Levels of PCBs in the Marine Ecosystem of the Gulf of Bothnia: A Chemometric Comparison of Different Sampling Sites.

Bert van Bavel[1], Bo Strandberg[1], Kjell Lundgren[1], Per-Anders Bergqvist[1],Douglas Zook[1], Dag Broman[2], Carina Näf[2], Johan Axelman[2], Carl Rolff [2]and Christoffer Rappe[1].

[1]Institute of Environmental Chemistry, Umeå University, S 901 87 Umeå, Sweden.

[2]Aquatic Chemical Ecotoxicology, Department of Zoology, University of Stockholm
S 106 91 Stockholm, Sweden.

INTRODUCTION

In the autumn of 1991 and the spring of 1992 more than 150 samples were collected throughout the Gulf of Bothnia in order to study the accumulation of organochlorine contaminants in benthic and pelagic marine food chains. For this survey the following matrices and species were selected from the marine ecosystem: sediments, amphipods (*Monoporeia affinis*), isopods (*Saduria entomon*), zoo- and phytoplankton, mysids , perch (*Perca fluviatilis*), fourhorned sculpin (*Oncocottus quadricornis*), herring (*Clupea harengus*), salmon (*Salmo salar*), salmon trout (*Salmo trutta*) and whitefish (*Coregonus lavaretus*). All specimen were analysed for a variety of chlorinated organic contaminants including PCDDs, PCDFs, PCNs, PCDTs, PCBs, Alkylated PCDFs, and pesticides including Chlordanes, DDTs, HCCHs, aldrin, dieldrin and mirex[1]. In this paper the congener specific GC/MS analyses of PCBs in sediment and the amphipod *Monoporeia affinis* is discussed as well as a chemometric comparison of the distribution of the different PCB congeners at the different sampling sites by principal component analysis (PCA).

MATERIAL and METHODS

Sediment and amphipod samples were collected from the second accumulation bottom at four different locations in the Gulf of Bothnia : Harufjärden (1) north in the Gulf of Bothnia outside the middle size town Luleå, an area merely dominated by the iron industry, Hornslandet (2) in the surroundings of a nature reserve, Gävlebukten (3) in the surroundings of middle size town Gävle, a merely agricultural dominated area with also paper industry and steel industry and Simpnäs (4,5) north of Stockholm (Figure 1).

After the homogenisation of 1-50 g of sample, the samples were soxhlet extracted with toluene (24h.) followed by an azotropic mixture of hexane and acetone (59/41, 24h.).
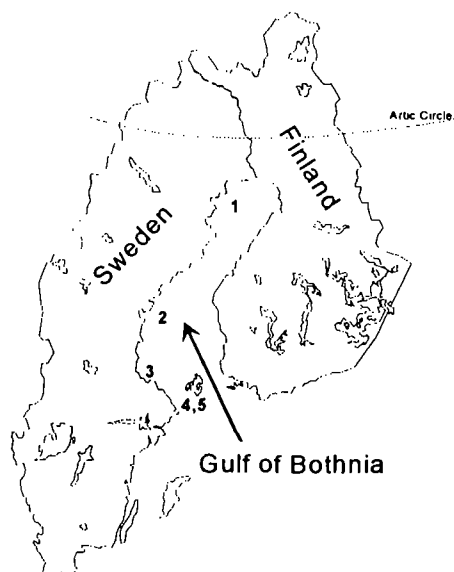Bulk removal of lipids was achieved by means of polyethylene film dialyses[2,3].

