Spatial Distribution of PCBs in Three-spined Stickleback from the Beach Zone in the Gulf of Gdańsk, Baltic Sea

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Abstract
The congener-specific data of PCBs including mono-*ortho* and non-*ortho* members are presented for three-spined stickleback collected from four spatially distant sites in the beach zone of the south-western part of the Gulf of Gdańsk. The lipid weight normalised concentration of total PCBs in sticklebacks ranged from 2700 to 4200 ng/g. The concentration of non- and mono-*ortho* PCBs when expressed as TCDD TEQs ranged from 0.054 to 0.087 pg/g lipids, and from 0.0015 to 0.0022 pg/g wet wt. Hexa-CBs (42-46%) were a dominating chlorobiphenyl homologue group in sticklebacks, followed by penta (31-39%), hepta- (10-14%), tetra- (6.9-8.5%), octa- (0.6-1.0%), tri- (0.7-0.8%) and nona-/deca-CB (<0.1%). The patterns of total PCBs displayed compositional similarity and implied on common source of pollution, possibly due to atmospheric deposition.

Key words: Polychlorinated biphenyls, chlorobiphenyls, PCBs, CBs, non-*ortho* PCBs, mono-*ortho* PCBs, stickleback, fish, Baltic Sea.

Introduction

Polychlorinated biphenyls (PCBs) are compounds that found numerous applications worldwide and become an ubiquitous contaminants in the environment. Nevertheless, there is lack a policy of identifying and controlling the sources of these chemicals on a global scale, and also at the national level in many countries. Up to 500 tonnes of PCBs (Sovol mixture) is produced annually by Russia till now (1). Since withdraw of PCBs from production and use in many countries, and also due to safe technologies of their disposal

developed mainly in the 1990s, a risk of adverse effects due to their presence in ecosystems should decrease (2).

Three-spined stickleback was selected as an indicator organism to investigate concentrations, spatial distribution and sources of man-made and persistent organochlorine compounds in coastal area of the Gulf of Gdańsk (3, 4), and in this communication are presented data on PCBs.

Materials and Methods

30 male and female adults of the three-spined sticklebacks (*Gasterosteus aculeatus*) were collected at four sites in the beach zone in the south-western part of the Gulf of Gdańsk from June 2 to July 1, 1992 (Figure 1).

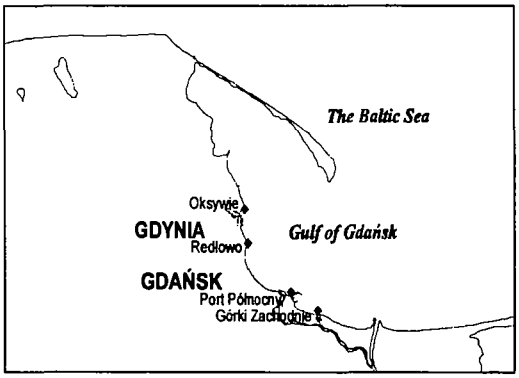

Figure 1. Sampling sites.

The analytical method used for determination of PCBs is a part of a multi-residue procedure allowing to determine simultaneously many organochlorines and polynuclear aromatic hydrocarbons (PAH) (5). After homogenization of the sample containing 30 whole individuals (145-310.6 g) with anhydrous sodium sulphate (1:7; baked at 550°C for 2 days), the powdered mixture was packed into a wide bore open glass column, spiked with an internal standard consisting of [$^{13}C_{12}$]- PCBs nos 77, 80, 118, 126, and 169, extracted with 500 ml of a mixture of acetone and *n*-hexane (2.5:1) followed by 500 ml of *n*-hexane and diethyl ether (9:1), to obtain a fat extract. The solvents were carefully evaporated on a water bath under vacuum pressure, using a rotary evaporator. Then, pure ethanol was added, to remove azeotropically co-extracted water, also under vacuum and using rotary evaporator. Bulk lipid removal was performed by means of the polyethylene film dialysis method (6). After dissolving the extracted lipids in cyclopentane, dialysis through the polymeric membrane was accomplished by changing the dialysate after 24, 48 and 72 hours. The three dialysate fractions, containing normally between 1-10% of the original

