(82) 51 - 15 28

NATURAL RESOURCES DEFENSE COUNCIL

June 14, 2004

VIA FACSIMILE (916-341-5620) AND U.S. MAIL

Arthur G. Baggett, Chair and Board Members
State Water Resources Control Board
1001 I Street
Sacramento, CA 95814

Re: Comments on "Notice of Public Solicitation of Water Quality Data and Information – 2004 Clean Water Act Section 303(d) List"

Dear Chairman Baggett and Board Members:

On behalf of the Natural Resources Defense Council, we are submitting these comments on the "Notice of Public Solicitation of Water Quality Data and Information – 2004 Clean Water Act Section 303(d) List" (Solicitation Notice). We also are including additional information and renewing our request that the San Francisco Bay be listed for a number of chemicals generally referred to as PBDEs.

I. Listing Solicitation and Board's 2004 Listing Process

In summary, we have significant concerns with regard to the legality of the Solicitation Notice, specifically with respect to its lack of compliance with Clean Water Act Section 303(d) and its implementing regulations. The failure of the Solicitation Notice to comply with these legal mandates makes it inherently flawed with respect to gathering information that could be relevant to decisions on the quality of the state's waters. As the agency charged with protecting the health of the waters of the state and cleaning up waters that fall through the cracks, the SWRCB should be particularly careful to comply with all statutory and regulatory mandates to cast a wide net to gather and use all existing and readily available information.

Legal Mandates

As the Solicitation Notice acknowledges, the SWRCB is required by Clean Water Act Section 303(d) and 40 C.F.R. § 130.7 to develop a list of water quality limited segments. Specifically, Section 303(d)(1) states that "[e]ach State shall identify those waters within its boundaries for which the effluent limitations required by section 1311(b)(1)(A) and section 1311(b)(1)(B) of this title are not stringent enough to implement any water quality standard applicable to such waters." (33 U.S.C.

§ 1313(d)(1)(A).) *Pronsolino*[1] made clear that Section 303(d)(1)(A) "appl[ies] to all waters in the state, not only to the subset covered by certain kinds of effluent controls," interpreting "not stringent enough" in Section 303(d)(1)(A) to mean "not adequate for" or "inapplicable to."[2]

> The regulations at 40 C.F.R. § 130.7(b)(5) add that:
>
> Each State shall assemble and evaluate all existing and readily available water quality-related data and information to develop the list required by §§130.7(b)(1) and 130.7(b)(2). At a minimum "all existing and readily available water quality-related data and information" includes but is not limited to all of the existing and readily available data and information about the following categories of waters:
>
> (i) Waters identified by the State in its most recent section 305(b) report as "partially meeting" or "not meeting" designated uses or as "threatened";
> (ii) Waters for which dilution calculations or predictive models indicate nonattainment of applicable water quality standards;
> (iii) Waters for which water quality problems have been reported by local, state, or federal agencies; members of the public; or academic institutions. These organizations and groups should be actively solicited for research they may be conducting or reporting. For example, university researchers, the United States Department of Agriculture, the National Oceanic and Atmospheric Administration, the United States Geological Survey, and the United States Fish and Wildlife Service are good sources of field data; and
> (iv) Waters identified by the State as impaired or threatened in a nonpoint assessment submitted to EPA under section 319 of the CWA or in any updates of the assessment.

(Emphasis added.) In addition, 40 C.F.R. § 130.7(b)(6) requires California to provide documentation to the EPA Region IX to support the State's determination to list or not to list its waters. This documentation must include a "rationale for any decision to not use any existing and readily available data and information for any one of the categories of waters as described in §130.7(b)(5)." (40 C.F.R. § 130.7(b)(6)(iii).) In other words, the state must explain why it did not seek out and assemble existing and readily available information.

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[1] *Pronsolino v. Nastri*, No. 00-16026, at 7929 (9th Cir., May 31, 2002).

[2] *Id.* at 7928; *see also Dioxin/Organochlorine Center v. Clarke*, 57 F.3d 1517, 1528 (9th Cir. 1995) ("since best practical technology effluent limitations do not apply to toxic pollutants, those limitations are, as a matter of law, 'not stringent enough' to meet water quality standards").

Inconsistencies Between Solicitation Notice and Legal Mandates

In a number of places, the Solicitation Notice sets limitations on the solicitation process such that the Notice violates the basic requirement to "assemble and evaluate all existing and readily available information" to develop the required list of "water quality-limited segments." (40 C.F.R. §§ 130.7 (b)(1), (b)(2) and (b)(5).) These include, but are not limited to, the following:

- The Solicitation Notice asks for information to "assess the State's water bodies for possible inclusion on or removal from the existing section 303(d) list," and then defines the list as including only those waters exhibiting "deleterious impacts from a pollutant or pollutants." However, nothing in Clean Water Act Section 303(d)(1)(A), which defines the scope of the list, or in the regulations limits the application of the listing requirement to only waters in which water quality standards are not met because of the presence of a "pollutant." The list must include all waters in which water quality standards are not achieved despite the application of effluent limitations, regardless of whether a pollutant is causing this failure to achieve water quality standards. Limitation of the Solicitation Notice in this way illegally limits the amount of information being solicited below the "all existing and readily available" threshold.

- The Solicitation Notice states that "[r]equirements for data and information from the Listing Policy – including those for quality control and assurance, temporal and spatial characteristics, and minimum sample sizes – will be followed when reviewing data and information." EPA Region IX's February 18, 2004 letter from Alexis Strauss to Art Baggett on the draft Listing Guidance makes clear that though "'high quality' data should be accorded the greatest weight . . . all data and information must be considered (see EPA, 1997a and EPA, 2003)" for listing decisions. (*See also* U.S. EPA, *Guidance for 2004 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d) and 305(b) of the Clean Water Act* (July 21, 2003) p. 25 (stating in response to the question "How should a State address data and information quantity?" that "All existing and readily available data and information must be considered during the assessment process") [hereafter "July 2003 Guidance"].)

- The Notice states that "[a]ll available data and assessment information generated since May 15, 2001 will be considered." This artificially short time constraint eliminates many potentially valuable pieces of information and again conflicts with the "all existing and readily available" standard. As EPA reiterated in July 2003 Guidance "[d]ata should not be excluded from consideration solely on the basis of age. . . . A State should consider all data and information." (July 2003 Guidance p. 25.) There are many situations in which information from before the last listing cycle would be submitted, including but not limited to: older data that

recently became relevant due to new scientific understandings about the relationships between the constituents at issue and impairment of beneficial uses, and older information that is meaningful and important in combination with more recent data.

- In paragraphs 6 through 9, the Notice states that "[a]ll" data and information submitted should be accompanied by numerous additional pieces of information and additional evaluations. Some of these additional requirements are simply unnecessary to the SWRCB's decision on whether a water body is impaired or threatened, and some represent tasks that even the regional water boards and SWRCB cannot currently perform. More importantly, virtually none, if any, of the additional information and evaluations called for in the Solicitation Notice is required under the broad "all existing and readily available" standard. Again, the end result is to severely discourage organizations and people from submitting what could be useful information, an extremely short-sighted decision given the paucity of SWRCB-collected and -organized data.[3] The SWRCB should instead indicate that such accompanying information and evaluations would be "welcome and useful," rather than require such additional information or evaluations or create the perception that such information and evaluations are required.

Finally, the Solicitation Notice states that the "final list will be based on data and information available to SWRCB" no later than June 14, 2004. (Emphasis added.) This language, which focuses updating the 303(d) list only on information *made available* to the SWRCB, makes it sound as if all that will be reviewed is the information handed to the SWRCB as part of the solicitation process. This, however, limits the data and information in a way that violates federal requirements and ignores the state's responsibility under federal regulations to seek out and use the myriad sources of information on water quality that are "existing and readily available." As set forth in those regulations, the State must base the 303(d) list on all existing and readily available data and information *that it has assembled.* (40 C.F.R. § 130.7(b)(5) "each state *shall assemble* . . . all existing and readily available . . . data and information" (emphasis added).) As such, the State is under a mandatory duty to collect, assemble and use all

[3] EPA Region IX commented on a similar approach in the state's draft Listing Policy, finding that "[t]he policy's minimum sample size and high quality data provisions and supporting rationale do not provide a 'good cause' rationale for excluding data and information from consideration (see 40 CFR 130.7(b)). These regulatory provisions create a rebuttable presumption that all readily available data and information will be used in the assessment process. A great deal of useful data from STORET, academic and agency reports, and volunteer monitoring groups would appear to be excluded from consideration under the proposed rule, an outcome which appears inconsistent with the federal requirements." (Letter from Alexis Strauss to Art Baggett, February 18, 2004.) EPA also noted in this letter that "the proposed policy appears to set a higher burden of proof than typically used in California's administrative proceedings." (Citations.) The onerous responsibilities for submitting information that the Solicitation Notice places on the public, many of which the SWRCB does not place on even itself, similarly appear to be more stringent that the principles governing the admissibility of evidence and opportunities for public participation typically used in California administrative proceedings.

readily available data and information. (*See Forest Guardians v. Babbitt* (10th Cir. 1999) 174 F.3d 1178, 1187 ("'shall means shall,' which imposes "a mandatory duty upon the subject of the command").)

It is insufficient, therefore, for the State to base the final 303(d) list merely on data and information that it has been handed. Rather, the State must complete its mandate and *actively gather and collect* all existing and readily available information from all potential sources, many of which are readily obvious to members of the public (who do not have the resources to do the state's job for them) and should be similarly obvious to the SWRCB. These include but are not limited to USGS data, DPR data, Monterey Bay Sanctuary data, DHS's Source Water Assessment database, and numerous other data sources, some of which are included in the state's draft Listing Policy. In its February 18th letter on the Policy, EPA Region IX specifically called on the state to "include all EPA monitoring data (not just EMAP) as well as other agencies that operate high quality sampling programs (*e.g.*, U.S. Fish and Wildlife Service, US Department of Agriculture, US Army Corps of Engineers, and National Oceanic and Atmospheric Administration)."

The apparent self-restriction on SWRCB data collection assembly activities is particularly problematic in light of the SWRCB's refusal to support the funding and implementation of a meaningful ambient monitoring program, or to effectively integrate the myriad databases that exist and that contain useful information. We would appreciate additional details from the SWRCB on its and the regional boards' activities to collect, assemble on their own initiative, and use to develop the 303(d) list "all existing and readily available information," over and above that provided as a result of the Solicitation Notice. (40 C.F.R. § 130.7 (b)(5).)

* * *

It is our understanding that the SWRCB is cutting $1.4 million in contract funds from the Surface Water Ambient Monitoring Program (SWAMP), which already is seriously under-funded. This is not the first time that this important program has been in jeopardy of near-collapse. The SWRCB must place monitoring information at a much higher priority if it is to adequately protect the health of the waters on which we all depend. Artificial and illegal constraints on the amount of information sought as part of the 2004 solicitation process, and continued assaults on SWAMP, appear to indicate that the SWRCB places a low value on obtaining the monitoring data its needs to do its job.

II. San Francisco Bay and Tributaries

NRDC requests that the SWRCB list the following chemicals under Section 303(d):

Chemical Name:	CAS Number:
Dibromobiphenyl Ether	2050-47-7
Tribromobiphenyl Ether	49690-94-
Tetrabromobiphenyl Ether	40088-47-9
Pentabromobiphenyl Ether	32534-81-9
Heptabromobiphenyl Ether	68928-80-3
Hexabromobiphenyl Ether	36483-60-0
Octabromobiphenyl Ether	32536-52-0
Nonabromobiphenyl Ether	63936-56-1
Decabromodiphenyl Ether	1163-19-5
Polybrominated Diphenyl Ether**	
Polybrominated Diphenyl Oxide**	

**Synonyms: Polybrominated Biphenyl Ether(s) = Polybrominated Biphenyl Oxide(s) = Polybrominated Diphenyl Ether(s) = Polybrominated Diphenyl Oxide(s)

The listing these compounds (collectively referred to as PBDEs) is justified due to an ongoing exponential increase of these substances in the water environment as evidenced by continuing increases in levels in bird eggs, fish, and seals. Over the past year, while the PBDEs have remained on the RMP analyte list, evidence has accumulated that concentrations are increasing in the Estuary: in water, in bird eggs, in fish, and in seals. Humans and wildlife in the Bay Area have some of the highest reported concentrations of these substances in the world.

In 2002, the RMP collected its 1st year of PBDE data in San Francisco Estuary water, sediments, and bivalve samples. Time series data for these three media are not yet available so it is not yet possible to determine temporal trends. The 2002 individual congener and total PBDE concentrations and distributions in San Francisco Estuary water, sediments, and bivalves are presented in the RMP's 2002 Annual Monitoring Results report.

Reports of PBDEs in environmental samples date back to the early 1980's in Sweden. Contamination by these chemicals has been reported in sediment, sewage sludge, pike, eel, sea trout, and human breast milk from locations throughout the world. Most researchers believe that a major exposure pathway of humans to these chemicals is through fish consumption.

Although the data on PBDE levels in the United States are less complete, the evidence shows that these chemicals are found in the environment and in human tissues at levels considerably higher than those reported in other countries. The Hazardous Materials Laboratory of the California Department of Toxic Substances Control has been tracking PBDE levels in harbor seal blubber from the San Francisco Bay, and breast and abdominal fat samples from women living in the San Francisco Bay Area.[i]

Thirty-four seal blubber samples were collected between 1989 and 1998 from eleven stranded, dead harbor seals found along the San Francisco Bay shoreline. Overall, levels of total PBDEs increased by nearly 100-fold during the two decades studied, implying a doubling of concentration every 1.8 years. Levels of total PBDEs in seals averaged 1730 ng/g fat with a range from 88 ng/g fat to 8325 ng/g fat. These levels are among the highest reported anywhere in the world.

The human fat levels were significantly higher than any levels previously reported anywhere in the world. The average level in San Francisco Bay area women was 86 ng/g fat. This level is ten times higher than the average level reported in Germany and Canada, three times higher than levels reported in Sweden, and twenty-five times higher than levels reported in human tissue in Spain. Levels of PBDEs tended to be higher in younger (premenopausal) women, implying that the exposures are likely to be a fairly recent phenomenon.

To further investigate the extent of contamination of the Bay area with PBDEs, this same group of researchers analyzed the eggs of four species of fish-eating shorebirds for levels of PBDEs. Shorebirds are an important ecosystem indicator due to their location at a high trophic level on the marine food chain, and the fact that they tend to breed in the same location every year. 53 individual eggs of four species (Least Tern, Clapper Rail, Forsters Tern, and Caspian Tern) and multiple nesting sites were tested. The results were compared with eggs gathered from these species in Washington state. The total PBDE levels measured in the California egg samples averaged 6.2 ppm (fat based), with a range of 0.30 to 62 ppm. Five PBDE congeners (PBDE 47, PBDE 99, PBDE 100, PBDE 153, and PBDE 154), were found in all egg samples from SF Bay and Washington state. When compared, the average level in eggs from San Francisco Bay birds was over 1000 ng/g fat higher than the average level measured in eggs from Washington state birds. As shown in Figure 1 below, the PBDE levels in bird eggs, seals, and humans all showed a similar congener pattern.

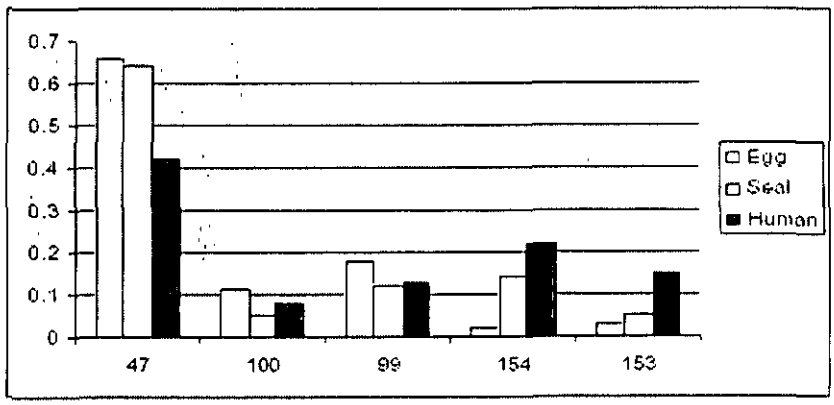


Figure 1. Relative congener patterns of PBDE in shorebird eggs, seals, and samples of human breast adipose, all from the San Francisco Bay Area.

