BFRs in California Women

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Introduction

Levels of PBDEs rose in the environment as a result of their widespread use, their persistence, and their tendency to bioaccumulate. High concentrations of PBDEs have been documented in California residents and wildlife (She et al., 2002; Petreas et al., 2003; Brown et al., 2006; She et al., 2007; Holden et al. 2009). Since the high use of BFRs is driven by the California's flammability standard, research continues as to the impact of the ban on Penta-BDEs and Octa-BDEs and their replacement with new products.

Our laboratory has performed measurements in human specimens (serum, milk, adipose) collected over the last 50 years in the course of a number of distinct studies, each with its own design and objectives. Whereas these samples were collected at different time periods, we analyzed them with similar methodologies. Since sample collection spans 50 years, results can be used to compare and contrast differences in POPs levels: over time; by age; ethnicity; country of birth; etc. In addition, such material can be used to explore the presence and time trends of new chemicals of emerging concern such as new BFRs, OH-PCBs, OH-PBDEs, Triclosan, PCP, BPA, etc.(Park et al., 2009)

Materials and Methods

Populations Studied: We only focus on studies of women (reproductive effects, breast cancer and healthy adults) to facilitate comparisons. All subjects had given written informed consent at the time of recruitment. Samples had been kept frozen at -20° C or -80° C and were analyzed by our laboratory using similar methods. Five distinct time periods are examined:

<u>1960s:</u>

- Serum samples from the California Health and Development Studies (CHDS), a longitudinal birth cohort of over 20,000 births in Northern California, recruiting pregnant women between 1959 and 1967. (N=1,200, Petreas et al. 2003).

<u>1980s</u>

- Serum samples from cancer-free women (controls) participating in a case-control study of breast cancer in the San Francisco area (N=30).

<u>1996-98:</u>

- Adipose samples from women participating in a breast cancer study in the San Francisco area (N=162, Petreas et al. 2008).
- Serum samples from pregnant Mexican women (N=30, Koepke et al. 2004).
- Serum samples from Laotian immigrants to California participating in a study of ovarian function (N=50, Windham et al. 2005).

<u>2003-05:</u>

- Milk samples from a community-based California study (N=82, She et al. 2007).
- Adipose samples from women participating in a breast cancer study in Brazil (N=25, Kalantzi et al. 2009).

2008-09:

- Serum samples from adult women from a California Biomonitoring pilot study (preliminary N=9).
- Serum samples from pregnant women from Northern California (preliminary N=18, Zota and Woodruff, work in progress).

Analytical Methods: Multiresidue analyses for the simultaneous extraction of PCBs, organochlorine pesticides, PBDEs and select new BFRs in serum, milk and adipose have been described in detail. In brief, for serum, after the extracts in neutral fractions were cleaned up using deactivated Florisil column chromatography, they were analyzed for PCBs, organochlorine pesticides and PBDE 47, 99, 100, 153 and 209 on a Varian 3800 GC-ECD (Varian Inc., Walnut Creek, CA) equipped with RTX-5MS capillary column (60m × 0.25 mm i.d., 0.25 um thickness, Restek, Bellefonte, PA) and DB-XLB capillary column (60 m × 0.25 mm i.d., 0.25 um thickness, J&W Scientific, Folsom, CA). Injection (2 uL) was made in split/splitless mode. External calibration standards were used for quantification. PBDEs analyzed by ECD were confirmed by high resolution mass spectrometry. Pooled serum samples were used as in-house control samples as described elsewhere (Rogers et al. 2004; Park et al. 2007). Recoveries from surrogate spikes, reference materials, and control samples were within reasonable analytical error ranges.
Adipose samples were spiked with ^{13}C-labeled internal standards, cleaned up with Gel Permeation Chromatography (GPC) and Florisil column; the extracts were reduced to 10 µL and recovery standards added. Analysis was carried out on a Varian 3800 GC with a deactivated injection port liner (Siltek, 4.0 mm id, with glass frit) in splitless mode, with no pressure pulse and a Varian VF-5ms column (30 m x 0.25 mm id x 0.25 µm). The 1200L mass spectrometer (Varian Inc., Walnut Creek, CA) was operated in extended dynamic range mode using electron impact ionization and MS/MS detection.

Milk samples were lyophilized, extracted using an ASE 200TM (Dionex, Sunnyvale, CA), cleaned up by mixed silica gel column, and GPC. The mixtures were spiked with ^{13}C internal standards of PBDEs and PCBs and analyzed on a Hewlett–Packard 6890 gas chromatograph, with a split/splitless injector and a DB-5 column (60m, 0.25 mm ID, 0.25 um). A Finnigan MAT95 high-resolution mass spectrometer (ThermoFinnigan, Bremen, Germany), operated in electron impact (EI) mode at 9000 resolution for PCBs and 6000 for PBDEs.

