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Catherine Ann Rice Date

Types and Preparations of Seafood in Diet, and Other Demographic Predictors of Persistent Organic Pollutants of Breast Milk in San Diego Women

By

Catherine Ann Rice

Degree to be awarded: Executive Master of Public Health

Epidemiology

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Associate Chair for Academic Affairs, Executive MPH

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Persistent Organic Pollutants of Breast Milk in San Diego Women

By

Catherine Ann Rice
Ecology and Evolutionary Biology
University of Arizona
1986

Thesis Committee Chair: Matthew O. Gribble, PhD DABT

An abstract of
A Thesis submitted to the Faculty of the
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Abstract

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Abstract

Background: This study seeks to describe Persistent Organic Pollutant (POP) levels in the breast milk of San Diego County women as a function of types of seafood consumed, cooking methods, and other lifestyle exposures.

Methods: Breast milk from 146 mothers in San Diego County were analyzed by GC/ECNI-MS for POP levels, and examined vis-à-vis self-reported questionnaire data using LASSO quantile regression methods.

Results: Among cooking methods, **baked, grilled and smoked seafood** were positively associated with median $\sum_{18}$PCBs. In contrast, **sautéed fish** was negatively associated with median PBDEs. **Dark fish, white fish** and **all seafood types combined** were positively associated with $\sum_{18}$PCBs (quantile 0.9), while **fried fish** was positively associated with median PBDE 47. Lifestyle factors positively associated with median POPs include: **time living in California** (PBDEs 28, 100, 153, 154; $\geq$ 223 months versus <76 months); **income** ($\sum_{18}$PCBs; \$40,000-75,000/year versus <\$40,000/year) and **education** ($\sum_{18}$PCBs*;* postgraduate/graduate degree versus $\leq$high school). Factors negatively associated with median POPs include: **income** ($\sum_{6}$PBDEs; >\$75,000 versus <\$40,000/year); **maternal age** ($\sum_{18}$PCBs; 25-29 versus <25 years) and **parity** ($\sum_{18}$PCBs; 2 versus 1 birth).

Conclusions: Consumption of higher trophic level fish (dark fish, white fish, all seafood types combined) was associated with higher levels of $\sum_{18}$PCBs at the 90th percentile whereas food preparation method (baked, grilled, smoked seafood) was associated with higher levels of $\sum_{18}$PCBs at the median.

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Finally, I would like to thank my husband, Bill and my family, Kayla, Rachel and Rebecca for their unwavering encouragement and support throughout this extended journey toward reaching my goal of becoming a public health professional.

Table of Contents

List of Tables and Figures

Supplemental Appendix: Materials and methods

Background and contributions prior to thesis

This study, which commenced in the fall of 2014, was a collaborative effort conducted by San Diego State University (SDSU) and the University of California, San Diego (UCSD) as part of Ocean and Human Health Project at Scripps Center for Oceans and Human Health (SCOHH). The study design, recruitment of subjects, collection of breast milk samples, data collection and analysis of the samples for levels of persistent organic pollutants (POPs) was conducted by students at SDSU under the supervision of Dr. Eunha Hoh. The data were initially analyzed by myself and colleagues for associations between POP levels in the breast milk and meat, dairy and seafood consumption, as well as personal and demographic characteristics of the mother and infant. This involved analysis of the data using parametric (ANOVA and two-sample t-test) and non-parametric (Kruskal-Wallis and Mann-Whitney) tests. In addition, multiple linear regression models were used to control for predictors of interest. This work is pending publication.

My thesis work will focus on the secondary analysis of this data, using bivariate and non-parametric LASSO quantile regression to evaluate associations in the data. I will first characterize the data from the questionnaire, which were not included in the initial analyses, using non-parametric Kruskal-Wallis and Mann-Whitney tests. These categorical variables will include fish parts consumed, fish cooking methods, fish source, smoking and other smoke exposures, use of medications and supplements, herbicides, pesticides and personal care products.

Secondly, using the method of non-parametric LASSO quantile regression, I will compare the levels of POPs in the breast milk samples associated with seafood cooking methods vs. types of seafood consumed, and demographic and maternal and infant characteristics at quantiles 0.5 and 0.9. This is a novel application to the analysis of this type of data.

All of the writing, data analysis, figure and table development within this manuscript are composed by the author, Catherine A. Rice. Dr. Eunha Hoh has served as Project Coordinator and Thesis Field Advisor on this project, with expertise in environmental pollutants and their impact on human health. Dr. Matthew Gribble has served as Thesis Committee Chair with expertise in methods of data analysis. Claire O'Brien and Chelsea Basirico contributed to the work leading up to this thesis, and have provided the supplemental information on the analytical methods used to implement this study.

Intended journal for publication: *Journal of Exposure Science and Environmental Epidemiology*

Introduction and Review of the Literature

Persistent organic pollutants (POPs) are lipophilic contaminants that persist in the environment and bioaccumulate and biomagnify in food chains and in humans, thereby posing a threat to public health.[1-5] Such halogenated organic compounds (HOCs) are primarily anthropogenic and include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs).[3,4] These contaminants are found throughout the world and are transported through wind and water where they accumulate in soils and sediments, often far from their original source.[3,5,6] POPs are assimilated into lower organisms in the food chain where they bioaccumulate in fatty tissues and are transported up the food chain and eventually into wildlife and humans, primarily through exposure from diet.[7-10]

More than 1 million tons of PCBs were produced commercially from the 1920s until the 1970s, and industrial applications included the use in plasticizers, paints, plastics, electronic appliances, hydraulic fluids, carbonless copy paper and heat transfer fluids.[2,3,5] Although manufacturing of PCBs has ceased since being banned in 2004 by the Stockholm Convention,[11,12] they persist in many products in wide use today and thus are continually released into the environment.[3,6] The adverse health effects caused by PCBs may include carcinogenesis, endocrine and immune system disruption, neurotoxicity and reproductive disorders.[13,14]

In the 1970s, widespread production of PBDEs, a class of organobromine compounds, was introduced for use as flame retardants in household products and building materials, including plastics, paints, furniture, polyurethane foams, textiles and carpeting.[6,15,16] These compounds do not bind chemically to the products in which they are used, allowing them to be leached into the environment where subsequent exposure to humans occurs primarily through dust inhalation, and diet.[7-9] California's strict anti-flammability requirements for household products culminated in a statewide furniture flammability standard (TB 117) in 1975 and the widespread use of PBDEs in the state.[17] Consequently, some of the highest levels of PBDEs in the world have been found in the breast adipose tissues of California residents [18-23] and in the blubber of harbor seals in the San Francisco Bay area.[23] Adverse health effects include reproductive disorders, hormone (thyroid) disruption and neurodevelopmental deficits.[24-27]

OCPs are a class of chlorinated hydrocarbon derivatives employed for their capacity to control insects, weeds, fungi, bacteria and other organisms.[3,28-29] They include compounds such as DDT and its metabolite, DDE; dibutyl phthalate (DBP); and chlordanes such as cis-nonachlor, trans-nonachlor and heptachlor. Although many OCPs, including DDT, were banned for agricultural use in the U.S. in 1972, their massive production and use from 1945 to 1972 in agriculture resulted in widespread environmental contamination of these POPs.[3,4,29] Legacy pollution and ongoing use in many countries has led to adverse health effects in humans including liver, kidney, thyroid and bladder damage, as well as central nervous system and reproductive disorders.[3,29]

The Stockholm Convention on Persistent Organic Pollutants sought to prohibit and/or eliminate the global manufacture and use of these and other POPs since 2004; however, these organic compounds continue to persist in the environment and are biomagnified in the food chains in terrestrial and aquatic species.[11,12,30] Because the marine environment represents a large reservoir for POPs, many fish and marine mammals contain high levels of PCBs, PBDEs and OCPs.[31-34] One such reservoir is the Southern California Bight (SBC), an area inhabited by more than 17 million people extending from Point Conception, CA to Ensenada, Mexico. There is abundant marine life, aquaculture and farming in this area, and studies have shown that many marine mammals and fish species in this coastal region contain high levels of PBDEs, PCBs and OCPs.[35] Insofar as exposure to POPs occurs primarily through diet, [7,9,36] consumption of seafood may contribute significantly to exposure levels and body burdens of POPs in this region. Due to the lipophilic nature of these contaminants, they bioaccumulate in the fatty tissues, including human breast tissue, and can be offloaded into breast milk consumed by infants.[37-39]

The objective of this study is to evaluate potential determinants of the levels of POPs in the breast milk of women in San Diego County.