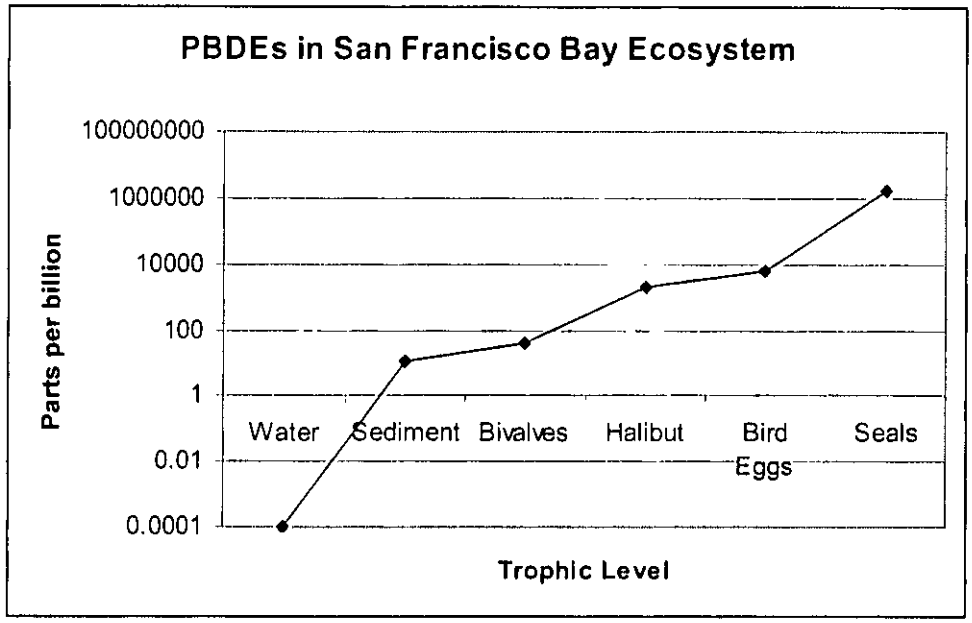
Data collected by the San Francisco Estuary Institute (SFEI) in 2001 on PBDE levels in bird eggs (double-crested cormorants and Samuels song sparrows), showed that these chemicals were present in all samples analyzed at levels ranging from 0-245 ug/kg depending on the congener, location, and species. (http://www.sfei.org/cmr/data/birdeggtables/CISNET_Egg_pbde.htm) SFEI also collected data on bivalves in the San Francisco Bay, detecting levels that averaged about 40 ppb in these animals. (http://www.sfei.org/rmp/2002/CSV%20FILES/PBDE%20TISSUE.csv) In addition, SFEI collected sediment samples from the San Francisco Bay in 2002, detecting BDE 99 in most of the samples tested, and reporting numerous detections of other congeners as well. (http://www.sfei.org/rmp/2002/CSV%20FILES/PBDE%20SEDIMENT.csv) As expected, detections of dissolved PBDEs in the water column were at far lower levels than the levels reported in sediment. (http://www.sfei.org/rmp/2002/CSV%20FILES/PBDE%20DISSOLVED.csv)

These data allow us to estimate the bioconcentration of the PBDEs in the San Francisco Bay. Table 1 below shows that these chemicals may concentrate by as much as 12 orders of magnitude from the water column to the blubber of seals.

Table 1: Bioconcentration of PBDEs in the San Francisco Bay Ecosystem

Trophic Level	Average PBDEs (2002)	Source:
Water column	0.000103 ug/L (ppb)	SFEI
Sediment	11.8 ug/kg (ppb)	SFEI
Bivalves	40.1 ug/kg (ppb)	SFEI
Halibut	2,000 ppb	EWG
Bird Eggs	6200 pg/g fat (ppb)	She et al.
Seals	1,730,000 pg/g fat (ppb)	She et al.

Figure 2



PBDEs Chemical Structure and Use

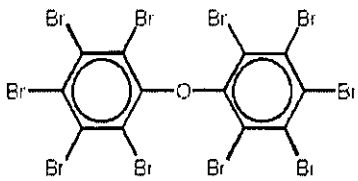
The polybrominated diphenyl ethers (PBDEs) are brominated organic compounds with chemical structures similar to dioxins and PCBs. Global production of these chemicals is approximately 40,000 tons per year for use as fire retardants in plastics and textiles. These chemicals are of major toxicological concern due to their environmental persistence and bioaccumulation, and due to available data on their toxicity. Environmental and human exposure studies worldwide are identifying dramatic increases in PBDE levels in sediments, biota, and mammalian tissues. The increases are generally logarithmic, indicating potential for significant health and environmental impairment in the relatively near future if the pollution from these chemicals is not addressed very soon. We are convinced that these chemicals will pose a threat to water on a par with the PCBs. Accordingly we ask the SWRCB to move rapidly to list these chemicals under Section 303(d) and immediately begin enhanced monitoring programs, identification of discharges, and pollution reduction activities. Waiting and watching will simply allow the health and environmental threat to grow.

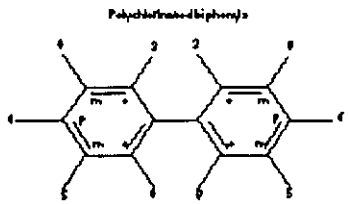
PBDEs in current widespread use have somewhat different environmental persistence and toxicity profiles. Deca-BDE accounts for the largest percentage of the market. However, the lower brominated congeners (hexa-BDE and below) are more readily absorbed by animals and their half-lives in living organisms are comparable to that of 2,3,7,8-TCDD (dioxin). In some test systems, tetra-and penta-BDE had higher bioaccumulation potential than the PCBs. Strategies aimed only at the penta-BDEs may

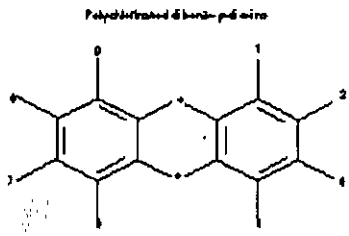
not solve the problem, because there is evidence that in the presence of sunlight, the higher brominated forms may degrade to the more readily absorbed, persistent and bioaccumulative lower-brominated forms.

Figure 3: Comparative Structures of PBDEs, PCBs, and Dioxins

Polybrominated diphenyl ethers







PBDEs Toxicity Summary

Dioxin-Like and PCB-Like Activity

The PBDEs are structurally very similar to the PCBs and the dioxins. The toxicological evidence to date indicates that these chemicals also share many common traits. The tetra- to hexa-BDEs are strong inducers of liver enzymes in the rodent. In particular, these chemicals induce the cytochrome p-450 (CYP) enzyme system in the liver, including CYP1A1 as assessed by the standard test using ethoxyresorufin-o-deethylase (EROD). EROD activity is considered a hallmark of dioxin-like compounds, and penta-BDE is a more powerful inducer of EROD activity than commercial PCB mixtures.[ii]

PBDEs also interact with the aryl hydrocarbon (Ah) receptor, another hallmark of dioxin-like activity. In a study of 17 PBDE congeners, seven acted as Ah receptor

agonists and nine acted as antagonists when co-treated with 2,3,7,8-TCDD. The potencies of the PBDE agonists were similar to some PCBs. When PBDEs and PCBs were administered together, the effects were additive, suggesting a similar mechanism of action.[iii]

Immune Suppression

Dioxins and PCBs are known to cause immunosuppression in laboratory animals, and probably in humans. In standardized tests of immune response, a mixture of PBDEs resulted in immune suppression in mice.[iv] In addition to reduction in immune function, cellular changes were observed in organs critical to immune function such as the spleen and thymus. The immunosuppressive effects of PBDEs have been reported to exceed the effects of PCBs in laboratory animals. Suppressed production of IgG antibodies after stimulation with chemicals that would normally enhance IgG production indicates that these chemicals may have significant adverse effects on immune function.[v] Negative findings reported from some *in vitro* standardized immunotoxicity tests are probably due to the fact that the immune effects are indirect and require interaction with other systems such as the Ah receptor. Simple *in vitro* systems fail to reflect the real effects of these chemicals in the body. This hypothesis is supported by the failure of these same systems to detect the known immunotoxic effects of the PCBs.[vi]

PBDEs and Cancer

Swedish hospital patients with non-Hodgkin's lymphoma were reported in one study to have higher concentrations of tetra-BDE in their fat.[vii] There are no other relevant cancer data from human epidemiologic studies. Mutagenicity studies have shown conflicting results, with several studies failing to show cell mutations in *in vitro* test systems, whereas other studies have shown evidence of an epoxide intermediate (a metabolite that would be expected to cause mutations) and of genotoxicity.[viii ix]

There is a major gap in the cancer toxicology database for the PBDEs. Only the deca-BDE form, which is poorly absorbed and rapidly eliminated, has been tested for carcinogenicity in the rodent. Nonetheless, in studies performed by the National Toxicology Program (NTP), deca-BDE produced statistically significant increases in hepatocellular adenomas and carcinomas in male mice, and marginal increases in thyroid follicular cell adenomas and carcinomas in both male and female mice. In rats, statistically significant dose-related increases in liver adenomas were seen in both males and females, and significantly increased numbers of pancreatic adenomas were seen in the males.[x]

Endocrine Disruption

The hormonal effects of the PBDEs, are of great toxicological concern. It is clear that the lower-brominated PBDEs disrupt thyroid hormone. In rats, penta-BDE reduces

thyroid hormone levels and increases thyroid hyperplasia even at the lowest doses tested - 2 mg/kg/day.[xi] In mice, one single dose of penta-BDE at only 0.8 mg/kg caused continued suppression of thyroid hormone levels more than a week later.[xii] When animals were exposed to both PBDEs and PCBs, the effect on thyroid hormone suppression was additive.[iii]

The hormonal effects of PBDEs are not expected to be peculiar to laboratory rats. In fact, men working in the production of deca-BDE were found to have a higher than expected rate of hypothyroidism. Four out of 35 exposed workers had clinically-significant hypothyroidism whereas no cases of thyroid dysfunction were identified among 89 age and sex-matched workers who were not exposed to these chemicals.[xiii]

Neuro-Behavioral and Developmental Effects

In standard developmental toxicology studies, the PBDEs cause increased fetal death, abnormal formation of the skull, enlarged heart, and subcutaneous edema. The doses that caused fetal toxicity were lower than the doses that affected the mothers.[xiv xv] It should be noted, however, that the standard developmental toxicology studies detect only obvious birth defects and toxicity, and are unable to detect more subtle alterations in neurologic function and behavior.

Single low doses of penta-BDE (only 0.8 mg/kg), administered to mice during the vulnerable period for brain development just after birth, resulted in permanent neurological dysfunction. These mice were permanently more sluggish and had decreased spontaneous activity levels throughout their lives, worsening with age, and permanent reductions in learning and memory.[xvi] These important findings have been confirmed in several different studies, and may be related to alterations in the cholinergic system or to suppression of thyroid hormone during a critical period of brain development.[xvii xviii xix] Thyroid suppression during development has been shown to cause permanent subtle impairment of neurological function.[xx]

Summary

The PBDEs are of serious toxicological concern on the basis of their environmental properties, their chemical structure, and the toxicological evidence to date. These chemicals are environmentally persistent and are known to bioaccumulate. The chemical structure of PBDEs is similar to the PCBs, dioxins, and other organohalogen compounds of serious concern. Although the toxicological database is incomplete, there is evidence that these chemicals have dioxin-like and PCB-like properties, including interference with enzyme systems and with the aryl hydrocarbon receptor. The effects of greatest concern to date are anti-thyroid effects and adverse effects on neurological development from early life exposures.

The levels of the PBDEs in biota in the San Francisco Bay is a serious cause for concern. The fact that the concentrations are among the highest reported anywhere in the world, combined with the evidence that the concentrations are increasing logarithmically, means that it is imperative to act quickly. A Section 303(d) listing will allow action to reduce exposures before the toxicity becomes severe enough to cause serious damage to the entire ecosystem in the San Francisco Bay.

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Thank you for the opportunity to provide these comments. Please do not hesitate to call if you have any questions.

Sincerely,

David S. Beckman
Senior Attorney and Director,
Coastal Water Quality Project

cc: Celeste Cantu, Executive Director, SWRCB
Craig Wilson, Chief Counsel, SWRCB
Craig J. Wilson, Chief, Monitoring and TMDL Listing Unit, SWRCB
Alexis Strauss, U.S. EPA, Region IX

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[i] She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D. PBDEs in the San Francisco Bay Area: measurements in harbor seal blubber and human breast adipose tissue. Chemosphere 46:697-707, 2002.
[ii] Von Meyerinck L, Hufnagel B, Schmoldt A, Benthe HF. Induction of rat liver microsomal cytochrome P-450 by the pentabromo diphenyl ether Bromkal 70 and half-lives of its components in the adipose tissue. Toxicology 61(3): 259-274, 1990.
[iii] Hallgren S, Darnerud PO. Effects of polybrominated diphenyle ethers (PBDEs), polychlorinated biphenyls (PCBs), and chlorinated paraffins (CPs) on thyroid hormone levels and enzyme activities in rats. Organohalogen Compounds 35:391-394, 1998.
[iv] Fowles JR, Fairbrother A, Baecher-Steppan L, Kerkvliet NI. Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. Toxicology 86:49-61, 1994.
[v] Darnerud PO, Thuvander A. Effects of polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) on some immunological parameters after oral exposure in rats and mice. Toxicol Environ Chem 70:229-242, 1999.
[vi] Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. Environ Health Perspect 109(Suppl 1):49-68, 2001.
[vii] Hardell L, Lindstrom G, van Bavel B, Wingfors H, Sundelin E, Liljegren G. Concentrations of the flame retardant 2,2', 4,4'-tetrabrominated diphenyl ether in human adipose tissue in Swedish persons and the risk for non-Hodgkin lymphoma. Oncol Res 10(8):429-432, 1998.
[viii] Orn U, Klasson-Wehler E. Metabolism of 2,2', 4,4'-tetrabromodiphenyl ether in rat and mouse. Xenobiotica 28(2): 199-211, 1998.

[ix] Helleday T, Tuominen KL, Bergman A, Jenssen D. Brominated flame retardants induce intragenetic recombination in mammalian cells. Mutat Res 439(2):137-147, 1999.
[x] NTP. Toxicology and carcinogenesis studies of decabromodiphenyl oxide (CAS No. 1163-19-5) in F344/N rats and B6C3F1 mice (feed studies). TR 309. Research Triangle Park, NC: National Toxicology Program, 1986.
[xi] U.S. EPA TSCA Section 8e report numbers 04760A, 04856A, 05420A.
[xii] Fowles JR, Fairbrother A, Baecher-Steppan L, Kerkvliet NI. Immunologic and endocrine effects of the flame-retardant pentabromodiphenyl ether (DE-71) in C57BL/6J mice. Toxicology 86(1-2): 49-61, 1994.
[xiii] Bahn AK, Mills JL, Synder PJ, Gann PH, Houten L, Bialik O, et al. Hypothyroidism in workers exposed to polybrominated biphenyls. N Engl J Med 302(1):31-33, 1980.
[xiv] WHO. Brominated diphenyl ethers. IPCS Environmental Health Criteria 162. Geneva: World Health Organization, 1994.
[xv] U.S. EPA. Brominated diphenyl ethers. Chemical Hazard Information Profile. Washington, DC, 1986.
[xvi] Eriksson P, Jakobsson E, Fredriksson A. Developmental neurotoxicity of brominated flame-retardants, polybrominated diphenyl ethers and tetrabromo-bisphenol A. Organohalogen Compounds 35:375-377, 1998.
[xvii] Eriksson P, Viberg H, Jakobssen E, Orn U, Fredriksson A. PBDE 2,2',4,4'-pentabromodiphenylether causes permanent neurotoxic effects during a defined period of neonatal brain development. Organohalogen Compounds 40:333-336, 1999.
[xviii] Viberg H, Fredriksson A, Jacobsson E, Ohrm U, Eriksson P. Developmental neurotoxic effects of 2,2',4,4',5,5'-pentabromodiphenyl ether (PBDE 99) in the neonatal mouse. Toxicologist 54(1):290, 2000.
[xix] Eriksson P, Jakobsson E, Fredriksson A. Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? Environ Health Perspect 109(9):903-908, 2001.
[xx] Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent psychological development in the child. N Engl J Med 341:549-555, 1999.