lipids, depending on sample size and matrix type, were combined and concentrated to a few milliliters using a rotary evaporator. The extract was split into two parts, of which of which 10% was used for the analysis of total PCBs and some organochlorine pesticides, while 90% was used for analysis of non- and mono-*ortho* PCBs and some other planar compounds. In the case of the 10% part of extract the remaining fat was removed on *n*-hexane wetted a Florisil gel column and PCBs were eluted with 32 ml *n*-hexane (fraction 1) and 38 ml 15% (v/v) methylene chloride in *n*-hexane (fraction 2). Before concentration of each fraction down to a final volume tetradecane (30 μl) was added to the vials as a keeper. An extract was than spiked with an internal standard 2 - recovery standard, consisting of 200 ng of [$^{13}C_{12}$]- labelled PCB no 101, and the results were corrected for recoveries. In the case of 90% part of the extract the remaining fat was removed on a combined column packed as follows from the bottom: glass wool, 10 ml potassium silica, a layer of neutral silica gel (1 g), 20 ml 40% sulphur acid silica gel, 10 ml 20% sulphuric acid silica gel, 20 ml neutral silica and a layer of sodium sulphate. The column length was 20 cm and the diameter was 38 mm. The gravimetric elution of planar organochlorines was done with 200 ml of *n*-hexane and 40 μl of tetradecane was added as a keeper before evaporation of the solvent. The extract was then fractionated by HPLC using an activated carbon column (Amoco PX-2; 2-10 μm, dispersed on LiChrospher RP-18; 15-25μm). Between the carbon column and the pre-column a filter valve (Valco Instruments Co. Inc., TX, US), was mounted enabling a backflush of the column. The elution from the HPLC carbon column was performed with 1% methylene chloride in *n*-hexane for 7.5 minutes (solvent 1), and then a gradient up to 10% toluene in *n*-hexane within 32.5 minutes (solvent 2). Solvents were of high purity grade (Burdick and Jackson, Muskegon, MI, US) and degassed with argon. Fraction one containing organochlorine pesticides and 2-4 *ortho* PCBs, was collected during the first 15 minutes and fraction two, containing mono-*ortho* PCBs, between 15 and 40 minutes. The total volume of solvents used was 160 ml, and the flow rate was 4 ml *per* min. Mono- and non-*ortho* PCBs together with PCDDs, PCDFs and R-PCDFs were eluted by backflush with 80 ml of toluene. The eluate was concentrated and spiked with $^{13}C_{12}$-2,2',4,5,5'-pentachlorobiphenyl (PCB no. 101) as recovery standard and evaporated to a final volume 30 μl after adding tetradecane as a keeper.

Analysis of the and detection of the bulk PCBs was accomplished by means of 60 m long capillary column (Rtx-5) gas chromatography and low resolution mass spectrometry (HRGC/LRMS). Additional procedural blanks were also performed and no interference were found. The mass spectrometer used was a Fisons MD 800 coupled to a Fison GC 8000 working in the Electron Impact (EI) ionisation mode using selected ion recording (SIR). For the confirmation/quantification of PCBs the two most abundant ions in the chlorine cluster of the molecular ion were monitored.

A gas chromatograph (Hewlett Packard 5890 GC) coupled to a high resolution mass spectrometer (VG Analytical 11-250 J, Altrincham, United Kingdom) was used for the determination of mono- and non-*ortho* PCB congeners. The mass resolution of the mass spectrometer (MS) was 8 000 amu, and the calibration gas was perfluorokerosone (PFK).

Injections were made in the splitless mode and the oven was temperature programmed as follows: initial temperature 180°C, initial time 2 min, 20°C/min to 200°C, then 4°C/min to 300°C (isothermal for 15 min). A Rtx-5 fused silica capillary column (60 m x 0.32 mm i.d.), coated with crossbond 5% diphenyl - 95% dimethyl polysiloxane with a film thickness of 0.25 μm was employed for the analysis. The ion source was kept at 250°C and operated under electron impact (EI) conditions at 70eV, and the MS was tuned in the selected ion recording (SIR) mode. For the confirmation/quantification of PCBs the two most abundant M^+ and $(M+2)^+$ ions in the chlorine cluster of the molecular ion were monitored at m/z 290 and 292 for tetra-CBs, m/z 324 and 326 for penta-CBs, *m/z* 358 and 360 for hexa-CBs. The ^{13}C- labelled PCBs internal and recovery standards were used to compensate for possible losses during the clean-up procedure. A procedural blank was performed with the every set of real samples examined.

