PCBs and OCPs in marine species from the Belgian North Sea and the Western Scheldt Estuary

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Introduction

The use and/or production of polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), such as 2,2-bis-(4-chlorophenyl)-1,1,1-trichloroethane (DDT), hexachlorobenzene (HCB) and lindane (γ-HCH) have been banned in most developed countries since the 1970's. Despite this measure, these compounds are among the most prevalent environmental pollutants and they can be found in various environmental compartments, both biotic[1] and abiotic[2]. Their widespread presence is due to their extremely persistant and lipophilic nature, resulting in enrichment throughout the food chain.[1]

Prolonged exposure to these pollutants can interfer with normal physiology and biochemistry[3], resulting in adverse effects in various organisms, including starfish, shrimp, crabs, and fish[4].

Because humans readily consume seafood, such as shrimp, crab and various fish species, these organisms are of great scientific value to estimate the possible exposure to PCBs and OCPs through marine food sources. The area studied in this investigation covered both commercial fishing grounds (Belgian North Sea – BNS) and a recreational fishing area (Western Scheldt Estuary – SE). The drainage basin of the SE covers a very densely populated and highly industrialised region, causing a high level of pollution in the SE[5,6].

In this work, PCBs and OCPs were determined in benthic invertebrates and different fish species from both BNS and SE in order to evaluate trends in levels, congener distribution, and geographical variation.

Materials and Methods

Sampling. Seven locations were selected in the BNS and 9 locations in the SE (Fig. 1). Samples were collected by the research vessel *Zeeleeuw*, provided by the Flemish Marine Institute (VLIZ) during October and November 2001. Three species of benthic invertebrate organisms were sampled: flying crab (*Lyocarcinus holsatus*), red starfish (*Asterias rubens*), and common shrimp (*Crangon crangon*). Three benthic flatfish species, namely common sole (*Solea solea*), dab (*Limanda limanda*), and plaice (*Pleuronectus platessa*), 2 gadoid fish species, namely whiting (*Merlangius merlangus*) and bib (*Trisopterus luscus*), and sand goby (*Pomatoschistus minutus*) were also sampled at the same locations. Except for starfish and goby, all organisms in this study are suited for human consumption.

Targeted compounds. Based on their abundance in the samples, the following PCB-congeners (IUPAC numbering), were targeted for analysis: 28, 44, 52, 74, 95, 99, 101, 105, 110, 118, 128/174, 138, 149, 153, 156, 163, 167, 170, 177, 180, 183, 187, 194, 196, and 199. The following OCPs were also determined: α -, β -, γ -, and δ -hexachlorocyclohexane (hereafter refered to as "HCHs"), hexachlorobenzene (HCB), 2,2-bis(4-chlorophenyl)-1,1,1-trichloroetane (*p,p'*-DDT), 2,2-bis(4-chlorophenyl)-1,1-dichloroetylene (*p,p'*-DDE), and 2,2-bis(4-chlorophenyl)-1,1-dichloroetane (*p,p'*-DDD) (hereafter referred to as "DDTs").
ε-HCH was used as internal standard (IS) for HCB and HCHs whereas CBs 46 and 143 were used as IS for the PCBs and DDTs.

Sample preparation and clean up. Prior to analysis, the samples were thawed and homogenised. For shrimp and crab only the soft parts were collected and for goby and starfish the whole body was used. Only liver was analysed for flatfish and gadoids. The samples were pooled based on species and location. The method used for the preparation and clean up of the samples has previously been described[7] and is briefly presented below. Between 1 and 10g of sample was spiked with internal standards and extracted for 2.5 h by hot Soxhlet with hexane/acetone. After lipid determination, the extract was cleaned-up on acid silica and PCBs and OCPs were eluted with hexane and dichloromethane.