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PBDEs in the San Francisco Bay Area: measurements in harbor seal blubber and human breast adipose tissue

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Abstract

To explore the levels of polybrominated diphenyl ethers (PBDEs) in California, samples from 11 archived harbor seals (*Phoca vitulina* Richardsi) from the San Francisco Bay and breast adipose tissue samples from 23 women were analyzed. The levels of PBDEs in human tissue samples were in the low ng/g fat range, with PBDEs 47, 153, 154, 99, and 100 as the major congeners. Average ∑PBDEs (86 ng/g fat) in these California women are the highest human levels reported to date. An inverse relationship between concentration of PBDEs and age of these women was apparent. The levels of PBDEs measured in harbor seal blubber were in the low ng/g to low μg/g fat range, with the same major congeners as those measured in the human tissues. PBDE 47 was the highest among all congeners measured in both human tissue and seal blubber samples. The concentrations of PBDEs in harbor seals in the San Francisco Bay have increased dramatically over the past decade, with current levels among the highest reported for this species.

Keywords: Seal; Blubber; Human Adipose; PBDEs; Time trends

1. Introduction

Unlike many of the historically common yet currently banned organochlorines, polybrominated diphenyl ethers (PBDEs) are unregulated chemicals in active use. Due to their high lipophilicity and persistence, PBDEs are expected to bioaccumulate. A substantial fraction of these compounds will eventually reach the marine environment and ultimately the human body. One of the first reports of PBDEs in the environment appeared in 1981, when PBDEs were found in pike, eel, and sea trout from a river in the western part of Sweden (Andersson and Blomkvist, 1981). Since then, residual levels of PBDEs have been found in sewage sludge (Hagenmaier et al., 1992; Nylund et al., 1992), sediment (Watanabe et al., 1995), biota (Jansson et al., 1993) and humans (Cramer et al., 1990; Stanley et al., 1991; Lindström et al., 1998; Meneses et al., 1999). PBDE analysis of archived human milk from Sweden showed a 60-fold increase from 1972 to 1997 (Norén and Meironyté, 1998). Since that report, increases in the levels of PBDEs were shown in human milk samples (Darnerud et al., 1998), human blood (Schröter-Kermani et al., 2000) and in lake trout (Luross et al., 2000). Most of the studies on levels of PBDEs have focused on northern Europe and Canada. Very few data are available on levels of PBDEs in the USA.

The sediments in the San Francisco Bay act as a secondary source of persistent organic pollutants (POPs) for the local environment, due to significant accumulations of persistent organochlorines, from both historical and on-going waste discharges. Harbor seals (*P. vitulina* Richardsi), as year-round residents of the Bay and upper trophic level piscivores, are exposed to those

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contaminants which bioaccumulate in the food web. Due to their position at the top of the aquatic food chain and their relatively long-life-spans, marine mammals are an important tool to monitor pollution of the marine environment worldwide. They can be used as global pollution indicators and can be considered as model systems for low-dose; long-term effects of environmental pollution (Reijenders, 1988; Hutchinson and Simmonds, 1994).

To explore the levels of PBDEs in marine mammals and humans in California, harbor seal blubber and human adipose tissue samples were analyzed. The aim of the study was to determine the level of PBDEs in seals and humans, assess time trends, and compare levels and profiles of PBDEs to results from other studies.

2. Materials and methods

2.1. Sample collection

Breast and abdominal adipose samples were collected in the late 1990s from San Francisco Bay Area women as part of a case-control study on organochlorines and breast cancer (Petreas et al., 2000). A random subset of 23 breast adipose tissue samples was selected for PBDE analysis. Women ranged from 28 to 62 years of age and were predominantly white (18/23) and born in the USA (19/23). Pathology reports indicated 12 women to be malignant, eight benign and three with ductal carcinomas in situ (DCIS), a condition considered by some as transitional to malignant.

Seal blubber samples were collected between 1989 and 1998 ($n = 34$) from stranded, dead harbor seals found along the San Francisco Bay shoreline; tissue collection was part of a larger study that examined harbor seal population dynamics, health, contaminant residues and prey selection (Kopec and Harvey, 1995). Gross blubber samples, from muscle to and including the skin, were collected in the field and later sub-sampled and archived at −20° until analysis. Blubber samples were taken from gender balanced, sexually mature adults, as defined by length and weight (Bigg, 1969), and included one mother and fetus pair that died during birth. In all, samples from 10 adults and one fetus were analyzed.

2.2. Analytical methods

Harbor seal blubber and human adipose tissue samples were processed in a similar way and analyzed for polychlorinated dibenzo-*p*-dioxins (dioxins), polychlorinated dibenzo furans (furans), polychlorinated biphenyls (PCBs), organochlorine pesticides and PBDEs (She et al., 2000). Only PBDEs will be discussed in this report. Briefly, samples (1–3 g) were homogenized and extracted in 1:1 hexane:methylene chloride, shaken and centrifuged to separate phases. Nine tenths of the extract were analyzed for dioxins and furans, and one tenth of the extract was cleaned up for PBDE, pesticide and PCB analyses. The samples were spiked with 10 ng each of $^{13}C_{12}$-PBDE 77, $^{13}C_6$-β-HCH, $^{13}C_4$-dieldrin and $^{13}C_8$-mirex, 20 ng each of $^{13}C_6$-HCB, $^{13}C_{12}$-*p,p'*-DDT and $^{13}C_{12}$-*p,p'*-DDE, 600 pg of $^{13}C_{12}$-dioxin and furan analogs (all 2,3,7,8-substituted), and 600 pg of $^{13}C_{12}$-PCB 28, 52, 47, 101, 105, 118, 153, 180, 194, 209. Initially, some of the human tissue samples were not spiked with $^{13}C_{12}$-PBDE 77, but $^{13}C_{12}$-PCB 180 was used for quanti- tation, based on its structural similarity and availability at the time when samples were processed. Fat was removed from the samples using gel permeation and Florisil chromatography in a single automated system (FMS, Waltham, MA). The analytes were concentrated in the presence of 4 ng of $^{13}C_{12}$-PCB 128 and 178 and $^{13}C_6$-α-HCH (recovery standards) to 10 μl for analysis. The analysis was carried out using a Finnigan- 4510 GC/MS system. Samples were introduced through a splitless injector connected to a 60 m × 0.25 mm, 0.25 μm film thickness, DB-5 ms column with helium as the carrier gas. The MS was operated in the electron capture, negative ionization (ECNI), multiple ion detection mode (MID). Research grade methane was used as the reagent gas. The ion source pressure was held at 0.6 torr and ion source temperature was 150 °C. The electron energy was typically 70 eV and the electron emission current was kept at 0.3 mA. Retention times for PBDEs 47, 99, 153 were established using standards (CIL, Andover, MA). PBDEs 100 and 154 were tentatively identified based on the literature retention times. The bromide ions of PBDEs were chosen as the quantitation ions. For quality control, a laboratory method blank was run with each batch of six samples. The samples were considered valid when analyte concentrations were at least three times higher than the respective blank value. No blank correction was used with these data.

The age for eight of the ten adult seals sampled, was determined by cementum analysis of the upper left canine or, for one seal, a premolar (Matson's Laboratory, LLC, Milltown, MT, USA).

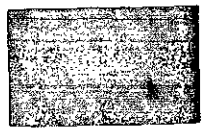
Lipid composition analyses were performed on six of the ten adult seal blubber samples to determine whether lipid composition varied with the total percent fat in the blubber. Lipid class composition was determined by Iatroscan TLC/FID (Ackman, 1981) at the Canadian Institute of Fisheries Technology, DalTech, Dalhousie University, Halifax, NS, Canada.

3. Results and discussion

Sample quantitation based on $^{13}C_{12}$-PBDE 77 did not differ from quantitation based on $^{13}C_{12}$-PCB-180 in terms of levels or patterns. Duplicate analyses of three

Table 1
PBDE concentrations in 23 human breast adipose samples (ng/g fat), and ratios of each congener to ∑PBDE

Sample ID	Age (years)	Fat (%) in sample	PBDE 47 (ng/g fat)	PBDE 99 (ng/g fat)	PBDE 100 (ng/g fat)	PBDE 153 (ng/g fat)	PBDE 154 (ng/g fat)	∑PBDE (ng/g fat)	Ratio PBDE#:∑PBDE				
									47	99	100	153	154
961429	28	9.9	48.6	21.7	12	33.8	41.7	158	0.31	0.14	0.08	0.21	0.26
961430	31	42.8	16.2	6.81	3.12	11.6	23.4	61.1	0.27	0.11	0.05	0.19	0.38
961431	40	45.6	29.9	11.6	8.26	14.2	22.6	86.6	0.35	0.13	0.10	0.16	0.26
961432	35	80.7	18.3	6.92	4.5	12.6	53.3	95.6	0.19	0.07	0.05	0.13	0.56
961433	41	93.6	196	72.2	59.4	63.5	70.5	462	0.42	0.16	0.13	0.14	0.15
961434	39	81.2	46.8	20	15.2	29.6	57	169	0.28	0.12	0.09	0.18	0.34
970036	45	44.6	23.1	7.3	3.17	2.3	3.76	39.6	0.58	0.18	0.08	0.06	0.09
970037	46	50.8	11.4	3.55	1.41	1.6	4.47	22.4	0.51	0.16	0.06	0.07	0.20
970038	54	26.8	11.4	6.75	1.89	1.98	9.42	31.4	0.36	0.21	0.06	0.06	0.30
970039	56	85.6	7.01	3.11	1.43	1.52	4.14	17.2	0.41	0.18	0.08	0.09	0.24
970040	48	89	28.2	6.59	4.14	2.37	4.15	45.5	0.62	0.14	0.09	0.05	0.09
970041	60	84.5	20.5	4.14	3.57	3.18	10	41.4	0.50	0.10	0.09	0.08	0.24
990592	50	87.8	26.6	8.16	3.07	3.54	5.23	46.6	0.57	0.18	0.07	0.08	0.11
990594	49	82.1	14.7	4.09	1.7	5.45	7.88	33.8	0.43	0.12	0.05	0.16	0.23
990595	55	92.9	8.11	2.78	1.01	1.6	4.8	18.3	0.44	0.15	0.06	0.09	0.26
990596	54	90.2	16.5	2.68	2.43	4.09	6.27	32.0	0.52	0.08	0.08	0.13	0.20
990790	42	89.8	149	29.8	60.6	124	16.3	380	0.39	0.08	0.16	0.33	0.04
990792	62	75.1	7.54	2.17	0.77	1.99	5.81	18.3	0.41	0.12	0.04	0.11	0.32
990794	45	66.3	22.9	3.29	4.56	14.8	7.26	52.8	0.43	0.06	0.09	0.28	0.14
990955	51	86.2	12.5	4.61	2.21	1.82	2.86	24.0	0.52	0.19	0.09	0.08	0.12
990956	51	85.1	10.7	2.77	1.88	2.54	5.22	23.1	0.46	0.12	0.08	0.11	0.23
990957	51	85.3	29.1	11.1	10.2	24	5.99	80.4	0.36	0.14	0.13	0.30	0.07
990960	48	84.5	10.9	3.64	3.4	9.89	6.4	34.2	0.32	0.11	0.10	0.29	0.19
Min	28	9.9	7.01	2.17	0.77	1.52	2.86	17.2	0.19	0.06	0.04	0.05	0.04
Max	62	93.6	196	72.2	60.6	124	70.5	462	0.62	0.21	0.16	0.33	0.56
Median	48	84.5	18.3	6.59	3.17	4.09	6.4	41.4	0.42	0.13	0.08	0.13	0.23
Mean	47	72.2	33.3	10.7	9.1	16.2	16.5	85.7	0.42	0.13	0.08	0.15	0.22
S.D.	8.6	23.4	45.9	15.1	16.5	27.7	19.7	114	0.11	0.04	0.03	0.08	0.12



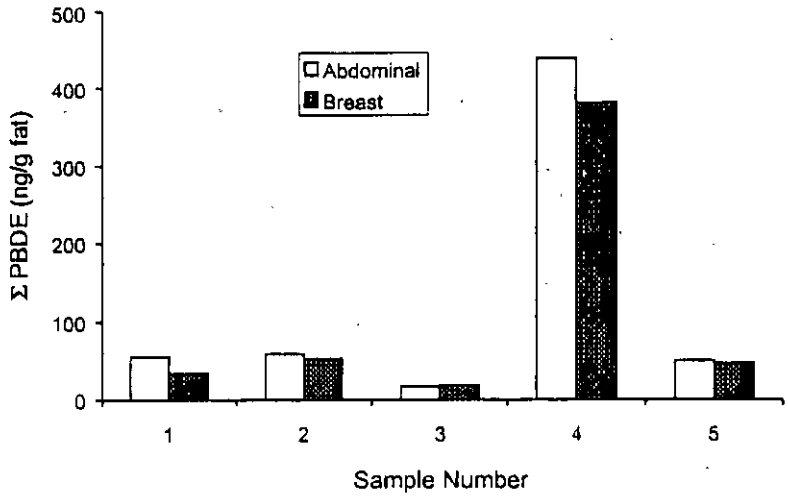


Fig. 1. Total PBDE concentrations (ng/g fat) in abdominal and breast adipose tissues of participating women.

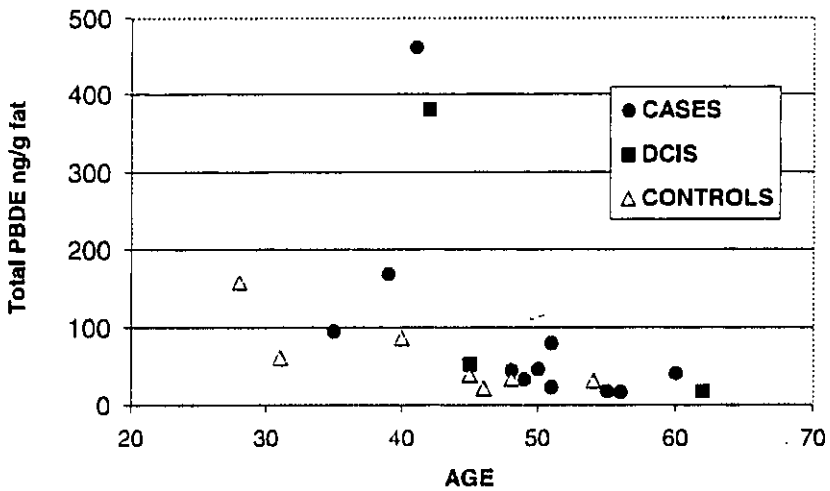


Fig. 2. Total PBDE concentration (ng/g fat) in human breast adipose as a function of disease status and age.

human samples showed an overall relative percent difference (RPD) of 15%, ranging from 7% for PBDE 154 to 35% for PBDE 99. Duplicate analysis of one seal sample showed an overall RPD of 11%, ranging from 8% for PBDE 99 and 153 to 19% for PBDE 47.

