* was calculated as three times the standard deviation of the mean of the blank measurements. For analytes that were not detected in procedural blanks,

LOQs were calculated for a ratio S/N equal to 10. LOQs ranged between 1 and 10 ng/g lipid weight.

Mean ± SD recoveries of the internal standards PCB 143 and BDE 77 were 86 ± 6% and 93 ± 10%, respectively. The analytical procedures were validated through the analysis of certified materials: SRM 1945 - PCBs, PBDEs and OCPs in whale blubber (NIST, National Institute of Standards and Technology) and CRM 536 – PCBs in freshwater sediment (BCR, Community Bureau of Reference of the European Commission). Measured values were deviating less than 20 % from the certified values.

The QC scheme is also assessed through regular participation to the Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues organized by the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA). Results for individual OCPs, PCB and PBDE congeners had a variation coefficient less than 15 % from the target values or z scores were always $-2 < z < 2$ (Kucklick et al., 2005, 2007, 2009).

Samples with concentrations below LOQ were calculated as f*LOQ with "f" being the fraction of samples above LOQ (or the detection frequency) as first described by James et al. (2002). This approach is a more realistic method of replacing non-detects with a certain value than the current procedures of lower bound (replacement with zero), medium bound (replacement with ½*LOQ) and upper bound (replacement with LOQ). All results were expressed as ng/g wet weight (ww).

References

James RA, Hertz-Picciotto I, Willman E, Keller JA, Charles MJ, 2002. Determinants of serum polychlorinated biphenyls and organochlorine pesticides measured in women from the Child Health and Development Study cohort, 1963-1967. Environ Health Perspect, 110, 617-624.

Kucklick JR, Pugh RS, Becker PR, Schantz MM, Wise SA, Rowles TK, 2005. Description and results of the 2003 NIST/NOAA interlaboratory comparison exercise program for organic contaminants in marine mammal tissues. NISTIR 7269, 157 pag.

Kucklick JR, Pugh RS, Becker PR, Schantz MM, Wise SA, Rowles TK, 2007. Description and results of the 2005 NIST/NOAA interlaboratory comparison exercise program for organic contaminants in marine mammal tissues. NISTIR 7410, 132 pag.

Kucklick JR, Pugh RS, Becker PR, Schantz MM, Wise SA, Rowles TK, 2009. Description and Results of the 2007 NIST/NOAA Interlaboratory Comparison Exercise Program for Organic Contaminants in Marine Mammal Tissues. National Institute of Standards and Technology, NISTIR 7642, 145 pag.

Table SI-1. Mean lipid content (%), weight (g) and total length (cm) of the collected species for POP analysis, together with median concentrations (ng/g ww, filamentous green algae and sediment in dw), separated per location (Terneuzen, Bath, Antwerpen**)**. N.d.= not detected

Sample		N (T/B/A)	Lipid (%)			Weight (g)*			Total length (cm)**			∑PCBs			∑PBDEs			∑DDT			∑HCH			∑chlordanes		
			T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A
Sediment		2/2/2		-								2.29	4.28	37.6	11.0	56.8	349	0.15	0.21	2.53	0.24	0.21	0.72	n.d.	n.d.	n.d.
Filamentous algae		1/1/1		-								32.7	2.50	32.7	1.52	0.41	0.96	2.83	0.30	2.51	1.64	0.83	1.05	0.15	0.07	0.13
Molluscs																										
Mytilus edulis	Blue mussel	4/0/0	2.55	-	-							94.9	-	-	0.59	-	-	3.75	-	-	0.22	-	-	0.23	-	-
Macoma balthica	Baltic tellin	0/2/0	-	2.80	-							-	249	-	-	1.71	-	-	11.2	-	-	0.38	-	-	0.87	-
Littorina littorea	Common periwinkle	4/0/0	0.85	-	-							22.8	-	-	0.01	-	-	0.70	-	-	0.16	-	-	n.d.	-	-
Polychaeta																										
Nereis diversicolor	Ragworm	0/2/3	-	1.15	1.54							-	55.1	110	-	0.48	1.20	-	2.93	6.08	-	0.44	0.40	-	0.22	0.62
Arenicola marina	Lugworm	3/0/0	1.00	-	-							22.4	-	-	0.24	-	-	0.85	-	-	0.10	-	-	n.d.	-	-
Crustacea																										
Chaetogammarus marinus		1/1/0	1.47	1.10	-							33.5	33.7	-	0.39	0.13	-	0.39	0.98	-	3.28	0.75	-	n.d.	n.d.	-
Crangon crangon	Brown shrimp	2/4/0	0.77	0.94	-							43.2	25.0	-	0.01	0.01	-	0.48	0.62	-	0.03	0.03	-	n.d.	n.d.	-
Carcinus maenas	Shore crab	2/11/0	0.68	0.64	-	41.1	38.5	-	5.40	5.40	-	39.0	88.8	-	0.15	0.26	-	0.73	1.82	-	0.25	0.06	-	n.d.	n.d.	-
Eriocheir sinensis	Chinese mitten crab	0/3/4	-	0.61	0.75	-	67.0	29.8	-	5.30	4.10	-	128	62.3	-	2.91	0.99	-	6.54	1.98	-	0.20	0.06	-	2.17	1.09
Fish																										
Platichthys flesus	European flounder	5/5/6	0.74	1.22	0.62	57.9	90.5	167	16.5	24.2	20	39.1	124.2	99.0	0.29	1.05	0.74	1.34	4.77	2.73	0.05	0.05	0.05	0.08	0.18	0.08