Results and Discussion

The lipid weight normalised non-, mono-*ortho* and total PCB concentrations is comparable at the Oksywie and Redłowo sampling sites, and a somewhat lower at the Port Północny and Górki Zachodnie sites (Table 1). The gradient of concentration of PCBs in sticklebacks is different than was observed for DDTs and hexachlorobenzene - displaying elevated levels at the Górki Zachodnie site (at the outlet of Brave Vistula River-Dead Vistula River Channel) (4). Sticklebacks collected at the Oksywie site were also more contaminated with chloronaphthalenes and pentachlorobenzene (3). The Oksywie and Redłowo sites are the areas under influence of the shipyards, navy and merchant port activity of the city of Gdynia from the 1920s. Apart from sticklebacks also flounder, perch and lamprey collected under Gdynia were more contaminated with PCBs than fish from the other sites in the coastal area of the Gulf of Gdańsk (7). Many tri- through deca-CB were found in sticklebacks. The patterns of three- to deca-CB homologue groups are shown in Figure 2. Hexachlorobiphenyls (42-46%) were dominating homologue group in sticklebacks, followed by penta- (31-39%), hepta- (10-14%), tetra- (6.9-8.5%), octa- (0.6-1.0%), tri- (0.7-0.8%) and nona- (<0.1%) and deca-CB (<0.1%).

Chlorobiphenyl congeners such as nos 132/153, 138/160/163/164 and 118 are a dominating members in total PCBs in sticklebacks from all four sites (Figure 3). The patterns of total PCBs displayed compositional similarity and implied a common source of pollution. A revalatilization of PCBs from the surface matrix around Gdańsk was suggested as a source of these chemicals in atmospheric air sampled in the city of Gdańsk in 1991/1992 (8).

Concluding, a site dependent similarities in the pattern of tri- through deca-CB in sticklebacks indicate on the same source of pollution with these substances in the beach zone in the south-western part of the Gulf of Gdańsk - probably because of atmospheric transport and subsequent deposition from the atmosphere.

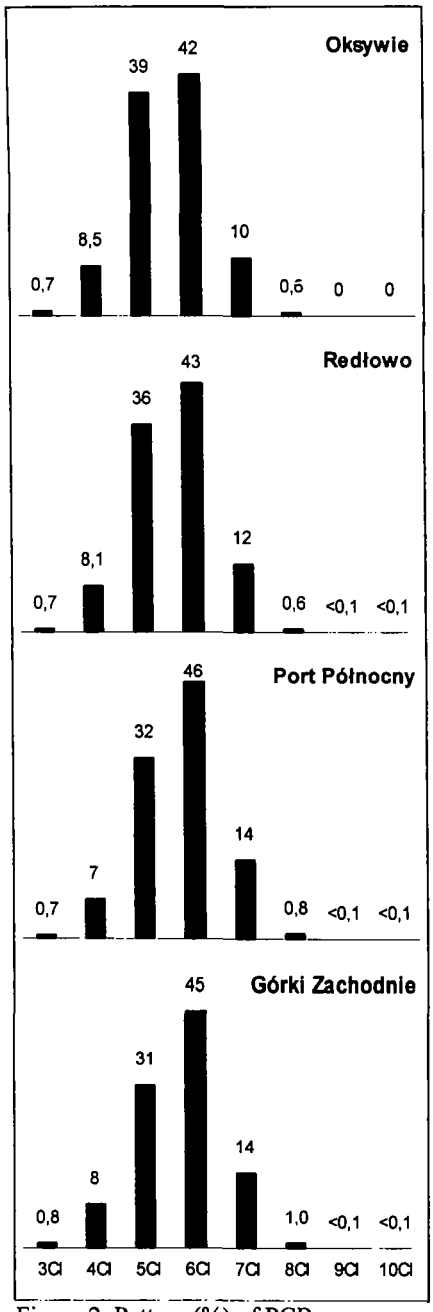

Figure 2. Pattern (%) of PCB homologue groups in three-spined stickleback.

