ANALYSIS OF PBDES, PCBS, ORGANOCHLORINE PESTICIDES, AND NEW BFR ALTERNATIVES IN CALIFORNIA PREGNANT WOMEN BY HIGH RESOLUTION MASS SPECTROMETRY

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Introduction

High levels of polybrominated diphenyl ethers (PBDEs) have been reported in humans and wildlife from California, USA[1, 2] As PBDEs have been or are being phased out in Europe and North America, other brominated flame retardants (e.g. DPTE, HBB, BTBPE, DBDPE, TDCPP) have been alternatively used [3].

We assessed levels of PBDEs, PCBs, selected organochlorine pesticides, and some new BFR alternatives in contemporary California human sera. In order to perform the analysis in a sensitive, accurate, and efficient manner, we adapted/modified our original blood extraction technique and coupled it with isotope dilution-GC/high resolution mass spectrometry based on techniques from the Centers for Disease Control. For this report we analyzed serum samples collected from 25 pregnant California women during 2008 – 2009 and compared the results with recent reports from the US and other countries.

Materials and Methods

Sample sources and Preparation

Serum samples were collected in 2008-09 from healthy pregnant women from Northern California [4] and were stored in a freezer at -20°C until analyzed.

Analytical method

Multiresidue analyses for the simultaneous extraction of PBDEs, PCB and organochlorine pesticides in serum have been published elsewhere [5, 6]. In this study, we used a simplified and optimized method. In summary, thawed serum samples (1mL) were fortified with a panel of nine ^{13}C labeled PBDE surrogate mix standards (BDE-28, 47, 99, 153, 154, 183, 197, 207, 209), sixteen ^{13}C labeled PCB and pesticides (PCB-118, 138, 153, 170, 180, 194, 101, 105, 156, 2,4'-DDT, hexachlorobenzene, oxychlordane, trans-nonachlor, 4,4'-DDE, 4,4'-DDT, and b-BHC), three ^{13}C labeled new BFR surrogate mix standards (HBB, BTBPE and DBDPE) and mixed well. The proteins in serum samples were denatured with 6 M hydrochloric acid and stabilized with isopropanol. The samples were extracted by a mixture of hexane and methyl *t*-butyl ether (1:1 v/v) and washed with 1% potassium chloride solution. Neutral and phenolic phases were separated by 0.5 M potassium hydroxide in 50% ethanol and water. The organic phase of the neutral fraction was concentrated on Turbo Vap (Labconco) with constant nitrogen flow. Removal of co-extracted lipid was performed on Florisil column. The Florisil was prebaked at 500^{o}C for 3 h and deactivated with 5% HPLC grade water. Hexane and subsequently hexane/dichloromethane (1:1 v/v) were used to elute all the analytes. The collected eluates were concentrated under nitrogen flow, and combined with recovery standard,^{13}C PCB-209.

Analytical determination of target analytes was performed by gas chromatography/high resolution mass spectrometry (GC-HRMS), (DFS, Thermo-Finnigan). For organochlorine pesticides and PCBs analyses, 2 uL of samples were injected by PTV (programmable temperature vaporizing) injector in splitless mode and separated using a HT8-PCB column (SGE International Pty Ltd. Australia & Pacific Region) (60 m × 0.25 mm I.D., 0.25 μm film) with helium carrier gas. The GC was programmed from 120 °C ramped to 180 °C at 20.0 °C/min, to 260 °C at 2.0 °C/min., to 300 °C at 5.0 °C/min and held for 5 mi at 300 °C. For PBDEs analyses, 2 uL of samples were injected and separated using a DB-5 MS column (J&W Scientific, USA) (15 m × 0.25 mm I.D., 0.10 μm film) with helium carrier gas. The GC was programmed from 175 °C for 2 min., ramped to 280 °C at 5.0 °C/min. to 310 °C at

7.0 °C/min., and held at 310 °C for 5 min. The MS was operated in electron impact ionization mode using multiple ion detection. The source temperature was set to 260°C, ionization energy was set to 42 V, and electron current was typically 0.5–0.6 mA, with a mass resolution of 10,000. Perfluorokerosene (PFK) was used as the mass reference. All seven pesticides (2,4'-DDT, 4,4'-DDT, 4,4'-DDE, b-BHC, Hexachlorobenzene, oxychlordane, trans-nonachlor) , fifteen PCBs (66, 74, 99, 101, 105, 118, 138, 153, 156, 170, 180, 183, 187, 194, 203), eighteen PBDE congeners (28, 47, 66, 85, 99, 100, 153, 154, 183, 196, 197, 201, 202, 203, 206, 207, 208, and 209) and eleven new BFR alternatives (ATE, α,β-TBECH, PBT, PBEB, HBB, TBPH, TBB, BTBPE, DPTE, DBDPE, and TDCPP) were measured.