Methods

Information on the materials used, sample preparation, instrumentation used for analysis, and quality assurance/quality control can be found in the Supplemental Appendix.

This study was conducted with the approval of the San Diego State University Human Research Protection Program. All participants in the study provided written informed consent.

Study Population

The exposure assessment survey was conducted by San Diego State University (SDSU) in collaboration with University of California, San Diego (UCSD) as part of the Ocean and Human Health Project at Scripps Center for Oceans and Human Health (SCOHH). Women at least 18 years old were recruited in the fall of 2014 from the UCSD Medical Newborn Center, as well as from other locations in San Diego such as the Women, Infants and Children (WIC) Program, breastfeeding support groups, lactation groups, and social media such as Facebook. Inclusion criteria were delivery of a full-term healthy infant; ability to express 50ml of breast milk, by hand, manual pump or electric pump; and ability to answer questions on a questionnaire in English, Spanish, Vietnamese or Arabic. Primiparity and the ability to exclusively breastfeed were not criteria for inclusion in this study.

The study sample consisted of 155 breastfeeding women in San Diego County age 20-44, 3-12 weeks postpartum. Nine participants were excluded from the data analyses for the following reasons: participant ID #12 and 14: incomplete questionnaire data or errors and inconsistencies in questionnaire information; participant ID #22: mistake made during sample preparation; participant ID #30 and 107: samples lost due to spillage during sample preparation; participant ID #91 and 94: samples were mixed up during processing; participant ID #95: error made during gas permeation chromatography processing. In addition, the baseline for sample participant ID #54 was too high to be able to detect any peaks or standards and therefore had to be excluded from analysis of the OCP data. Two of the infants were twins (participant ID #96) and were excluded from analyses involving gender, birth weight, age of infant and parity. The final analyses were therefore performed on a total of n=146 individuals, 80 of whom were primiparous.

Analytical Methodology and Quantitation of POPs in Breast Milk

Study participants provided 50ml aliquots of breast milk collected by hand expression, or by manual or electric breast pump, at 3-12 weeks postpartum. Samples were stored in the home freezer until collection by researchers, at which time they were transported on ice to the laboratory at SDSU and stored at -20ºC. A 10-ml portion of each sample was analyzed by gas chromatography in combination with electron capture negative ion mass spectrometry (GC/ECNI-MS) to quantitate levels (ng/g lipid) of PCB, PBDE and OCP contamination.[40] Each breast milk sample was analyzed for levels of PCB congeners 28, 52, 60, 101, 81, 77, 123, 118, 114, 153, 105, 138, 126, 187, 128, 167, 156, 157, 180, 169, 170, 189, 195, 206 and 209.[41] The PBDE congeners analyzed were PBDE 28, 47, 100, 99, 153, 154 and 183.[42] The OCPs DBP (p,p-DBP), HEPT (Heptachlor epoxide), *trans*-nonachlor, DDE (p,p-DDE) and *cis*-nonachlor, were also analyzed in this study.[29] Congeners with a frequency of detection <60% were excluded from statistical analyses: PCB 28, 52, 60, 77, 81, 101 and 126 and PBDE 183. In total, 18 PCBs, 6 PBDEs, and 5 OCPs were included for statistical analyses.

Detailed information on the sample preparation, instrumentation and methods used in the analysis of each sample are provided in the Supplemental Appendix: Materials and Methods.

Behavioral and Demographic Data Questionnaire

A questionnaire was self-administered to each study participant at the time of sample collection, 3-12 weeks postpartum, to obtain current and retrospective personal information. (See Supplemental Appendix: Maternal Questionnaire – Diet and Breast Milk)

Statistical Analysis

PCB, PBDE and OCP concentrations in the breast milk were corrected for lipid content and are presented throughout this paper as ng/g lipid. The distributions of each contaminant are summarized throughout this paper by medians, except in the case of quantile regression. The quantile regression analysis results are presented for two quantiles: 0.5 (median) and 0.9.

Bivariate Data Analysis

Bivariate relationships with POPs were analyzed using Mann-Whitney non-parametric tests. These included variables related to smoking history and smoke-related products, exposure to candles, incense, soap, perfume, sunscreen and makeup, fish body part consumed (body, fillet, brain, liver, head, heart and skin), fish source (store, fishing, restaurant), and fish origin (wild, farm-raised, ocean or lake).

For bivariate associations, each lipid-corrected POP concentration (continuous, ng/g lipid) was natural-log transformed to approximate a lognormal distribution. Median levels of POPs in the breast milk samples were qualitatively analyzed with SAS version 9.4 for bivariate relationships with each explanatory variable of interest by histogram, Q-Q plots and the Shapiro-Wilk test for goodness-of-fit. Non-parametric Mann-Whitney tests were used to determine which categorical variables were crudely associated with fish parts consumed, fish source, supplements and medications taken, smoking history and use of smoke-related products, use of personal care products, and pesticides and herbicides.

Cross-validated LASSO-penalty Quantile Regression Analysis

The data related to methods of fish preparation and types of fish consumed were analyzed using quantile regression models with Least Absolute Shrinkage and Selection Operator penalty (LASSO), adjusting for demographic characteristics and potential confounders that are known to associate with increased POP body burdens.[9]

LASSO-penalty quantile regression models were utilized to analyze the association of each independent variable with the conditional quantiles 0.5 (median) and 0.9 of the response variable (i.e., POP biomarker).[43-46] This method of cross-validated quantile regression, implemented in R version 1.1.383 (R package rqPen, version2, cv.rq.pen),[47] makes no distributional assumptions about the outcomes, is robust to outliers in POP levels, and calculates a range of 'lambdas' (which control the strength of the LASSO penalty) with the penalty of choice.[48,49] Due to our relatively small sample size (n=146) and large number of predictors, we chose the LASSO penalty function to improve predictive power and reduce Type I error by shrinking large regression coefficients toward zero, thereby improving predictions.[46,48,49] Standard errors and percentile-based 95% confidence intervals of each LASSO regression coefficient were obtained by bootstrap (boot package in R) methods.[50]

The LASSO quantile regression models' candidate predictors included: cooking methods (baked, fried, grilled, sautéed, steamed, broiled, smoked or raw fish), total combined amounts of seafood consumed (grams/day of dark fish, white fish, canned tuna, raw shellfish, squid/octopus, lobster/crab), as well as the individual types of fish consumed (dark fish, white fish and canned tuna), pesticide and herbicide use, maternal age, infant gender, infant age at time of sampling (lactation duration), months living in California, BMI (kg/m^2), income, ethnicity, maternal education, and marital status.