3.1. PBDEs in human breast adipose tissue

Table 1 shows the lipid concentrations of PBDE congeners in 23 human tissue samples. Tetra- to hexa-BDEs were measured in all samples and among them, PBDE 47 was the highest. PBDE 47 also appears to be the predominant congener in all human studies reported, with the exception of occupationally exposed electronics dismantlers (Sjödin et al., 1999). The average levels in our human adipose samples are higher than levels reported for Swedish and Spanish tissue samples (Lindström et al., 1998; Meneses et al., 1999). The mean concentration of PBDE 47 in our human tissue samples (33 ng/g fat) is about 10 times higher than serum from Germany (Schröter-Kermani et al., 2000) and human milk from Canada (Ryan and Patry, 2000); three times higher than tissue samples from Sweden; and 25 times higher than human tissues from Spain (Meneses et al., 1999). Further, the levels of penta- and hexa-BDEs are higher in San Francisco Bay women than in any of the above human samples from Europe and Canada. The levels of hexa-BDE in our human tissue samples were higher than previously estimated levels in the USA in the late 80s (Stanley et al., 1991). While these are limited and preliminary data, it appears that the levels of PBDEs in the general California population are the highest

Table 2
Concentrations (ng/g fat) of PBDE congeners in harbor seal blubber, and ratios of each congener to ∑PBDE

Sample ID	Date collected	Age (years)	Sex	Fat (%) in lipid	(ng/g) Lipid weight						Ratio PBDE#:∑PBDE				
					PBDE 47	PBDE 99	PBDE 100	PBDE 153	PBDE 154	∑PBDE	47	99	100	153	154
981044	04/14/92	0	Fetus	66.9	277	85.6	12.4	10.5	44.6	430	0.64	0.2	0.03	0.02	0.10
981049	04/25/89	8	M	98.6	45.6	16.2	4.16	4.06	17.7	88	0.52	0.18	0.05	0.05	0.20
981050	04/08/91	12	M	72.4	87.4	23.5	5.82	6.75	38.0	161	0.54	0.15	0.04	0.04	0.24
981047	11/22/91	10	F	95.8	308	149	33.6	41.8	230	763	0.40	0.2	0.04	0.05	0.30
981045	04/14/92	25	F	73.5	350	151	26.4	35.4	220	782	0.45	0.19	0.03	0.05	0.28
981046	02/22/93	5	F	96.9	331	51.6	47.7	15.5	26.7	473	0.70	0.11	0.10	0.03	0.06
981051	04/27/93	10	M	74.4	299	95.4	26.9	25.9	51.0	498	0.60	0.19	0.05	0.05	0.10
981052	05/10/93	8	M	94.3	956	102	67.5	59.7	91.4	1276	0.75	0.08	0.05	0.05	0.07
981053	04/11/97	9	M	39.6	1633	60.6	121	74.0	55.0	1944	0.84	0.03	0.06	0.04	0.03
981048	10/31/98	NA	F	47.2	2343	172	231	160	79.1	2985	0.79	0.06	0.08	0.05	0.03
981054	10/31/98	NA	M	27.9	6682	303	307	649	384	8325	0.80	0.04	0.04	0.08	0.05
All adults															
Mean				72.1	1304	112	87.1	107	119	1730	0.64	0.12	0.05	0.05	0.14
S.D.				25.8	2032	86.0	103	196	120	2481	0.16	0.07	0.02	0.01	0.11
Females															
Mean				85.1	198	85	17.5	22.0	126	449	0.48	0.18	0.04	0.05	0.25
S.D.				14.1	153	75.3	14.8	19.3	114	375	0.06	0.02	0.01	0.01	0.05
Males															
Mean				63.4	2041	131	133.5	164	115	2583	0.75	0.08	0.06	0.05	0.06
S.D.				29.3	2405	94	112	243	134	2968	0.09	0.06	0.02	0.02	0.03

reported to date. These high PBDE levels may be partially explained by the fact that California regulations require all furnishings to pass flammability tests for fire safety (State of California, 1992). Although no specific flame retardants are mandated, it is quite likely that PBDEs are added to polyurethane foam used in furnishings.

3.2. Distribution of PBDEs in breast and abdominal tissue

In order to assess contaminant distribution in breast and abdominal adipose tissue, we collected samples of both these tissues from 21 women. Five of these pairs were analyzed for PBDEs. Overall RPDs were 25%, ranging from 17% for PBDE 47 to 32% for PBDE 154. Abdominal and breast concentrations were highly correlated with no bias (Wilkoxon signed rank test $p > 0.05$) and, therefore, these types of tissues may be used interchangeably in future studies. Levels of $\sum$PBDEs in human abdominal and breast adipose tissue are shown in Fig. 1.

3.3. The relationship between PBDE levels, disease status and age

Fig. 2 shows the $\sum$PBDE levels in breast adipose tissues of 23 women as a function of their age. Disease status (malignant, benign or DCIS) is indicated on the graph. Whereas, disease status does not appear to correlate with $\sum$PBDEs, there appears to be an inverse relationship with age. In fact, when women were grouped using the median age as the cutoff, women younger than 48 had significantly higher $\sum$PBDE levels than women older than 48 (Mann–Whitney test, $p < 0.05$). Others have reported no association of PBDEs with age (Meneses et al., 1999), consistent with the assumption of a recently introduced POP. As expected, levels of dioxins and furans have a positive correlation with age in the same population (Petreas et al., 2000). This may imply that different activities may expose different age groups more than others, or that some compounds may accumulate differently with age. Multi- variate analysis of the entire population, currently underway, should provide a more definitive picture.

3.4. PBDEs in pinniped blubber

Published reports on PBDEs in pinnipeds refer to various species from different geographic locations, and to regional subspecies of the harbor seal (*P. vitulina*). Therefore, comparisons should be made with caution. Female ringed seals (*P. hispida*) collected in 1981 from Svalbard contained $\sum$PBDE levels of 40–51 ng/g fat (de Wit, 1999). Baltic Sea harbor seals (*P. vitulina*) contained 90 ng/g fat and harbor seals from the North Sea contained 10 ng/g fat $\sum$PBDE (de Wit, 1999). Female gray seals (*Halichoerus grypus*) from the Baltic Sea collected in 1979–1985 contained 730 ng/g fat (de Wit, 1999). Blubber from Baltic gray and ringed seals collected between 1981 and 1988 contained 419 and 350 ng/g fat, respectively (Haglund et al., 1997). Harbor seals from the Dutch coast contained a range of 605–6010 ng/g fat PBDEs (de Boer et al., 1998).

Table 2 shows the concentration of PBDEs in 11 harbor seal samples. $\sum$PBDE blubber concentrations in the adult seals varied by two orders of magnitude over the nine-year sample period, ranging from 88 to 8325 ng/g fat, with a mean of 1730 ng/g fat and a standard deviation of 2480 ng/g fat. PBDE 47 was significantly greater than all other residue concentrations ($p < 0.001$). Numerous factors may contribute to PBDE residue variability as will be discussed below.

3.5. Age and sex

Age, determined for 8 of the 10 adult seals, averaged 10.9 years, with a S.D. of 6.1 years. Most were between 5 and 12 years old, with one female 25 years old at the time of death (during parturition). Since a positive correlation has been reported between organochlorine residues and age at time of sampling (Addison et al., 1973), partial regression analysis was used to compare PBDE residue levels to age, while controlling for date of sampling. Neither $\sum$PBDE nor individual PBDE congeners were significantly correlated with seal age ($n = 8$, $p > 0.05$). Nor was any correlation found between age and PBDE residue level in males alone ($n = 5$, $p > 0.05$). The absence of a correlation between age and residue level, when controlling for date of sample collection, may be due to our small sample size or the limited age range in our current data set.

Sex has been reported to influence organochlorine accumulations in mammals with males accumulating higher concentrations than reproductively active females (Addison and Smith, 1974; Ikonomou et al., 2000). Only one female in this sample set, which died during parturition, was confirmed to be reproductively active. No significant difference was found between the six adult males and four adult females in this sample set. Analysis of covariance (ANCOVA) was used to compare mean residue levels between genders while controlling for date sampled. No significant interaction was found between gender and date sampled ($p > 0.05$), nor was any correlation present between gender and PBDE residue level ($p > 0.05$). This finding may be due to a limited sample size ($n = 10$), or some other factor.

3.6. Blubber lipids

In marine mammals, blubber serves as insulation from cold marine waters and as a lipid storage depot. In pinnipeds metabolic consumption of depot fat in the

blubber follows an annual cycle, reaching its highest level during the spring breeding season and summer molt, when peak metabolic rates are reported. In addition, reduced prey consumption, resulting from injury, disease or limited prey availability can stimulate metabolism of blubber lipids (Davis et al., 1993).

Annual changes in blubber thickness are also observed, yet blubber thickness is not directly correlated with lipid content of the tissue. In the blubber, metabolized lipids are initially replaced with water, in order to maintain blubber thickness, and so maintain insulation properties (Aguilar, 1985). Prolonged metabolism of blubber lipids eventually reduces blubber thickness.

A wide range of blubber fat content was present in our data set. Percent fat comprised an average of 72.1 with a S.D. of 25.8 of the blubber of 10 adult seals. Mean fat content did not vary significantly between males and females (t-test, $n = 10$, $p = 0.56$). No seasonal variation in percent fat was found in our sample set of 10 adult seals. Six of the seals were sampled during the spring pupping season and the remaining four were sampled during the fall and winter months. No significant difference was found in percent fat values between these two seasons (t-test, $n = 10$, $p = 0.64$). Similarly, percent fat was not correlated with the month the sample was collected ($R^2 = 0.135$, $p = 0.30$). This finding was not unexpected, since, as discussed above, stranded marine mammals may metabolize blubber lipids for energy when injured or weakened by disease prior to death.

Past studies indicate that the polarity differences in the primary lipid fractions influence the partitioning of organochlorines in blubber tissue. Triglycerides (tryacylglycerides), the dominant lipid fraction in seal blubber, also comprise the most non-polar fraction and the primary site of organochlorine accumulations in blubber (Aguilar, 1985).

Lipid metabolism that creates reduced levels of total lipids in blubber tissue may theoretically influence triglyceride levels through selective metabolism of specific lipid fractions. Organochlorine residues, that partition to the triglyceride fraction, are expected to vary in relation to the volume of the triglyceride fraction. A reduced concentration of triglycerides in the blubber lipids could lower organochlorine residue levels reported on a lipid weight basis. Organochlorines bound to metabolized lipids entering the bloodstream for transport, may increase the circulating levels of these trace residues (Findlay and de Freitas, 1971). Alternately, trace residues may further concentrate in the remaining lipid tissue, serving to increase the organochlorine residues in the remaining blubber (Addison and Brodie, 1987).

Lipid composition analyses were performed on a subset ($n = 6$) of the adult seals included in this study, to determine if a correlation existed between triglyceride levels and the total lipid content of individual blubber samples within our data set. Blubber from three seals with lipid values below 50% and three seals with blubber lipids greater than 95% were analyzed to determine whether triglyceride concentrations varied with total lipid concentration in the blubber. Triglyceride levels in the blubber lipids in this data set did not vary with total blubber lipids. The mean triglyceride level was 81.7%, with a S.D. of 6.5%. No significant difference in triglyceride levels was found between blubber samples with greater than 95% vs. lower than 50% lipids (t-test, $n = 6$, $p = 0.71$), nor did triglyceride levels correlate with total lipid levels ($R^2 = 0.20$, $p = 0.37$). Despite a wide variation in the percent total lipid in these blubber samples, the percent triglyceride fraction did not vary significantly. The magnitude of the total triglyceride fraction is reflected in the total percent lipids reported in each sample.

3.7. Time trend

Numerous recent reports indicate increasing concentrations of PBDE residues in both human (Norén and Meironyté, 1998; Ryan and Patry, 2000; Schröter-Kermani et al., 2000) and wild animal populations. In biota, results from a temporal study in Lake Ontario indicated an increase in the concentration of $\sum$PBDEs in lake trout over time, from 3 ng/g fat in 1978, to 945 ng/g fat in 1998 (Luross et al., 2000). Similarly, the $\sum$PBDE levels in ringed seals collected from Holman Island (NW Territories, Canada) have increased from 1981 to 1996 (Ikonomou et al., 2000). Since 1982, the levels of the major PBDE homologue groups and congeners in the beluga (*Delphinapterus leucas*) have increased significantly. Concentrations of PBDE 47, the most predominant PBDE congener residue in the beluga blubber, increased 6.5-fold over that 15-year time period (Stern and Ikonomou, 2000).

In our study, the three highest concentrations (8325, 1944 and 2986 ng/g fat) of $\sum$PBDEs were found in seals with the lowest fat content (28%, 40% and 48%). Because these three samples were also collected later in time (1997–1998), the correlation of date of collection and lipid content was examined. The lipid concentration in the blubber was correlated with sample collection date (1989–1998) ($n = 10$, $R^2 = 0.74$, $p = 0.001$). This correlation was driven by the three samples collected in 1997–1998, and was no longer significant when those samples were removed from the analysis (1989–1993) ($n = 7$, $p > 0.05$). The cause of the reduced lipid concentration in the blubber of the three later samples (e.g., a sampling artifact, or a reflection of changing environmental conditions in San Francisco Bay) is unknown.

As shown in Fig. 3, concentrations of $\sum$PBDEs increased almost two orders of magnitude in our entire population of adult seals ($n = 10$, sampled between 1989 and 1998). This significant increase ($R^2 = 0.833$,

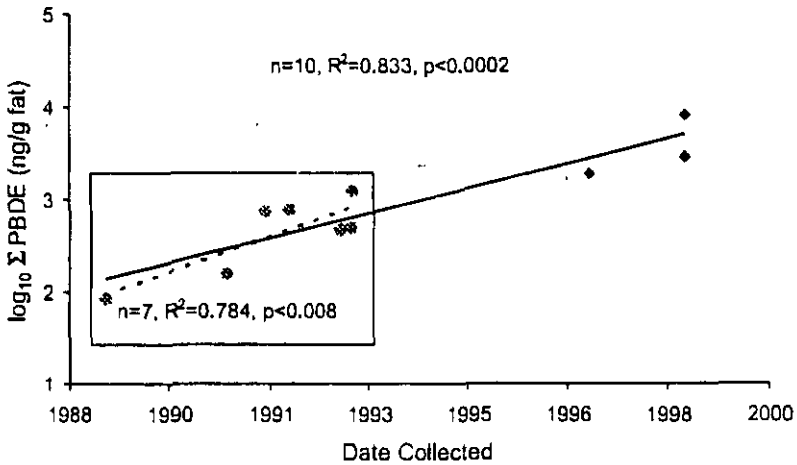


Fig. 3. ∑PBDEs (ng/g fat) in Harbor Seal Blubber, time trend. Regression includes all 10 animals (solid line), or the seven animals prior to 1994 (dashed line).

$p < 0.0002$) translates into a doubling of the concentration of PBDEs in seal blubber every 1.8 years. When the three seals with the highest PBDEs and lowest lipids were removed, ∑PBDEs in the seven seals sampled during the four-year period (1989–1993) increased by a factor of 14.5, a doubling of the concentration level every 1.3 years ($R^2 = 0.784$, $p < 0.008$). This confirms that concentrations in San Francisco Bay seals have increased dramatically over the past decade, in spite of any interaction between lipid content and collection date.

This study provides the first data on concentrations of PBDEs in the San Francisco Bay. The level of PBDEs in the harbor seals can be seen as an indicator of environment pollution by flame retardants in fish eating marine mammals in the Bay Area.

3.8. Comparison of PBDE levels in mother seal and fetus

Trans-placental transfer of chlorinated hydrocarbons has been suggested in seals (Tanabe et al., 1982), but little is known in regard to PBDEs. In our study, one mother–fetus pair of harbor seals was sampled in 1992. Since the full-term pup was found dead inside the birth canal, the PBDEs in the pup should have come only from in utero exposure. The ∑PBDE residue level in the mother was 782 and 430 ng/g fat in the fetus.

