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Environmental Contamination and Toxicology

PCB Residues in a Breast Cancer Patient Population

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Polychlorinated biphenyls (PCBs) are a complex mixture that comprise theoretically 209 congeners, some of which can cause a wide range of effects in experimental animals, wild life and humans. PCBs have been marketed for use in hydraulic fluids, printing inks, paints, transformers, and many other applications. Some PCBs were used as pesticides. With organochlorine pesticides, these chemicals constitute what is called Persistent Organic Pollutants (POPs), due to their great chemical stability, their lipid solubility, their ubiquitous prevalence in environment (Pauwels et al.2000; Asplund et al.1994).

The presence of POPs in human serum and adipose tissue has been reported in many studies over the last three decades. Recently, many epidemiological studies have suggested that exposure to POPs may increase the risk of endocrine disruption by interference with estrogen metabolism (Bradlow et al. 1995), elevation of the amount of bioavailable estradiol (Reickman et al. 1993), or synergistic effects on estrogen responsive genes (Arnold et al. 1996). POPs have been reported to cause a variety of effects including hypofertility in man (decrease in semen quality), carcinogenesis in man (prostate, testis) and woman (breast, ovary), immunological perturbations and neurological effects (Kodovanti et al.1998; Demers et al. 2000).

Breast cancer is the most common cancer in women, affecting one out of every 10 women. Breast carcinogenesis is a matter of debate. The aetiology of breast cancer is multifactorial, including : genetic predisposition to breast cancer, damage to breast cell DNA by environmental or metabolic toxins, epigenetic stimulation by endogenous and exogenous hormones (Mc Mahon et al. 1973). Hormones may act to promote the late stages of carcinogenesis and to facilitate the proliferation of malignant cells and it seems admitted that long-term use of postmenopausal hormones increases the risk of breast cancer (Colditz et al. 1995). The question of whether environmental exposure to POPs, particularly to PCBs, increases the risk of breast cancer has been debated in the literature with controversial results (Colditz et al. 1998; Charlier et al. 2003a). Few epidemiologic studies have examined the association between PCBs and breast cancer risk by congener.

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In the present study, we have compared the blood levels of seven PCBs congeners (IUPAC n° 28, 52, 101, 118, 138, 153 and 180) in 100 women at the time of breast cancer discovery and in 100 age-matched healthy controls.These pollutants were quantified simultaneously using a gas chromatographic analyser coupled to an ion-trap mass spectrometer detector. The variety and serum levels of detected PCBs in cases and controls were compared.

MATERIALS AND METHODS

The present case-control study involved 100 women (55.63 ± 10.23 yrs) suffering from breast cancer with a surgical issue were considered as cases. Control subjects (100) were selected at random in a population of presumably healthy women (54.69 ± 12.30 yrs) consulting for routine vaginal cytological examinations. Both groups were similar with respects to age ($p = 0.52$), smoking habits (33.33 % smokers in controls vs 35.60 % in cases, $p = 0.63$), living environment (48.56 % urban living in controls vs 50.80 % in cases, $p = 0.56$) and BMI (21.54 vs. 23.60, $p = 0.49$). All patients gave their informed consent for participating in the study. For controls, blood specimens were taken at the time of the examination, whereas for women with breast cancer, samples were collected prior to surgery. Blood samples were drawn in the early morning after overnight fasting and were immediately centrifuged with serum specimens kept frozen at −18°C until assay (within one week).

The identification and quantification of seven PCBs in serum were done using a gas chromatographic analyser coupled to an ion-trap mass spectrometer detector (Saturn 2000, Varian) (Charlier et al. 2003b). Calibration samples or serum samples (1 ml) were extracted with 1 ml acetonitrile, 1 ml K_2CO_3 and 5 ml petroleum ether / diethyl ether (98:2), v/v) after addition of 10 µl of internal standard (aldrin, 10 mg/l). The samples were vortex mixed, then centrifuged (2500 rpm, 5 min) and the supernatant was transferred to a collection vial. A repeated extraction with another 5 ml of petroleum ether / diethyl ether is performed and supernatant is added to the first one. The two fractions are simultaneously transferred to the pre-conditioned extraction column. The solution drawn through the cartridge is collected and evaporated under mild nitrogen at 35°C. The residue was reconstituted in 100 µl hexane and 1 µl was injected in split/ splitless (ratio : 35) mode onto the GC. Analysis was performed on an ion-trap mass spectrometer coupled to a gas chromatography (Saturn 2000, Varian, Harbor City, CA). The samples were eluted through a HP5 Trace (30m, 0.25 mm id, 0.25 µm film) column (Agilent, Waldbronn, Germany). For detection, the MS was operated in the electronic impact mode (70 eV). Selected ion storage was used for identification and quantification. Limits of quantification (LOQ) were approximately 1 ppb for PCBs 28, 52, 138 and 180 and 2 ppb for PCBs 101, 118 and 153.