Results

Participant Characteristics

Approximately 93.9% of the mothers in this study were 25 years or older and 63.7% had a pre-pregnancy BMI between 18.6 and 24.9 kg/m^2 (Table 1). The majority of the mothers (75.3%) resided in California for at least 6.3 years. Slightly over half (55%) of the women were primiparous and breastfeeding for the first time. White or Caucasian race/ethnicity accounted for 65.8% of the participants and 13.7% were Latino or Hispanic. Nearly all (96.6%) reported having an education higher than high school and most (84.9%) of the participants were married; 56.2% reported income levels at >$75,000/ year while 20.5% had incomes <$40,000/year. In this sample, 52.4% of the infants were male and 47.6% were female, with 24.1% exhibiting a birth weight greater than 3.7 kg.

Table 1. Maternal and infant characteristics

Maternal Demographics (n=146)					
	n	**Percentage (%)**		**n**	**Percentage (%)**
Maternal Age (years)			**Ethnicity**		
<25	9	6.2	Latino or Hispanic	20	13.7
25-29	36	24.7	White or Caucasian	96	65.8
30-34	60	41.1	Other	30	20.5
≥35	41	28.1	**Marital Status**		
Pre-pregnancy BMI			Single	19	13
≤18.5	7	4.8	Married	124	84.9
18.6-24.9	93	63.7	Other	3	2.1
25-29.9	27	18.5	**Education**		
≥30	19	13	High School equivalent or less	5	3.4
Months living in California			Some college, associate's degree, trade school	33	22.6
0-75	36	24.7	College degree	49	33.6
76-222	37	25.3	Postgrad or graduate degree	59	40.4
223-360	38	26	**Income**		
>360	35	24	<$40,000	30	20.5
Parity			$40,000-$75,000	27	18.5
1	80	55.2	>$75,000	82	56.2
2	48	33.1	Don't know/decline to answer	7	4.8
3+	17	11.7			
Number of breastfed children					
1	81	55.5			
2	48	32.9			
3+	17	11.6			
Infant Demographics (n=145)					
	n	**Percentage (%)**		**n**	**Percentage (%)**

Sex			**Birth weight (kg)**		
Male	76	52.4	2.38-3.09	37	25.5
Female	69	47.6	3.10-3.37	37	25.5
Lactation duration at sample collection (weeks)			3.38-3.69	36	24.8
0.86-5.43	38	26.2	>3.69	35	24.1
5.44-7.86	39	26.9			
7.87-10.14	33	22.8			
>10.14	35	24.1			

Participants' POP levels

All of the breast milk samples collected in the study contained detectable levels of one or more congeners of PCB, PBDE and OCP contaminants (Table 2). The median $\sum_{25}$PCB level among all participants (n=146) was 46.4 ng/g lipid. PCB 153 was the most prevalent congener in the samples tested, accounting for 24.8% of total median PCB levels followed by (in descending order), PCB 138, 180, 118, 170, 187, 156, 105, 114, 157, 167, 206, 195, 169, 128, 123, 209, 189, 52, 126, 28, 60, 101, 81 and 77. The median $\sum_{7}$PBDE level among all participants (n=146) was 48.6 ng/g lipid. PBDE 47 accounted for 34.6% of the total median PBDE levels in the breast milk samples followed by (in descending order), PBDE 154, 153, 99, 100, 28, 183. DDE was the most prevalent OCP (n=145) at 320ng/g lipid followed by (in descending order), *trans*-nonachlor, heptachlor, *cis*-nonachlor and DBP. The following congeners with <60% of samples below detection limit were excluded from data analysis: PCB 28, 52, 60, 77, 81, 101 and 126 and PBDE 183.

Table 2. Distribution of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) in breast milk samples.

PCBs (ng/g lipid)	**Congener Name**	**N**	**Lower Quartile**	**Median**	**Upper Quartile**
PCB 77	(3,3',4,4'-tetrachlorobiphenyl	146	0.0027	0.0036	0.0060
PCB 81	(3,4,4',5-tetrachlorobiphenyl	146	0.0028	0.0040	0.0250
PCB 101	(2,4,5,2',5'-pentachlorobiphenyl)	146	0.0030	0.0074	0.0361
PCB 60	(2,3,4,4'-tetrachlorobiphenyl)	146	0.0122	0.0297	0.0446
PCB 28	(2,4,4'-trichlorobiphenyl)	146	0.0040	0.0380	0.2990
PCB 126	(3,3',4,4',5-pentachlorobiphenyl	146	0.0033	0.2208	0.4201
PCB 52	(2,5,2',5'-tetrachlorobiphenyl)	146	0.0380	0.2833	0.4020
PCB 189	(2,3,3',4,4',5,5'-heptachlorobiphenyl)	146	0.0671	0.2867	0.4320
PCB 209	(2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl)	146	0.1688	0.2907	0.4127
PCB 123	(2,3',4,4',5'-pentachlorobiphenyl)	146	0.0058	0.2951	0.4440
PCB 128	(2,2',3,3',4,4'-hexachlorobiphenyl)	146	0.2401	0.3201	0.4514
PCB 169	(3,3',4,4',5,5'-hexachlorobiphenyl	146	0.2132	0.3321	0.4349
PCB 195	(2,2',3,3',4,4',5,6-octachlorobiphenyl)	146	0.3206	0.4317	0.6447
PCB 206	(2,2',3,3',4,4',5,5',6-nonachlorobiphenyl)	146	0.3419	0.4825	0.6924
PCB 167	(2,3',4,4',5,5'-hexachlorobiphenyl)	146	0.3849	0.5443	0.8019
PCB 157	(2,3,3',4,4',5'-hexachlorobiphenyl)	146	0.4486	0.6579	1.0389
PCB 114	(2,3,4,4',5-pentachlorobiphenyl)	146	0.3781	0.6769	1.0281
PCB 105	(2,3,3',4,4'-pentachlorobiphenyl)	146	0.8343	1.2909	2.1018
PCB 156	(2,3,3',4,4',5-hexachlorobiphenyl)	146	1.1152	1.8317	3.5046
PCB 187	(2,2',3,4',5,5',6-heptachlorobiphenyl)	146	1.2467	2.2939	3.6632
PCB 170	(2,2',3,3',4,4',5-heptachlorobiphenyl)	146	1.8521	3.0312	4.9812
PCB 118	(2,3',4,4',5-pentachlorobiphenyl)	146	3.0276	4.7894	6.8679
PCB 180	(2,2',3,4,4',5,5'-heptachlorobiphenyl)	146	3.7227	6.0832	10.1469
PCB 138	(2,2',3',4,4',5-hexachlorobiphenyl)	146	6.1678	10.7266	15.3863
PCB 153	(2,2',4,4',5,5'-hexachlorobiphenyl)	146	6.8005	11.5008	18.2543
Sum PCBs		146	29.2627	46.3989	73.7959
BDEs (ng/g lipid)					

BDE 183	(2,2',3,4,4',5',6-heptabromodiphenyl ether)	146	0.0033	0.0082	0.0808
BDE 28	(2,4,4'-tribromodiphenylether)	146	1.0550	1.6856	2.8746
BDE 100	(2,2',4,4',6-pentabromodiphenylether)	146	2.0578	3.5772	5.8681
BDE 99	(2,2',4,4',5-pentabromodiphenylether)	146	2.8200	4.4536	6.6219
BDE 153	(2,2',4,4',5,5'-hexabromodiphenylether)	146	4.8689	8.0090	14.7933
BDE 154	(2,2',4,4',5,6'-hexabromodiphenylether)	146	3.6871	8.1277	11.4957
BDE 47	(2,2',4,4'-tetrabromodiphenylether)	146	8.9228	16.7938	29.7843
Sum BDEs		146	30.5048	48.6044	72.4997
OCPs (ng/g lipid)					
DBP	p,p-DBP	145	0.5065	0.9150	1.7414
NON-CIS	Nonachlor-cis	145	1.3116	2.0328	2.8504
HEPT	Heptachlor epoxide	145	5.9197	9.8084	16.0694
NON-TRANS	Nonachlor-trans	145	10.1438	14.9176	24.2103
DDE	p,p-DDE	145	187.1544	320.0369	535.0569

Bivariate data analysis

The results of the bivariate data analysis are shown in Table 3 (PCBs) and Table 4 (PBDEs and OCPs), with a nominal significance threshold of alpha=0.05.

