Biomagnification of mono-ortho and non-ortho PCBs in a benthic food chain in the Baltic Sea.

Kjell Lundgren[1], Mats Tysklind[1], Rasha Ishaq[2]

[1]Environmental Chemistry, Department of Chemistry, Umeå University, Umeå
[2]Institute of Applied Environmental Research (ITM), Stockholm University, Stockholm

Introduction

When PCBs were identified as environmental pollutants and quantified in marine species from Swedish waters in the late 1960s[1,2], the biomagnification of PCBs in the Baltic Sea food web became evident. The total PCB concentrations in white tailed eagles were approximately three orders of magnitude higher than in the fish feed. Since then, thousands of analyses of environmental samples of air, water, sediment and biota have been performed. However, the analytical techniques used were initially inadequate for the determination of individual non-*ortho* PCBs and several important mono-*ortho* PCBs, PCBs that have been assigned toxic equivalency factors (TEFs) by the world health organisation (WHO)[3]. Improvements of separation methods and analytical techniques made it later possible to individually identify and quantify low levels of these important substances in the environment[4,5]. For example, the use of activated charcoal chromatography for the separation of planar substances from non-planar ones, including non-*ortho* PCBs, was demonstrated[6,7]. In 1991, a multi-residue method allowed analysis of the above-mentioned congeners in biological samples[8]. Furthermore, Järnberg et al. presented concentration data from a wide-range of environmental samples in an extensive study, including the toxic mono- and non-*ortho* PCBs[9]. In burbot muscle from the Bothnian Bay and herring muscle from the Baltic proper, average total non-*ortho* PCB concentrations were approximately 1.5 and 4.8 ng/g lw, respectively. In Guillemot eggs and in Grey seal from the Baltic proper, the corresponding concentrations were 150 and 2.0 ng/g lw, respectively, indicating that the biomagnification of these congeners is species-dependent. In this paper, coplanar PCB concentrations (Mono-*ortho* PCB 123, 118, 114, 105, 167, 156, 157, and 189; Non-*ortho* PCB 77, 126, and 169), measured in a benthic food chain consisting of surface sediments, amphipods (*Monoporeia affinis*), isopods (*Saduria entomon*), and fourhorned sculpins (*Oncocottus quadricornis*), are reported. The obtained PCB concentrations in sediments and biota enabled calculations of biota to sediment accumulation factors (BSAFs) and biomagnification factors (BMFs). All samples were collected in the Gulf of Bothnia, northern Baltic Sea, reflecting the PCB pollution of a remote semi-arctic region.

Methods and Materials

Samples: Surface sediments, amphipods, isopods, and sculpins were sampled at five different coastal locations in the Gulf of Bothnia (Bothnian Bay and Bothnian Sea), northern Baltic Sea (Fig. 1). The sampling locations were as follows: Harufjärden (HF), Umeå (UM), Hornslandet (HL) Gävlebukten (GB), and Simpnäs (SN). The samples were collected, from the second accumulation depression from the coastline at all locations in order to establish background PCB concentrations, and in the autumn during the years 1991, 1992, or 1993. Surface bottom sediment samples were taken with a modified Ponar grab sampler and the amphipods were extracted from the sediments by sieving. Isopods were collected in cages placed on the bottom and sculpins were caught in fishing-nets by local fishermen. In total, 13 surface sediment samples, 12 pooled whole-body amphipod, 13 isopod, and 11 sculpin samples were analysed. The biological tissues were initially homogenized and sub-sampled into replicates. The sub-samples were stored at −20 ºC until analysis.

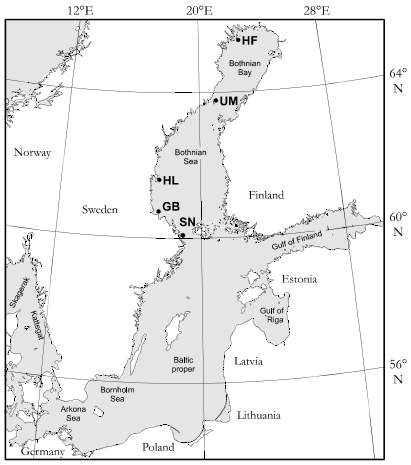

Figure 1: Sampling locations in the Gulf of Bothnia, northern Baltic Sea.