As shown in Fig. 4, lower brominated PBDEs were more likely to be transferred from the mother to the offspring than the higher brominated ones. Fetus to mother ratios decreased from 0.79 for the tetra-BDE, to 0.57 and 0.47 for the penta-BDEs, to 0.3 and 0.2 for the

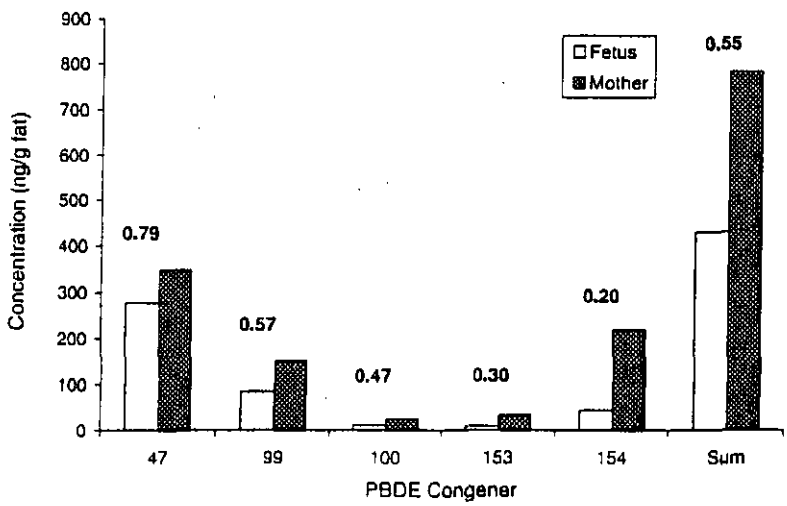


Fig. 4. PBDE concentrations (ng/g fat) in fetus and mother blubber, and fetus-to-mother ratios.

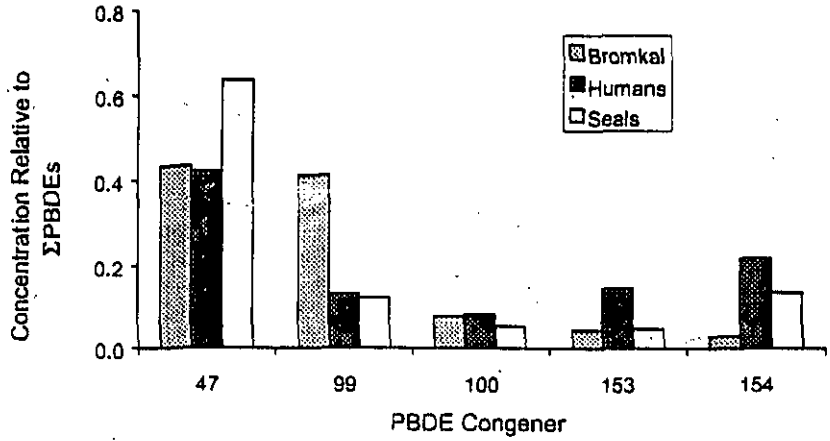


Fig. 5. Relative congener patterns of PBDE in Bromkal, human breast adipose, and seal blubber.

hexa-BDEs. Such a trend of trans-placental transfer has been observed for PCB homologues. Addison and Brodie (Addison and Brodie, 1987), present a formula for predicting PCB transfer during gestation that inversely relates transfer efficiency to lipid solubility of individual PCB congeners.

3.9. Comparison of levels and profiles of PBDEs in human and seal samples

The major congeners found in human tissue and seal blubber were the same: PBDE 47, 99, 100, 153, and 154. Whereas concentrations were orders of magnitude higher in seals than in humans, PBDE 47 was dominant in both human tissue and seal blubber. In the seal blubber, the average PBDE profile consisted of 64% PBDE 47, 12% PBDE 99, 5% PBDE 100, 5% PBDE 153, and 14% PBDE 154 (Table 2). In human adipose, the average profile consisted of 42% PBDE 47, 13% PBDE 99, 8% PBDE 100, 15% PBDE 153, and 22% PBDE 154 (Table 1). PBDE 154 was the dominant congener in 3 of the 23 women, all 3 under 40 years of age. This observation mainly underscores the greater variability observed in humans as compared to the seals. To assess human differences by age, disease status, or other variables, a greater number of samples is planned (Petreas et al., 2000).

The dominance of PBDE 47 is consistent with other studies. For example, the PBDE 47 content was 49–51% in long finned pilot whales from the Atlantic (Lindström et al., 1999), and approximately 70% in ringed seals from the Baltic Sea (Haglund et al., 1997) and beluga from Canada (Stern and Ikonomou, 2000). In fish, PBDE 47 was also dominant in Lake Michigan salmonids (Manchester-Neesvig et al., 2001) and several fish species from Virginia rivers (Hale et al., 2000), while in humans, PBDE 47 was the dominant congener in milk from Canada (Ryan and Patry, 2000) and Finland (Strandman et al., 2000), and in serum from Germany (Schröter-Kermani et al., 2000).

Major amounts of tetra-, penta- and hexa-congeners have been found in both human tissue and seal blubber. Most studies find that the lower brominated PBDEs are present in tissues at higher concentrations than the more highly brominated congeners. This may be caused by a preferential uptake of the lower brominated congeners, or by their presence at higher levels in the environment, the latter perhaps resulting from breakdown of the more highly brominated major commercial products.

Human and seal profiles were compared to Bromkal 70-5DE (de Boer et al., 2000). Whereas, Bromkal consists mainly of PBDE 47 and 99, higher congeners appear enriched in tissues, particularly in humans. When congeners were expressed as a percentage of $\sum$PBDEs (Fig. 5), the contribution of PBDEs 153, 154, 99, and 100 was quite small in the seals but more prominent in the humans. This may be partly explained by selective bioaccumulation through the trophic web of the lower brominated congeners in seals, whereas routes other than, or in addition to, diet may be contributing to human exposures. Differences in human and seal metabolism may also contribute to the different patterns.

4. Conclusions

- GC/MS in ECNI mode provided a very sensitive tool for monitoring trace levels of PBDEs in biological matrices.
- For the first time, PBDEs were analyzed and detected in human breast adipose tissue and seal blubber from the San Francisco Bay Area of California. $\sum$PBDE levels in seals averaged 1730 ng/g fat, while levels in human adipose tissue averaged 86 ng/g fat.
- PBDE levels in San Francisco Bay harbor seals are among the highest reported, and PBDE levels in

humans from the San Francisco Bay Area are the highest reported to date.
- The dominance of PBDE 47 (tetra-) over the other PBDE congeners may indicate that tetrabrominated biphenyl ethers bioaccumulate more than the higher brominated congeners.
- A dramatic increase in PBDEs in San Francisco Bay harbor seals was observed over the last decade.

Acknowledgements

The harbor seal samples are being studied for dioxin, PCB and organochlorine pesticide levels in a project funded by the San Francisco Regional Water Quality Control Board. The carcasses had been archived and stored at the UC Berkeley Museum of Vertebrate Zoology by Dr. Robert Jones. The human breast adipose samples were collected by Drs. Jeffrey and Mahoney of Stanford University Hospital and Dr. O'Neal of Kaiser Oakland Hospital, as part of a breast cancer study conducted in collaboration with Drs. Reynolds, Smith and Gilliss of the California Department of Health Services. The breast cancer study was partially funded by the US Department of the Army (DAMD-17-94-J-4429) and by the California Breast Cancer Research Program (Grant #2RB-0054).

References

Ackman, R.G., 1981. Flame ionization detection applied to thin-layer chromatography on coated quartz rods. In: Lowenstein, J.M. (Ed.), Methods in Enzymology, vol. 72. Academic Press, New York, pp. 205–252.

Addison, R.F., Brodie, P.F., 1987. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating grey seal (*Halichoerus grypus*). Can. J. Fish Aquat. Sci. 44, 782–786.

Addison, R.F., Kerr, S.R., Dale, J., 1973. Variation of organochlorine levels with age in Gulf of St. Lawrence harp seals (*Pagophilus groenlandicus*). J. Fish. Res. Board Can. 30 (5), 595–600.

Addison, R.F., Smith, T.G., 1974. Organochlorine residue levels in Arctic ringed seals: variation with age and sex. Oikos 25, 335–337.

Aguilar, A., 1985. Compartmentation and reliability of sampling procedures in organochlorine pollution surveys of cetaceans. Residue Rev. 95, 91–114.

Andersson, Ö., Blomkvist, G., 1981. Polybrominated aromatic pollutants found in fish in Sweden. Chemosphere 10, 1051–1060.

Bigg, M.A., 1969. The harbour seal in British Columbia. Fish. Res. Board Can. Bull. 172, 33p.

Cramer, P.H., Ayling, R.E., Thornburg, K.R., Stanley, J.S., Remmers, J.C., Breen, J.J., Schwemberger, J., 1990. Evaluation of an analytical method for the determination of polybrominated dibenzodioxins/dibenzofurans in human adipose. Chemosphere 20, 821–827.

Darnerud, P.O., Atuma, S., Aune, M., Cnattingios, S., Wernroth, M.-L., Wicklund-Glynn, A., 1998. Polybrominated diphenyl ethers (PBDEs) in breast milk from primiparous women in Uppsala country Sweden. Organohalogen Compounds 35, 411–414.

Davis, R.W., Beltz, W.F., Peralta, F., Witztum, J.L., 1993. Role of plasma and tissue lipids in the energy metabolism of the harbour seal. Symp. Zool. Soc. London 66, 369–382.

de Boer, J., de Boer, K., Boon, J.P., 2000. New types of persistent halogenated compounds. In: Paasivirta, J., Hutzinger, O. (Eds.), The Handbook of Environmental Chemistry, vol. 3. Springer, Berlin, Part K.

de Boer, J., Wester, P.G., Klamer, H.C., Lewis, W.E., Boon, J.P., 1998. Do flame retardants threaten ocean life? Nature 394, 28–29.

de Wit, C.A., 1999. Brominated flame retardants in the environment – an overview. Organohalogen Compounds 40, 329–332.

Findlay, G.M., de Freitas, A.S., 1971. DDT movement from adipocyte to muscle cell during lipid utilization. Nature 229, 63–66.

Hagenmaier, H., She, J., Benz, T., Dawidowsky, N., Düsterhöft, L., Lindig, C., 1992. Analysis of sewage sludge for polyhalogenated dibenzo-*p*-dioxins, dibenzofurans and diphenylethers. Chemosphere 25, 1457–1462.

Haglund, P.S., Zook, D.R., Buser, H.-R., Hu, J., 1997. Identification and quantification of polybrominated diphenyl ethers and methoxy-polybrominated diphenyl ethers in Baltic biota. Environ. Sci. Technol. 31, 3281–3287.

Hale, R.C., Guardia, M.J., Harvey, E.P., Mainor, T.M., Duff, W.H., Gayor, M.O., Jacobs, E.M., Mears, G.L., 2000. Comparison of brominated diphenyl ether fire retardant and organocholrine burdens in fish from Virginia rivers (USA). Organohalogen Compounds 47, 65–68.

Hutchinson, J.D., Simmonds, M.P., 1994. Organochlorine contamination in pinnipeds. Rev. Environ. Contam. Toxicol. 36, 123–167.

Ikonomou, M.G., Fischer, M., He, T., Addison, R.F., Smith, T., 2000. Congener patterns, spatial and temporal trends of polybrominated diphenyl ethers in biota samples from the Canadian west coast and the Northwest Territories. Organohalogen Compounds 47, 77–80.

Jansson, B., Andersson, R., Asplund, L., Ltizén, K., Nylund, K., Sellström, U., Uvemo, U.-B., Wahlberg, C., Wideqvist, U., Odsjö, T., Olsson, M., 1993. Chlorinated and brominated persistent organic compounds in biological samples from the environment. Environ. Toxicol. Chem. 12, 1163–1174.

Kopec, A.D., Harvey, J.T., 1995. Toxic pollutants, health indices and population dynamics of harbor seals in San Francisco Bay, 1989–1992. Moss Landing Marine Laboratories Technical Report 96-4. Moss Landing, CA, pp. 168.

Lindström, G., Hardell, L., Bavel, B., Wingfors, H., Sundelin, E., Liljegren, G., Lindholm, P., 1998. Current levels of 2,2′,4,4′-tetrabrominated diphenyl ether in human adipose tissue in Sweden – a risk factor for non-Hodgkin's lymphoma? Organohalogen Compounds 35, 431–434.

Lindström, G., Wingfors, H., Dam, M., van Bavel, B., 1999. Identification of 19 PBDEs in long-finned pilot whale (*Globicephala melas*) from the Atlantic. Arch. Environ. Contam. Toxicol. 36, 355.

Luross, J.M., Alaee, M., Sergeant, D.B., Whittle, D.M., Solomon, K.R., 2000. Spatial and temporal distribution of polybrominated diphenyl ethers in lake trout from the Great Lakes. Organohalogen Compounds 47, 73–76.

Manchester-Neesvig, J.B., Valters, K., Sonzogni, W.C., 2001. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. Environ. Sci. Technol. 35, 1072–1077.

Meneses, M., Wingfors, H., Schuhmacher, M., Domingo, J.L., Lindström, G., van Bavel, B., 1999. Polybrominated diphenyl ethers detected in human adipose tissue from Spain. Chemosphere 39, 2271–2278.

Norén, K., Meironyté, D., 1998. Contaminants in Swedish human milk. Decreasing levels of organochlorine and increasing levels of organobromine compounds. Organohalogen Compounds 38, 1–4.

Nylund, K., Asplund, L., Jansson, B., Jonsson, P., Litzén, K., Sellström, U., 1992. Analysis of some polyhalogenated organic pollutants in sediment and sewage sludge. Chemosphere 24, 1721–1730.

Petreas, M., She, J., Winkler, J., Visita, P., McKinney, M., Reynolds, P., Smith, D., Gilliss, D., Hurley, S., Jeffrey, S., Mahoney, E., 2000. Body burdens of organohalogens in California populations. Organohalogen Compounds 48, 17–20.

Reijenders, P.J.H., 1988. Exocotoxicological perspectives in marine mammology: research principles and goals for a conservation policy. Mar. Mamm. Sci. 4, 91–102.

Ryan, J.J., Patry, B., 2000. Determination of brominated diphenyl ethers (BDEs) and levels in Canadian human milks. Organohalogen Compounds 47, 57–60.

Schröter-Kermani, C., Helm, D., Hermann, T., Päpke, O., 2000. The German environmental specimen bank – application in trend monitoring of polybrominated diphenyl ethers in human blood. Organohalogen Compounds 47, 49–52.

She, J., Petreas, M., Winkler, J., Visita, P., McKinney, M., Jones, R., Kopec, A.D., 2000. Harbor seals as indicators of halogenated contaminants in San Francisco Bay. Organohalogen Compounds 49, 422–425.

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Kronholm-Diab, K., Jakobsson, E., Bergman, Å., 1999. Polybrominated diphenyl ethers (PBDEs) in blood from Swedish workers. Environ. Health Perspec. 107, 643–648.

Stanley, J.S., Cramer, P.H., Thornburg, K.R., Remmers, J.C., Breen, J.J., Schwemberger, J., 1991. Mass spectral confirmation of chlorinated and brominated diphenylethers in human adipose tissues. Chemosphere 23, 1185–1195.

State of California, Department of Consumers Affairs, Bureau of Home Furnishings and Thermal Insulation, 1992. Technical Bulletins, 117; 133.

Stern, G.A., Ikonomou, M.G., 2000. Temporal trends of polybrominated diphenyl ethers in SE Baffin Beluga: increasing evidence of long range atmospheric transport. Organohalogen Compounds 47, 81–84.

Strandman, T., Koistinen, J., Vartiainen, T., 2000. Polybrominated diphenyl ethers (PBDEs) in placenta and human milk. Organohalogen Compounds 47, 61–64.