- **Fish parts:** Among fish parts consumed, the head and skin were the most strongly associated with increased median $\sum_{18}$PCB levels compared to those who did not consume these body parts (P values 0.057 and 0.03, respectively). Skin consumption was also strongly associated with OCPs DBP, HEPT and trans-nonachlor and cis-nonachlor (P values 0.007, 0.055, 0.01 and 0.003, respectively).

- **Fish source:** Store-bought fish consumption was associated with increased median $\sum_{18}$PCB levels (P value 0.026), compared to those who did not purchase fish in stores, and this association was true whether participants reported having consumed fish that was purchased as wild-caught (P value 0.003) or as farm-raised (P value 0.01). $\sum_{18}$PCB and $\sum_{6}$PBDE levels were not significantly influenced with the consumption of restaurant fish. However, median levels of heptachlor, trans-nonachlor and cis-nonachlor were significantly higher among women who reported consuming restaurant fish (P values 0.001, 0.003 and 0.003, respectively), compared to women who did not eat restaurant fish. Interestingly, the consumption of wild-caught fish, whether caught in lakes or in the ocean, was associated with lower overall levels of contaminants.

- **Medications:** Medications for both high blood pressure and heart disease showed associations with increased median levels of heptachlor (P value 0.034), whereas medications for cancer or other indications did not show significant associations with increased POP levels in breast milk compared to women who did not take these medications.

- **Candles and incense:** Exposure to burned candles inside the home in the past year was associated with an increase in median $\sum_{18}$PCB levels (P value 0.025), whereas exposure to incense in the home in the past year was associated with an increase only in DDE (P value 0.02), compared to those with no exposure to these sources of smoke.

- **Smoking:** Exposure to second-hand smoke outside of the home was associated with higher levels of median PBDE 153 and 154 (P values 0.042 and 0.045) and OCP heptachlor (P value 0.02) compared to women with no exposure to second-hand smoke outside of the home.

- **Personal care products (antibacterial soap, perfume, sunscreen lotion or cream and makeup):** Sunscreen lotion or cream use was associated with increased $\sum_{18}$PCB levels (P value 0.03), whereas no significant associations in increased POP levels were observed with the use of antibacterial soap, perfume or makeup compared to those with no exposure to these products.

Table 3. Mann-Whitney test for bivariate relationships between explanatory variables and lipid-adjusted concentrations (ng/g lipid) of polychlorinated biphenyls (PCBs) in the breast milk of San Diego California women (n=146).

	Polychlorinated biphenyls																		
	∑	**123**	**118**	**114**	**153**	**105**	**138**	**187**	**128**	**167**	**156**	**157**	**180**	**169**	**170**	**189**	**195**	**206**	**209**
Variable	**Chi-square**																		
Body part eaten (yes/no)																			
Body	929	1011	1017	853	964	901	960	973	871	995	825	768	885	615	870	764	823	791	842
Fillet	333	389	359	311	343	348	336	337	247	340	299	280	298	139	296	251	280	285	234
Brain	53	2	78	1	68	1	75	75	27	68	43	36	48	3	46	46	26	14	3
Liver	272	203	250	165	300	162	298	326	232	257	217	176	289	119	279	258	267	238	230
Head	445	283	429	319	476◆	344	467◆	477◆	226	440	405	344	453◆	120◆	450◆	366	373	291	269
Heart	53	2	78	1	68	1	75	75	27	68	43	36	48	3	46	46	26	14	3
Skin	1414◆	1226	1462◆	1377	1446◆	1369	1451◆	1487◆	1197	1406◆	1290	1226	1341	908	1321	1112	1277	1234	1086
Fish Source (yes/no)																			
Source: store	435◆	560	439◆	608	429◆	547	441◆	421◆	307◆	414◆	465◆	615	427◆	683	432◆	702	472	575	589
Source: fishing	1459	1592	1470	1394	1463	1417	1467	1503	1482	1548	1336	1354	1456	1285	1452	1438	1587	1467	1544
Source: restaurant	1270	1114	1165	1248	1275	1296	1310	1211	1246	1230	1347	1387	1294	1369	1349	1420	1172	1189	1346
Source: store, wild	2880◆	2604	2910◆	3070◆	2877◆	2878◆	2931◆	2863◆	3392	2936◆	2933◆	3044◆	2949◆	3664	3041◆	3110◆	2959◆	2834◆	3205
Source: store, farmed	5377◆	5237	5504◆	5154	5401◆	5367◆	5424◆	5384◆	5008	5410◆	5227	5052	5256◆	4548	5235◆	4866	5319◆	5302◆	4603
Source: fishing, ocean	15	15	13	16	14	16	15	14	13	14	13	21	13	16	13	6	14	25	13
Source: fishing, lake	35	41	37	32	36	35	33	37	37	32	33	24	35	42	33	29	38	35	33
Medications (yes/no)																			
High blood pressure	144	200	167	149	149	166	158	143	241	165	128	150	133	229	136	187	190	186	106
Heart disease medication	144	200	167	149	149	166	158	143	241	165	128	150	133	229	136	187	190	186	106
Cancer medication	88	88	74	85	94	54	96	108	54	84	92	72	92	29	83	59	82	73	70
Other medication	1922	1735	1899	2012	1926	1860	1893	1994	1661	2008	1921	1925	1983	1887	1933	1814	2118	2072	1902
No medication	2572	2520	2526	2812	2554	2513	2552	2617	2499	2660	2619	2612	2624	2801	2588	2754	2833	2756	2584
Smoking, smoke																			
Ever smoked 100 cigarettes in lifetime	2048	2140	1934	2383	2005	1926	2015	2042	1987	2036	2180	2312	2048	2320	2065	2050	2130	2309	2144
Ever smoked daily for 6 months	1760	1857	1616	2073	1723	1702	1755	1762	1731	1712	1875	2015	1743	2001	1761	1885	1834	2011	1915

Secondhand smoke in home (past year)	1231	1494	1324	1300	1227	1429	1238	1213	1369	1332	1260	1220	1246	1350	1239	1516	1235	1198	1411
Second hand smoke outside home (past year)	3588	3247	3512	3458	3634	3464	3634	3603	3356	3582	3542	3553	3604	3641	3625	3595	3556	3463	3558
Burned candles in home (past year)	2562◆	3001	2847	2579◆	2596◆	2902	2628◆	2644	3091	2612◆	2337◆	2393◆	2472◆	2840	2489◆	2791	2484◆	2399◆	3162
Burned incense in home (past year)	1257	1313	1314	1059	1271	1226	1295	1226	1373	1369	1218	1261	1204	1212	1237	1147	1204	1207	1063
***Personal Care** Products*																			
Antibacterial soap	1191	1270	1178	1276	1184	1151	1195	1166	902	1137	1314	1265	1227	956	1222	1045	1045	1066	1252
Perfume	3491	2891	3297	3595	3462	3446	3511	3515	3099	3316	3539	3632	3523	2931	3490	3021	3387	3219	3183
Sunscreen lotion or	389◆	647	351◆	425	409◆	454	401◆	423	687	403◆	403◆	449	414	559	385◆	403◆	462	506	776
Makeup	711	814	730	751	706	867	727	672	967	706	727	842	656	805	667	548◆	674	587◆	909

◆ P value <0.05

Table 4. Mann-Whitney test for bivariate relationships between explanatory variables and lipid-adjusted concentrations (ng/g lipid) of polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) in the breast milk of San Diego California women (n=146).

