PERSISTENT ORGANIC POLLUTANTS IN CALIFORNIA WOMEN: A BASELINE FOR THE CALIFORNIA BIOMONITORING PROGRAM

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Introduction

In 2006, California passed legislation establishing the first State Biomonitoring Program in the USA[1]. The main goals are to: 1) Determine levels of environmental chemical contaminants in a representative sample of Californians; 2) Establish trends in the levels of these chemicals over time; 3) Assess the effectiveness of public health efforts and regulatory programs to decrease exposures to specific chemicals.

Prior to this new Biomonitoring Program, our laboratory had conducted a number of epidemiologic studies using blood, milk or adipose collected from the 1960s to the present. Samples were analysed for Persistent Organic Pollutants (POPs) such as organochlorine pesticides (OCPs), PCBs, polybrominated diphenyl ethers (PBDEs), perfluorinated chemicals (PFCs), triclosan, phenols, OH-PCBs and OH-PBDEs. Following standard conventions, results are expressed on a lipid basis (PCBs, PBDEs, OCPs), or on a serum volume basis (PFCs, Triclosan, Phenols, OH-PCBs, OH-PBDEs). A Quality Management system tracks all laboratory work.

In addition to addressing the research hypotheses of each epidemiologic study, data compiled across studies can show trends such as the emergence of PBDEs, and the decline in PCBs, phenols, OCPs over time. Moreover, significant determinants of exposures (age, country of birth, ethnicity and reproductive history) can be identified. This information would allow for optimal sampling designs to account for the population diversity in California and can also be used in questionnaires to assess exposures. These data help establish a baseline of chemical contaminants before the new Biomonitoring Program launches its surveys.

Materials and Methods

Populations Studied:

We only focus on studies of women (recruited from studies on 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 at -20^{o} C or -80^{o} C and were analyzed by our laboratory using similar methods. Five distinct time periods of recruitment are examined:

1960s:

- Serum samples[2] from the California Health and Development Studies, a longitudinal birth cohort of over 20,000 births in Northern California, recruiting pregnant women between 1959 and 1967. (N=1,200).

1980s

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

1996-98:

- Adipose samples[3] from women participating in a breast cancer study in the San Francisco area (N=162).
- Serum samples from Laotian immigrants to the San Francisco area participating in a study of ovarian function[4] (N=50).

2003-05:

- Milk samples[5] from a community-based California study (N=82).

2008-09:

- Serum samples from adult California women from a pilot study (preliminary N=9).
- Serum samples from a pilot study on pregnant women[6] from Northern California (preliminary N=25)

Analytical Methods: Our methods for multiresidue analyses for the simultaneous extraction of PCBs, organochlorine pesticides, PBDEs, select new BFRs and PFCs in serum, milk and adipose have been described in detail [7-10].

Serum: Serum samples were extracted and the 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[7, 10]. Online Solid Phase Extraction - High Performance Liquid Chromatography - Turbo ion Spray - Tandem Mass Spectrometry (online SPE-HPLC-TIS-MS/MS) was used to analyze 12 PFCs in serum samples[11]. Briefly, 100 uL of serum sample is diluted with formic acid and injected into the online SPE-HPLC-TIS-MS/MS system. The analytes of interest are first purified and concentrated in the SPE column (C18), then separated by a C8 HPLC column before entering the MS/MS system operating in negative-ion spray ionization mode. A calibration curve is constructed with each batch of samples, and the ratio of the area of target Q1/Q3 ions over internal standards area is used to quantitate each analyte.

Adipose: 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). A 1200L mass spectrometer (Varian Inc., Walnut Creek, CA) was operated in extended dynamic range mode using electron impact ionization and MS/MS detection[8].

Milk: 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[9].

