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Application of validated method for determination of selected polychlorinated biphenyls in human adipose tissue samples

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Abstract

The validation method has been employed to determine PCBs in human female adipose tissue and in different tissue samples (brain, kidney, liver and adipose tissue) collected from five donors from the Wielkopolska region, Poland. The contents of 15 PCB congeners have been determined in the tissues (brain, kidney, liver and adipose tissue) of 5 donors aged 18–78. The highest PCB concentrations have been found in the adipose tissue, in which the total of 15 congeners occur in the amount 78–591 ng/g tissue, and in the liver tissue in the amount 16–94 ng/g tissue. In 16 samples of adipose tissue taken from women aged 25–36, 4 PCB congeners (PCB 105, 138, 150 and 180) have been determined. The mean content of the total of these congeners has been 41 ng/g tissue. This result is lower than the concentration of analogous PCB in the tissues collected from women from the other European countries, which well correlates with the low content of PCB in the food produced in Poland.

Keywords: PCB; Human adipose tissue; Gas chromatography; Quality assurance

1. Introduction

Polychlorinated biphenyls (PCB) along with polychlorinated dibenzo-*p*-dioxins, dibenzo-furans, polychlorinated naphthalenes and some organochlorine pesticides have been included in the list of 12 persistent organic pollutants (POPs) established at the Stockholm convention in 2001 (UNEP, 2003). The toxicity of PCB depends to a significant degree on the percent content of chlorine and the degree of pollution with other aromatic hydrocarbons. Because of the hydrophobic and lipophilic properties, PCBs tend to accumulate in lipid rich tissues (Crouch and Barker, 1997; Albaigés, 1999).

The literature provides many reports on the level of PCBs in adipose tissue of inhabitants of different countries (Japan, Belgium, Turkey, Finland, Italy and Chile) (Mariottini et al., 2000; Smedes and Saukko, 2001; Takenaka et al., 2002; Choi et al., 2002; Çok and Şatiroğlu, 2004; De Saeger et al., 2005), although there are no current data on the content of PCB in tissues from the Polish subjects. The reports on the content of PCB in human tissue in the population of Poles provide total PCBs determination in adipose tissue expressed in Aroclor and not the contents of individual congeners (Tanabe et al., 1993; Falandysz et al., 1994; Ludwicki and Góralczyk, 1994; Struciński et al., 2002). To the best of our knowledge no reports have been published on the determination of individual PCB congeners in human tissue of the subjects from the Wielkopolska region. Similarly, no data have been found on the contents of PCB in different tissues coming from the same donor.

To ensure high quality of results of exposure and risk assessment, a specific analytical method used for monitoring PCBs should be demonstrated in laboratory experiments. Credible results of PCB determination mainly in biological samples can be obtained only after validation of the analytical procedures used.

The aim of this study was to validate the method proposed for determination of selected PCB congeners in human adipose tissue and the application of this method for determination of PCB congeners in adipose tissue samples collected from 16 women aged 25–36, from the Wielkopolska region. Moreover, for the first time the content of PCB in the tissues from four organs collected from each of the donor has been determined.

2. Materials and methods

On the basis of the reported abundance and toxicity (Albaigés, 1999), the following congeners, chlorine position at biphenyl molecule are listed in Table 1,

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(IUPAC nos.) 28, 52, 101, 105, 114, 118, 128, 138, 149, 153, 156, 170, 180 and 187 were targeted for validation of the method for determination of selected PCB in adipose tissues. PCB 30 and 209 were used as internal standards (IS). All PCBs used were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany).

In accordance with the demands of ethic, the biological samples of human tissues were collected on a formal consent of the Bioethical Commission at the University of Medical Sciences in Poznań. Human tissue samples were collected from January 2000 to December 2001 from dead bodies at the Department of Forensic Medicine in Poznań University of Medical Sciences. The donors were three women and two men aged 18–78. Samples of the human adipose tissue were also obtained from the Department of Perinatology and Women's Diseases of the Poznań University of Medical Sciences from Polish women living in Wielkopolska region and were collected in 2004. All samples were wrapped with aluminium foil and stored in freezer at −20 °C until the analysis. The collected tissue samples directly prior to analysis were stored at room temperature and then were homogenized in a homogenizer Ultra Turax T25 for 5 min at a speed of 11,000 rotations/min.

Unspiked and spiked vegetable oil was used for the method validation. The samples of tissues, reference material and vegetable oil used for the validation of the method were subjected to KOH in the process of saponification. The extractions were performed using hexane. The hexane solution was concentrated and the extracts were cleaned-up with concentrated H_2SO_4. After that the extracts were quantitatively transferred to a Florisil SPE cartridge. The internal standards (IS) (PCB 30 and 209) were added to concentrated extracts before their analysis by gas chromatography.