Figure 1 Sampling sites in the Gulf of Bothnia

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Dissolving the extracts in cyclopentane, dialysis over the polymeric film was accomplished by changing the outer cyclopentane (dialysate) after 12, 40 and 64 hours. The three dialysate fractions, containing about 1% of the original lipids, were collected and evaporated to a few mL's. The extract was split into two parts, 9/10 of the extract for the analysis of non- and mono-*ortho* PCBs, PCDDs, PCDFs, PCDTs and PCNs and 1/10 of the extract for the analysis of 'bulk' PCBs and pesticides. The PCBs and pesticides fraction was further separated on polarity on a 40 cm long 1.0 cm ID glass column packed with 8 g of methanol washed, baked (550°C, 3 days), activated (130°C, 2 days) and deactivated (1.2 % wt wt[-1] water) Florisil gel[4]. After addition of the fraction on top, the Florisil column is eluted in steps of increasing polarity of the mobile phase. This resulted in three fractions: fraction 1 (F1) eluted with 32 mL hexane and 38 mL 15% (v/v) methylene chloride in hexane, fraction 2 (F2) eluted with 56 mL 50 % (v/v) methylene chloride in hexane and fraction 3 (F3) eluted 66 mL methanol. As a keeper 30 uL tetradecane was added and all the fractions were evaporated down to this volume.

Before extraction several ^{13}C labelled internal standards (IS) were added to the samples. For PCB and pesticide analysis the IS contained 100 ng PCB #80, 200 ng PCB #153, 300 ng lindane, 400 ng HxCBz, 500 ng dieldrin and 5000 ng p,p-DDT. After clean up completement a recovery standard (RS) was added to the final volume of 30 uL containing ^{13}C labelled PCB #101 (100 ng), PeCBz (100 ng) and native OCN (200 ng).

PCB and pesticide analyses of the first Florisil fraction (F1) were carried out by HRGC/LRMS using selected ion recording (SIR). Two mass spectrometers were used: a VG 12-250 and a Fisons MD 800, the VG 12-250 coupled to a HP5890 GC, the MD 800 coupled to a Fisons GC 8000. Sample introduction was achieved using splitless injection at 250 °C using Helium (99.996%, Aga Gas, Sweden) as a carrier gas. A non polar capillary column (Supelco PTE-5, 60m x 0.32mm i.d.,0.25µm film thickness Bellefonte, PA, USA or J &W DB-5, 60m x 0.32 mm i.d., 0.25µm film thickness Folsom, CA USA) was temperature programmed as follows: 180°C initial hold for 2 min, increase at a rate of 15°C per min to 205°C followed by an increase of 2°C per min to 300°C. The GC-MS interface was kept at a temperature of 270°C and the ion source at 250°C. In the SIR mode the two most abundant ions in the chlorine cluster of the molecular ion (PCBs) or the most abundant fragments exhibiting a chlorine cluster (pesticides) of the native compounds were monitored. One ion was recorded for the ^{13}C-labelled internal and recovery standards. Identification of the different PCB isomers at each chlorine level was made after Schwartz and Stalling[5].

Before each set of samples 1 µl of 5 calibration solutions was injected, containing the RS, IS and native ^{12}C standards containing all target pesticides and at least one PCB at each chlorination level at 5 different concentrations: 10 ng, 50ng, 100ng, 500ng and 1000ng in 500µl tetradecane.
From the calibration solutions injections average relative response factors
$((\text{area}^{12}C_{st}/\text{area}^{13}C_{is})*(\text{amount}^{13}C_{is}/\text{amount}^{12}C_{st})$ or
$(\text{area}^{13}C_{rs}/\text{area}^{13}C_{is})*(\text{amount}^{13}C_{is}/\text{amount}^{13}C_{rs}))$ were calculated at each concentration level. When the standard deviation of the mean of the relative response factors (RRFs) was below 15%, this average RRF was used in the calculation of the concentration for the samples
$((\text{area}^{12}C_{sa}/\text{area}^{13}C_{is})*(\text{amount}^{13}C_{is}/\text{RRF}))$.
Other quality control criteria for positive identification of the target compounds were: 1.) Signal to noise ratio over three (S/N >3). 2.) Isotope ratios of the two most abundant ions monitored within 15 % of the theoretical chlorine cluster values. 3.) Relative retention time difference related to the internal standard within 2 seconds. 4.) Recovery of the internal ^{13}C labelled standards between 50 and 120 %.