Extraction and cleanup: A multi-residue non-destructive analytical procedure was applied to all the samples[10]. Sediment and biological samples was placed in pre-extracted cellulose thimbles and extracted wet in a Soxhlet apparatus, equipped with a Dean Stark trap for the collection of water. The homogenate was extracted with toluene for 24 h followed by acetone:*n*-hexane (59:41) for another 24 h. After solvent reduction, the lipid content in each sample was determined gravimetrically. The total organic carbon content (TOC) in the sediment samples was determined for a sub-sample using a high-temperature combustion elemental analyzer following standard procedures. Prior to extraction, four $^{13}C_{12}$-labelled coplanar PCBs ($^{13}C_{12}$-PCB 77, 118, 126, and 169) were added as internal standards. Cleanup was achieved by dialysis through a semi-permeable membrane (SPM), using cyclopentane, to reduce the bulk of the lipids[11]. The dialysate was further cleaned-up by elution on a silica column with *n*-hexane and fractionated on an HPLC aminopropylsilica column[12]. A fraction from the amino-column containing di- and tricyclic aromatic compounds was then introduced onto an HPLC column containing PX-21 activated carbon[13].The fractionation on the carbon column resulted in a final separation of coplanar PCBs from less planar compounds (e.g. poly-*ortho* PCBs) and other interfering compounds. This was achieved by gradient elution with a mixture of dichloromethane (DCM, 1%) in *n*-hexane and toluene (0-10%). Non-*ortho* PCBs were backflushed from the column with pure toluene and mono-*ortho* PCBs were collected in a fraction nearest to the final backflush sequence. A tetradecane keeper and a recovery standard ($^{13}C_{12}$-labelled PCB 101) were added to the fractions containing the mono-*ortho* and non-*ortho* PCBs prior to evaporation and the final analysis.

HRGC-MS analysis: The extracts were injected in splitless mode on a Hewlett Packard 5890 high-resolution gas chromatograph coupled to a VG 12-250 low-resolution (HRGC-LRMS) or a VG Analytical 70-250S double focusing high-resolution (HRGC-HRMS) mass spectrometer system, analysing mono-*ortho* and non-*ortho* PCBs, respectively. PCB separation was performed on an Rtx-5 capillary column (60 m, 0.32 mm i.d., 0.25 μm film thickness) using the following temperature program: 180 ºC (2 min), 20 ºC/min to 200 ºC, then 4 ºC/min to 300 ºC (held for 15 min). Electron ionisation was used at 70 eV (LRMS) or 35 eV and the HRMS instrument operated at a mass resolution of 8000. The detection of PCB ions was carried out in SIM-mode and the two most abundant ions in the molecular ion chlorine distribution cluster for each PCB homologue (tetra- through hepta-CBs) were monitored. The identification of PCBs was based on added standards and retention data quoted in the literature[14].

Results and Discussion

Coplanar PCB concentrations in the benthic food chain: Average mono-*ortho* and non-*ortho* PCB concentrations, PCB-TEQs (PCB-TEQ = $\Sigma(TEF_i \times C_i)$), number of samples, and content of carbon and lipids in the analysed benthic food chain samples are presented in Table 1 and 2. The PCB-TEQ calculations were based on assigned fish sample TEFs of individual congeners[3] (Table 1). The analyses of the surface sediment samples showed that the average concentrations of total coplanar PCBs were lowest at the locations UM and HL (230 and 404 pg/g dw) and highest at the locations HF, GB, and SN (509, 677, and 1019 pg/g dw). These results are probably related to distance from more or less industrialized and populated regions along the Swedish east coast. The total coplanar PCB concentrations in the biological samples increased from the bottom trophic level (amphipods: 28-39 ng/g lw) to the top predators (isopods: 105-315 ng/g lw; sculpins: 71-252 ng/g lw) of the food chain demonstrating that the analysed PCB congeners biomagnified in this type of benthic food chain.

