POPS IN HUMAN BREAST CANCER ADIPOSE TISSUE FROM CENTRAL CHILE

Mariottini Michela[1], Pozo Karla[1], Bolados Aliro[2], Barra Ricardo[3], Guerranti Cristiana[1], Corsolini Simonetta[1], Focardi Silvano[1]

[1]University of Siena, Department of Environmental Sciences "G. Sarfatti", Via Mattioli 4, 53100 Siena, Italy
[2]Medical School, Concepción, Chile
[3]EULA-Chile Environmental Science Centre, University of Concepción

Introduction

Persistent organic pollutants (POPs) are chemicals that persist in the environment, bioaccumulate through the food web, and exhibit toxic effects that may threaten the health of humans and wildlife[1]. Since they are lipophilic, POPs tend to accumulate in fatty tissues where they have toxicological implications for human health. International efforts such as the Stockholm Convention under the auspices of the United Nations Environmental Program (UNEP), seek to reduce emissions of POPs into the environment and risks to the human health. Under this convention, twelve compounds commonly known as the "dirty dozen" should be eliminated from the environment world wide[1]. The dirty dozen includes nine organochlorine pesticides (OCPs) and industrial compounds such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and –furans (PCDD/Fs). Emerging compounds like polybrominated diphenyl ethers (PBDEs) are also currently being considerated as candidate substances. Several studies have reported POP levels in organisms, [2,3,4] adipose tissues[5,6] and abiotic matrices in central Chile[3,7,8]. In 2002, action was taken by the Chilean National Environmental Commission (CONAMA) to identify emission sources of POPs through PCB and PCDD/F inventories[9]. In the last ten years, breast cancer has threatened public health in Chile becoming the second cause of death for women. Epidemiological studies suggest a relationship between cancer and exposure to POPs which act as direct carcinogens[10]. The WHO recently recommended that these compounds be studied due to increasing evidence that they are a threat for human health[11]. In Chile there is little information about levels of POPs in humans. In the past ten years only two studies have reported pesticide levels in human tissues[5,6].

The aim of this study was 1) to determine a wide range of organohalogen compounds (HCB, DDTs, HCHs, PCBs, PBDEs and PCDD/Fs) in adipose tissue of Chilean women with breast cancer and 2) to evaluate the toxicological risk associated with levels of PCDD/F and dioxin-like PCBs in human tissues, using the Toxic Equivalency Factor (TEF).

Materials and Methods

Seventeen adipose tissue samples were obtained from women (average age: 46±10) in Concepción city in 2007 (Table 1). Concepción is on the delta of the Biobio river. The Biobio Region is one of the most industrialized regions of Chile, supporting 83% of Chilean pulp production, petrochemical and steel industries. According to the inventory[9], it receives the highest load of dioxins in the country.

Peritumoral breast adipose specimens (1-2 g) were collected during surgery from patients with benign or malignant tumours. The tissue was freeze-dried, packed in sealed containers and shipped to the Department of Environmental Science, University of Siena (Italy) where it was stored at –20°C until chemical analysis. Samples were targeted for seven PCB congeners (PCB-28, -52, 101, -118, -153, -138, -180), nine OCPs (HCB, α-HCH, β-HCH, γ-HCH (lindane), δ-HCH, *p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE), seventeen PCDD/Fs and three non-*ortho* PCBs (PCB-77, -126, -169, Wellington Laboratories Inc.). Extraction of adipose tissues was carried out with an Accelerated Solvent Extractor system (ASE 200, Dionex)[12], using toluene (60 ml) (US EPA 3545 A method revision B)[2]. A mix of $^{13}C_{12}$ labelled PCB congeners (Cambridge Isotope Laboratories) was added as recovery standard before extraction (mean recovery: 110%). The extracts were purified using Dioxin-prep (Supelco), Florisil version. The final extracts containing PCBs, OCPs and PBDEs were concentrated to 50 µl using nonane as keeper solvent, and injected in a gas chromatograph (Mod. Trace^{TM} GC 2000 with AS3000 autosampler, ThermoFinnigan) with an ion trap mass spectrometer (Mod. *Polaris* MS). Analytical conditions are reported elsewhere[13,14]. For determination of PCDD/Fs we used isotope dilution according to US EPA 1613 method revision B[15] with PCDD/F standard specific for that method ($^{13}C_{12}$, 99% Cambridge Isotope Laboratories). Two µl of extract was injected in a gas chromatograph with *Polaris* MS ion trap detector. The