Sample		N (T/B/A)	Lipid (%)			Weight (g)*			Total length (cm)**			∑PCBs			∑PBDEs			∑DDT			∑HCH			∑chlordanes		
			T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A	T	B	A
Solea solea	Common sole	1/4/6	0.59	0.70	0.59	19.0	169	19.4	13.2	23.4	13.5	37.4	44.2	47.7	0.13	0.24	0.18	0.88	1.60	1.25	0.05	0.05	0.05	n.d.	n.d.	n.d.
Osmerus eperlanus	Smelt	2/7/0	1.45	1.21	-	50.4	36.2	-	19.3	17.3	-	139	76.1	-	0.98	0.67	-	6.63	3.05	-	0.05	0.05	-	0.24	0.08	-
Sprattus sprattus	European sprat	0/1/0	-	0.96	-	-	4.93	-	-	10.6	-	-	210	-	-	1.00	-	-	8.47	-	-	0.17	-	-	n.d.	-
Sander lucioperca	Pike-perch	0/2/3	-	0.49	0.72	-	49.8	55.3	-	17.6	16.7	-	116.8	152.7	-	0.80	1.77	-	3.96	5.20	-	0.11	0.15	-	0.08	0.16
Trisopterus luscus	Pouting	3/4/0	0.51	0.68	-	7.17	11.3	-	8.50	9.90	-	24.5	26.6	-	0.13	0.14	-	0.84	0.86	-	0.05	0.05	-	0.08	0.08	-
Myoxocephalus scorpius	Shorthorn sculpin	2/6/0	0.72	0.90	-	61.6	74.9	-	16.5	16.5	-	31.2	28.7	-	0.19	0.22	-	1.16	0.88	-	0.05	0.05	-	n.d.	n.d.	-
Anguilla anguilla	European eel	0/0/6	-	-	17.7	-	-	273	-	-	52.2	-	-	1290	-	-	8.76	-	-	49.3	-	-	2.24	-	-	5.67

*some crabs were missing legs, so crab weight may not be accurate

**for crabs, total lenght was the length of the carapax

Table SI-2. Statistics for all significant correlations (Pearson's Product Moment) between POP concentrations (ww) and lipid content in biota.