Table 1: Average mono-*ortho* and non-*ortho* PCB concentrations in sediment (pg/g dw) and amphipod (ng/g lw) samples collected in the Gulf of Bothnia[a]. PCB-TEFs used are in brackets[3].

Type of sample	**Sediment**					**Amphipod**				
Location[b]	**HF**	**UM**	**HL**	**GB**	**SN**	**HF**	**UM**	**HL**	**GB**	**SN**
Mono-*ortho* PCB (TEF[c])										
123 (0.000005)	11	3.6	11	14	19	0.9	0.84	0.80	1.5	1.5
118 (0.000005)	260	110	170	340	550	14	14	16	16	19
114 (0.000005)	5.8	2.5	7.0	6.5	7.6	0.27	0.39	0.35	0.45	0.44
105 (0.000005)	72	36	60	100	170	4.4	4.4	5.3	5.1	5.7
167 (0.000005)	50	24	53	73	88	3.4	2.7	3.9	4.7	4.6
156 (0.000005)	49	35	63	77	110	3.9	3.3	4.5	5.0	4.7
157 (0.000005)	14	6.4	11	16	20	0.58	0.66	1.1	1.0	0.89
189 (0.000005)	n.d.	4.2	4.8	5.0	7.2	0.23	0.33	0.40	0.46	0.37
Non-*ortho* PCB										
77 (0.0001)	25	3.4	12	27	34	0.88	0.38	0.56	0.65	1.2
126 (0.005)	13	1.6	6.1	8.7	12	0.59	0.24	0.25	0.37	0.68
169 (0.00005)	3.9	0.51	1.6	4.3	6.5	0.15	0.065	0.074	0.092	0.19
Mono-*ortho* PCBs	**467**	**224**	**384**	**637**	**967**	**28**	**27**	**32**	**34**	**37**
Non-*ortho* PCBs	**42**	**5.5**	**20**	**40**	**52**	**1.6**	**0.67**	**0.87**	**1.1**	**2.0**
Coplanar PCBs	**509**	**230**	**404**	**677**	**1019**	**30**	**28**	**33**	**35**	**39**
Mono-*ortho* PCB-TEQ	0.002	0.001	0.002	0.003	0.005	0.14	0.13	0.16	0.17	0.18
Non-*ortho* PCB-TEQ	0.126	0.017	0.032	0.046	0.061	4.43	1.91	2.79	3.27	5.81
PCB-TEQ (pg/g)	**0.128**	**0.018**	**0.034**	**0.049**	**0.066**	**4.57**	**2.04**	**2.95**	**3.44**	**5.99**
No. of samples	3	2	3	3	2	3	2	2	2	3
Carbon content (%)	3.1	1.5	3.3	3.2	3.1	-	-	-	-	-
Lipid content (%)	-	-	-	-	-	52	48	53	43	40

[a] n.d., not detected. [b] HF, Harufjärden; UM, Umeå; HL, Hornslandet; GB, Gävlebukten; SN, Simpnäs
[c] TEF = Toxic equivalency factor

Table 2: Average mono-*ortho*- and non-*ortho* PCB concentrations (ng/g lw) in isopod and fourhorned sculpin samples collected in the Gulf of Bothnia.