five-point calibration curve was provided by Cambridge Isotope Laboratory. The quality assurance and quality control (QA/QC) of the procedure were tested by analyzing two replicates Certified Reference Material WMF-01 (freeze-dried fish tissue), from Wellington Laboratories Inc. Recovery results agreed well with certified values with an average error of 5% for PCBs, 7% for PBDEs and 10% for dioxins. Limits of detection (LOD) calculated as mean blank +3SD, were 0.8 and 0.01 ng/g dry weight (d.w.) for OCPs and PCBs, respectively, and 150 pg/g d.w. for PBDEs. LOD for non-*ortho* PCBs and PCDD/Fs was 2 and 0.4-1 pg/g d.w., respectively. Statistical analysis was performed using Kruskal-Wallis one-way ANOVA by ranks and Pearson correlation coefficient (r) (Statistica 6.0).

Results and Discussion

In this study OCPs showed the highest concentration followed by PCBs, PBDEs and PCDD/Fs. *p,p'*-DDE was the predominant compound. Average concentrations (ng/g d.w) of target compounds in breast adipose tissues were 21.9± 16.9 for HCB, 1457±1314 for DDTs, 3.8 ± 5.8 for HCHs, 29±13.9 for PCBs, 2.1± 2.2 for PBDEs and 228.7±173.2 pg/g d.w. for PCDD/Fs (Figure 1).

From all seven PCBs studied, PCB-153 and -118 were the predominant congeners, as reported in other studies analysing human samples [16,17,5,6]. The most abundant PBDE congener was BDE-153; BDE-66, -85 and -138 were below the LOD in all samples (Figure 1). A high proportion of BDE-153 has also been observed in human adipose tissue from the Japanese population[18].

PCB concentrations were low and PBDE levels were similar to those reported in European countries[19] and much lower than those in USA[20]. Statistical analysis revealed a positive correlation between DDTs, PCBs and age of women (r= 0.51 and 0.39, respectively, $p<0.05$) while POP levels were not correlated with breast feeding or number of pregnancies. Significant correlations were found between between PCBs, DDTs and HCB (r=0.69 and 0.57, respectively; $p<0.05$); no correlation with PBDEs was found.

Our results are in line with the industry (paper mills) and intensive forestry (*Pinus radiata* and *Eucalyptus* plantations) in this region. Chlorinated pesticides have been widely used in this part of Chile. Comparing our results with previous studies[6], there has been an increase in DDT and a decrease in PCB levels, in line with a decline in global emissions of PCBs in the past 10 years[21]. However, agricultural use of DDT has been forbidden in Chile since the 1980's and the levels detected in the environment were quite low[22]. Other potential sources of DDTs in humans (such as dietary intake) need to be investigated.

Toxic Equivalents (TEQs)

The Toxic Equivalency Factor (TEF)[23], based on the toxicity of single PCB and PCDD/F congeners relative to that of 2,3,7,8-TCDD, was used to evaluate the toxicological risk associated with dioxin-like PCBs and PCDD/Fs in human tissues.

Total TEQs indicated a high toxic potential of about 7.2±10.5 pg/g d.w. of 2,3,7,8-TCDD in adipose tissue (Table 1). These values are similar to those found in a previous study on Chilean women from the same region carried out ten years ago[5] and lower than those reported in adipose tissue samples[24] from subjects living in a non industrialized part of Italy (average TEQ values: 54.32 pg/g w.w.), in 1991-92. Although the pathophysiological significance of dioxins cannot be evaluated, total TEQ values were much lower than those obtained for "Yusho" poisoning victims (10-20 ng/g of dioxins) in 1969[25].

OCs and flame retardants are highly persistent and toxic substances and therefore further monitoring and additional follow up studies are necessary not only to evaluate the current presence of such compounds in biological matrixes, but also to identify new relationships between risk of breast cancer and organohalogen concentrations in breast adipose tissue.