Results and Discussion

We had first reported the absence of PBDEs in serum samples from 1960s California populations as opposed to their presence in samples collected in the late 1990s[2]. We confirmed this observation with the analysis of over 1200 serum samples from the 1960s. The abundance of PBDE congeners was in the order of BDE-47>153>99>100, while BDE-209 was measurable in only a few of the contemporary serum samples. Some of the new BFRs (e.g., ATE, α,β-TBECH, PBT, PBEB, HBB, DPTE, DBDPE) were not detected or detectable in contemporary serum but at levels below quantitation. Analyses are underway for additional BFR replacements of PBDEs (e.g., TBPH, TBB, BTBPE, and TDCPP).

PBDE levels (BDE 47 shown in Fig 1) in California samples exceed levels in the US population (NHANES, 2003-04)[12]. As expected, younger women (participants in reproductive studies) have higher levels than older women (participants in breast cancer studies, pilot study). With our limited preliminary data set it is not clear whether PBDEs have peaked in the mid 2000s and started declining, possibly as a result of the ban. A clear difference appears between samples from pregnant and younger women and those from older women. This may be a function of age or of changes during pregnancy; additional data are being generated to test that hypothesis. 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. Similarly, predictors of exposures to PCBs, OCPs and PFCs are being investigated.

On the other hand, levels of legacy contaminants such as PCBs and chlorinated pesticides in California women (serum, adipose, milk) showed declining temporal changes over the past 50 years (Fig 2).

Conclusions

Californians continue to have high PBDE levels, exceeding US averages (NHANES).
PBDEs were not measurable in the 1960s, or 1980s, and were first observed in the mid- to late- 1990s.
All new BFRs analyzed were below their respective reporting limits in all the contemporary serum samples analyzed so far.

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Fig. 1. BDE-47 in California Women

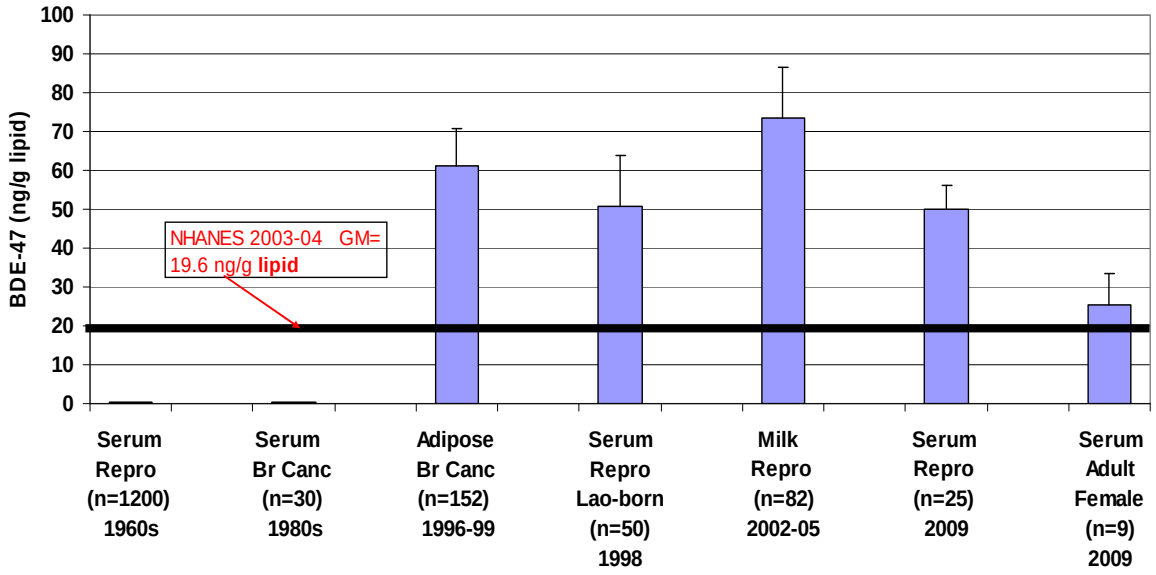
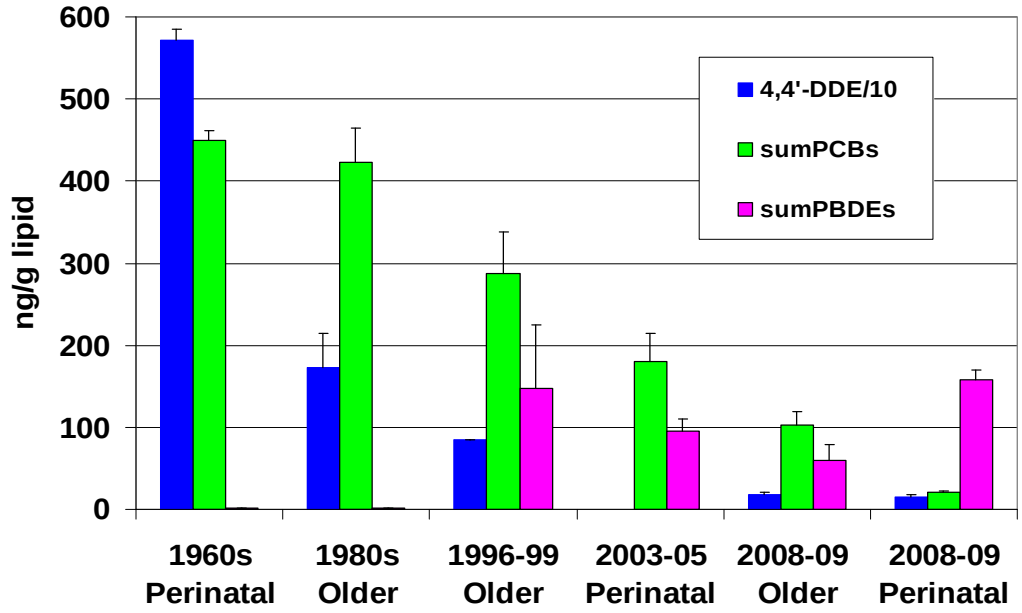