Type of sample	**Isopod**				**Fourhorned sculpin**				
Location[a]	**HF**	**UM**	**HL**	**SN**	**HF**	**UM**	**HL**	**GB**	**SN**
Mono-*ortho* PCB									
123	0.59	0.24	0.92	0.43	0.97	2.4	0.61	2.6	2.5
118	130	51	72	180	75	130	35	110	74
114	2.5	0.83	1.4	2.8	1.3	2.2	0.64	1.5	1.2
105	30	15	23	36	27	41	12	37	28
167	28	10	14	30	14	20	6.8	28	9.8
156	44	20	22	44	34	43	12	50	16
157	8.7	3.5	4.6	9.0	6.4	8.4	2.4	10	3.5
189	2.8	0.99	2.0	2.3	2.2	2.8	0.80	4.2	1.4
Non-*ortho* PCB									
77	3.2	2.5	2.6	3.7	0.18	0.28	0.19	1.5	0.43
126	3.0	0.74	1.4	2.2	0.60	1.1	0.36	0.64	0.55
169	0.79	0.18	0.40	0.55	0.16	0.37	0.12	0.22	0.17
Mono-*ortho* PCBs	**243**	**102**	**139**	**309**	**160**	**250**	**70**	**240**	**136**
Non-*ortho* PCBs	**7.0**	**3.4**	**4.4**	**6.4**	**0.94**	**1.8**	**0.67**	**2.4**	**1.1**
Coplanar PCBs	**250**	**105**	**143**	**315**	**161**	**252**	**71**	**242**	**137**
Mono-*ortho* PCB-TEQ	1.3	0.51	0.70	1.54	0.80	1.25	0.35	1.20	0.68
Non-*ortho* PCB-TEQ	16.0	12.5	13.1	18.5	0.91	1.45	0.97	7.68	2.16
PCB-TEQ (pg/g lw)	**17.3**	**13.0**	**13.8**	**20.0**	**1.71**	**2.70**	**1.32**	**8.88**	**2.84**
No. of samples	5	1	2	5	3	1	2	2	3
Lipid content (%)	11	14	20	13	18	18	35	26	33

[a] HF, Harufjärden; UM, Umeå; HL, Hornslandet; GB, Gävlebukten; SN, Simpnäs

A minor fraction, 1-14% of the coplanar PCBs, was non-*ortho* PCBs in the sediment and biota samples. On the contrary, the non-*ortho* PCB-TEQs exceeded the mono-*ortho* PCB-TEQs in all samples depending on the low TEF values (0.000005) that have been assigned for the mono-*ortho* PCBs. The PCB-TEQs in biological samples were highest in the isopods (13-20 pg/g lw) and lowest in the sculpins (1.3-8.9 pg/g lw). When moving upwards in the food chain, the non-*ortho* PCB pattern became less dominated by lower chlorinated PCB congeners. For example, the tetra chlorinated PCB 77 contributed less to the total non-*ortho* PCB concentration (PCB 77, 126 and 169) in the sculpins compared to the concentration in the amphipods (Fig. 2). This might be due to the limited capacity of the amphipods and isopods to excrete or eliminate/metabolize the coplanar PCBs. The low concentration of PCB 77 in the sculpins was also the major reason why the PCB-TEQs in the sculpins were lower than in the amphipods and isopods. Furthermore, the found maximum PCB-TEQ in the sculpins was less than 4 pg/g fresh weight, which have been set as the maximum level in consumer fish by the European Commission[15].

