Polybrominated diphenyl ethers (PBDEs) in human breast adipose tissue samples from Brazil

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Introduction

Polybrominateddiphenyl ethers (PBDEs) are a widely used class of flame retardants[1]. Due to their lipophilicity and low vapour pressures, they tend to bioaccumulate through the foodchain[2]. PBDEs were first detected in the environment in Sweden, in 1981 by Andersson and Blomkvist[3] and have since been found in many environmental matrices including human body fat[4].

PBDEs have been previously reported in humans from North America[4,5] and Europe[6], but there is a distinct lack of data for South America. Using specimens from a case-control breast cancer study, this exploratory study was undertaken to investigate the distribution of PBDEs and PCBs in human breast adipose from Brazil, and explore possible regional and temporal differences[7].

Materials and methods

Participants for the study were recruited in Porto Alegre, Brazil where high levels of industrial and agricultural chemical exposures have been documented[8] and where our pilot data[7] indicated higher PCB body burdens than in contemporary US populations. Out of the targeted 120, a total of 32 samples were collected in 2004, with more being collected in 2005. The age of donors ranged from 40 to 73 years old. For this report, samples from both cancer cases and controls are examined. Samples were frozen below -20°C until analysis.

All solvents used were of pesticide grade. Silica gel (EMD, 100-200 Mesh) was activated at 130^{o}C overnight for the acidified silica chromatography and silica-gel fractionation chromatography. Sodium sulphate was baked at 130^{o}C overnight and all sorbents were stored in sealed containers after activation. Extracts were stored in amber glassware and precautions were taken not to expose the samples to light.

Adipose samples were thawed, about 0.3 g was homogenized with sodium sulphate and extracted with 1:1 hexane:dichloromethane. A sub-sample of the extract was taken for lipid determination and the remaining sample was spiked with internal standards ($^{13}C_{12}$-labelled BDEs 77 and 209 and $^{13}C_{12}$-PCB-118, 138, 153 and 180 in tetradecane). The extract was then cleaned-up using acidified silica gel chromatography followed by activated silica-gel fractionation chromatography. The extracts were concentrated and recovery standards added ($^{13}C_{12}$-PCB-128 and 178 in tetradecane). Adipose samples were analyzed in batches of six, with a reagent blank per batch. Standard Reference Materials (SRM 1945, whale blubber; National Institute of Standards and Technology, Gaithersburg, MD) were analyzed in duplicate with every third batch to assess accuracy and precision. Lipid content of the adipose samples was determined gravimetrically in an aliquot of the extract, and PBDE results were reported as nanograms per gram lipid.

The samples were analyzed on a Varian (Walnut Creek, CA, USA) 1200L GC/MS system (Varian 3800 GC, Varian 1200L quadrupole mass spectrometer) using a 5% diphenyl/95% dimethylpolysiloxane column (Restek, Bellefonte, PA, USA, 60m x 250 mm i.d. x 0.25 mm film). The mass spectrometer was operated in ECNCI mode using methane as the reagent gas (approximately 6.5 Torr source pressure). The GC was operated in constant flow mode of 1.0

mL/min of He as carrier gas. The injector and transfer line temperatures were 280 °C, and the source temperature was 200 °C. The GC temperature program was: 180 °C (2 minute hold time), followed by an increase to 300 °C (10 ° C/min, 30 minute hold time), with a total run time of 45 minutes. BDEs 17 and 28 (tribromo), 47, 66, and 71 (tetrabromo), 85, 99, and 100 (pentabromo), and 153, 154, 138 (hexabromo) were monitored using masses 79/81. PCBs 118, 138, 153, and 180 were monitored using their molecular masses: 326/338 (PCB-118 / $^{13}C_{12}$ PCB-118), 360/372 (PCB-138/-153, $^{13}C_{12}$ PCB-138/-153), and 394/406 (PCB-180 / $^{13}C_{12}$ PCB-180). PBDE and PCB standards were obtained from Cambridge Isotope Laboratories (Andover, MA, USA) and Wellington Laboratories (Guelph, ON, Canada).

Results and discussion

Preliminary data on 11 samples indicate that BDE-47 dominates the congener profile, followed by BDEs 99 and 100, all of which were detected in all adipose samples. BDEs 153, 154 and 28 were also detected in most adipose samples, in order of abundance. Lipid content ranged from 66% to 95% with a mean of 81%, so results are expressed on a lipid weight basis. Total PBDE concentrations (sum of all measurable congeners) ranged from0.73 to 3.69 ng/g lipid, with a mean of 1.89 ng/g lipid and a median of 1.86 ng/g lipid. A summary of the PBDE data can be seen in Table 1. Comparing these PBDE data to other studies of human adipose we can observe that the values are much lower for Brazilian women than women from Singapore[9] (by about 2 times), Belgium[10] (by about 3 times) or the USA[4] (by about 30 times). As no PBDE consumption and usage data are available for Brazil, we can only assume that these lower levels in Brazilian women reflect lower usage of PBDE-containing products or lower exposures.

PCBs 118, 153, 138 and 180 were detected in all samples. Total PCB concentrations ranged from 98.9-314 ng/g lipid, with a median of 125 ng/g lipid. The results can be seen in Table 2. When comparing these results to our previous pilot study in the area[7] we notice that PCBs are more than three times lower than what they were in that 1998 study, which could be a reflection of decreasing environmental levels or lower exposures. Correlations among chemicals and age effects are currently under investigation.

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Table 1 - Summary of the main PBDE congener concentrations observed in human adipose samples (in ng/g lipid) (*n*=11). ΣBDE represents the sum of all measurable congeners.

BDE congener	median	minimum	maximum	% detected in samples
BDE-47	0.52	0.27	1.54	100
BDE-100	0.12	0.06	0.35	100
BDE-99	0.34	0.03	0.82	100
BDE-154	0.07	0.03	0.11	27
BDE-153	0.19	0.11	0.37	73
ΣBDE	1.36	0.73	3.69	

Table 2 - Summary of the PCB congener concentrations observed in human adipose samples (in ng/g lipid) (*n*=11) collected in 2004 and 1998.

PCB congener	median 2004	mean 2004	minimum 2004	maximum 2004	mean 1998[7]
PCB-118	10.2	11.5	4.83	19.8	38
PCB-153	54.2	61.1	37.2	116	206
PCB-138	44.8	45.3	24.3	81.4	175
PCB-180	36.5	51	2.51	144	282