Terneuzen (N=30)			Bath (N=52)			Antwerp (N=26)			Antwerp (N=26)		
Compound	p-value	r^2	Compound	p-value	r^2	Compound	p-value	r^2	Compound	p-value	r^2
PCB 28	0.0014	0.311	PCB 18	< 0.0001	0.581	PCB 28	< 0.0001	0.958	pp-DDE	< 0.0001	0.934
PCB 52	0.0015	0.306	PCB 52	< 0.0001	0.366	PCB 52	< 0.0001	0.973	op-DDD	< 0.0001	0.590
PCB 49	0.0003	0.383	PCB 44	< 0.0001	0.652	PCB 49	< 0.0001	0.823	pp-DDD	< 0.0001	0.964
PCB 44	0.0004	0.364	PCB 95	< 0.0001	0.563	PCB 44	< 0.0001	0.933	pp-DDT	< 0.0001	0.925
PCB 95	< 0.0001	0.537	PCB 101	0.0004	0.225	PCB 95	< 0.0001	0.872	OxC	< 0.0001	0.790
PCB 101	0.0006	0.348	PCB 87	< 0.0001	0.371	PCB 101	< 0.0001	0.875	TN	< 0.0001	0.927
PCB 99	0.0045	0.254	PCB 110	< 0.0001	0.544	PCB 99	< 0.0001	0.926	CN	< 0.0001	0.921
PCB 87	0.0003	0.381	PCB 151	< 0.0001	0.474	PCB 87	< 0.0001	0.855	TC	< 0.0001	0.836
PCB 110	0.0001	0.422	PCB 149	< 0.0001	0.390	PCB 110	< 0.0001	0.917	CC	< 0.0001	0.888
PCB 118	0.0031	0.272	PCB 174	< 0.0001	0.504	PCB 118	< 0.0001	0.950	b-HCH	< 0.0001	0.990
PCB 105	0.0034	0.268	PCB 171	< 0.0001	0.330	PCB 105	< 0.0001	0.952	g-HCH	< 0.0001	0.986
PCB 151	0.0009	0.332	PCB 195	< 0.0001	0.374	PCB 151	< 0.0001	0.771	BDE 28	< 0.0001	0.827
PCB 149	< 0.0001	0.538	PCB 194	0.0004	0.223	PCB 149	< 0.0001	0.867	BDE 47	< 0.0001	0.867
PCB 146	0.0007	0.344	PCB 206	< 0.0001	0.557	PCB 146	< 0.0001	0.941	BDE 100	< 0.0001	0.911
PCB 153	0.0018	0.298	op-DDE	< 0.0001	0.699	PCB 153	< 0.0001	0.952	BDE 99	0.0008	0.381
PCB 138	0.0009	0.328	pp-DDE	< 0.0001	0.293	PCB 138	< 0.0001	0.950	BDE 154	< 0.0001	0.475
PCB 128	0.0004	0.361	op-DDD	< 0.0001	0.786	PCB 128	< 0.0001	0.921	BDE 153	< 0.0001	0.862
PCB 187	< 0.001	0.423	pp-DDD	< 0.0001	0.725	PCB 156	< 0.0001	0.895	Antwerp (eel excluded, N=20)		
PCB 183	0.0038	0.262	TC	< 0.0001	0.739	PCB 187	< 0.0001	0.923	Compound	p-value	r^2
PCB 177	< 0.0001	0.518	BDE 28	0.0006	0.212	PCB 183	< 0.0001	0.936	PCB 18	0.0009	0.469
PCB 171	0.002	0.292	BDE 100	< 0.0001	0.363	PCB 174	< 0.0001	0.835	PCB 28	0.0011	0.455
pp-DDE	0.0027	0.279	BDE 154	< 0.0001	0.438	PCB 177	< 0.0001	0.951	PCB 44	0.0391	0.216
op-DDD	< 0.0001	0.747				PCB 171	< 0.0001	0.939	HCB	< 0.0001	0.669
TN	0.0003	0.377				PCB 172	< 0.0001	0.941	op-DDD	< 0.0001	0.700
CN	< 0.0001	0.431				PCB 180	< 0.0001	0.872	pp-DDD	0.0013	0.444
TC	< 0.0001	0.693				PCB 170	< 0.0001	0.885	TN	0.0016	0.435
BDE 28	< 0.0001	0.451				PCB 199	< 0.0001	0.951	TC	< 0.0001	0.766
BDE 100	0.0026	0.281				PCB 195	< 0.0001	0.949	CC	< 0.0001	0.643
BDE 99	< 0.0001	0.568				PCB 194	< 0.0001	0.946	a-HCH	0.0022	0.414
						PCB 205	< 0.0001	0.585	b-HCH	0.0095	0.319
						PCB 206	< 0.0001	0.926	g-HCH	0.0004	0.516
						PCB 209	< 0.0001	0.866	BDE 28	0.0009	0.464
						HCB	< 0.0001	0.948	BDE 99	0.0243	0.252

Only correlations with $r^2 > 0.2$ are shown.