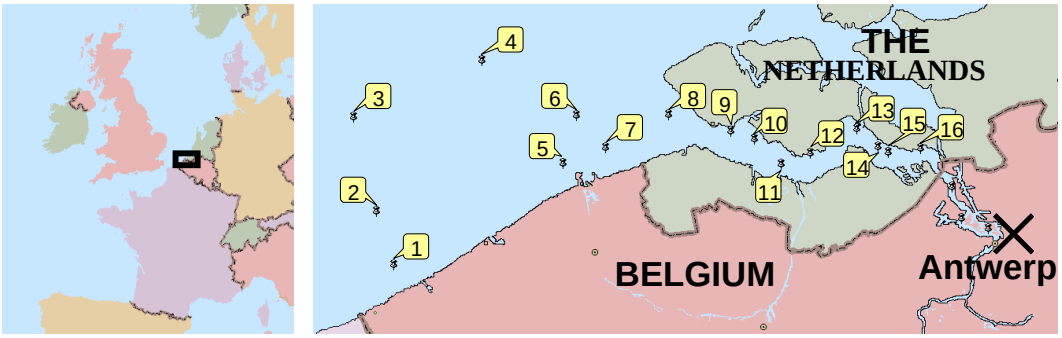


Fig. 1. Sampling locations

Chemical analysis. PCB quantification was performed using a HP 6890 GC (Palo Alto, CA, USA) coupled with a μ-ECD detector and equipped with a 50m × 0.22mm × 0.25μm HT-8 (SGE) capillary column. OCP measurements were performed using a HP 6890 GC equipped with a 25m x 0.22mm x 0.25μm HT-8 capillary column connected with a HP 5973 mass spectrometer that was operated in Electron Capture Negative Ionisation (ECNI) mode. A more detailed description of the instrumental set-up can be found elsewhere[8].

Quality Assurance. The quality control was performed by regular analyses of procedural blanks, blind duplicate samples, certified reference material CRM 349 (PCBs in cod liver oil), and by random injection of standards and solvent blanks.
The sample intake was adapted to the expected pollution load of the sample. The limit of quantification (LOQ) for PCBs and OCPs was established 0.01 ng/g wet weight (ww). Procedural blank values were consistent (RSD < 30%) and were therefore subtracted from the results.

Statistical analysis. For samples with concentrations below LOQ, zero was used in the calculations. Simple linear regression was used also to test the correlation between PCBs and OCPs. The Mann Whitney U-test was used to compare the mean concentrations in BNS and SE and to test the profile differences. Tests were considered significant if p was lower than 0.05.

Results and Discussion

The lipid content of fish tissue is influenced by several factors, such as sex, age, species, nourishment and spawning status[9,10]. In the present study, all samples were taken prior to spawning, resulting in maximum seasonal lipid levels. Nevertheless, wet weight based results are preferred and therefore, lipid based results are given only for comparison with other studies.

PCB and OCP levels. PCB and OCP data are summarised in Table 1. The PCB congeners that could be detected and their frequency of detection were species- and location-dependent. Shrimp showed very low concentrations for all congeners analysed. The low extractable lipids of shrimp (Table 1) were probably related to this observation[11]. In the other benthic invertebrate samples and goby most congeners could be measured.

In liver samples of gadoid fish (whiting and bib) all congeners could be measured. These samples also showed the highest lipid content (Table 1). Although total PCB levels in dab (flatfish) were the lowest among all fish livers analysed, most congeners could be determined. Sole liver displayed the highest number of not detects. The lipid content in sole liver was lower than in the other samples (Table 1), which can explain the high frequency of congeners below LOQ.

Similar to PCBs, the lowest OCP concentrations were found in shrimp. Only HCB, *p,p'*-DDE, and γ-HCH could be detected in shrimp samples from both BNS and SE. All crab and most goby samples contained measurable concentrations of all OCPs. All OCPs were consistently detected in liver samples of dab, plaice, bib, and whiting and most compounds were found in sole samples.