RESULTS and DISCUSSION

Table 1 the average relative response factors related to their respective ^{13}C internal standards are presented. These RRFs are used to calculate the amount of the target compounds in the samples. For all target compounds the % RSD of the RRFs of the calibration curve at five different concentration levels were well within the quality control limit of 15%.

Table 1. Relative Response Factors and average RRF of the five points calibration curve.

Target compound	CAL 1	CAL 2	CAL 3	CAL 4	CAL 5	RRF	%RSD
HCBz	1.01	1.06	1.01	0.95	0.86	0.98	7%
13C PCB #80	0.48	0.47	0.47	0.47	0.48	0.47	1%
PCB #28	0.95	1.00	0.97	1.00	1.03	0.99	3%
PCB #52	0.62	0.65	0.62	0.64	0.65	0.64	2%
13C PCB #153	1.05	1.04	1.03	1.03	1.08	1.05	2%
PCB #101	1.00	1.03	1.01	1.07	1.10	1.04	4%
PCB #153	0.86	0.90	0.87	0.91	0.95	0.90	4%
PCB #138	0.85	0.86	0.82	0.86	0.90	0.86	3%
PCB #180	0.76	0.80	0.78	0.82	0.86	0.81	4%
P,P-DDE	1.42	1.54	1.52	1.60	1.67	1.55	5%

Results for the amphipod *Monoporeia Affinis* are presented in table 2. All results are normalised to the total amount of extractable organic matter. Double and triple sediment and amphipod samples from the different locations displayed small relative standard deviations, a RSD over 25% is an exception.

Levels of PCBs from the autumn samples of both amphipods and sediment are lowest in Hornslandet (2) and clearly highest in Simpnäs(4). The samples from Gävlebukten(3) and Harufjärden (1) contain somewhat higher levels than Hornslandet (2). As for the other compounds presented in this paper: p,p-DDE levels are the highest in the south as the levels decrease to the north for both matrices. Surprisingly high levels of HCBz were found in the amphipods from Harufjärden (1) but this high concentration was not confirmed in the sediments from the same sampling site where the level was found to be lower than the other sampling sites.
Samples from Simpnäs collected in spring (5) show clearly lower levels than samples collected in the autumn for both the amphipods as well as the sediment samples. The percentage organic matter differed from 0.13-0.19% (autumn) to 0.20-0.22 (spring) for the sediment samples and from 33-40% (autumn) to 41-56% (spring) for the amphipod samples.

For further evaluation of the congener distribution at the different sampling sites multivariate data analysis (MVDA) was used. In MVDA the samples (objects *k*) are seen as points in a multidimensional space with as many dimensions as different PCB congeners (variables *i*) measured on the samples. Calculating principal components (PCs) the points in this multidimensional space are projected down onto a line, a plane or a hyperplane to give the best low-dimensional representation of the data. The data is so approximated by a systematic part (the loading and the score vectors) and a random part (the residuals) which consists of errors in the approximation. The dimension of the principal component analyses (PCA) is determined by cross-validation. Before the calculation of PCs the data set was centred and scaled to unit variance. All calculations were performed on a PC operating in a MS DOS environment using the SIMCA-S package (Umetri AB, Box 1456, S-901 24 Umeå, Sweden). The principles of PCA are extensively discussed elsewhere [6,7] and used on a number of occasions to characterise sediment [8,9] and other matrices [5,10] containing organochlorine residues.