	Polybrominated diphenyl ethers							**Organochlorine pesticides**					
	∑	**28**	**47**	**100**	**99**	**153**	**154**	**DBP**	**HEPT**	**Non-trans**	**DDE**	**Non-cis**
Variable	**Chi-square**							**Chi-square**					
Body part eaten (yes/no)													
Body	1003	893	963	819	928	986	665	733	883	915	821	992
Fillet	368	288	399	313	355	326	271	291	243	242	384	321
Brain	108	21	46	17	31	139	41	140	67	54	93	63
Liver	202	170	181	124	98	291	88	216	206	267	156	285
Head	326	233	288	213	198	354	194	263	312	340	293	382
Heart	108	21	46	17	31	139	41	140	67	54	93	63
Skin	1237	1188	1261	1191	1203	1188	1038	1483◆	1364	1469◆	1281	1527.5◆
Fish Source (yes/no)													
Source: store	832	612	768	900	887	937	840	597	459◆	435◆	539	543
Source: fishing	1546	1555	1559	1530	1547	1555	1295	1374	1352	1409	1329	1425
Source: restaurant	1171	1184	1254	1312	1234	1473	1220	1267	857◆	915◆	1342	922◆
Source: store, wild	3892	3784	3889	4201◆	3831	3740	3659	3528	3174	3049◆	3645	3077◆
Source: store, farmed	4467	4602	4532	4230◆	4338	4719	4435	4739	5324.5◆	4984	4891	5083
Source: fishing, ocean	24	15	18	20	22	30	27	24	28	20	15	14
Source: fishing, lake	25	23	23	25	22	42	30	43	53	41	44	27
Medications (yes/no)													
High blood pressure medication	182	174	180	174	166	212	202	197	269◆	222	162	146
Heart disease medication	182	174	180	174	166	212	202	197	269◆	222	162	146
Cancer medication	105	64	29	14	8	141	89	77	48	91	79	115
Other medication	1675	1548	1619	1511	1705	1896	1654	1543	1812	1740	1693	1900
No medication	2628	2207	2598	2483	2669	2660	2635	2431	2828	2601	2476	2641
Smoking, smoke products (yes/no)													
Ever smoked 100 cigarettes in lifetime	2396	2005	2238	2374	2400	2512	2390	2021	2435	2222	1944	2217

Ever smoked daily for 6 months	2092	1712	1943	2124	2103	2154	2138	1792	2147	1933	1709	1926
Secondhand smoke in home (past year)	1519	1242	1533	1679	1556	1538	1431	1169	1660	1407	1575	1362
Second hand smoke outside home (past year)	3109	3234	3165	3123	3313	3036◆	3041◆	3436	2889◆	3275	3093	3365
Burned candles in home (past year)	3240	3453	3296	3246	3070	2771	3254	3103	2828	2694	3160	2857
Burned incense in home (past year)	1271	1176	1291	1253	1256	1214	1301	1317	1208	1198	1452◆	1272
Personal Care Products (yes/no)												
Antibacterial soap	1033	1064	1086	1026	1104	832	1076	1102	1183	1336	1038	1191
Perfume	3088	3148	3010	2926	2905	3135	3185	3532	3210	3293	3107	3232
Sunscreen lotion or cream	759	592	632	678	489	701	784	682	511	578	579	615
Makeup	1072	897	1091	1003	981	794	1087	1124	785	732	821	707

DBP = p,p-DBP; HEPT = Heptachlor epoxide; NON-TRANS = *trans*-nonachlor; DDE = p,p-DDE; NON-CIS = *cis*-nonachlor

◆ P value <0.05

Multivariable quantile regression with LASSO penalty

The type of seafood consumed as well as seafood preparation methods were associated with the level of POP contaminants in the breast milk of women in San Diego County (Heat Maps, Figures 1-2 and Appendix Tables 1-5). ***Total consumption of seafood*** [(including dark fish, white fish, canned tuna, raw shellfish, squid/octopus, lobster/crab), (4.05 - 10.125g/day compared to <4.05g/day)] were associated with higher PCB 123 (β = 0.03, 95%CI: -0.07, 0.26) and PCB 156 (β = 0.05, 95% CI: 0, 1.5) at quantile 0.5, and associated with increased $\sum_{18}$PCBs (β = 9.54, 95%CI: 0, 32.14) and PBDE 153 (β = 6.34, 95% CI: 0, 32.5) at the 90th percentile. ***Dark fish consumption*** (>0-4.05 g/day compared to 0g/day) was negatively associated with medians of PCB 123 (β = -0.03, 95% CI: -0.17, 0.04) and positively associated with medians of PBDE 28 (β = 0.20, 95% CI: -0.07, 0.75), PBDE 153 (β = 0.02, 95% CI: 0, 3.05) and PBDE 154 (β = 2.61, 95% CI: -0.74, 4.65), and at the 90th percentile was associated with higher $\sum_{18}$PCBs (β = 15.15, 95% CI: 0, 33.16) and lower OCP DBP (β = -0.23, 95% CI: -1.01, 0). ***White fish consumption*** (2.026-4.05g/day compared to 0g/day) was negatively associated with median PCB 123 (β = -0.15, 95% CI: -0.24, 0.02) and PCB 128 (β = -0.01, 95% CI: -0.08, 0), but at the 90th percentile was positively associated with $\sum_{18}$PCBs (β = 0.83, 95% CI: 0, 31.55). ***Canned tuna consumption*** (2.026-4.05 g/day compared to 0g/day) was positively associated with medians of PCB 123 (β = 0.07, 95% CI: 0, 0.22), PCB 128 (β = 0.02, 95% CI: 0, 0.11), PCB 138 (β = 0.85, 95% CI: 0, 3.21), PCB 153 (β = 0.18, 95% CI: 0, 3.28), PBDE 154 (β = 3.17, 95% CI: -0.77, 5.6), heptachlor (β = 0.10, 95% CI: -0.72, 2.75), and *cis*-nonachlor (β = 0.05, 95% CI: 0, 0.56), but had mixed associations at the 90th percentile (>0-2.025 g/day compared to 0 g/day) with PCB 123 (β = 0.002, 95% CI: 0, 0.10), PCB 114 (β = -0.12, 95% CI: -0.48, 0) and PCB 157 (β = -0.19, 95% CI: -0.45, 0).