Petroleum ether, n-hexane and diethyl ether were obtained from Baker (Phillipsburg, USA). Potassium carbonate and sodium sulfate were from Sigma (Steinheim, Germany). Methanol and acetonitrile were from Labscan (Dublin,

Ireland). All reagents were of the highest purity available. For SPE, Bond Elut Certify® (130 mg/3 ml) extraction columns from Varian (Harbor City, CA) were used. All PCBs and internal standard (aldrin) were purchased from Dr Ehrenstorfer (Augsburg, Germany). Samples were analysed in a blind procedure together with controls consisting of samples spiked with 2 or 5 ppb of each PCB.

Serum levels of PCBs were expressed as mean ± SD. When lower than LOQ, serum levels of PCBs were recorded as "0" value for further calculation. Comparison between cases and controls was performed using a Student's *t*-test. Relationship between age and total PCB content in serum was tested by linear regression. All results were considered to be significant at the 5% critical level.

RESULTS AND DISCUSSION

The distribution of all detected compounds is presented in Table 1.

Only 10% of the controls and 7% of the case were free of any PCB residue. The most frequently detected residue was PCB153, with a serum concentration higher than LOQ in 42% of the controls and 59% of the cases.

PCBs 28, 118 and 180 were never detected. PCBs 52, 101, 138 and 153 have been found positive both in controls and cancer women.

Only one residue was detected in 65% of all samples. Two PCB residues were found simultaneously in 25% of samples and three residues were detected together in 4% of the cases (2 % of total samples).

Table 1. Distribution of PCBs in controls and cases

	Controls	Cases	Frequency (%)
No residue	10	7	8.5
PCB 52, alone	12	5	8.5
PCB 101, alone	10	8	9.0
PCB 138, alone	12	6	9.0
PCB 153, alone	37	39	38.5
PCBs 52 + 101	4	4	4.0
PCBs 52 + 138	2	1	1.5
PCBs 52 + 153	2	7	4.5
PCBs 101 + 138	8	10	9.0
PCBs 101 + 153	0	4	2.0
PCBs 138 + 153	3	5	4.0
PCBs 52 + 138 + 153	0	4	2.0

Linear regression analysis of total PCB content in serum (obtained by addition of all PCB congeners) revealed a significant correlation with age (Figure 1).

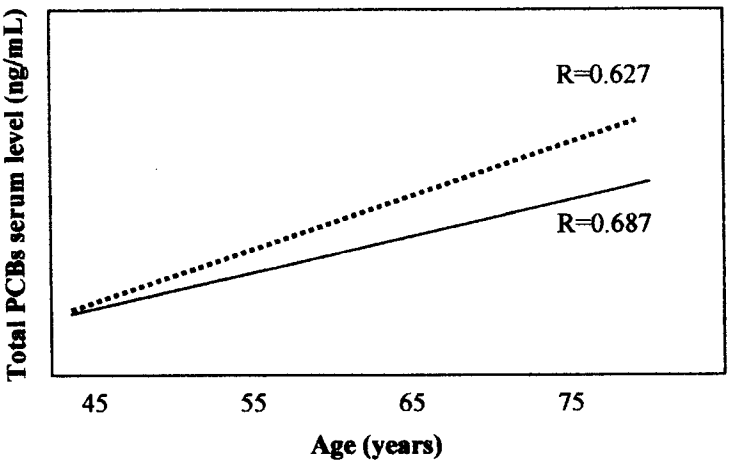

Figure 1 : Relationship between total PCB content in controls (—) and cases (···) and women age. The regression lines show a significant correlation coefficient for both controls and cases ($p < 0.05$).

Mean concentrations of PCBs 52 and PCB 138 were not significantly different between cases and controls, whilst PCB 101 and PCB 153 levels were increased in cases when compared to controls (Table 2).

Table 2. PCBs prevalence (%) and concentrations (ng/mL) in cases and controls. Values are mean ± SD.

	PCB 52		PCB 101		PCB 138		PCB 153	
	%	ng/mL	%	ng/mL	%	ng/mL	%	ng/mL
Controls	20	1.62 ± 3.47	22	0.68 ± 1.36	25	0.79 ± 1.68	42	0.92 ± 1.31
Cases	21	1.79 ± 3.29	39	1.61 ± 3.68	26	0.85 ± 1.71	59	1.47 ± 1.52
p		0.36		0.009		0.42		0.003

The dispersion of PCBs residues in the population has been frequently described. The present results can be added to the general evidence that contamination is ubiquitous.

Chronic effects of PCBs are a public health concern, since a potential relationship with breast cancer has been postulated (Falck et al. 1992; Lucena et al. 2001).

The results presented here support the hypothesis of a different pattern of PCBs residues in the breast cancer population. Further data analysis are needed, integrating common risk factors, such as family history of breast cancer, nulliparity, age at menarche and menopause, alcohol, and other POPs identification and quantification, given the complexity of PCB mixtures in environmental exposures.

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