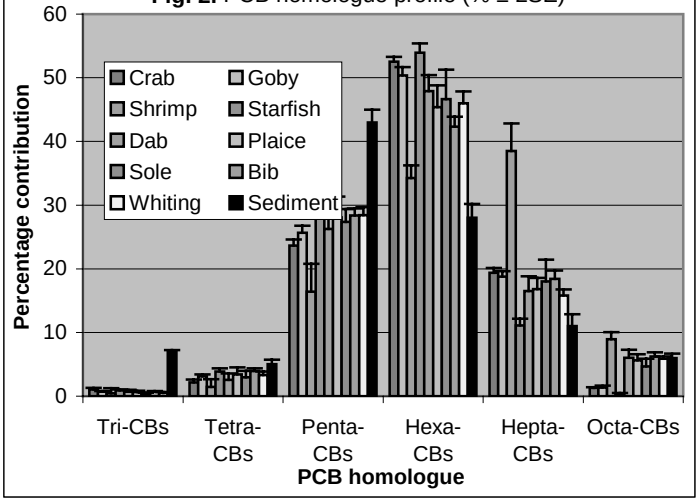
Interspecies variation of PCB and OCP levels was rather limited. However, shrimp showed significantly lower levels at the BNS locations. These lower PCB levels in shrimp can be partially explained by their low trophic level.[12] Crab and starfish contained relatively high pollutant levels, that can also be explained by their feeding habit. Crabs and starfish, are scavengers that feed partially on decaying organic material, which can bear relatively high pollutant loads.[13]

PCB and OCP levels in other studies. Based on data provided by other studies, we can conclude that biota from the SE are rather highly contaminated. Levels of PCBs and OCPs found in dab, plaice, and sole liver from near the Norwegian coast[14] were comparable with levels of the BNS livers from the present study. Similar CB 153 levels in fish liver as in the samples from the SE in the present study were reported from a relatively highly polluted area with high degree of industrialisation and harbour activities (Rotterdam habour, The Netherlands)[2].

In general, total POP levels of the BNS samples were almost one order of magnitude higher than those of Greenland[15], while fish livers from the SE surpassed the Greenland values by more than two orders of magnitude.

PCB profiles. Contribution of the lower chlorinated congeners (tri- and tetra-CBs) to the sum of PCBs was very low (Fig. 2). Although the lower chlorinated biphenyls have an increased mobility from the substrate to water and are therefore more available to aquatic organisms[2], they are very susceptible to metabolism and are eliminated rapidly in the marine environment. The most obvious statistically significant deviating pattern was found in shrimp, where levels of tetra-, penta-, and hexa-CB congeners were relatively lower, while the concentrations for hepta- and octa-CB congeners were relatively higher (Fig. 2). The different levels of nearly all PCB homologue groups found in shrimp compared to the other benthic invertebrates are not likely to be solely dependent on the bioavailability, but probably also on metabolism and elimination. Nevertheless, deducting metabolic capacities from tissue profile should be done with great caution.

Fig. 2. PCB homologue profile (% ± 2SE)



Because contaminants in the sediments are bioavailable to sediment dwelling organisms[16], 4 sediment samples from the SE have also been analysed to be able to compare the PCB profile in the sediment and in biota (Fig. 2).

OCP profiles. OCPs were divided into 3 groups, namely HCHs, HCB, and DDTs. No location dependent profile differences were seen in neither species. Between the small benthic organisms and the larger fishes and among the small benthic organisms themselves, statistically significant profile differences of HCH-isomers and DDT-metabolites were observed. The relative contribution of *p,p'*-DDD to the total OCP load was virtually equal in most species of this study. This metabolite is mainly formed in the environment by anaerobic degradation of *p,p'*-DDT.[17] The *p,p'*-DDD concentrations found in these samples are due to uptake from the environment (water, sediment, etc.) or by ingestion with food. Compared to flatfish and gadoids, the contribution of *p,p'*-DDT to the total sum of DDTs in the small benthic organisms was higher, while the contribution of *p,p'*-DDE was lower. Benthic invertebrates have a lower metabolic rate[18], which can explain this observation.

Crab showed significantly higher contribution of *p,p'*-DDE, which seems contradictory to the above proposed explanation of low metabolic rate.[18] The relatively higher *p,p'*-DDE levels in crab may however be explained by the species' feeding habit. The contribution of the β-HCH isomer was quite similar for most species. However, in the small benthic species the α-isomer contribution was higher and γ-isomer contribution was lower compared to the flatfish and gadoids. The high contribution of α-HCH, which was in the HCH technical mixture, to the total sum of HCHs in the

benthic invertebrates from this study is consistent with the limited metabolic abilities of these organisms.
Selective organ distribution may also explain the profile differences between the small benthic organisms and fish.[19] For the crab, goby, shrimp, and starfish samples, the whole bodies or soft parts were used, while for fish, only liver tissue was analysed.