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Table 2 PCB levels in Monoporeia Affinis normalised to lipid weight (ng/g).

| PCB congener	1. Harufjärden Autumn (n=3)		2.Hornslandet Autumn (n=2)		3.Gävlebukten Autumn (n=2)		4.Simpnäs Autumn (n=4)		5.Simpnäs Spring (n=3)	
	ng/g	%RSD	ng/g	%RSD	ng/g	%RSD	ng/g	%RSD	ng/g	%RSD
PCB#28,31	6.0	3%	4.9	0%	6.2	4%	9.4	5%	6.2	14%
PCB#74	7.1	11%	6.6	0%	6.8	6%	11.1	6%	5.0	9%
PCB#70,76	13.5	5%	13.3	9%	14.9	9%	22.5	3%	10.2	6%
PCB#66	12.4	11%	8.7	6%	11.1	8%	18.3	5%	8.4	6%
PCB#56,60	5.5	12%	3.6	11%	4.6	9%	6.8	5%	3.6	16%
PCB#88,95	14.1	6%	17.8	7%	20.5	10%	22.2	11%	10.4	10%
PCB#91	1.6	0%	1.8	3%	2.4	9%	2.8	12%	1.3	12%
PCB#84,89,92	3.2	4%	4.7	2%	5.7	10%	6.9	9%	2.9	13%
PCB#101	29.9	4%	32.1	8%	39.2	13%	47.7	7%	20.4	10%
PCB#99,113	9.9	3%	12.5	2%	14.2	10%	20.5	7%	8.4	12%
PCB#83	4.0	4%	5.2	1%	6.4	9%	8.1	7%	3.4	12%
PCB#111	3.4	1%	3.4	1%	4.1	8%	5.9	4%	2.3	13%
PCB#110	23.6	7%	25.4	0%	32.6	10%	39.2	5%	17.5	14%
PCB#123	3.3	10%	2.0	0%	2.7	2%	3.9	6%	1.6	8%
PCB#118	56.3	14%	29.4	2%	34.5	2%	55.6	6%	23.2	5%
PCB#105,127	18.9	15%	7.2	0%	9.5	1%	14.5	10%	6.5	7%
PCB#136,148	5.7	2%	4.6	1%	5.7	12%	7.1	8%	3.1	9%
PCB#151	8.2	4%	4.8	2%	11.2	3%	9.1	9%	4.8	10%
PCB#135,144	6.1	5%	6.3	2%	8.8	6%	9.4	4%	4.0	4%
PCB#149	**30.4**	**4%**	31.1	2%	43.1	9%	48.7	2%	20.8	2%
PCB#143,134	2.9	6%	1.9	2%	2.1	11%	3.7	3%	1.6	10%
PCB#146	9.8	5%	9.5	1%	12.4	8%	16.3	3%	6.2	4%
PCB#132.153	63.4	2%	59.9	0%	73.9	4%	100.5	4%	38.6	2%
PCB#168	11.8	9%	10.1	1%	15.2	10%	16.1	2%	7.0	10%
PCB#141	10.1	2%	8.6	1%	12.3	4%	12.6	5%	5.0	4%
PCB#138,160,163,164	79.5	3%	67.5	1%	86.2	0%	116.0	6%	42.6	4%
PCB#158	6.5	8%	5.1	0%	7.4	1%	9.0	11%	3.2	10%
PCB#128	13.9	7%	7.3	6%	8.9	4%	13.1	11%	5.0	1%
PCB#156	22.9	15%	7.0	7%	8.4	9%	13.1	17%	5.1	19%
PCB#179	6.8	3%	5.9	1%	8.6	4%	10.6	2%	4.5	8%
PCB#176	1.9	3%	2.0	1%	3.0	2%	3.5	4%	1.4	6%
PCB#178	4.2	2%	3.9	2%	4.9	1%	6.8	7%	2.5	6%
PCB#182,187	22.6	3%	19.0	1%	25.2	3%	33.4	8%	13.0	10%
PCB#183	9.2	3%	9.0	2%	12.3	3%	15.8	9%	6.0	8%
PCB#185	1.3	7%	1.1	0%	1.8	3%	1.9	8%	0.7	5%
PCB#174	16.2	2%	11.9	4%	16.9	7%	18.2	11%	6.9	11%
PCB#177	10.6	4%	8.3	5%	11.4	6%	14.9	5%	5.2	8%
PCB#171	4.3	8%	3.7	6%	5.2	7%	7.1	5%	2.3	7%
PCB#172	3.7	10%	2.8	10%	3.6	11%	4.0	13%	1.3	0%
PCB#180,193	54.0	4%	42.9	11%	52.2	12%	66.2	14%	23.6	12%
PCB#170,190	25.5	11%	20.8	17%	23.9	14%	27.5	18%	8.6	3%
PCB#202	1.5	14%	1.5	8%	1.7	11%	3.3	15%	1.2	5%
PCB#200	1.1	0%	0.9	8%	1.1	10%	1.9	14%	0.7	0%
PCB#196,201,203	5.2	11%	3.9	21%	4.2	18%	6.2	21%	2.0	2%
PCB#195	1.1	0%	1.0	0%	1.4	20%	1.8	22%	0.5	2%
PCB#194	4.7	21%	3.8	34%	4.0	23%	5.1	28%	1.9	15%
total PCBs	659.9		545.1		692.4		898.3		360.7	
HCBz	336.2	5%	187.9	3%	92.8	5%	137.9	9%	72.0	36%
P,P-DDE	48.9	9%	105.4	4%	101.8	11%	165.6	6%	65.6	11%
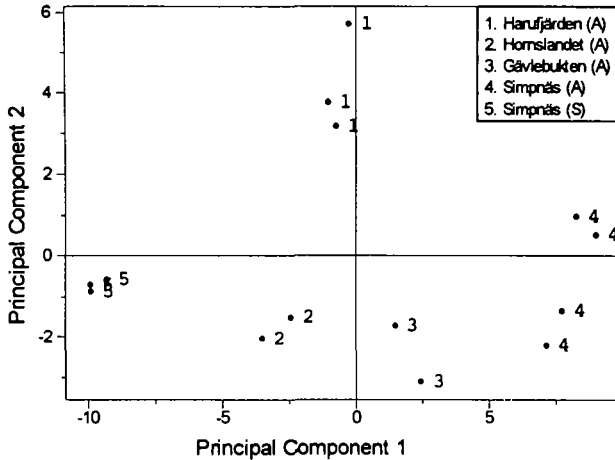
Figure 2 Score plot of PC 1 versus PC 2 together explaining 85% of the variance in the data.