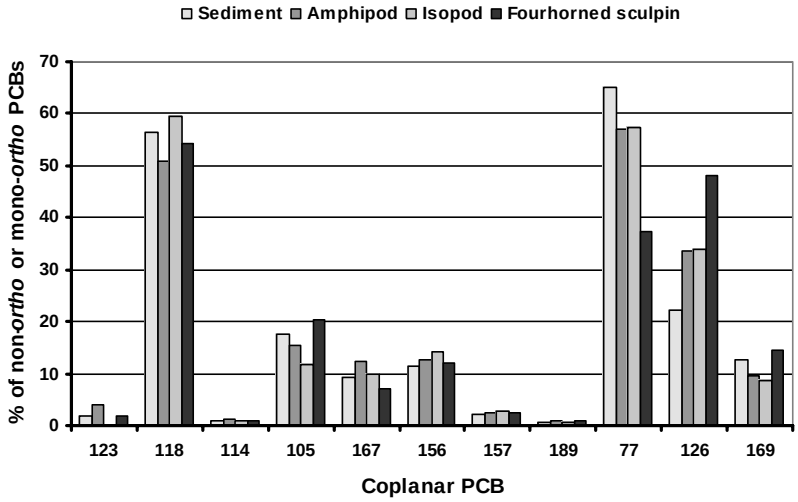

Figure 2: Composition of PCB congeners (% of non-*ortho* or mono-*ortho* PCBs) in sediment, amphipod, isopod, and fourhorned sculpin collected at Simpnäs, SN.

Biomagnification of coplanar PCBs: BSAF/BMFs (BMF_1-BMF_3) for the studied benthic food chain are depicted in Figure 3 and calculated values are listed in Table 3. The magnitudes of the BSAFs/BMFs indicated that all the analysed coplanar PCBs biomagnified to some extent in the food chain. The BSAFs for mono-*ortho* and non-*ortho* PCBs were approximately 2 and 1.5, respectively. Similar BSAFs for polychlorinated naphthalenes (PCNs) have been reported for the same food chain in the literature[16]. These BSAF values demonstrate that the uptake of PCNs and coplanar PCBs from sediment into amphipods is equivalent. In general, the coplanar PCB concentrations in the higher trophic levels (isopods and sculpins) were higher than the concentrations in the lowest trophic level (amphipods) resulting in BMFs greater than one. The average BMF values were 5.5 (isopod/amphipod) and 5.2 (sculpin/amphipod) for the mono-*ortho*

PCBs and 4.2 and 1.4, respectively, for the non-*ortho* PCBs. Similar BMF values have been reported in the food web of Lake Ontario[17].

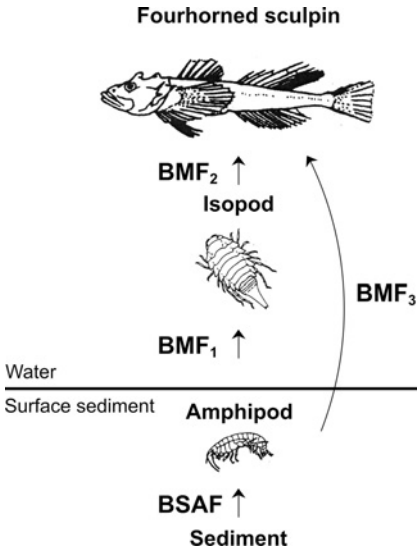

Figure 3: The benthic food chain (sediment, amphipod, isopod, and fourhorned sculpin) investigated in the Gulf of Bothnia. The possible BSAF and BMFs for the food chain are indicated between trophic levels.

Table 3: Calculated average BSAF and BMF values for mono-*ortho* and non-*ortho* PCBs in the benthic food chain[a].

PCB	BSAF (C_{amp}/C_{sed})	BMF_1 (C_{iso}/C_{amp})	BMF_2 (C_{scu}/C_{iso})	BMF_3 (C_{scu}/C_{amp})
123	2.8	0.5	3.3	1.6
118	1.8	6.9	0.8	5.4
114	1.9	5.0	0.7	3.6
105	1.9	5.2	1.1	5.8
167	2.0	5.3	0.8	4.1
156	1.9	7.6	1.0	7.2
157	1.9	7.6	1.0	7.3
189	2.0	5.6	1.1	6.4
Mono-*ortho* PCBs	**1.9**	**5.5**	**1.2**	**5.2**
77	1.2	4.1	0.2	0.7
126	1.6	4.3	0.4	1.5
169	1.2	4.2	0.4	1.8
Non-*ortho* PCBs	**1.3**	**4.2**	**0.3**	**1.4**
Coplanar PCBs	**1.8**	**5.1**	**1.0**	**4.1**