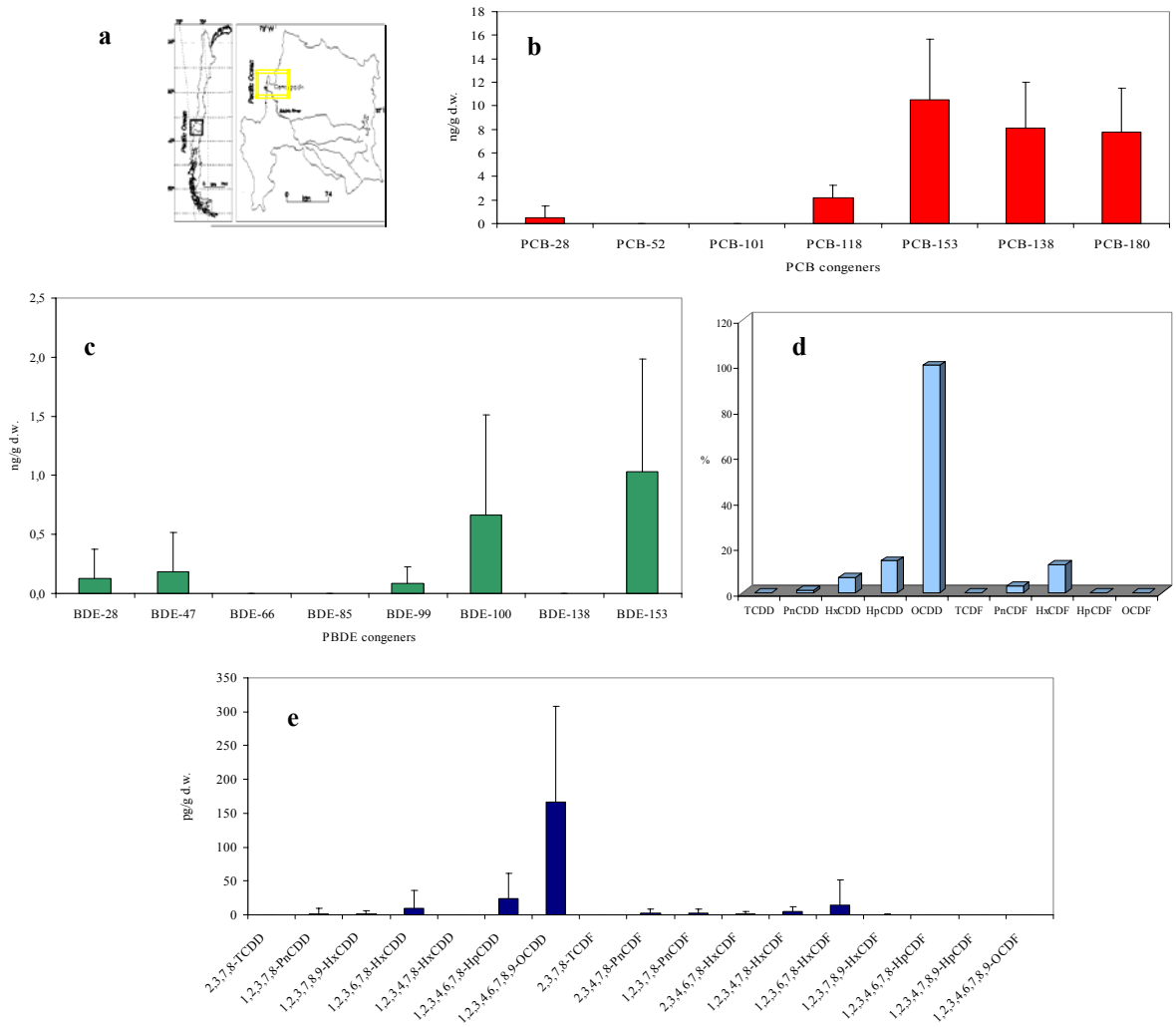

Figure 1. Study area (a), PCB and PBDE fingerprints (b, c), PCDD/F isomer and congener pattern (d, e) in Chilean breast adipose tissue from women living in Concepción.

Table 1. Concentration (pg/g d.w.) and TEQs of PCDD/Fs in breast adipose tissue from women living in Concepción.

	Mean conc. pg/g d.w.	**TEQs pg/g d.w.**
PCDDs	203.0 (173.7)	3.2 (8.1)
PCDFs	25.7 (41)	3.0 (4.3)
ΣPCDD/F	228.7 (173.2)	6.1 (10)
Σ non-*ortho* PCB	23.1 (47.5)	0.7 (1.3)
PCDD/Fs + PCB non-*ortho*	265.2 (158.9)	7.2 (10.5)

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