The methods of fish preparation were associated with breast milk POP levels at the 50th and 90th percentiles. Specifically, ***baked fish*** preparation (yes versus no) was associated with median $\sum_{18}$PCBs (β = 3.66, 95% CI: 0, 14.66), PBDE 28 (β = 0.30, 95% CI: 0, 1.09) and PBDE 154 (β = -0.82, 95% CI: -5.03, 1.84), whereas the 90th percentile associated with PCB 114 (β = 0.13, 95% CI: 0, 0.40), PCB 157 (β = 0.17, 95% CI: 0, 0.38), PCB 206 (β = 0.10, 95% CI: 0, 0.21) and PBDE 100 (β = 0.39, 95% CI: 0, 0.87). ***Smoked fish*** (such as smoked salmon, lox or other seafood) preparation (yes versus no) was positively associated with median $\sum_{18}$PCBs (β = 10.48, 95% CI: 0, 27.73) and negatively associated with median PBDE 28 (β = -0.02, 95% CI: -0.32, 0.47), PBDE 153 (β = -0.56, 95% CI: -3.11, 0) and PBDE 154 (β = -3.26, 95% CI:-5.65, 0.49), while only negative associations were observed at the 90th percentile with PCB 114 (β = -0.02, 95% CI: -0.23, 0) and PBDE 100 (β = -1.54, 95% CI: -2.15, 0). ***Grilled fish*** preparation (yes versus no) was associated with median $\sum_{18}$PCB levels (β = 0.25, 95% CI: 0, 13.49) and median PBDE 154 (β = 1.46, 95% CI: -0.16, 5.18), whereas no associations were observed at the 90th percentile. Other methods of fish preparation (yes versus no) that were associated with POPs at the median included ***fried fish*** (yes versus no): PCB 123 (β = -0.07, 95% CI: -0.15, 0.05) and PCB 128 (β = -0.02, 95% CI: -0.09, 0); ***steamed fish*** (yes versus no) PCB 123 (β = 0.07, 95% CI: -0.08, 0.14), PCB 118 (β = 0.25, 95% CI: 0, 1.33), PCB 156 (β = 0.26, 95% CI: -0.08, 1.02), PBDE 28 (β =0.23, 95% CI: -0.11, 0.68), PBDE 99 (β = -0.18, 95% CI: -0.80, 0), PBDE 154 (β = 1.87, 95% CI: -0.3, 6.14), and heptachlor (β = 0.60, 95% CI: 0, 6.44) and ***broiled fish*** (yes versus no): PCB 123 (β = 0.07, 95% CI: -0.04, 0.18), PCB 157 (β = 0.07, 95% CI: 0, 0.29), PBDE 28 (β = -0.09, 95% CI: -0.65, 0.23), PBDE 154 (β = -1.06, 95% CI: -4.48, 1.15) and heptachlor (β =0.89, 95% CI: 0, 3.47).

Fried fish (including fish sticks, fish sandwich, and fried shellfish, shrimp and oysters) consumption (yes versus no) was positively associated with medians of PBDE 28 (β = 0.27, 95% CI: 0, 0.98), PBDE 47 (β = 2.72, 95% CI: 0, 7.21), PBDE 100 (β = 0.20, 95% CI: 0, 0.78), PBDE 154 (β = 1.33, 95% CI: -0.83, 5.23), and with median DBP (β = 0.01, 95% CI: 0, 0.21), whereas only PBDE 154 was positively associated at the 90th percentile (β = 2.84, 95% CI: 0, 5.08). ***Fried fish*** consumption was not associated with increased median PCB levels but was associated with PCB 189 at the 90th percentile (β = 0.02, 95% CI: 0, 0.13). ***Sushi*** (all types, including raw fish or seafood, and shellfish) consumption (yes versus no) was positively associated with medians of PCB 114 (β = 0.04, 95% CI: 0, 0.24) and PCB 156 (β = 0.05, 95% CI: -0.26, 0.97), as well as median PBDE 28 (β = 0.15, 95% CI: -0.16, 0.63), PBDE 47 (β = 0.24, 95% CI: 0, 6.86) and heptachlor (β = 0.01, 95% CI: -0.08, 4.27), whereas the 90th percentile associated with PCB 128 (β = 0.04, 95% CI: 0, 0.11), and PCB 169 (β = 0.04, 95% CI: 0, 0.19). In contrast to the positive associations of other food preparation methods with POPs, ***sautéed*** fish preparation (yes versus no) methods were negatively associated with median levels of PBDE 28 (β = -0.01, 95% CI: -0.66, 0.16), PBDE 47 (β = -3.10, 95% CI: -7.85, 0), PBDE 99 (β = -0.27, 95% CI: -1.67, 0), PBDE 100 (β = -0.49, 95% CI: -1.30, 0) and PBDE 154 (β= -3.0, 95% CI: -5.15, 0.26) and with $\sum_6$PBDEs at the 90th percentile (β = -6.72, 95% CI: -40.25, 0).

Lifestyle factors associated with higher median POPs included: ***time living in California*** (223-360 months versus <76 months); PBDE 28 (β = 0.43, 95% CI: 0, 1.35), PBDE 100 (β = 1.47, 95% CI: 0, 3.17), PBDE 153 (β = 1.37, 95% CI: 0, 6.16), PBDE 154 (β = 1.92, 95% CI: -1.68, 4.91), ***BMI*** ($\geq$30 kg/m^2 versus < 18.6 kg/m^2); PBDE 28 (β = 1.02, 95% CI: 0, 1.38), PBDE 47 (β = 5.04, 95% CI: 0, 12.47), PBDE 100 (β = 0.55, 95% CI: 0, 2.0), ***income*** ($40,000 - 75,000/year versus <$40,000/year); $\sum_{18}$PCBs (β = 14.68, 95% CI: 0, 29.23), and ***education*** (postgraduate/graduate degree versus $\leq$high school); $\sum_{18}$PCBs (β = 1.77, 95% CI: 0, 16.85). Factors negatively associated with median POPs included: ***income*** (>$75,000 versus <$40,000/year); $\sum_6$PBDEs (β = -5.42, 95% CI: -21.3, 0), ***maternal age*** (25-29 versus <25 years); $\sum_{18}$PCBs (β = -10.04, 95% CI:-18.54, 0), and ***parity*** (2 versus 1 birth); $\sum_{18}$PCBs (β = -0.99, 95% CI: -14.94, 0).

Figure 1. Heat map of LASSO quantile regression analysis at quantile 0.5, showing polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

