TWO METHODS OF SAMPLE PREPARATION FOR ANALYSIS OF NON-ORTHO AND MONO-ORTHO PCB CONGENERs IN THE MUSCLES OF SELECTED FISH SPECIES

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are aromatic halide compounds, which consist of two phenyl rings saturated with chloride atoms. Specific physico-chemical properties of PCBs (low electrical conductivity, high thermal conductivity, high lipid solubility, low flammability) contributed to their wide industrial application, especially in electrotechnics. However, their slow biodegradation and high persistency resulted in accumulation of the compounds in various compartments of the environment for long periods, even years (Brzeziński 2002). PCBs have permeated to food products and their biggest amounts are accumulated in aquatic animals, especially fish (Atuma et al. 1998, Ciereszko 2002, Ciereszko and Witzcak 2002, Falandysz et al. 2002, Ciereszko et al. 2004, Baars et al. 2004, Davis et al. 2007). In the recent years, a decreasing trend has been observed for PCBs levels in fish from the Netherlands’s inland waters, the Belgian continental shelf and the North Sea. However, in fish from the Netherlands’s inland waters, concentrations of the more highly chlorinated congeners have remained on a constant level. No changes have been observed in the concentrations of chlorobiphenyls in fish from the Arctic Sea and the Baltic Sea (Paasivirta et al. 1994, Roose et al. 1998).

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During analytical procedure of PCB residues determination losses of these compounds may occur, which contribute to obtaining underestimated analytical results. Adequate analytical method of chlorobiphenyls determination should be applied to avoid the losses and obtain high recoveries, and furthermore to enable accurate estimation of the risk of consuming contaminated food.

The aim of this study was to determine if the method of sample preparation influences the recovery of non-ortho (PCB 77, 81, 126, 169) and mono-ortho (PCB 105, 114, 156, 157) PCB congeners (Fig. 1) in the muscles of selected fish species. Two methods of sample drying have been applied: rubbing in a mortar with anhydrous sodium sulphate and freeze drying.

Fig. 1. Chemical structures of non-ortho (a, b, c, d) and mono-ortho (e, f, g, h) PCB congeners
The study involved muscle tissue collected from edible portions of flounder, *Platichthys flesus* (L.); Atlantic halibut, *Hippoglossus hippoglossus* (L.); Atlantic mackerel, *Scomber scombrus* L.; Atlantic herring, *Clupea harengus* L.; and Atlantic salmon, *Salmo salar* L. The fish were bought as skinned fillets at retail in Szczecin in March 2006.

The fish tissues were blended in a homogenizer and analytical samples were taken for analyses of PCBs (10 g) and dry mass content (1 g). To examine recoveries of the analyzed compounds, the samples were fortified with a known amount of the internal standard solution Pesticides Surrogate Spike Mix (SUPELCO, USA, 4-8460), which was a solution of decachlorobiphenyl and 2,4,5,6-tetrachloro-m-xylene (100 µL of concentration 0.32 ppm). Some samples were additionally fortified with the standard solution of 8 PCB congeners (PCBMix-8 CERTAN, LGC Promochem, NE 90152) (50 µL of concentration 0.1 ppm). To prepare samples for gas chromatographic determination two methods were applied. Fish muscle tissues were dried by rubbing with anhydrous sodium sulphate or freeze dried (lyophilised). The samples were prepared according to the flowchart (Fig. 2).

Operating conditions of the chromatographic analysis were, as follows: column temperature program: 130°C (hold 0.5 min) → increase rate 7°C · min⁻¹→ 200°C (hold 5 min) → increase rate 4°C·min⁻¹→ 280°C (hold 10 min); single sample analysis time: 45.5 min; carrier gas (helium); flow rate: 1.1 mL · min⁻¹; pressure: 0.18 MPa (26.5 psi); detector MSD (HP 5973); column: HP-5MS (60.0 m; ID 250 µm; film thickness 2.25 µm).

Statistical treatment of the results was performed using the STATISTICA® 6.1 software. The statistical analysis included analysis of variance (ANOVA) and determination of correlation and variability coefficients. Toxic equivalents (TEQs) were calculated as a sum of products of the determined PCB concentrations in the analyzed samples and their toxic equivalency factors (TEFs) (Safe 1994, Van den Berg et al. 2006).