The identification and quantification of the analytes in purified extracts were carried out by gas chromatography (GC) with electron capture detection (ECD) (Shimadzu GC-14) equipped with an Rtx5 column (Restek 60 m × 0.25 mm ID, 0.25 μm coating) fused silica capillary column (5% diphenyl polysiloxane, 95% diethyl polysiloxane) and PE-XLB column (60 m × 0.25 mm ID, 0.25 μm coating). The content of PCB congeners mentioned above was calculated using chromatographic peak highs provided by a CR 6A Chrompack Shimadzu integrator.

The quality of the method examined was verified in an interlaboratory exercise. Within the framework of 36 rounds of Quasimeme (Quality Assurance of Information in Marine Environmental Monitoring) two samples: fish muscle (Limanda limanda from Netherlands sea-coast (QOR078BT)) and shellfish (Mytilus edulis from Striven Lake in Scotland (QOR079BT)) were analyzed. The nine congeners PCB (28, 52, 101, 105, 118, 138, 153, 156 and 180) were analyzed in the test materials. The estimated levels of PCB ranged from 0.14 ppb (PCB 156) to 7.27 ppb (PCB 153). To assess the participant's results (33 laboratories) the z-score was calculated. The assigned value on which the z-score is based is derived from the Cofino model mean (Report, 2004). From 20 results only one was in $2 < |Z| < 3$ range (PCB 105) as a questionable performance, the rest were in the satisfactory performance range i.e. $|Z| < 2$.

3. Results and discussion

Identification of the compounds analyzed was performed by a comparison of the relative retention times (RRT_{30} or RRT_{209}) corresponding to the peaks from the calibration standards with the peaks from purified extracts of all samples examined.

The intermediate precision of the RRT_{30} or RRT_{209}, calculated from 17 replicate analyses of a standard mixture of the PCB congeners was between 0.013 and 0.124% or 0.007 and 0.070%, respectively. The following elution order of the congeners was established: 30 IS, 28, 52, 74, 101, 149, 118, 114, 153, 105, 138, 187, 128, 156, 180, 170 and 209 IS.

The limit of detection (signal-to-noise ratio = 3) and quantification limit (signal-to-noise ratio = 10) for the specific compounds depended significantly on the detection method in the GC analysis. As follows from the data presented in Table 1, the method's limit of detection ranged between 0.03 and 0.08 ng/g oil for ECD, and the quantification limit of the congeners for ECD was 0.14–0.27 ng/g oil.

The linearity of the ECD response for each congener was determined by plotting calibration graphs of peak height/mass injected versus mass injected (Wells et al., 1992). The linear range for the PCB congeners was between 20 and 350 ng/mL. The repeatability of the peak heights calculated from five replicate analyses of a standard mixture of the PCB congeners was between 3.82 and 6.51%. The linearity of the method was assessed on the basis of the oil samples spiked with increasing amounts of particular congeners in the range from ∼50 to 200% (2.4 ng/g oil for PCB 74–124 ng/g oil for PCB 153) of their levels recently reported which have been determined in human adipose tissue samples in different countries (Mariottini et al., 2000; Holoubek et al., 2000). The calibration parameters and the correlation coefficients for all congeners studied were calculated from the equation: $y = a + bx$, and listed in Table 2. The data from Table 2 confirmed statistical significance of the regression coefficient "b" ($t_b > t_{\alpha f}$) of linear calibration equation and insignificance of the coefficient "a" ($t_a < t_{\alpha f}$).

To complete the validation of the method, the recoveries of the analytes from the sample of spiked brain tissue of an inhab-

Table 1
Detection and quantification limit of the method for determination of selected polychlorinated biphenyls, PCB, congeners in oil

PCB		Detection limit (ng/g oil)	Quantification limit (ng/g oil)
IUPAC no.	Structure	GC-ECD	GC-ECD
28	2,4,4′-Cl_3	0.08	0.25
74	2,2′5,5′-Cl_4	0.08	0.27
101	2,2′4,5,5′-Cl_5	0.08	0.25
105	2,3,3′4,4′-Cl_5	0.05	0.17
114	2,3,4,4′,5-Cl_5	0.05	0.17
118	2,3′4,4′,5-Cl_5	0.06	0.21
128	2,2′,3,3′,4,4′-Cl_6	0.05	0.18
138	2,2′,3,4,4′,5′-Cl_6	0.05	0.18
149	2,2′,3,4′,5′,6-Cl_6	0.08	0.27
153	2,2′,4,4′5,5′-Cl_6	0.08	0.25
156	2,3,3′,4,4′,5-Cl_6	0.05	0.15
170	2,2′,3,3′,4,4′,5-Cl_7	0.03	0.14
180	2,2′,3,4,4′,5,5′-Cl_7	0.05	0.18
187	2,2′,3,4′,5,5′,6-Cl_7	0.06	0.19