	Polychlorinated biphenyls																			Polybrominated diphenyl ethers							Organochlorine pesticides				
50% QUANTILE	Sum PCBs	PCB 123	PCB 118	PCB 114	PCB 153	PCB 105	PCB 138	PCB 187	PCB 128	PCB 167	PCB 156	PCB 157	PCB 180	PCB 169	PCB 170	PCB 189	PCB 195	PCB 206	PCB 209	Sum BDEs	BDE 28	BDE 47	BDE 100	BDE 99	BDE 153	BDE 154	OCP DBP	OCP Hept	OCP Non-Trans	OCP DDE	OCP Non-Cis
	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β
Baked fish (Yes, ref=No)	3.661		0.210		0.071		0.658		0.034		0.141	0.001									0.296					-0.816					
Fried fish (Yes, ref=No)		-0.074							-0.019																		0.130	0.014			
Grilled fish (Yes, ref=No)	0.253	0.153			0.706		1.008				0.055								0.039							1.456					
Sauteed fish (Yes, ref=No)							0.083				0.160										-0.013	-3.098	-0.492	-0.268		-2.995					
Steamed fish (Yes, ref=No)		0.069	0.250								0.255										0.228			-0.176		1.865		0.597			
Broiled fish (Yes, ref=No)		0.072																			-0.089					-1.062		0.889			
All Seafood types* (4.051-10.125 g/day, ref=<4.05g/day)		0.026									0.050	0.069														-0.762					
All Seafood types* (10.126-21.768 g/day, ref=<4.05g/day)		0.028									0.440										-0.374					1.652					
All Seafood types* (>21.768 g/day, ref=<4.05g/day)		0.004																			-0.082					2.887					
Dark Fish (>0-4.05 grams/day, ref=0)		-0.027																			0.197				0.0244	2.605					
Dark Fish (4.051-8.1 grams/day,ref=0)																															
Dark Fish (>8.1 grams/day,ref=0)											0.035	0.007										-0.477	-0.775								
White fish (>0-2.025 grams/day,ref=0)		0.070									0.330															-0.674					
White fish (2.026-4.05grams/day,ref=0)		-0.151							-0.014																	-0.940					
White fish (>4.05 grams/day,ref=0)		0.025																								0.918					
Canned Tuna (>0-2.025grams/day, ref=0)		0.027							0.024		-0.457										-0.054										
Canned Tuna (2.026-4.05grams/day, ref=0)		0.067			0.175		0.851		0.022												-0.220					3.170		0.095			0.045
Canned Tuna (>4.05grams/day, ref=0)		0.023									-0.407										-0.156										
Sushi (Yes, ref=No)		-0.111		0.039							0.051	0.159									0.149	0.235				-1.392		0.010			
Fried fish (Yes, ref=No)																					0.270	2.716	0.199			1.327	0.009				
Smoked fish or seafood (Yes, ref=No)	10.478	0.046			2.600		2.288				1.073	0.028									-0.024				-0.5618	-3.260					
Gender (Male, ref=female)		-0.101							-0.023														0.192	0.385		-0.403					
Months living in California (76-222 months, ref=<76 months)											-0.066										-0.005					-2.052					
Months living in California (223-360 months, ref=<76 months)		-0.029									0.043										0.429		1.470		1.3734	1.924		-1.179			
Months living in California (>360 months, ref=<76 months)			-0.386						0.001												0.618		0.148			2.527			1.748		0.032
Maternal age (25-29 years, ref=<25 years)	-10.039	-0.003			-1.945		-1.325	-0.461	-0.004		-0.479	-0.089					-0.064	-0.081	-0.004												
Maternal age (30-34 years, ref=<25 years)			0.258									0.002														2.580					
Maternal age (≥35 years, ref=<25 years)				0.024														0.025				-0.822			-1.702						
BMI (18.6-24.9 kg/m2, ref=<18.6kg/m2)											0.091					0.028			0.008				-0.428			0.322		-4.147			
BMI (25-29.9 kg/m2, ref=<18.6kg/m2)		0.015																								-0.489					
BMI (≥30 kg/m2, ref=<18.6kg/m2)		-0.038									-0.586	-0.069									1.020	5.036	0.546								
Baby age (5.44-7.86 months, ref=<5.44 months)		0.073									-0.007															-0.295					
Baby age (7.87-10.14 months, ref=<5.44 months)			-0.081				-0.633														0.786					4.536					
Baby age (>10.14 months, ref=<5.44 months)																										4.166					
Income ($40,000-$75,000/year, ref=<$40,000/year)	14.684	0.110	1.563	0.036	1.723	0.326	2.784		0.013	0.054	1.021	0.143									0.430	1.565				6.457					
Income (>$75,000/year, ref=<$40,000/year)		-0.010																		-5.419	-0.619	-10.287	-2.247	-1.696	-0.2804	-2.123					0.028
Ethnicity (Latino/Hispanic,ref=Other)		-0.004																													
Ethnicity (White/Caucasian, ref=Other)		-0.028																								-0.339				-81.029	
Education (SomeCollege/AssocDegree, ref=≤High School)		-0.032	-0.857	-0.022		-0.023															-0.229					1.370		-4.180	-1.302		-0.507
Education (CollegeDegree, ref=≤High School)											0.289										0.069										
Education (PostGrad/GradDegree, ref=≤High School)	1.774	0.010	1.137	0.079	0.408	0.041	0.525																			-1.479		0.691	1.172		0.519
Marital (Married, ref=Single)																															
Pesticide use (Wkly-Qtly, ref=Never/rarely)			0.251																			0.253	0.286			-0.755					
Herbicide use (Wkly-Qtly, ref=Never/rarely)		-0.026							0.018													0.002				-2.135					
Fish Oil Supplements Taken (Yes,ref=No)		-0.161	-0.695			-0.038																0.571				-1.126					
Parity (2)	-0.991		-0.504	-0.141		-0.013	-0.867			-0.035											0.143				-0.8552			-0.981			
Parity (≥3)		0.097									-0.036															-1.020		-0.355			

*All seafood types includes dark fish, white fish, canned tuna, raw shellfish, squid/octopus, lobster/crab

Figure 2. Heat map of LASSO quantile regression analysis at quantile 0.9, showing polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

	Polychlorinated biphenyls																			Polybrominated diphenyl ethers							Organochlorine pesticides				
90% QUANTILE	Sum PCBs	PCB 123	PCB 118	PCB 114	PCB 153	PCB 105	PCB 138	PCB 187	PCB 128	PCB 167	PCB 156	PCB 157	PCB 180	PCB 169	PCB 170	PCB 189	PCB 195	PCB 206	PCB 209	Sum BDEs	BDE 28	BDE 47	BDE 100	BDE 99	BDE 153	BDE 154	OCP DBP	OCP Hept	OCP Non-Trans	OCP DDE	OCP Non-Cis
	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β	β
Baked fish (Yes, ref=No)				0.126								0.165						0.102					0.393								
Fried fish (Yes, ref=No)																															
Grilled fish (Yes, ref=No)																															
Sauteed fish (Yes, ref=No)																				-6.721			-0.998								
Steamed fish (Yes, ref=No)																															
Broiled fish (Yes, ref=No)				-0.061																						-0.695					
All Seafood types* (4.051-10.125 g/day, ref=<4.05g/day)	9.54	0.017				0.32								0.192		0.244		0.047	0.119						6.344						
All Seafood types* (10.126-21.768 g/day, ref=<4.05g/day)		-0.05				-0.402																	-0.987								
All Seafood types* (>21.768 g/day, ref=<4.05g/day)							0.931																								
Dark Fish (>0-4.05 grams/day, ref=0)	15.15				2.857		4.638						1.943														-0.225				
Dark Fish (4.051-8.1 grams/day,ref=0)																															
Dark Fish (>8.1 grams/day,ref=0)													-1.128																		
White fish (>0-2.025 grams/day,ref=0)							1.424																								
White fish (2.026-4.05grams/day,ref=0)	0.825				1.171	0.188	2.184			0.034						0.001		0.067													
White fish (>4.05 grams/day,ref=0)																															
Canned Tuna (>0-2.025grams/day, ref=0)		0.002		-0.116								-0.192																			
Canned Tuna (2.026-4.05grams/day, ref=0)																															
Canned Tuna (>4.05grams/day, ref=0)																															
Sushi (Yes, ref=No)									0.036					0.035																	
Fried fish (Yes, ref=No)																0.02										2.841					
Smoked fish or seafood (Yes, ref=No)				-0.021																			-1.541		-2.801						
Gender (Male, ref=female)						-1.376	-4.184																0.283			-1.285					
Months living in California (76-222 months, ref=<76 months)														-0.068																	
Months living in California (223-360 months, ref=<76 months)												0.047				0.03		0.064					5.571								
Months living in California (>360 months, ref=<76 months)												-0.075		0.012												1.174					
Maternal age (25-29 years, ref=<25 years)	-36.01				-12.72		-13.11																			-1.932					
Maternal age (30-34 years, ref=<25 years)		0.088			0.087																					1.129					
Maternal age (≥35 years, ref=<25 years)																							-0.386								
BMI (18.6-24.9 kg/m2, ref=<18.6kg/m2)	6.401												0.585												0.718						
BMI (25-29.9 kg/m2, ref=<18.6kg/m2)																															
BMI (≥30 kg/m2, ref=<18.6kg/m2)																							0.276								
Baby age (5.44-7.86 months, ref=<5.44 months)													1.087	-0.118											-2.685						
Baby age (7.87-10.14 months, ref=<5.44 months)		0.02																				1.799	2.234			1.487					
Baby age (>10.14 months, ref=<5.44 months)																0.014										0.108					
Income ($40,000-$75,000/year, ref=<$40,000/year)		0.226							0.061	0.082																4.06					
Income (>$75,000/year, ref=<$40,000/year)				-0.101		-0.763	-6.04		-0.09			-0.164										-17.24	-4.206	-0.565		-3.749					
Ethnicity (Latino/Hispanic,ref=Other)																															
Ethnicity (White/Caucasian, ref=Other)																															
Education (SomeCollege/AssocDegree, ref=≤High School)														0.185																	
Education (CollegeDegree, ref=≤High School)				-0.142														0.035													
Education (PostGrad/GradDegree, ref=≤High School)		0.091												-0.019								-12.76	-1.027		0.431						
Marital (Married, ref=Single)																															
Pesticide use (Wkly-Qtly, ref=Never/rarely)																															
Herbicide use (Wkly-Qtly, ref=Never/rarely)																															
Fish Oil Supplements Taken (Yes,ref=No)																									6.683						
Parity (2)	-13.9	-0.005	-1.237	-0.367	-1.814	-0.265	-6.402					-0.161													-11.41	3.419	0.219				
Parity (≥3)																															