Tanabe, S., Tatsukawa, R., Maruyama, K., Miyazaki, N., 1982. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin (*Stenella coeruleoalba*) to her fetus. Agric. Biol. Chem. 46 (5), 1249–1254.

Watanabe, I., Kawano, M., Tatsukawa, R., 1995. Polybrominated and mixed polybromo/chlorinated dibenzo-*p*-dioxins and -dibenzofurans in the Japanese environment. Organohalogen Compounds 24, 337–340.

High PBDE Levels in Shorebird Eggs from the San Francisco Bay and Washington State

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Abstract
Concentrations of contaminants have been measured for decades and used as meaningful indicators of the relative health of populations of various species, and the ecosystems in which they live, across both space and time. The goal of this study was to assess aspects of the current health of nearshore coastal marine waters of the western United States by measuring various organic and heavy metal contaminant levels in appropriate trophic-level species Seabirds are a particularly useful group of species to assess ecosystem health at various temporal and geographic scales because many species feed at high trophic levels, are long-lived, and typically are faithful to their breed sites. Therefore, in this study, we measured some heavy metal and organic contaminants in three upper trophic level obligate fish-eating seabirds (Forsters, Least and Caspian Terns) at various colonies along the west coast of the United States to: (1) assess differences in contaminant levels among different geographic breeding populations, and (2) compare these levels to historical contaminant data for these species. Specifically, we measured various polychlorinated biphenyls (PCBs), organochlorines, dioxins, a relatively new class of compounds of concern known as polybrominated biphenyl ethers, and three heavy metals (mercury, arsenic and lead). These data will be presented and discussed.

Introduction
High levels of polybrominated biphenyl ethers (PBDEs) have been found in humans and wildlife from the San Francisco Bay area [1,2], with levels in women among the highest in the world, and levels in harbor seals doubling every 2-3 years[2]. To further investigate the extent of contamination of the Bay area with PBDEs and their associated co-pollutants, 73 eggs of four species of fish-eating shorebirds were analyzed for levels of PBDEs, PCBs, PCDD/Fs, and PBDD/Fs. Shorebirds are useful for assessing ecosystem health at various times and places because they are at a high trophic level atop the marine food chain, are long-lived, and are faithful to their breeding sites. For comparison, measurements were made on eggs of shorebird species from the State of Washington.

Materials and Methods
53 individual eggs of four species and multiple nesting sites were provided by USFWS. Table 1 summarizes the species studied, the location of their nesting sites and the egg selection criteria.

Table 1. Species studied and the location of nesting sites

Common Name	Scientific Name	Nesting sites	Selection Criteria	Number of samples
Caspian Tern	*Sterna caspi*	Napa Marsh, Brooks Is., CA WA (4 sites)	Random	34
Forsters Tern	*Sterna forsteri*	Napa Marsh, Brooks Is., CA	Random	29
Least Tern	*Sterna antillarum*	Alameda NAS	Fail-to-hatch eggs	6
Clapper Rail	*Rallus longorostrus obsoletus*	North and South Bay sites	Fail-to-hatch eggs	4

The eggs were received frozen and were stored at -20° C until analyzed. The eggs were lyophilized, and moisture content was calculated by weight difference. The dried eggs were homogenized with a glass rod, and an aliquot representing 0.2 to 0.4 g of fat was spiked with nine ^{13}C-PCBs; 15 ^{13}C-PCDDs/Fs, and ^{13}C- PBDE77 and then extracted by sonication and standing (3x) with 1:1 hexane: methylene chloride. A fraction of the extract was centrifuged, and "fat content" was determined by evaporating a known volume of supernatant extract to dryness. The remaining extract was passed over an mixed silica gel column and a carbon column (AX-21) in series. The eluate was labeled fraction 1, containing PCBs and PBDEs. The carbon column was eluted in the reverse direction with warm toluene, and this fraction, containing PCDD/Fs and coplanar PCBs, was labeled fraction 2. Both fractions were reduced to 5-7 ml and passed over an ABC Gel Permeation chromatographic column containing 60 g of BioBeads SX-2 with 357 ml of 1:1 hexane:methylene chloride, the final 170 ml of which was collected. This fraction was reduced to dryness with tetradecane keeper. After addition of recovery standard the samples were analyzed by HRGC/HRMS (Finnigan Mat 95). HRGC/MS was operated in EI multiple ion-monitoring mode with 9000 resolution. A 1μL sample was injected onto a 60 meter, 0.25 μm film thickness DB 5 ms column in pulsed splitless mode.

Results and Discussion

∑PBDE (of tetra to hepta-PBDEs) in the California egg samples averaged 6.2 ppm (fat based), with a range of 0.30 to 62 ppm. Five PBDE congeners (PBDE 47, PBDE 99, PBDE 100, PBDE 153, and PBDE 154), were found in all egg samples from SF Bay and Washington state. However, the hepta PBDE (PBDE 183) was not detected in any of the egg samples. In SF Bay samples, PBDE 47 was the predominant congener, averaging 4.1 ppm (66% of the total), followed by PBDE 99 (18%), PBDE 100 (11%), PBDE 153 (3%) and PBDE 154 (2 %). Analytical results are summarized in Table 2.

Table 2. PBDE levels (ng/g fat) in shorebird eggs from San Francisco Bay, CA (based on 53 Califonia egg samples).

	Min	Max	Average	Median	STDEV	Ratio of PBDE#: ∑PBDE
Moisture%	74.0	79.1	77.1	77.4	1.19	
Fat%	30.7	41.5	38.25	38.43	2.21	
PBDE 47	129	52600	4116	2425	7260	0.66
PBDE 100	52.7	2920	658	452	630	0.11
PBDE 99	68.2	5650	1120	798	1110	0.18
PBDE 154	3.86	579	150	116	127	0.02
PBDE 153	1.67	895	163	115	172	0.03
Total PBDE	291	62400	6200	4410	8830	

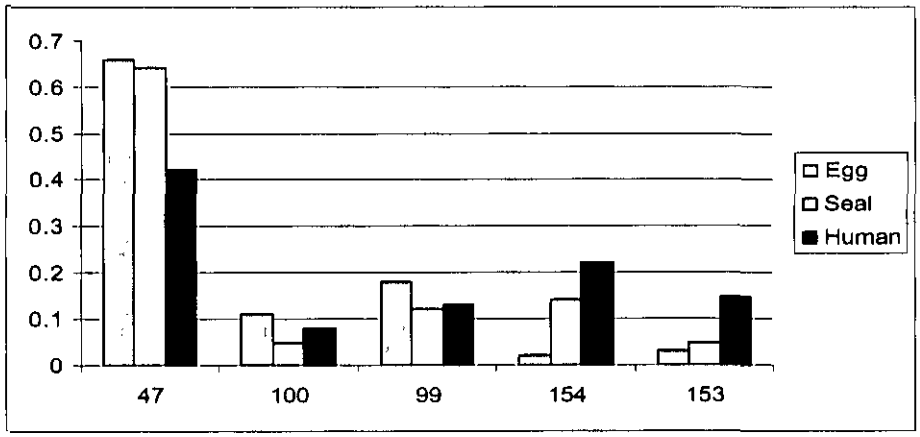

Figure 1. Relative congener patterns of PBDE in shorebird eggs, seals, and samples of human breast adipose, all from the San Francisco Bay Area.

Comparison of levels and profiles of PBDEs in humans, shorebird eggs, and seals from San Francisco Bay Area
The major congeners found in humans, shorebird eggs, and seals were the same: PBDE 47, 99, 100, 153 and 154. PBDE 47 was the dominant congener in all matrices. ΣPBDE concentrations were orders of magnitude higher in eggs (6 ppm) and seals (2 ppm) than in humans (86 ppb)[1,2].

PBDE 47 averaged about 65% of ΣPBDEs in both egg and seal samples, but only 42% in human adipose tissue samples, as can be seen in Figure 1. Hexa congeners (PBDE 153 and 154) appear proportionally lower in eggs and seals than in humans.

A subset of egg samples were tested for PBDDs, PBDFs and methoxy-PBDEs. No PBDDs, PBDFs and/or methoxy-PBDEs were found in the tested egg samples.

Comparison of levels and profiles of PBDEs in eggs from different species
Among eggs from the San Francisco Bay, 14 samples are from Caspian tern, 29 are from Fosters tern, 6 are from Least tern, and 4 are from Clapper Rail. The comparison of the levels and patterns of PBDE congeners from four species is shown in Fig. 2. Out of the four species, Fosters terns had the highest ∑PBDE, whereas the Clapper rail had the lowest ∑PBDE.

Comparison of levels and profiles of PBDEs in eggs from different locations
In addition to eggs from the San Francisco Bay, 20 eggs of Caspian terns from Washington State were also analyzed for PBDEs. ΣPBDEs in eggs from Washington State were lower than ΣPBDEs in eggs from San Francisco Bay. Table 3 compares the levels of PBDEs in eggs of Caspian terns from the San Francisco Bay and the State of Washington.

Table 3. Comparison of PBDE Levels in Caspian Tern Eggs from SF Bay and Washington State.

Washington State Eggs (n=20)						
	Min	Max	Average	Median	Std Dev	Ratio
Moisture %	75.8	79.1	77.5	77.5	0.79	
Fat %	30.7	41.5	38.5	39.0	2.31	
PBDE 47	1050	8840	2660	2070	1710	0.57
PBDE 100	266	1760	564	474	318	0.12
PBDE 99	542	3200	1210	1100	640	0.26
PBDE 154	44.2	349	96.8	80.4	65.3	0.02
PBDE 153	43.7	843	145	102	169	0.03
Total PBDE	1970	15000	4670	3920	2820	1.00

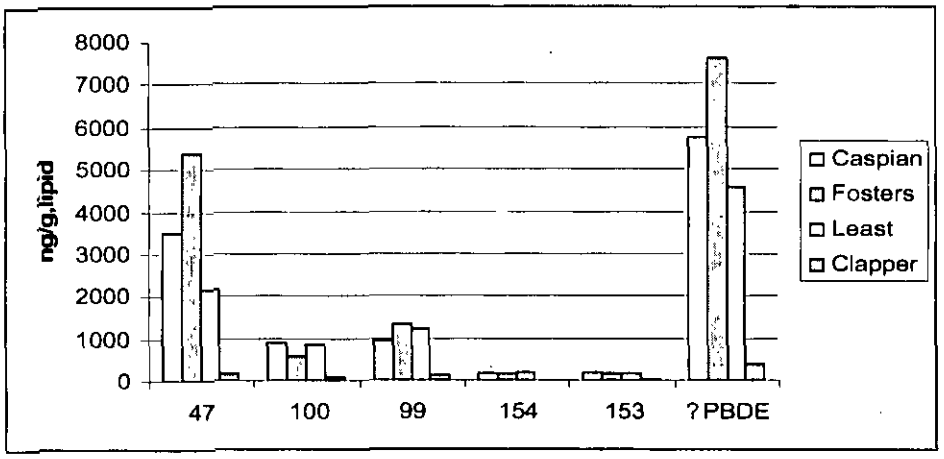

Figure 2. Comparison of levels of PBDEs in eggs from different species.

San Francisco Bay Area Eggs (n=14)						
	Min	Max	Average	Median	Std Dev	Ratio
Moisture %	71.2	78.2	75.9	76.0	1.64	
Fat %	22.3	45.1	37.7	38.7	5.76	
PBDE 47	744	10500	3490	3100	2740	0.61
PBDE 100	185	2770	919	660	796	0.16
PBDE 99	238	2910	956	730	798	0.17
PBDE 154	39.2	499	168	124	137	0.03
PBDE 153	61.0	733	200	140	205	0.03
Total PBDE	1340	17300	5730	4680	4620	1.00

Comparison of levels of PBDEs and PCBs

Among the PCB congeners analyzed, levels of PCB 153 were the highest, averaging 4.4 ppm (fat based). As noted above, PBDE 47 averaged 4.1 ppm. These results suggest that the PBDE brominated flame retardants are fast becoming a newer version of the 'PCB problem'.

References

1. Jianwen She, Jennifer Winkler, Pat Visita, Michael McKinney, Myrto Petreas (2000), Analysis of PBDEs in Seal Blubber and Human Breast Adipose Tissue Samples, Organohalogen Compounds, 47, 53, 2000

2. Jianwen She, Myrto Petreas, Jennifer Winkler, Patria Visita, Michael McKinney, Dianne Kopec (2002), PBDES in the San Francisco Bay Area: measurements in the harbor seal blubber and human breast adipose tissue, 46, 697, 2002

High Body Burdens of 2,2′,4,4′-Tetrabromodiphenyl Ether (BDE-47) in California Women

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Following our first report on elevated polybrominated diphenyl ether (PBDE) concentrations in California women, we expanded our investigation to include diverse groups of local women. We analyzed additional adipose and serum samples collected in the late 1990s from San Francisco Bay Area women participating in a breast cancer study and in a reproductive study, respectively. Adipose samples (n = 32) were analyzed by low-resolution mass spectrometry in negative-ion chemical ionization mode, whereas serum samples (n = 50) were analyzed by dual-column gas chromatography with electron capture detection. The results confirmed our earlier findings. Concentrations of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) in contemporary California women ranged between 5 and 510 ng/g lipid, with a median (16.5 ng/g lipid) 3–10 times higher than those reported from Europe. In contrast, PBDEs were not measurable in any of 420 archived serum samples collected in the 1960s from San Francisco Bay Area women participating in a study of child development. BDE-47 concentrations did not increase with age or with concentrations of a polychlorinated biphenyl (PCB-153), suggesting other routes of exposure in addition to diet. Rising body burdens of endocrine-disrupting chemicals such as PBDEs may pose a potential public health threat. *Key words:* adipose tissue, BDE-47, body burdens, California, PBDEs, persistent organic pollutants, polybrominated diphenyl ethers, serum, time trends. *Environ Health Perspect* 111:1175–1179 (2003). doi:10.1289/ehp.6220 available via *http://dx.doi.org/* [Online 10 March 2003]

Persistent organic pollutants (POPs) enter the natural environment via a multitude of pathways. Body burdens reflect cumulative exposures to such chemicals and can be used to assess temporal and spatial trends. Body burdens of organochlorine compounds [polychlorinated dibenzo-*p*-dioxins, polychlorinated biphenyls (PCBs), pesticides] are declining in most of the industrialized countries (Liem et al. 1995; Noren and Meironyte 1998, 2000; Smith 1999) as a result of source reduction measures. Polybrominated diphenyl ethers (PBDEs), on the other hand, show increasing trends worldwide (de Wit 2002). Three industrial formulations of PBDEs are used widely as flame retardants [Bromine Science and Environmental Forum (BSEF) 2001]. Deca-BDE (consisting almost completely of BDE-209) is used mainly in thermoplastics and textiles. In 1999, its use in the United States was estimated at 25,000 metric tons, or 44% of its global use (Hale et al. 2002). Octa-BDE (a mixture of hexa- to octa-BDE congeners) is used in acrylonitrile/butadiene/styrene (ABS) plastics. Its use in the United States was estimated at 1,400 metric tons in 1999, corresponding to about 36% of its global use (Hale et al. 2002). Penta-BDE (a mixture of tetra- and penta-BDE congeners) is used mainly in polyurethane foam. The U.S. market used about 8,000 metric tons in 1999, which is approximately 98% of the global production of penta-BDE (Hale et al. 2002). Although the more brominated formulations are used more extensively worldwide than is penta-BDE, the tetra- and penta-congeners bioaccumulate to a greater degree than do the higher homologues (World Health Organization 1994). In fact, the congener pattern found in biota closely matches the pattern of the penta-BDE formulation (Hale et al. 2002), with 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47) as a dominant congener. Production of all PBDEs has increased over the last 20 years, accompanied by their emergence in environmental and biologic samples (de Boer et al. 1998; de Wit 2002). Hepatotoxicity, embryotoxicity, thyroid, and behavioral effects have been reported in animal studies (Darnerud et al. 2001; McDonald 2002). Of particular concern is the ability of PBDEs to disrupt thyroid hormone balance and to cause behavioral and learning deficits in rodents exposed *in utero* or postnatally (Eriksson et al. 2001). Elevated PBDE body burdens in women of childbearing age could therefore be an important public health issue.