One reagent black, one standard reference material (NIST 1589a) and one in-house control sample (bovine serum pre-spiked with known amounts of targeted analytes) were processed with each batch of 9 human sera samples. The lipid content was calculated using Philip's formula [7] from the total cholesterol and triglycerides determined by Boston's Children's Hospital.

Results and Discussion

This HRMS method was set up using selected molecular fragmentation ions in order to enhance the selectivity and sensitivity. All targeted PBDEs and 8 out of 12 targeted alternative BFRs were successfully measured with our method. Recoveries from surrogate spikes, reference materials (NIST 1589a), and in-house control samples were within reasonable analytical error ranges. Fig 1 is an example of our QC procedures showing the percent recoveries of the in-house control sample ranging from 90±4% (BDE-153) to 118±19 % (DPTE).

Compared to our data from California serum collected in the 1960s and 1980s [8], levels of legacy contaminants (PCBs and chlorinated pesticides) in California serum decreased over the past 30 years (not shown), while the PBDE levels have risen, consistent with our California wildlife studies [1, 2]. The abundance of PBDE congeners was in the order of BDE-47, 153, 99, and 100, which is generally found in human serum [9]. Eight new BFRs (ATE, α,β-TBECH, PBT, PBEB, DPTE, HBB, DBDPE) measured in these contemporary human serum were not measurable above the quantitation level.

As shown in Fig 2, PBDE levels in California pregnant women (2008-2009) exceed levels from other regions of the U.S [10], and the general US female population (NHANES, 2003-04) [9], and far exceed those recently reported from China [11] and France [12]. This is of concern because the high PBDE exposures in mothers may affect the health of the fetus and newborn at early developmental stages. Work is under way to improve the resolution of those new BFRs not being measured here and to explore possible exposure pathways for these contaminants (e.g. house dust) and their relationship to potential adverse health effects.

Acknowledgement and Disclaimer

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Fig 1. Percent Recovery in In-house Control samples (Bovine Serum) for PBDEs and new BFRs

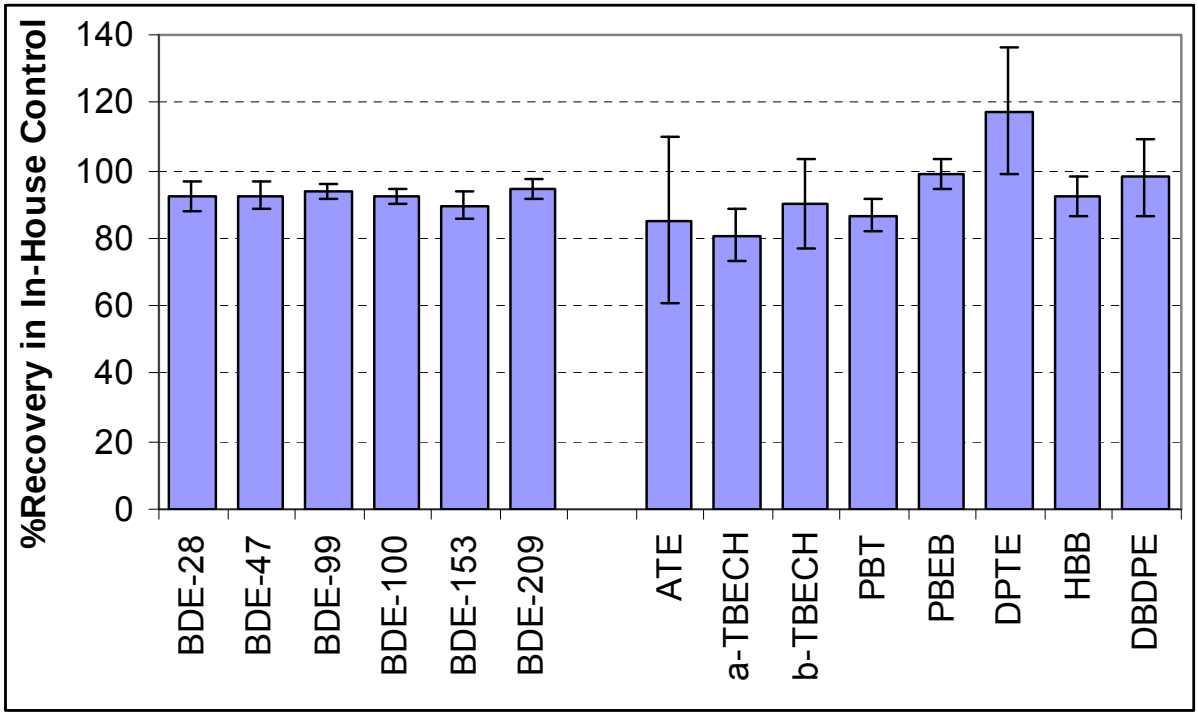

Fig 2. Comparison of levels of BDE congeners in different populations