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**Fig. 2.** The flowchart of PCB analysis in fish samples.
RESULTS

Raw muscle tissue of the examined fish contained from 25.3% to 37.2% of dry weight, and from 3.09% to 17.3% of lipids. After freeze drying, the dry weight content in the samples ranged from 99.0% to 99.9% (Table 1).

Concentrations of the toxic PCB congeners (non-ortho: PCB 77, 81, 126, 169 and mono-ortho: PCB 105, 114, 156, 157) in the fish muscle tissue samples, dried with two different methods, are presented in Tables 2 and 3. The data were calculated as arithmetic means and standard deviations of PCB concentrations reported as ng·g⁻¹, on a dry or lipid weight basis.

The determined PCB concentrations ranged from 0.029 ± 0.0034 ng·g⁻¹ d.w. (dry weight) (PCB 157, salmon) to 5.035 ± 0.0207 ng·g⁻¹ d.w. (PCB 157, mackerel) in the lyophilisates and from 0.025 ± 0.0021 (PCB 126, salmon) to 5.118 ± 0.0547 ng·g⁻¹ d.w. (PCB 157, mackerel) in the samples rubbed with Na₂SO₄. Having calculated PCB concentrations in samples prepared with either the first or the second method, the largest concentrations were found for PCB 157 in mackerel (above 5 ng·g⁻¹ d.w.), while the lowest were observed for PCB 126 in all the examined fish species (Table 2, 3).

Recoveries of the internal standard (surrogate) in the freeze dries samples ranged from 57.6% for herring to 88.8% for salmon, and in the samples rubbed with anhydrous Na₂SO₄ from 63.8% for halibut to 97.5% for salmon (Fig. 3).

Comparison of the results of analytical samples and fortified samples determinations revealed that mean recoveries of PCBs ranged from 59.2% to 102.1%. In the freeze dried samples the recoveries were from 68.9% (PCB 157) to 79.2% (PCB 114), and in the samples rubbed with Na₂SO₄—from 72.4% (PCB 77) to 83.5% (PCB 156) (Fig. 4). Significance of differences among the obtained results was tested with Student’s t-test.

Statistically significant differences (P ≤ 0.05; Student’s t-test for independent samples, n = 5) between PCB concentrations in the samples prepared with the above mentioned methods were not observed. Only in herring, the differences were observed for all the examined PCB congeners, excluding PCB 81.

Table 1

| Fish species | Dry weight in raw sample | Lipids in raw sample | Dry weight after freeze drying | Lipids in dry weight content [%] |
|--------------|--------------------------|----------------------|-------------------------------|---------------------------------|
| Mackerel     | 37.24 ± 0.96*            | 17.34 ± 0.54         | 99.94 ± 0.07                  | 46.56                           |
| Flounder     | 25.31 ± 0.49             | 6.9 ± 1.0            | 99.25 ± 0.49                  | 27.26                           |
| Salmon       | 25.56 ± 0.46             | 3.09 ± 0.31          | 99.0 ± 0.14                   | 12.10                           |
| Halibut      | 29.09 ± 0.15             | 14.94 ± 1.33         | 99.55 ± 0.21                  | 51.36                           |
| Herring      | 31.92 ± 0.56             | 13.83 ± 0.9          | 99.9 ± 0.001                  | 43.34                           |

* Arithmetic mean ± standard deviation (n = 5).