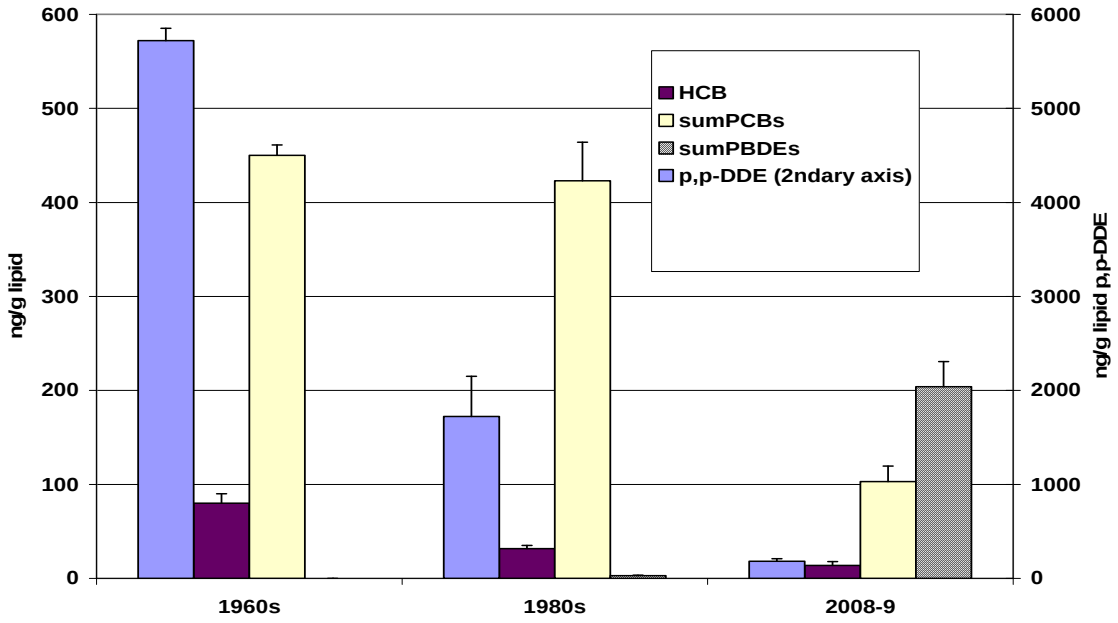
Results and Discussion

We had first reported the absence of PBDEs in serum samples from 1960s California populations (Petreas et al. 2003) as opposed to their presence in samples collected in the late 1990s. We confirmed this observation with the analysis of over 1200 serum samples from the 1960s. Levels of legacy contaminants (PCBs and chlorinated pesticides) in California women sera showed declining temporal changes over the past 50 years while the PBDE levels have risen and now exceed PCB levels in contemporary serum samples (Fig 1). The abundance of PBDE congeners was in the order of BDE-47, 153, 99, and 100, while BDE-209 was measurable in only a few of the contemporary serum samples. Some of the new BFRs (e.g., TBB) were detectable in contemporary serum but at levels below quantitation. Analyses are underway for additional BFR replacements of PBDEs (e.g., TDCPP).

PBDE levels (BDE 47 shown in Fig 2) in California samples exceed levels in the US population (NHANES, 2003-04) (Sjodin et al. 2008). They are also higher than levels in samples collected at corresponding times from Brazil and Mexico. As expected, younger women (participants in reproductive

studies) have higher levels than older women (breast cancer studies, Biomonitoring pilot). As more samples are collected, work is under way to explore determinants of exposures to PBDEs and new BFRs, and whether the lower levels observed in contemporary adult women are a reflection of age, or a result of the recent ban of Penta- and Octa-BDEs.

Fig 1. Temporal Trends in California Women Serum



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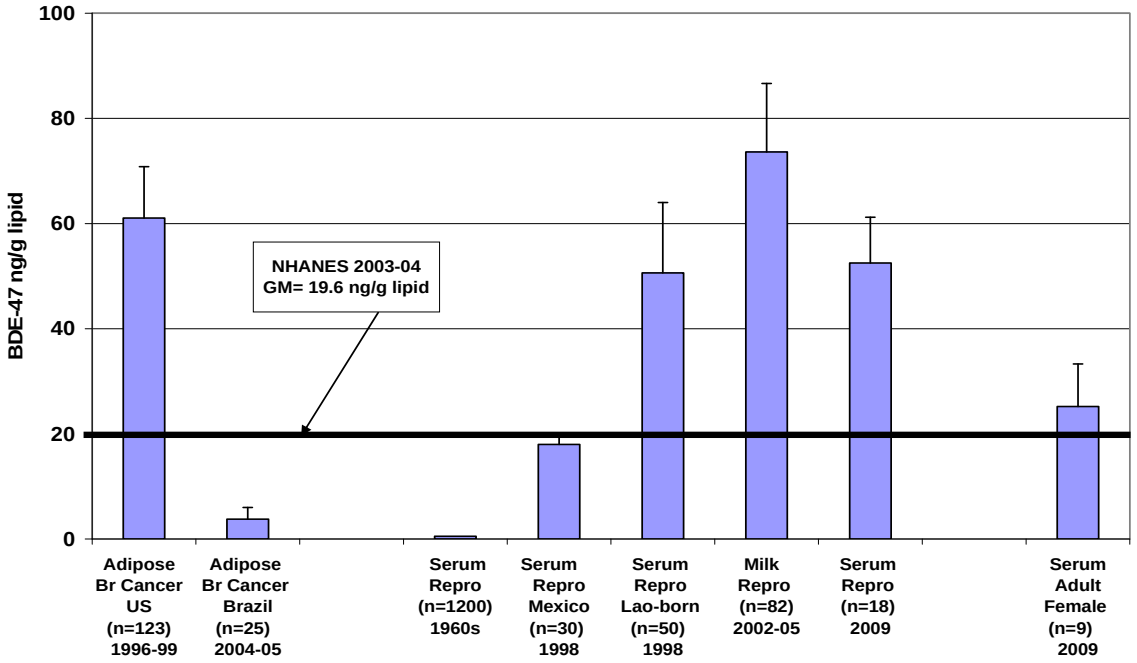
Fig 2. BDE 47 in California Women