Concentrations (mean, 86 ng/g lipid) of ΣPBDEs (sum of BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154) in adipose tissues from a group of 23 California women (She et al. 2002) appear to be 3–10 times higher than concentrations reported from other parts of the world (Darnerud et al. 1998; de Wit 2002; Noren and Meironyte 2000; Ohta et al. 2002; Ryan and Patry 2000; Schroeter-Kermani et al. 2000). In addition, concentrations in archived blubber from San Francisco Bay harbor seals demonstrate a 100-fold increase over the last decade (She et al. 2002). In this article, we report on additional PBDE measurements in adipose and serum from diverse groups of California women, in an effort to better understand sources and pathways leading to the observed high levels.

Materials and Methods

Subjects. The PBDE analyses were performed on adipose and serum samples collected in the course of three separate epidemiologic studies. All three studies involved women living in the San Francisco Bay Area of California.

Adipose from the late 1990s. Breast adipose samples from a group of 32 women, residents of the San Francisco Bay Area, were analyzed for PBDEs. This group was a random subsample from a case–control study on breast cancer and organochlorine exposures (Petreas et al. 2000), and it included women with malignancies, ductal carcinoma *in situ*, and benign breast disease. Participants of the original case–control study were recruited among women undergoing biopsies or lumpectomies at Stanford University Hospital or Kaiser-Oakland Hospital (both in the general San Francisco Bay Area). Eligibility criteria included age between 25 and 65 years, no prior cancer, and not taking tamoxifen or undergoing chemotherapy. Breast adipose tissue (~1 g) was collected during biopsy or

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breast surgery between 1996 and 1998 and archived at –20°C until analysis. The group of women discussed in this study includes the 23 women whose PBDE concentrations have already been reported (She et al. 2002).

Serum from the late 1990s. Between 1997 and 1999, blood was collected from a group of 50 Laotian immigrant women who lived in the San Francisco Bay Area and were participating in a study of organochlorine exposures and menstrual cycle function (Windham et al. 2002). Convenience sampling was used with trained Laotian community field workers, recruiting participants from Asian markets, cultural events, friends, health clinics, and English language classes. Eligibility criteria included reproductive age (19–40 years), recent menstrual period, birth in Southeast Asia, and regular consumption of fish. Blood was drawn at a local clinic and serum was separated and archived at –20°C until analysis.

Serum from the early 1960s. For a historic comparison population, archived serum was obtained from a case–control study of cryptorchidism and hypospadias and *in utero* exposure to organochlorine pesticides (OCPs) nested within the historic Child Health and Development Studies (CHDS) cohort. The CHDS (van den Berg 1979), a longitudinal study of 20,000 pregnancies among Northern California Kaiser Foundation Health Plan members, enrolled subjects between 1959 and 1966, a time of unrestricted use of PCBs and OCPs. Mothers were followed during pregnancy, and multiple samples of serum from each pregnancy were collected and archived. A subset of these children was followed for subsequent follow-up examinations. Subjects selected for this study included 155 male infants with hypospadias or cryptorchidism and surviving for 2 years, as well as twice that number of randomly selected controls. Maternal serum from the second or third trimesters of pregnancy was retrieved from archived frozen samples stored at the National Cancer Institute (Frederick, MD). Serum from 420 women was available for this comparison.

Analytical methods. Serum and adipose samples were kept frozen below –20°C until analysis. Serum was thawed, and 1 mL was pipetted into a 15-mL test tube. Internal standards [PCB congeners 14, 65, and 166 and tetrachloro-*m*-xylene (TCMX)] were added before denaturing the proteins with 1 mL acetic acid (California Department of Toxic Substances Control 2003). The analytes in the serum were then extracted four times with 3 mL hexane/dichloromethane (90:10, vol/vol), and the extract was passed through a glass column (15 × 250 mm) filled with 11.5 g Florisil. The Florisil was baked at 575°C for 4–6 hr, deactivated with distilled HPLC-grade water, and conditioned with 6 mL hexane before use. The analytes were eluted with 60 mL hexane followed by 6 mL hexane/dichloromethane (1:1, vol/vol) The eluates were combined and reduced to 75 µL. Twenty-five microliters of a 61.9 pg/µL solution of the recovery standards [pentachloronitrobenzene (PCNB), PCB-30, PCB-204, and PCB-209] were added to the extract to achieve a final concentration of 15.5 pg/µL. Calibration solutions ranged in concentration from about 0.2 pg/µL to 50 pg/µL of BDE-47 and 15.5 pg/µL of PCNB, PCB-30, PCB-204, and PCB-209. Analysis was performed by gas chromatography/electron capture detection (GC/ECD) equipped with 60-m DB-XLB (Agilent Technologies, Wilmington, DE) and Rtx-5ms (Restek Corporation, Bellefonte, PA) capillary GC columns. In previous work (James et al. 2002), 60-m DB-17 and Rtx-5ms columns were used to provide congener separation of the PCBs. However, in this project, we discovered that using the combination of DB-XLB and Rtx-5ms columns provided better resolution of the PCB congeners. By using a long temperature program, the retention time of BDE-47 exceeded the retention time of most PCBs.

Quantitation for BDE-47 was accomplished by using the data obtained on the DB-XLB column. The serum samples were processed in batches of nine. With each batch, we processed 1 mL HPLC-grade water (reagent blank) and 1 mL bovine serum fortified with BDE-47 (among other analytes) to evaluate background contributions from the reagents, precision, and analyte recovery. In addition, samples of pooled human serum were interspersed blindly among the samples (for quality control) to assess accuracy and precision across all serum batches. Total cholesterol and triglycerides were determined enzymatically in a small aliquot of serum at the Clinical and Epidemiological Research Laboratory, Boston Children's Hospital (Boston, MA). Total lipids were calculated from total cholesterol and triglycerides as described by Phillips et al. (1989), and results were reported as nanograms per gram lipid.

As described in detail previously (She et al. 2002), adipose samples were homogenized and internal standards were added, including $^{13}C_{12}$-labeled BDE-77. Although we could have used unlabeled standards for this work, $^{13}C_{12}$-labeled BDE-77 allowed us a comparison of instrumental techniques (not reported here). Samples were extracted with 1:1 hexane:dichloromethane, and PBDEs were isolated by passing the extract through a gel permeation chromatographic column and a glass column packed with Florisil in a single automated step (FMS, Waltham, MA). The extracts were concentrated and recovery standards added ($^{13}C_{12}$-PCB-128, $^{13}C_{12}$-PCB-178, and $^{13}C_6$ α-hexachlorocyclohexane). BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 were analyzed by low resolution mass spectrometry (Finnigan 4510; Finnigan MAT, San Jose, CA) in the negative ion chemical ionization mode using a DB-5ms column (60 m, 0.25 mm inner diameter, 0.25 µm film thickness; J&W Scientific, Folsom, CA). Methane was used as the reagent gas; the ion source pressure was 0.6 Torr, and the ion source temperature was 100°C. The electron energy was typically 70 eV, and the electron current was kept at 0.3 mA. We monitored *m/z* 79 and 81, corresponding to bromide. Adipose samples were analyzed in batches of six, with a reagent blank per batch. Samples of certified reference material (SRM 1945, whale blubber; National Institute of Standards and Technology, Gaithersburg, MD) were analyzed 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.

Statistical analyses to examine correlations between analytes and to compare BDE-47 body burdens among groups and by age were performed using STATA 7 (Stata Corp., College Station, TX).

Results

Serum and adipose samples from all three studies (Petreas et al. 2000, 2002; Windham et al. 2002) were originally scheduled for analysis of PCBs and OCPs (She at al. 1997), with the PBDEs added on as a secondary objective after analytical methods were developed. Only 1 mL of serum was available for analysis, limiting our ability to detect low levels. Therefore, only

Table 1. Demographic characteristics of the three groups of California women.

Group	Breast cancer	Reproductive (Laotian)	Child development
No.	32	50	420
Years sampled	1996–1998	1997–1999	1959–1967
Age (years)			
Mean ± SD	47.4 ± 7.7	31.4 ± 6.2	26.8 ± 6.2
Median (range)	48 (28–62)	32 (19–40)	26 (15–44)
Race (%)			
White	62.5	0	63.6
African American	25.0	0	26.4
Asian	12.5	100	4.1
U.S. born (%)	84.4	0	79.0
Prior pregnancy (%)	75	88	56[a]

[a]All participants in this group were pregnant during blood draw; value refers to previous pregnancy.

BDE-47 could be measured in serum without interference from the blank (signal in the sample should be at least 3× the signal in the blank), resulting in a much higher reporting limit for BDE-47 in serum (10 ng/g lipid) than in adipose tissue (< 0.5 ng/g lipid). BDE-47 was the major congener in adipose samples, and BDE-99, BDE-100, BDE-153, and BDE-154 were also measurable in all adipose samples. Table 1 shows demographic characteristics and Table 2 shows concentrations of BDE-47 and PCB-153 in the three groups of California women.

As shown in Table 1, the 32 women in the breast cancer subsample examined here were 28–62 years of age; most (84.4%) were born in the United States, and 62.5% were white. More than 95% had a college education, and 75% had at least one prior birth. Of the immigrant group, most participants were born in Laos, where they had spent the first part of their lives (mean ± SD, 11.8 ± 6.1 years), and had immigrated to the United States within the previous 2–20 years (14.4 ± 4.5 years), after various lengths of time (5.4 ± 4.8 years) in Thailand, presumably in refugee camps. Half the women had only an elementary school level education, and 18% had completed high school; most (88%) had a prior pregnancy. Among the women from the CHDS, the age ranged from 15 to 44 years (Table 1); 56% had one or more prior pregnancies, and 56% had graduated from high school and 14% from college. Most women were white (63.6%) or African American (26.4%). About 79% were born in the United States, 9.3% were foreign born (about 2% were born in Asia), and the birthplace was unknown for 11.7%.

We analyzed 32 adipose samples and 50 serum samples from the late 1990s and 420 serum samples from the 1960s. All samples were analyzed individually. We had no coelutions on the DB-XLB column with any PCB or OCP expected in human serum (James et al. 2002). Recoveries of internal standards (PCB congeners 14, 65, and 166 and TCMX) were used to gauge overall data quality for all analytes across all serum batches. Recoveries were between 81% and 99%, and no corrections were made to the measurements. In addition, BDE-47 recoveries in fortified bovine serum included with every batch were between 93% and 113%.

As shown in Table 2, BDE-47 was measurable in all adipose samples (100% above the reporting limit), with concentrations ranging from 5.2 ng/g lipid to 196 ng/g lipid, and a mean of 28.9 ng/g lipid. Concentrations in the 1990s serum ranged from < 10 ng/g lipid (reporting limit) to 511 ng/g lipid, with a mean of 50.6 ng/g lipid (median, 16.5 ng/g lipid). Summary statistics were calculated using the reporting limit for all samples at or below that limit. BDE-47 was not measurable in any of the 1960s serum samples. Despite the limitation of an elevated reporting limit, it is clear that none of the 420 serum samples from the early 1960s contained BDE-47 above the reporting limit of 10 ng/g lipid, whereas BDE-47 was measurable in 24 of the 50 serum samples from the late 1990s with the same reporting limit. PCB-153 was measurable in all specimens (adipose and serum) from all three groups of women (Table 2).

The Spearman correlation coefficient (r) between BDE-47 concentrations and age for the 32 mostly U.S.-born women (adipose samples) was $r = -0.413$ ($p = 0.019$), indicating a significant negative association. The correlation was not significant for the 50 Laotian serum samples ($r = 0.079$, $p = 0.589$) or for the two 1990s groups combined ($r = 0.058$, $p = 0.606$). In contrast, for the same two 1990s groups, a significant correlation was found for PCB-153 and age ($r = 0.619$, $p < 0.001$). No correlation was found between BDE-47 and PCB-153 ($r = 0.062$, $p = 0.647$) for the same two groups of women combined.

Discussion

Noren and Meironyte (1998) reported that concentrations of PBDEs (BDE-28, BDE-47, BDE-66, BDE-85, BDE-99, BDE-100, BDE-153, and BDE-154) in human milk from Sweden were increasing exponentially over time. Several reports on PBDEs in biota followed (Alaee et al. 1999; Darnerud et al. 1998; de Boer et al. 1998; Ryan and Patry 2000; Schroeter-Kermani et al. 2000), including ours (She et al. 2000, 2002), which showed an exponential increase of BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 in archived blubber from San Francisco Bay harbor seals. In the same study, we reported high concentrations of BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154 in adipose tissues collected from San Francisco Bay Area women in the late 1990s. BDE-47 was the dominant PBDE congener in all of our samples, in agreement with other human studies. Because our current work focuses on BDE-47, we will limit our discussion to that congener in our comparisons with other studies. Soon after our first study (She et al. 2000), a single composite human milk sample collected in 2000 from the United States showed high concentrations of several BDE congeners, including BDE-47 (120 ng/g lipid) (Papke et al. 2001), confirming our finding that U.S. levels appeared higher than levels reported in European studies, whose averages ranged within 1–10 ng/g lipid (Darnerud et al. 1998; Noren and Meironyte 2000; Schroeter-Kermani et al. 2000; Thomsen et al. 2002; van Bavel et al. 2002). Conversely, when 12 serum samples collected in 1988 at an Illinois blood bank were analyzed for PBDEs, the median concentration of BDE-47 was 0.6 ng/g lipid, with a range of < 0.4–24 ng/g lipid (Sjodin et al. 2001). According to the authors, these 1988 U.S. levels were considered equivalent to levels measured in Swedish blood collected in 1995 (Sjodin et al. 2001). When we compare these 1988 Illinois serum concentrations (Sjodin et al. 2001) with those we reported in our 1996–1998 California adipose samples (She at al. 2002) and in the composite milk sample (Papke et al. 2001), we can see an increase in U.S. body burdens of BDE-47 over the last 10–15 years. Our newest data support this observation. As shown in Table 2, concentrations of BDE-47 were below the reporting limit (10 ng/g lipid) in all the serum samples from the 1960s, whereas concentrations in 1990s adipose tissues averaged 28.9 ng/g lipid (median, 16.5 ng/g lipid), and concentrations in the late 1990s serum averaged 50.6 ng/g lipid (median, 10 ng/g lipid). A similar increase has been reported for human milk from Canada, where median PBDE levels have increased from 1.7 ng/g lipid in 1992 to 25.4 ng/g lipid in 2001 (Ryan et al. 2002). Although differences in PBDE partitioning in the various matrices examined (adipose, serum, milk) may confound precise comparisons, it is clear that for samples collected in the 1990s from the United States and Canada, body burdens are 3–10 times higher than those reported from Europe (Darnerud et al. 1998; Noren and Meironyte 2000; Schroeter-Kermani et al. 2000; Thomsen et al. 2002; van Bavel et al. 2002) or Japan (Ohta et al. 2002). This observation may be consistent with California regulations mandating that all polyurethane foam and textiles

Table 2. Concentrations of BDE-47 and PCB-153 in the three groups of California women.

Group	Breast cancer	Reproductive (Laotian)	Child development
No.	32	50	420
Years sampled	*1996–1998*	*1997–1999*	*1959–1967*
Matrix	Breast adipose	Serum	Serum
PBDE-47 (ng/g lipid)			
Percent > reporting limit	100%	48%	0%
Mean ± SD	28.9 ± 39.8	50.6 ± 94.6	—
Median (range)	16.5 (5.2–196)	10 (< 10–511)	< 10
PCB-153 (ng/g lipid)			
Percent > reporting limit	100%	100%	100%
Mean ± SD	158.8 ± 68.5	52.7 ± 38.3	82.9 ± 43.8
Median (range)	157.9 (61–321)	41.2 (5.6–195)	72.5 (7–409)

used in furnishings pass a flammability test (not necessarily requiring use of PBDEs) (State of California 1991, 2000). It is possible that these unique California regulations drive the consumer product market across the United States and, perhaps, Canada. Regional differences have also been reported in sewage sludge, where PBDE concentrations appeared 10 times higher in the United States than in Europe (Hale et al. 2001, 2002).