Fig. 3. Recoveries of surrogate (decachlorobiphenyl, PCB 209) in the muscle tissue of various fish species prepared for analysis with two methods: freeze drying and rubbing with anhydrous sodium sulphate, (n = 5)
| PCB Congener | Non-ortho PCB | Mono-ortho PCB | Mackerel | Herring | Halibut | Salmon | Flounder |
|--------------|---------------|----------------|----------|---------|---------|--------|----------|
| PCB 77       | FD 0.228 ± 0.0030 | 0.311 ± 0.0008  | 1.254 ± 0.0082  | 2.746 ± 0.0032  | 0.205 ± 0.0319  |
| AS 0.222 ± 0.0968 | 0.666 ± 0.1043  | 1.548 ± 0.0495  | 2.310 ± 0.5192  | 0.216 ± 0.0570  |
| PCB 81       | FD 0.244 ± 0.034 | 0.694 ± 0.0038  | 1.1900 ± 0.0007 | 0.650 ± 0.0020  | 0.282 ± 0.0201  |
| AS 0.245 ± 0.0700 | 0.802 ± 0.0576  | 1.220 ± 0.0357  | 0.634 ± 0.0781  | 0.349 ± 0.0230  |
| PCB 126      | FD 0.081 ± 0.003 | 0.069 ± 0.0082  | 0.035 ± 0.0013  | 0.0475 ± 0.0095 | 0.181 ± 0.1471  |
| AS 0.075 ± 0.0020 | 0.062 ± 0.004   | 0.025 ± 0.0021  | 0.049 ± 0.0326  | 0.203 ± 0.0139  |
| PCB 169      | FD 0.148 ± 0.0195 | 0.3280 ± 0.0099 | 0.034 ± 0.0101  | 0.157 ± 0.0125  | 0.382 ± 0.0830  |
| AS 0.184 ± 0.0260 | 0.367 ± 0.0724  | 0.282 ± 0.0033  | 0.170 ± 0.008   | 1.395 ± 0.2499  |
| PCB 105      | FD 0.756 ± 0.0195 | 2.797 ± 0.0289  | 0.263 ± 0.0002  | 1.407 ± 0.0105  | 2.186 ± 0.0073  |
| AS 0.680 ± 0.0062 | 2.815 ± 0.0803  | 0.252 ± 0.0270  | 1.609 ± 0.0923  | 2.072 ± 0.0478  |
| PCB 114      | FD 0.175 ± 0.0116 | 0.1530 ± 0.0022 | 0.249 ± 0.0191  | 0.0635 ± 0.0095 | 0.801 ± 0.0192  |
| AS 0.277 ± 0.1007 | 1.970 ± 0.1755  | 0.269 ± 0.0961  | 0.709 ± 0.2000  | 0.787 ± 0.0629  |
| PCB 156      | FD 0.147 ± 0.0041 | 0.795 ± 0.0030  | 0.025 ± 0.0034  | 1.390 ± 0.1178  | 1.156 ± 0.0263  |
| AS 0.231 ± 0.0279 | 0.847 ± 0.0578  | 0.027 ± 0.0140  | 1.519 ± 0.0783  | 1.301 ± 0.0432  |
| PCB 157      | FD 0.098 ± 0.0075 | 0.893 ± 0.0069  | 0.029 ± 0.0034  | 2.295 ± 0.0316  | 5.035 ± 0.0207  |
| AS 0.107 ± 0.0393 | 0.824 ± 0.0319  | 0.032 ± 0.0023  | 2.817 ± 0.0521  | 5.118 ± 0.0547  |
| TEQs [ng-TEQ/g dry weight] | FD 0.0127 ± 0.0009 | 0.0171 ± 0.0011 | 0.0050 ± 0.0004 | 0.0101 ± 0.0013 | 0.0299 ± 0.0072 |
| AS 0.0132 ± 0.0031 | 0.0177 ± 0.0063 | 0.0055 ± 0.0003 | 0.0106 ± 0.0007 | 0.0326 ± 0.0089 |
| TEQs [ng-TEQ/g wet weight] | FD 0.00405 | 0.00497 | 0.00128 | 0.00256 | 0.01113 |
| AS 0.00421 | 0.00515 | 0.00141 | 0.00268 | 0.01214 |
| Dietary intake [Pg-TEQ/kg body weight/day] | FD 0.8398* | 1.03 | 0.2647 | 0.5295 | 2.306 |
| AS 0.8727 | 1.0666 | 0.2912 | 0.5558 | 2.515 |