Table SI-3. Mean (±SD) of δ13C and δ15N values (‰) of all samples and number of measurements (N) per location.

Sample		N (T/B/A)	$\delta^{13}C$ Terneuzen	$\delta^{13}C$ Bath	$\delta^{13}C$ Antwerpen	$\delta^{15}N$ Terneuzen	$\delta^{15}N$ Bath	$\delta^{15}N$ Antwerpen
SPM		1/1/1	-25,3	-26,3	-27,7	2,79	4,59	2,50
Filamentous algae		2/2/2	-16,5 ± 0,08	-19,0 ± 0,00	-14,0 ± 0,51	22,3 ± 0,39	15,3 ± 0,43	15,0 ± 1,07
Oligochaeta		1/2/2	-19,9	-22,9 ± 0,21	-26,5 ± 0,03	15,9	17,5 ± 0,31	17,1 ± 0,09
Molluscs								
Mytilus edulis	Blue mussel	10/0/0	-24,4 ± 0,78	/	/	14,1 ± 0,49	/	/
Macoma balthica	Baltic tellin	2/10/0	-20,9 ± 0,04	-23,8 ± 0,71	/	14,2 ± 0,20	17,7 ± 0,88	/
Littorina littorea	Common periwinkle	10/0/0	-17,3 ± 0,23	/	/	16,6 ± 0,53	/	/
Polychaeta								
Nereis diversicolor	Ragworm	0/11/9	/	-18,2 ± 0,6	-19,0 ± 1,36	/	17,5 ± 0,41	21,6 ± 0,74
Arenicola marina	Lugworm	9/0/0	-18,1 ± 1,01	/	/	17,5 ± 0,78	/	/
Crustacea								
Chaetogammarus marinus		2/5/0	-19,2 ± 1,09	-19,4 ± 0,15	/	16,2 ± 0,49	18,8 ± 0,68	/
Corophium volutator		1/0/1	-17,3	/	-26,3	13,7	/	17,6
Crangon crangon	Brown shrimp	8/13/0	-19,1 ± 1,09	-22,3 ± 1,36	/	18,2 ± 1,20	19,8 ± 0,61	/
Carcinus maenas	Shore crab	3/12/0	-20,6 ± 0,92	-21,8 ± 1,09	/	18,0 ± 0,63	19,0 ± 0,91	/
Eriocheir sinensis	Chinese mitten crab	0/3/4	/	-27,0 ± 1,05	-26,7 ± 18,0	/	17,7 ± 3,62	21,0 ± 0,71
Fish								
Platichthys flesus	European flounder	5/8/8	-20,7 ± 3,30	-22,6 ± 1,71	-25,4 ± 0,82	18,2 ± 0,57	21,0 ± 1,04	21,5 ± 0,48
Solea solea	Common sole	1/4/6	-22,0	-20,3 ± 1,25	-22,7 ± 1,10	17,7	19,2 ± 1,22	20,0 ± 0,69
Osmerus eperlanus	Smelt	2/7/0	-20 ± 0,2	-22,9 ± 2,10	/	20,6 ± 0,57	21,5 ± 0,91	/
Sprattus sprattus	European sprat	0/2/0	/	-23,3 ± 2,25	/	/	20,9 ± 1,56	/
Sander lucioperca	Pike-perch	0/3/3	/	-26,0 ± 2,3	-27,6 ± 1,51	/	21,6 ± 1,08	20,6 ± 0,59
Trisopterus luscus	Pouting	3/4/0	-18,4 ± 0,4	-21,8 ± 1,84	/	17,2 ± 0,40	18,8 ± 0,88	/
Myoxocephalus scorpius	Shorthorn sculpin	2/6/0	-19,3 ± 1,1	-18,9 ± 1,68	/	20,3 ± 0,21	19,5 ± 1,04	/
Anguilla anguilla	European eel	0/0/6	/	/	-27,4 ± 1,09	/	/	21,1 ± 2,05