The Laotian immigrant women in this study had much higher dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyltrichloroethane (DDT) concentrations (as expected) and quite lower PCB levels in their serum than those measured in the adipose tissue of the mostly U.S.-born women sampled in the same period (Petreas et al. 2002; Windham et al. 2002). It is therefore noteworthy that the Laotians showed serum BDE-47 concentrations equivalent to (or even greater than) adipose concentrations of the contemporary group that was mostly born in the United States. We should note, however, that comparing these two groups is complicated for several reasons: not only are we comparing serum with adipose levels but we are also comparing women of different ages who were born and raised in different continents with different lifestyles. Although we are not familiar with blood/adipose partition coefficients for PBDEs, it is well established that, for most POPs, lipid-adjusted concentrations in serum and adipose tissue are not exactly the same (Archibeque-Engle et al. 1997; Lopez-Carrillo et al. 1999; Mussalo-Rauhama 1991; Needham et al. 1990). As shown in Figure 1, there is little age overlap between the two study populations, and the younger Laotian women appear to have higher and more variable levels of BDE-47 than do the older, mostly U.S.-born women. Because penta-BDE formulations are not widely used in Asia (BSEF 2001), it seems unlikely that these Laotian women were exposed in Laos or in the Thai refugee camps. Thus, it is more likely that practices and lifestyles acquired more recently in the United States are the main exposure pathways. Fish consumption was one of the eligibility criteria for the Laotian women; therefore, this is another source of exposure that may differ from the other populations. These Laotian women consumed fish from the San Francisco Bay in amounts similar to those of an earlier study of anglers fishing in the bay [California Department of Health Services (CDHS) 2001)]. Although we do not have data on PBDE levels in bay fish, we have reported an exponential increase in PBDEs in harbor seals (She et al. 2002) from the San Francisco Bay. In addition, there are fish advisories recommending limited bay fish consumption because of contamination with PCBs and mercury. Similar to PCBs, PBDEs would be expected to accumulate in fatty fish. The angler report (CDHS 2001) noted that Asians are more likely than other ethnic groups to prepare and consume fish in a manner that is likely to increase their exposure to contaminants. Almost all of the Laotian women consumed at least some marine fish, compared with about 30% of the U.S. population (U.S. EPA 1997). On the other hand, given the relatively low PCB body burdens in our Laotian women, fish consumption could not be a major pathway for PBDEs. At this time, we can only speculate on possible explanations for these differences, including different activities or lifestyle factors affecting exposure, age effects on metabolic clearance of BDE-47, or greater BDE-47 partitioning in serum than in adipose tissue.

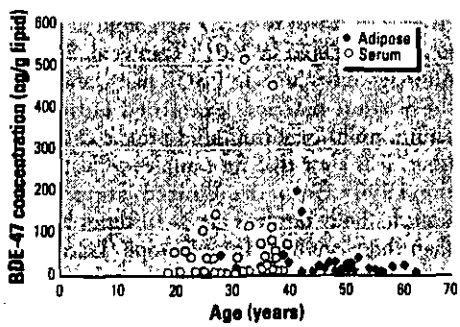

Figure 1. BDE-47 concentrations (ng/g lipid) in serum samples from Laotian women and adipose samples from mostly U.S.-born women. All samples were collected between 1996 and 1999.

A lack of a significant increase with age, in contrast to other POPs, has been reported for PBDEs (Darnerud et al. 1998; Thomsen et al. 2002), and our data appear to support this. The correlation between BDE-47 and age was not significant for both groups combined. When we examined the two groups separately, the correlation was similarly not significant for serum alone, but a significant negative correlation in the adipose was seen. Thomsen et al. (2002) reported higher PBDE concentrations in young children compared with other age groups in Norway, but no differences among age groups corresponding to those seen in our study. We had reported higher ΣPBDE concentrations ($p < 0.05$) in the adipose of women younger than 48 years (median age) compared with those older than 48 years in a subset of 23 women (She at al. 2002). Expanding the sample size to 32 women retained the difference between young and old and revealed this significant inverse correlation with age. Given the small number of samples, however, and the presence of outliers, this association may be spurious. Regardless, the important finding is that the association between BDE-47 and age is not positive, a departure from other POPs. When we examined the two 1990s groups of women combined, the concentration of PCB-153 (the most prominent PCB congener in humans) increased with age. On the other hand, there was no correlation between BDE-47 and PCB-153 concentrations in our samples, possibly indicating different exposure pathways for BDE-47 from those for other POPs, which are driven by diet. A strong possibility is inhalation or ingestion of indoor air dust from PBDE-treated consumer products, particularly polyurethane foam. PBDEs have been reported in dust collected with vacuum cleaners from European offices (Leonards et al. 2001) and homes (Knoth et al. 2002). Although BDE-209 was the dominant congener in office and house dust, BDE-47 was also present, ranking second or third in concentration.

Our data indicate the presence of outliers. These high BDE-47 values did not parallel high levels of PCBs or pesticides measured in the same samples (Petreas et al. 2002). High BDE-47 levels were not explained by any of the common POPs predictors such as age, parity, lactation, occupation, country of birth, socioeconomic status, or medical history. Because the study questionnaires were not designed to assess PBDE exposures, questions that might elucidate exposure pathways were not included. Outliers in PBDE distributions have also been reported recently for Swedish blood (van Bavel et al. 2002) and Canadian milk (Ryan et al. 2002). It is possible that these outliers reflect hot spots originating from the relatively recent introduction of PBDEs and its still inconsistent presence in the food web, as well as from the selective use of various PBDE-treated consumer products.

Our analysis showed the emergence of BDE-47 in residents of the San Francisco Bay Area by the late 1990s, compared with the 1960s, confirming similar trends from Canada (Ryan et al. 2002), Germany (Schroeter-Kermani et al. 2000), Sweden (Noren and Meironyte 1998, 2000), and Norway (Thomsen et al. 2002). Our study has several weaknesses stemming from the fact that the three epidemiologic studies we used were not designed to assess PBDE exposures. Sample volumes were small, raising the reporting level and limiting the numbers of detectable samples, which in turn did not allow analysis of subgroups at higher risk of exposure. In addition, only the most dominant PBDE, BDE-47, could be measured in the serum samples, precluding analysis of PBDE profiles. Nevertheless, BDE-47 concentrations were high, pointing to the need for follow-up studies designed to investigate PBDE exposures. Increasing body burdens, particularly in young women of reproductive age, pose a potential public health threat to future generations. PBDE sources need to be recognized, evaluated, and controlled to minimize exposures. At the same time, the systematic monitoring of

body burdens of known and emerging POPs should become a high priority for our public health system (Hooper and McDonald 2000).

References

Alaee M, Luross J, Sergeant DB, Muir DCG, Whittle DM, Solomon K. 1999. Distribution of polybrominated diphenyl ethers in the Canadian environment. Organohalogen Compounds 40:347–350.

Archibeque-Engle SL, Tesseri JD, Winn DT, Keefe TJ, Natt TM, Zheng T. 1997. Comparison of organochlorine pesticide and PCB residues in human breast adipose tissue and serum. *J Toxicol Environ Health* 52:285–293.

Bromine Science and Environmental Forum. 2001. Major Brominated Flame Retardants Volume Estimates. Total Market Demand by Region in 2001. Available: http://www.bsef-site.com/docs/BFR_vols_2001.doc [accessed 18 February 2003].

CDHS. 2001. San Francisco Bay Seafood Consumption Study. Oakland, CA:California Department of Health Services.

CDTSC. 2003. Preparation and Analysis of Blood Serum for PCBs *OCPs and PBDEs. Standard Operating Procedure 891-S.* Berkeley, CA:California Department of Toxic Substances Control.

Darnerud PO, Atuma S, Aune M, Cnattingius S, Wernroth ML, Wicklund GA. 1998. Polybrominated diphenyl ethers (PBDEs) in breast milk from primiparous women in Uppsala County, Sweden: Organohalogen Compounds 35:411–414.

Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. 2001. Polybrominated diphenyl ethers: occurrence, *dietary exposure and toxicology. Environ Health Perspect* 109(suppl 1):49–68.

de Boer J, Wester PG, Klamer HC, Lewis WE, Boon JP. 1998. Do flame retardants threaten ocean life? Nature 394:28–29.

de Wit CA. 2002. An overview of brominated flame retardants in the environment. Chemosphere 46:583–624.

Eriksson P, Viberg H, Jakobsson E, Orn U, Fredriksson A. 2001. Brominated flame retardants: a novel class of developmental neurotoxicants in our environment. Environ Health Perspect 109:903–908.

Hale R, La Guardia M, Harvey E, Mainor M. 2001. Brominated diphenyl ethers in land-applied sewage sludges in the US. In: Proceedings of the Second International Workshop on Brominated Flame Retardants. 14–16 May 2001, Stockholm, Sweden. Stockholm:Swedish Chemical Society, 149–152.

———. 2002. Potential role of fire retardant-treated polyurethane foam as a source of brominated diphenyl ethers to the US environment. Chemosphere 46:729–735.

Hooper NK, McDonald TA. 2000. The PBDEs: an emerging environmental challenge and another reason for breast milk monitoring programs. Environ Health Perspect 108:387–392.

James RA, Hertz-Picciotto I, Willman E, Keller JA, Charles MJ. 2002. Determinants of serum polychlorinated biphenyls *and organochlorine pesticides measured in women from* the Child Health and Development Study cohort, 1963–1967. Environ Health Perspect 110:617–624.

Knoth W, Mann W, Meyer R, Nabbuth J. 2002. Polybrominated *diphenylether in house dust. Organohalogen Compounds* 58:213–216.

Leonards PEG, Santillo D, Brigden K, van der Veen I, Hesselingen JV, de Boer J, et al. 2001. Brominated flame retardants in office dust samples. In: Proceedings of the Second International Workshop on Brominated Flame Retardants. 14–16 May 2001, Stockholm, Sweden. Stockholm:Swedish Chemical Society, 299–302.

Liem AKD, Albers JMC, Baumann RA, van Beuzekom AC, den *Hartog RS, Hoogerbrugge R, et al. 1995. PCBs, PCDD/PCDFs* and organochlorine pesticides in human milk in The Netherlands. Levels and trends. Organohalogen Compounds 26:69–74

Lopez-Carrillo L, Torres-Sanchez L, Lopez-Cervantes M, Blair A, Cebrian M, Uribe M. 1999. The adipose tissue to serum DDE ratio: some methodological considerations. Environ Res 81:142–145.

McDonald TA. 2002. A perspective on the potential health risks *of PBDEs. Chemosphere 46:745–755.*

Mussalo-Rauhama H. 1991. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue. Sci Total Environ 103:159–175.

Needham LL, Burse VW, Head SL, Korver MP, McClure PC, Andrews JS, et al. 1990. Adipose tissue/serum partitioning of chlorinated hydrocarbon pesticides in humans. Chemosphere 20:975–980.

Noren K, Meironyte D. 1998. Contaminants in Swedish human *milk. Decreasing levels of organochlorine and increasing* levels of organobromine compounds. Organohalogen Compounds 38:1–4.

———. 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. Chemosphere 40:1111–1123.

Ohta S, Ishizuka D, Nishimura H, Nakao T, Aozasa O, Shimidzu Y, et al. 2002. Comparison of polybrominated diphenyl ethers in fish, vegetables and meats and levels in human milk of nursing mothers in Japan. Chemosphere 46:689–696.

Papke O, Bathe L, Bergman A, Furst P, Meironyte-Guvenius D, Herrmann T, et al. 2001. Determination of PBDEs in human milk from the United States—comparison of results from three laboratories. Organohalogen Compounds 52:197–200.

Petreas MX, Rogers E, Zhao G, Windham G, Bhatia R, Charles MJ. 2002. Organohalogen body burdens in California women. Organohalogen Compounds 55:259–262.

Petreas MX, She J, Winkler J, Visita P, McKinney M, Reynolds P, et al. 2000. Organochlorine body burdens in California populations. Organohalogen Compounds 48:17–21.

Phillips DL, Prickle JL, Burse VW, Bernert JT, Henderson O, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ *Contam Toxicol 18:495–500.*

Ryan JJ, Patry B. 2000. Determination of brominated diphenyl ethers (BDEs) and levels in Canadian human milk. Organohalogen Compounds 47:57–60.

Ryan JJ, Patry B, Mills P, Beaudoin G. 2002. Recent trends in levels of brominated diphenyl ethers in human milks from Canada. Organohalogen Compounds 58:173–176.

Schroeter-Kermani C, Hei D, Hermann T, Päpke O. 2000. The *German environmental specimen bank—application in* trend monitoring of PBDEs in human blood. Organohalogen Compounds 47:49–52.

She J, Petreas M, Winkler J, Visita P, McKinney M, Jones R, Kopec D. 2000. Harbor seals as indicators of halogenated contaminants in San Francisco Bay. Organohalogen Compounds 48:422–425.

She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D. 2002. Polybrominated diphenyl ethers (PBDEs) in the San *Francisco Bay Area: measurements in harbor seal blubber* and human breast adipose tissue. Chemosphere 46:697–707.

She J, Winkler J, McKinney M, Visita P, Petreas M. 1997. Development of an isotope dilution GC/NCI-MS method for the analysis of organochlorine pesticides in human breast adipose tissue. Organohalogen Compounds 31:272–275.

Sjodin A, Patterson DG, Bergman A. 2001. Brominated flame retardants in serum from US blood donors. Environ Sci Technol 35:3830–3833.

Smith D. 1999. Worldwide trends in DDT levels in human breast milk. Int J Epidemiol 28:179–188.

State of California, Department of Consumers Affairs, Bureau of Home Furnishings and Thermal Insulation. 1991. Flammability Test Procedure for Seating Furniture for Use in Public Occupancies. Technical Bulletin 133. North Highlands, CA. Department of Consumers Affairs, Bureau of Home Furnishings and Thermal Insulation. Available: http://www.bhfti.ca.gov/techbulletin/tb133.pdf [accessed 8 *May 2003].*

———. 2000. Requirements, Test Procedure and Apparatus for Testing the Flame Retardance of Resilient Filling Materials Used in Upholstered Furniture. Technical Bulletin 117. North Highlands, CA. Department of Consumers Affairs, Bureau of Home Furnishings and Thermal Insulation. Available: http://www.bhfti.ca.gov/techbulletin/117.pdf [accessed 8 May 2003].

Thomsen C, Lundanes E, Becher G. 2002. Brominated flame retardants in archived serum samples from Norway: a study on temporal trends and the role of age. Environ Sci Technol 36:1414–1418.

U.S. EPA. 1997. Food Ingestion Factors. Exposure Factors Handbook, Vol. II. EPA/600/P-95/002Fb. Washington, DC:U.S. *Environmental Protection Agency, Office of Research and* Development.

van Bavel B, Hardell L, Kitti A, Lijedahl M, Karlsson M, Pettersson A, et al. 2002. High levels of PBDEs in 5% of 220 blood samples from the Swedish population. Organohalogen Compounds 58:161–164.

van den Berg BJ. 1979. The California Child Health and Development Studies: twenty years of research. World Health Stat Q 32:269–286.

WHO. 1994. Brominated Diphenyl Ethers. Environmental Health Criteria 162. Geneva:World Health Organization.

Windham G, Mitchell P, Petreas M, Lee D, Lasley B. 2002. Exposure to DDT and metabolites in relation to menstrual cycle length among Laotian immigrants [Abstract]. Epidemiology 13:S198.