* average body weight 70 kg.
FD, Freeze dried samples.
AS, Samples rubbed with anhydrous sodium sulphate.
Residues of PCB congeners in various fish species (ng \cdot g^{-1} lipid weight), prepared for analysis with two methods: freeze drying and rubbing with anhydrous sodium sulphate

|              | Herring | Halibut | Salmon | Flounder | Mackerel |
|--------------|---------|---------|--------|----------|----------|
|              | arithmetic mean ± standard deviation, \(n = 5\) for each species |         |        |          |          |
| PCB 77 Non-ortho PCB |         |         |        |          |          |
| FD           | 1.649 ± 0.022 | 2.082 ± 0.005 | 40.583 ± 0.265 | 39.797 ± 0.046 | 1.182 ± 0.184 |
| AS           | 1.605 ± 0.699 | 4.458 ± 0.698 | 50.097 ± 1.602 | 33.478 ± 7525 | 1.246 ± 0.329 |
| PCB 81 Non-ortho PCB |         |         |        |          |          |
| FD           | 1.765 ± 0.244 | 4.645 ± 0.026 | 38.511 ± 0.023 | 9.420 ± 0.029 | 1.626 ± 0.116 |
| AS           | 1.772 ± 0.506 | 5.368 ± 0.386 | 39.482 ± 1.155 | 9.188 ± 1.132 | 2.013 ± 0.133 |
| PCB 126 Non-ortho PCB |         |         |        |          |          |
| FD           | 0.586 ± 0.024 | 0.462 ± 0.055 | 1.133 ± 0.042 | 0.674 ± 0.137 | 1.044 ± 0.084 |
| AS           | 0.542 ± 0.053 | 0.412 ± 0.080 | 0.809 ± 0.068 | 0.710 ± 0.047 | 1.171 ± 0.080 |
| PCB 169 Non-ortho PCB |         |         |        |          |          |
| FD           | 1.071 ± 0.141 | 2.195 ± 0.066 | 1.1003 ± 0.322 | 2.275 ± 0.180 | 2.203 ± 0.475 |
| AS           | 1.330 ± 0.6019 | 2.456 ± 0.485 | 0.913 ± 0.108 | 2.464 ± 0.116 | 2.278 ± 0.5367 |
| PCB 105 Mono-ortho PCB |         |         |        |          |          |
| FD           | 5.466 ± 0.141 | 18.722 ± 0.193 | 8.511 ± 0.0002 | 20.391 ± 0.152 | 12.607 ± 0.042 |
| AS           | 4.917 ± 0.044 | 18.842 ± 0.538 | 8.155 ± 0.874 | 23.319 ± 1.338 | 11.949 ± 0.276 |
| PCB 114 Mono-ortho PCB |         |         |        |          |          |
| FD           | 1.266 ± 0.084 | 10.241 ± 0.015 | 8.058 ± 0.618 | 9.203 ± 0.136 | 4.619 ± 0.111 |
| AS           | 2.003 ± 0.728 | 13.186 ± 1.175 | 8.706 ± 1.794 | 10.275 ± 2.898 | 4.539 ± 0.363 |
| PCB 156 Mono-ortho PCB |         |         |        |          |          |
| FD           | 1.063 ± 0.029 | 5.321 ± 0.020 | 0.809 ± 0.110 | 20.145 ± 1.707 | 6.667 ± 0.152 |
| AS           | 1.670 ± 0.202 | 5.669 ± 0.387 | 0.874 ± 0.116 | 22.015 ± 1.135 | 7.503 ± 0.249 |
| PCB 157 Mono-ortho PCB |         |         |        |          |          |
| FD           | 0.709 ± 0.054 | 5.977 ± 0.046 | 0.939 ± 0.109 | 33261 ± 0.458 | 29.037 ± 0.119 |
| AS           | 0.774 ± 0.184 | 5.515 ± 0.214 | 1.036 ± 0.192 | 40.826 ± 0.755 | 29.516 ± 0.316 |
| TEQs [ng-TEQ/g lipids in dry weight] |         |         |        |          |          |
| FD           | 0.0916 ± 0.0067 | 0.1149 ± 0.0075 | 0.1624 ± 0.0141 | 0.1449 ± 0.0192 | 0.1727 ± 0.0993 |
| AS           | 0.0951 ± 0.0015 | 0.1182 ± 0.0424 | 0.1257 ± 0.0107 | 0.1539 ± 0.0095 | 0.1877 ± 0.0514 |

FD, Freeze dried samples.
AS, Samples rubbed with anhydrous sodium sulphate.
Coefficients of variation (CV) were calculated for the data obtained in the study (Table 4):

\[
CV = \frac{SD}{\bar{x}} \times 100\% ,
\]

where SD is standard deviation; \( \bar{x} \) is mean concentration of PCB congener.