Acknowledgements

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Table 1. The concentrations of non-*ortho*, mono-*ortho*, total PCBs (ng/g lipids) and TCDD TEQs of non- and mono-*ortho* PCBs (pg/g lipids) in three-spined stickleback

Compound	Site			
	Oksywie	Redłowo	Port Północny	Górki Zachodnie
Non-*ortho* PCBs				
No. 77	14	14	8.6	8.7
No. 126	3.9	4.0	2.5	2.7
No. 169	0.092	0.12	0.088	0.13
Mono-*ortho* PCBs				
105	310	330	180	210
114	18	17	9.5	11
118	890	760	480	540
123	36	5.4	17	38
156	130	130	82	93
157	30	33	19	20
167	100	110	70	84
189	4.1	4.4	2.9	3.9
Total PCBs	3800	4200	2700	3200
*TEQs (lipids)	0.087	0.087	0.054	0.060
*TEQs (wet weight)	0.0022	0.0021	0.0015	0.0015
Lipids (%)	2.4	2.4	2.7	2.4

*based on 1997 World Health Organization (WHO) TCDD TEFs for fish

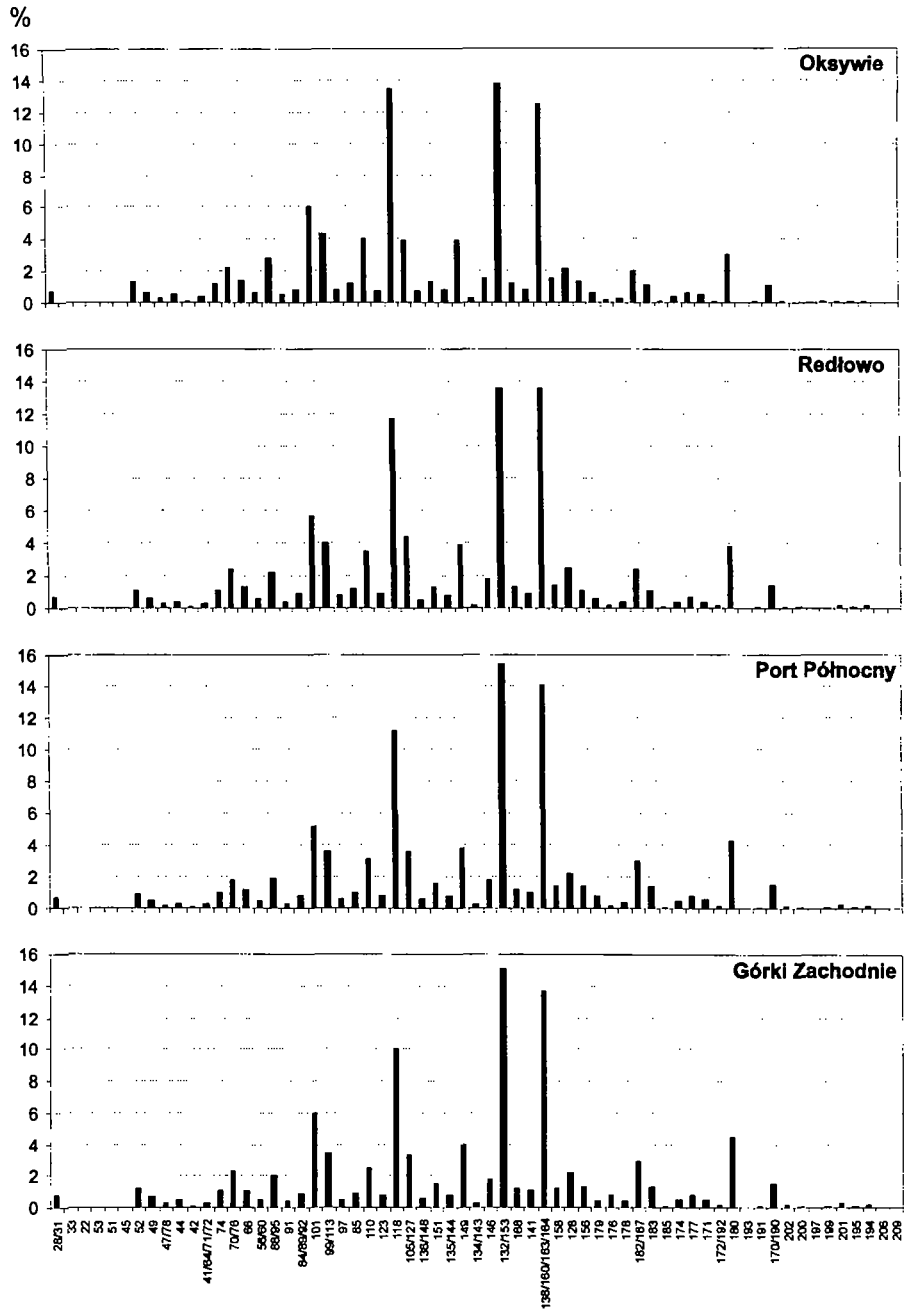

Figure 3. Pattern (%) of PCBs in three-spined stickleback.