[a] sed = sediment; amp = amphipod; iso = isopod; scu = fourhorned sculpin

The obtained BMFs in this study exceeded the established BMFs for total PCBs[18]. These results demonstrate that the coplanar toxic PCBs biomagnified to a higher degree than the non-toxic PCBs. Furthermore, biological processes seem to alter the composition and PCB pattern in predators such as sculpins higher up in the food chain. The difference in PCB patterns may reflect a congener-specific rapid excretion, intestinal absorption, and/or metabolic transformations in the marine species. This is especially true for PCB 77, the only tetra-chlorinated PCB among the analysed coplanar PCBs (Fig. 2).

Acknowledgments
The financial support from the Kempe Foundation, the Swedish Forest Industries, the Swedish EPA, the Finnish National Board of Water and the Environment, and the Boliden Mineral AB are gratefully acknowledged. Thanks go to Ourania Papacosta (Institute of Applied Environmental Research, ITM, Stockholm University, Sweden) and Bo Strandberg (Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden) for the cleanup of the samples.

References

1. Jensen S. (1966) New Sci. 32, 612.
2. Jensen S., Johnels A.G., Olson M., and Otterlind G. (1969) Nature 224, 247-250.
3. Van den Berg M., Birnbaum L., Bosveld A.T.C., Brunström B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., Larsen J.C., van Leeuwen F.X.R., Liem A.K.D., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillit D., Tysklind M., Younes M., Wærn F., and Zacharewski T. (1998) Environ. Health Perspect. 106, 775-792.
4. Creaser C.S., Krokos F., and Startin J.R. (1992) Chemosphere 25, 1981-2008.
5. Kannan N. (1999) In: Paasivirta J. (ed) New Types of Persistent Halogenated Compounds, The Handbook of Environmental Chemistry Vol 3 Part K. Springer-Verlag, New York, pp. 127-156
6. Stalling D.L., Smith L.M., and Petty J.D. (1979) In: Van Hall C E (Ed.) ASTM STP 686, American Society for Testing and Materials.
7. Smith L.M., Stalling D.L., and Johnson, J.L. (1984) Anal Chem 56, 1830-1842.
8. Jansson B., Andersson R., Asplund L., Bergman Å., Litzén K., Nylund K., Reutergårdh L., Sellström U., Uvemo U.-B., Wahlberg C., and Wideqvist U. (1991) Fres. J. Anal. Chem., 340, 439-445.
9. Järnberg U., Asplund L., de Wit C., Grafström A.-K., Haglund P., Jansson P., Lexén K., Strandell M., Olsson M., and Jonsson B. (1993) Environ. Sci. Technol. 27, 1364-1374.
10. Lundgren K. (2003) *Thesis*, Environmental Chemistry, Umeå University, Sweden, ISBN 91-7305-366-X.
11. Strandberg B., Bergqvist P.-A., and Rappe C. (1998) Anal. Chem. 70, 526-533.
12. Colmsjö A.L., Zebühr Y., and Östman C.E. (1987) Chromatographia 24, 541-544.
13. Lundgren K., van Bavel B., and Tysklind M. (2002) J. Chromatogr. A 962, 79-93.
14. Ballschmiter K. and Zell M. (1980) Fresenius Z. Anal. Chem. 302, 20-31.
15. Council Regulations (EC) No 2375/2001 setting maximum levels for certain contaminants in foodstuffs.
16. Lundgren K., Tysklind M., Ishaq R., Broman D., and van Bavel B. (2002) Environ. Sci. Technol. 36, 5005-5013.

17. Metcalfe T.L. and Metcalfe C.D. (1997) Sci. Total Environ. 201, 245-272.
18. Van Bavel B., Näf C., Bergqvist P.-A., Broman D., Lundgren K., Papakosta O., Rolff C., Strandberg B., Zebühr Y., Zook D.R., and Rappe C. (1996) Mar. Pollut. Bull. 32, 210-218.