In the freeze dried samples CV varied between 0.11% (PCB 81, halibut) and 7.14% (PCB 157, halibut). In the samples rubbed with \( \text{Na}_2\text{SO}_4 \) CV ranged from 0.54% (PCB 126, mackerel) to 7.27% (PCB 157, salmon).

Additionally, toxic equivalents TEQs were calculated on the basis of the toxic equivalency factors TEFs (Van den Berg et al. 2006) and PCB concentrations in the samples. For the freeze dried samples, TEQs ranged from 0.0050 ng-TEQ \( \cdot \text{g}^{-1} \) d.w. in salmon to 0.0299 ng-TEQ \( \cdot \text{g}^{-1} \) d.w. in mackerel, and for the samples rubbed with anhydrous sodium sulphate they were from 0.0055 ng-TEQ \( \cdot \text{g}^{-1} \) d.w. in salmon to 0.0326 ng-TEQ \( \cdot \text{g}^{-1} \) d.w. in mackerel (Fig. 5). When converted into lipids, the highest TEQ values were found, in case of both methods, in mackerel, and the lowest in herring (Fig. 5).

**DISCUSSION**

PCBs penetrate into fish body mainly through the gills, and also through the alimentary tract, their bioaccumulation coefficients in organs being high, even to several thousands (Kulkarni and Kavara 1990, Strandberg et al. 1998, Ruus et al. 1999, Crimmins et al. 2002). The real toxicological hazard is posed by dioxin-like PCBs, and fortunately these PCB congeners occur in fish bodies in

| Fish species | PCB 77 | PCB 81 | PCB 126 | PCB 169 | PCB 105 | PCB 114 | PCB 156 | PCB 157 |
|--------------|--------|--------|---------|---------|---------|---------|---------|---------|
| Herring      | 1.30   | 6.66   | 2.38    | 1.29    | 2.64    | 6.59    | 3.65    | 5.89    |
| Halibut      | 2.63   | 0.11   | 1.94    | 4.07    | 3.62    | 1.23    | 1.07    | 7.14    |
| Salmon       | 3.25   | 2.93   | 3.82    | 4.77    | 6.02    | 4.13    | 3.53    | 4.37    |
| Flounder     | 0.43   | 4.70   | 1.57    | 4.58    | 2.59    | 0.70    | 1.72    | 4.01    |
| Mackerel     | 3.72   | 4.81   | 1.02    | 4.68    | 0.26    | 1.71    | 1.60    | 2.91    |

**Table 4** Coefficients of variation CV [%] in the muscle tissue of various fish species prepared with two methods: freeze drying and rubbing with anhydrous Na\(_2\)SO\(_4\), (n = 5 for each species)

| Fish species | CV [%] | **Freeze dried samples** | | | | | |
|--------------|--------|--------------------------|--|--|--|--|
| Herring      |        |                          | | | | |
| Halibut      |        |                          | | | | |
| Salmon       |        |                          | | | | |
| Flounder     |        |                          | | | | |
| Mackerel     |        |                          | | | | |
| Herring      |        |                          | | | | |
| Halibut      |        |                          | | | | |
| Salmon       |        |                          | | | | |
| Flounder     |        |                          | | | | |
| Mackerel     |        |                          | | | | |

**Fig. 4.** Mean recoveries (±standard deviation) of non-ortho and mono-ortho PCB congeners in the muscle tissue of five fish species prepared for analysis with two methods: freeze drying and rubbing with anhydrous sodium sulphate, (n = 5); CV, coefficient of variation.
much lower concentrations than the indicator PCBs (i.e., PCB 180, PCB 153) (Falandysz 2002). Adequate procedures of sample preparation, assuring the lowest possible losses of analytes, are necessary for accurate estimation of the risk of consuming contaminated food products. Special attention should be focused on fish, as the aquatic animals are particularly exposed to PCB accumulation in their tissues.

The study of Berdié and Grimalt (1998) revealed, that PCB content in freeze dried fish muscle tissue was significantly lower than in the same tissue dried by rubbing with anhydrous sodium sulphate. Moreover, Falandysz (1982) observed that freeze drying reduced PCB content in contaminated eggs by 25 percentage points, and in contaminated shrimps by nearly 50 percentage points. These data imply, that during freeze drying significant amounts of PCB compounds evaporate from the dried matrices.