Fig 2. Temporal Trends in California Women



References

1. California Environmental Contaminant Biomonitoring Program. www.oehha.ca.gov/Biomonitoring
2. Petreas M, She J, Brown F, Winkler J, Windham G, Rogers E, Zhao G, Bhatia R, Charles M. *Env Health Persp*.111:1175-1179, 2003
3. Petreas M, Brown FR, Goldberg D, Hurley S, Nelson D, Reynolds P. *Organohalogen Compounds*. 70 1888-1891, 2008
4. Windham G, Lee D, Mitchell P, Anderson M, Petreas M, Lasley B. *Epidemiology*. 16:182-190, 2005
5. Hooper K, J She, M Sharp, J Chow, N Jewell, R Gephart, A Holden. *Environ Health Perspect* 115:1271–1275, 2007
6. Zota A, Schwartz JM, Steinauer J, Petreas M, Pasternack T, Woodruff T. *2010 Joint ISES-ISEE Conference*, Seoul, Korea
7. Rogers E, Petreas M, Park J, Zhao G, Charles M. *Journal of Chromatography B*. 813:269-285, 2004
8. Brown, FR, Winkler J, Visita P, Park JS, Petreas M. *Organohalogen Compd*. 69:1199-1202; 2007
9. She J, Holden A, Sharp M, Tanner M, Williams-Derry C, Hooper K. *Chemosphere*. 67:S307-317, 2007
10. Park J-S, Guo W, Rhee A, Harwani S, Wang Y, Baek J-S, Wang M, Cohn BA, Cirillo PM, Factor-Litvak P, Petreas M. *Organohalogen Compounds*. 71: 999-1002, 2009
11. Wang M, JS Park, M Petreas. *58th ASMS Conference on Mass Spectrometry and Allied Topics*. Salt Lake City, UT, 2010
12. Sjodin A, Wong L, Jones R, Park A, Zhang Y, Hodge C, DiPietro E, McClure C, Turner W, Needham L, Patterson D. Envir Sci Technol. 42:1377-1384, 2008