Correlations between compounds. Levels of PCBs and OCPs were significantly correlated in all species. This correlation was greatly influenced by CB 153, which constituted almost 20 % of the total PCB load, and by *p,p'*-DDE, which contributed approximately for 50% to the total OCPs. Details of correlations between CB 153 and *p,p'*-DDE are given in Table 2. The high correlation between these compounds (mean $r > 0.93$; $p < 0.05$) indicates that they are likely to originate from the same source and that they represent the background pollution in this area; the presence of a point-source of one of the compounds is highly unlikely.

Table 2. Correlation between CB 153 and *p,p'*-DDE levels

	Crab	Shrimp	Starfish	Goby	Dab	Plaice	Sole	Bib	Whiting
N	14	13	10	7	6	7	12	10	9
r	0.9893	0.9526	0.9653	0.9996	0.8783	0.8952	0.9713	0.8359	0.9063
p	0.000	0.000	0.000	0.000	0.021	0.006	0.000	0.003	0.001

Geographical variation. The mean concentrations of PCBs and OCPs were significantly higher in the SE compared to the BNS (Mann-Whitney U test). Only species of which more than 3 sampling locations for each area were available were included in the calculations. Recently similar conclusions were drawn concerning levels of polybrominated diphenyl ethers (PBDEs) in biota from these two areas.[5]
Apart from the concentration difference between BNS and SE, a correlation was observed between pollutant concentration and the distance to Antwerp. Considering samples from location 7 to 16, a statistically significant inverse correlation between the distance to Antwerp and concentration was observed for both PCBs and OCPs. This correlation was highly significant for both PCBs and OCPs in crab, shrimp, sole, and bib (r between 0.850 and 0.893; $p < 0.05$). The results of all other samples clearly indicated that pollution was higher more upstream. Recently the same inverse correlation with the distance to Antwerp was observed concerning PBDE levels in biota.[5]

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References

1. de Voogt P., Wells D.E., Reutergardh L. and Brinkman U.A.Th. (1990) Int. J. Environ. An. Ch. 40, 1-46.
2. de Boer J., van der Zande T.E., Pieters H., Ariese F., Schipper C.A., van Brummelen T. and Vethaak D. (2001) J. Environ. Monitor. 3, 386-393.
3. Picard A., Palavan G., Robert S., Pesando D. and Ciapa B. (2003) Toxicol. Sci. 73, 141-148.
4. Sleiderink H.M., Oostingh I., Goksøyr A. and Boon J.P. (1995) Arch. Environ. Con. Tox. 287, 423-430.
5. Voorspoels S., Covaci A. and Schepens P. (2003) Environ. Sci. Technol. 37, 4348-4357.
6. Steen R.J., van der Vaart J., Hiep M., Van Hattum B., Cofino W.P. and Brinkman U.A. (2001) Environ. Pollut. 115, 65-79.
7. Jacobs M., Covaci A. and Schepens P. (2002) Environ. Sci. Technol. 36, 2797-2805.
8. Voorspoels S., Covaci A., Maervoet J., De Meester I., and Schepens P. (2004) Mar. Poll. Bull. *In press*.
9. Kozlova T. A. (1997) J. Fish Biol. 50, 734-743.
10. Larsson P., Okla L. and Collvin L. (1993) Environ. Toxicol. Chem. 12, 855-861.
11. Roose P., Cooreman K. and Vyncke W. (1998) Chemosphere 37, 2199-2210.
12. Oh C.W., Hartnoll R.G. and Nash R.D.M. (2001) Mar. Ecol. – Prog. Ser. 214, 211-223.
13. Everaarts J.M., den Besten P.J., Hillebrand M.Th.J., Halbrook R.S. and Shugart L.R. (1998) Ecotoxicology 7, 69-79.
14. Green N.W. and Knutzen J. (2003) Mar. Pollut. Bull. 46, 362-377.
15. Cleeman M., Riget F., Paulsen G.B., Klungsøyr J. and Dietz R. (2000) Sci. Total Environ. 245, 87-102.
16. Pruell R.J., Rubinstein N.I., Taplin B.K., LiVolsi J.A. and Bowen R.D. (1993) Arch. Environ. Con. Tox. 24, 290-297.
17. Walters G.W. and Aitken N.D. (2001) Water Environ. Res. 73, 15-23.
18. Borgå K., Gabrielsen G.W. and Skaare J.U. (2001) Environ. Pollut. 113, 187-198.
19. Inomata O.N.K., Montone R.C., Lara W.H., Weber R.R. and Toledo H.H.B. (1996) Antarct. Sci. 8: 253-255.