De Voogt et al. (2000) obtained PCB recoveries in freeze dried bivalves on the level of 53 ± 36%. However Thomas et al. (1998) reported, that recoveries of 53 PCB congeners in freeze dried milk samples amounted from 69 to 96 (±10) percentage points.

Losses of PCBs during sublimation drying depend mainly on the properties of particular PCB congeners and their concentrations in the dried product. Other important factors are the properties of the product: fat content, physicochemical properties (e.g., density, viscosity) and changes of the product’s structure produced by freeze drying process (Falandysz 1982).

Our study confirmed previous reports of other researchers to a large extent. The obtained values are especially similar to PCB recoveries reported by Thomas et al. (1998). The authors also claim, that reliability of an analytical method is confirmed by coefficients of variation lower than 30%. In our study CV values were lower than 8%.

Toxic equivalents TEQs calculated for the freeze-dried samples ranged from 0.0050 ng-TEQ · g⁻¹ dry weight (d.w.) in salmon to 0.0299 ng-TEQ · g⁻¹ dry weight in mackerel. In the samples rubbed with anhydrous sodium sulphate, the highest TEQ values were also in mackerel (0.0326 ng-TEQ · g⁻¹ d.w.), and the lowest in salmon (0.0055 ng-TEQ · g⁻¹ d.w.).

According to the Commission Regulation (EC) No. 1881/2006 of 19 December 2006 (Anonymous 2006), the maximum level of dioxin-like PCBs, calculated as the difference between the level of the sum of dioxins and dioxin-like PCBs (WHO-PCDD/F-PCB-TEQ) and the level of dioxins (WHO-PCDD/F-TEQ), in the muscle meat of fish and fishery products, excluding eel, is set at 4.0 pg-TEQ · g⁻¹ fresh weight, and in the muscle meat of eel and products thereof—8 pg-TEQ · g⁻¹ fresh weight. A comparison of the data obtained in the study and the maximum levels set by the Regulation indicated, that only the muscle meat of mackerel contained more than 4 pg-TEQ · g⁻¹ fresh weight (Table 2).

Tolerable weekly intake (TWI) for dioxins and dioxin-like PCBs is 14 pgWHO-TEQ · kg⁻¹ body weight, which establishes TDI (tolerable daily intake) on the level of 2 pgWHO-TEQ · kg⁻¹ body weight (OJ L 364 2006). In Poland, annual consumption of fish (marine and freshwater) and fishery products per one person averaged 4.8 kg in 2002, and 5.3 kg in 2003 (Anonymous 2004). This establishes daily consumption on the level of 0.0145 kg per day per person. On the basis of the calculated TEQs and the daily consumption of fish and fishery products per person (0.0145 kg), were estimated the PCB intake from the consumed fish. For the freeze dried fish the dietary intake amounted to 0.2647–2.306 pg-TEQ · kg⁻¹ body weight per day, and for the samples dried with anhydrous sodium sulphate 0.2912–2.515 pg-TEQ · kg⁻¹ body weight per day.

CONCLUSIONS

The analysed non-ortho and mono-ortho PCB congeners have been detected in all the examined fish species. PCB 157 was the most abundant congener, its concentrations (dry weight basis) being the highest in mackerel muscle meat. The lowest concentrations (dry weight basis) were observed for PCB 126 in the salmon meat.

Toxicity equivalents (TEQs) calculated for the examined fish species ranged between 1.278 pg-TEQ · g⁻¹ w.w. in mackerel (Fig. 5, a) and 0.0044 pg-TEQ · g⁻¹ w.w. in salmon (Fig. 5, b).
for salmon (freeze dried) and 12.14 pg-TEQ·g⁻¹ w.w. for mackerel (rubbed with Na₂SO₄).

The results obtained with both methods have not differed significantly (P < 0.05), although freeze drying resulted in slightly higher losses of PCB congeners. The values of coefficient of variation below 8% confirm good precision of both methods applied. Despite of slightly smaller recoveries, freeze drying can be applied because of solvent saving and easier sample preparation.

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