In figure 2 the two significant PCs, according to cross validation, are plotted. Both PCs describe 85 % of the variance of the data set (PC1 74%, PC2 11%).
PC 1 describes a concentration gradient from the low concentration in Simpnäs in the spring (5) , to a high concentration in Simpnäs in the autumn (4) and a gradually higher level from Hornslandet (2) to Harufjärden (1) to Gävlebukten (3).
The differences in levels between Harufjärden (1), Hornslandet (2) and Gävlebukten (3) are small, according to PC1 and also the total PCB concentration given in table 2, but PC 2 reveals a different PCB pattern for the sample from Harufjärden (1). This difference can be an indication of different sources of exposure in Harufjärden compared to the other three sampling sites or a result of the fact that Harufjärden is located in a sub artic region where run off water from the artic region is collected.

CONCLUSIONS

In the quantitative analysis of halogenated organic compounds is GC/MS analysis combined with extensive sample clean up essential to provide high quality data.

Levels of PCBs in sediment and amphipods differ throughout the Gulf of Bothnia. The highest levels are seen in Simpnäs south in the Gulf of Bothnia, the lowest levels are seen in Hornslandet, a nature reserve.

Large seasonal fluctuations in PCB levels are seen in samples from the autumn (high) and the spring (low) for both sediment and amphipod samples.

PCA revealed differences in the PCB isomer pattern between Harufjärden, an area located in a sub artic region, and the other three locations.

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