Table 1. Total PCB and OCP levels for each species expressed in ng/g ww (in ng/g lw) at the different locations.

		Benthic invertebrates			Benthic fish	Benthic flatfish liver			Gadoid fish liver	
Location		Crab	Shrimp	Starfish	Goby	Dab	Plaice	Sole	Bib	Whiting
PCBs BNS	1	36 (2900)	1.5 (330)	26 (680)	25 (860)	310 (790)	190 (840)	200 (670)	810 (1400)	780 (2100)
	2	29 (2200)		30 (1200)		250 (760)	110 (570)			730 (1800)
	3	23 (2100)		30 (1200)		89 (420)		74 (480)		
	4	53 (4400)	2.5 (430)	45 (2500)	25 (2100)			140 (1500)	1500 (2600)	1300 (2800)
	5	32 (3100)	2.4 (380)	44 (2300)	27 (2200)			20 (1100)	940 (1600)	1600 (3500)
	6	27 (2200)	1.8 (380)	30 (710)	23 (2400)	160 (420)	1100 (2300)	68 (1300)	650 (1200)	230 (1300)
	7	47 (3100)	2.6 (360)	29 (1100)	13 (1700)	290 (810)	96 (640)	57 (910)		
	8	47 (3400)	2.6 (420)	46 (1900)	97 (3200)				1700 (3100)	1700 (10900)
PCBs SE	9	210 (6200)	3.1 (470)					230 (2300)	1300 (2200)	
	10	200 (11700)							1400 (2900)	
	11	190 (8600)	7.3 (1000)	83 (5200)			1400 (2900)	480 (3700)	2900 (4900)	
	12	200 (6700)	6.5 (1200)		120 (4000)	260 (620)	980 (3900)	450 (3600)	2700 (4800)	3100 (10100)
	13	280 (24200)	19 (3100)					300 (2100)	3200 (7300)	2800 (5400)
	14	270 (5800)	37 (5300)				1200 (4300)	680 (5200)		1400 (14400)
	15		39 (6200)					800 (7700)		
	16		34 (4800)							
OCPs BNS	1	3.3 (270)	0.43 (91)	2.6 (67)	3.2 (110)	34 (86)	26 (120)	23 (76)	89 (160)	100 (280)
	2	2.8 (220)		2.8 (110)		23 (72)	15 (79)			63 (150)
	3	3.0 (270)		2.8 (120)		13 (59)		9.7 (62)		
	4	4.6 (380)	0.27 (46)	4.5 (250)	3.4 (290)			16 (170)	270 (480)	160 (340)
	5	3.0 (280)	0.61 (97)	4.4 (230)	3.6 (300)			6.7 (370)	120 (200)	280 (620)
	6	2.6 (220)	0.28 (58)	3.3 (80)	3.0 (320)	31 (83)	160 (350)	7.5 (150)	75 (140)	22 (130)
	7	4.4 (290)	0.47 (66)	2.6 (100)	1.8 (220)	38 (110)	8.7 (58)	6.0 (96)		
	8	4.4 (320)	0.67 (110)	4.9 (200)	14 (460)				190 (350)	210 (1400)
OCPs SE	9	18 (510)	0.60 (91)					23 (230)	150 (250)	
	10	16 (920)							78 (160)	
	11	14 (660)	0.71 (100)	10 (650)			130 (270)	51 (390)	280 (460)	
	12	18 (600)	0.66 (120)		16 (550)	56 (130)	110 (440)	45 (370)	310 (540)	410 (1300)
	13	21 (1800)	0.91 (150)					26 (180)	360 (810)	380 (750)
	14	23 (480)	1.6 (230)				100 (370)	54 (410)		140 (1500)
	15		1.3 (200)					57 (550)		
	16		1.7 (240)							
Lipid %		1.9 (1.2)	0.6 (0.1)	2.7 (1.1)	2.0 (1.1)	35 (7.1)	27 (12)	15 (9.4)	55 (7.8)	34 (14)