*All seafood types includes dark fish, white fish, canned tuna, raw shellfish, squid/octopus, lobster/crab

Discussion

This study highlights the associations of seafood with the body burden of POPs in women who had recently given birth in San Diego County.

There is potential for selection and information bias in any convenience sample survey with self-reported data. In order to reduce selection bias for inferences about San Diego County women from a sampling at a single venue, participants in this study were recruited from a variety of locations throughout San Diego County, and from different ethnic and cultural groups with varying incomes, education levels, and parity. Nonetheless, a higher percentage of the participants in this study were of White/Caucasian origin (65.5%) compared to the county statistics reported by the U.S. Census Bureau in 2016 (46%). The median income in San Diego in 2016 was $66,529, whereas 56.5% of the participants in this study reported having an annual income over $75,000. The majority of participants in this study had attained a college degree or post graduate degree (74%) compared to 36.5% in San Diego County.[(56)] Thus, this study sample over-represents individuals of White/Caucasian descent with higher level education and household income than the county average. However, this sampling bias will only introduce a selection bias to our associations of seafood with body burden of POPs if the relationships between seafood and body burden are differential by those socio-demographic features.

We attempted to control for potential confounding by measurement of many potential predictors. All measured demographic characteristics and potential confounders that were associated in the bivariate analyses, or were hypothesized *a priori* based on other studies to be associated with POP body burdens, were included in the quantile regression models. These included maternal age, infant age at time of sampling (lactation duration), months living in California, BMI (kg/m^2), income, ethnicity, education, marital status, infant gender and pesticide and herbicide use in the home. Although our association estimates from the LASSO quantile regression are shrunk (e.g., biased toward the null), and therefore lack a direct causal interpretation, the predictors significantly associated with body burden in this analysis are likely to reflect a real rather than confounded (by measured variables) relationship.

The LASSO quantile regression methods revealed distinct associations at the median and 90th percentiles of the POP distributions. POP exposure levels were related both to cooking methods and the types of seafood being consumed. LASSO regression at the 90th percentile, revealed which predictors were most strongly associated with the highest POP body burdens among women in this study. This study revealed that the consumption of higher trophic level fish (all seafood types combined, dark fish and white fish) was most strongly associated with increased $\sum_{18}$PCB levels at the 90th percentile. This is consistent with the bioaccumulative and biomagnification properties of POPs in higher food chains and in humans with primary exposures occurring through dietary sources.[6-10,14-15,30]

Regression at the 50th percentile revealed which predictors were most strongly associated with median POP levels. This study revealed that cooking methods were most strongly associated with median $\sum_{18}$PCB levels. Many studies have reported the effect of various cooking methods on POP levels in various foods.[51-54] In this study, we found that baked, grilled and smoked cooking methods, which utilize dry heat, were most strongly associated with increased median $\sum_{18}$PCB levels. Conversely, the method of sautéing, in which oil is used to cook the fish at low temperatures, resulted in decreased overall PBDE levels. This is consistent with previous studies reporting that cooking fish with oil may indeed affect the bioaccessibility of POPs in seafood by lowering PCB, PBDE and DDT levels.[52] Consumption of deep-fried seafood (fried fish, fish sticks, fish sandwiches and fried shrimp or oysters) resulted in increased PBDE and DBP levels. This is consistent with reports that deep-frying "seals in", rather than releases, the POP-laden fats, thereby trapping the contaminants in the flesh.[55] Sushi contained one or more of all of the contaminants, PCBs, PBDEs and OCPs, consistent with other studies that reported uncooked or raw fish contains higher levels of contaminants than cooked fish.[51,52]

In short, the consumption of higher trophic level seafood was positively associated with $\sum_{18}$PCBs at the tail of the POP distributions (quantile 0.9), while smoked, baked and grilled seafood were positively associated with median $\sum_{18}$PCB contaminants. The use of oils (with the exception of deep-frying) in cooking methods, such as sautéing (and possibly pan-frying), was negatively associated with overall median levels of POP contaminants. In conclusion, both seafood type and seafood preparation technique may contribute to the body burden of POPs in the breast milk of women in San Diego.

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The authors declare they have no actual or potential competing financial interests.

Supplemental Appendix

Materials and Methods

Quality Assurance and Quality Control

All glassware was covered in aluminum foil and baked in a muffle furnace at 450°C for 6 hours and all caps were rinsed with methanol and air-dried prior to use to remove traces of organic contamination. All work was done in a Labconco Protector Laboratory Hood (Labconco Corporation, Kansas City, Missouri, USA). Breast milk samples were kept in a -20°C walk-in freezer. No plastic was used during sample preparation unless otherwise noted. Laboratory equipment, such as solid phase extraction (SPE) manifolds, pipettes, and gas tight syringes were washed three times with HPLC grade acetone and three times with GC grade hexane prior to use. The manual disperser used for initial breast milk homogenization was washed by running the instrument at 12,000rpm for 30 seconds using LC/MS grade water and isopropanol and the exterior was rinsed with methanol and allowed to air dry prior to use. One laboratory blank with LC/MS grade water was prepared for every 7 samples.

Materials:

Solvents:

Pesticide grade methanol, LC/MS grade isopropanol, GC grade acetone, HPLC grade ethyl acetate (Fisher Scientific, Pittsburg, PA, USA). Cyclohexane for residue analysis, dichloromethane (Acros Organics, Fair Lawn, NJ, USA).

Standards:

Compound Name	Abbreviation	Vendor	Use
6-flouro-2,2',4,4'-tetrabromodiphenyl ether	6 FBDE 47	AccuStandard, New Haven, CT, USA	Internal standard
2,3,4,4',5,6-hexabromodiphenyl ether	BDE 166	AccuStandard, New Haven, CT, USA	Internal standard
3,3',4,4'-tetrabromodiphenyl ether	BDE 77	AccuStandard, New Haven, CT, USA	Internal standard
tris(4-chlorophenyl)methane-[13C19], 2,2',4,4',5,5'-hexachloro[13C12]biphenyl	13C TCPM	Wellington Laboratories, Guelph, Ontario, Canada	Internal standard
2,2',4,4'-tetrabromo-6-methoxy[13C12]diphenyl ether	13C 6MeOBDE 47	Wellington Laboratories, Guelph, Ontario, Canada	Internal standard
2,2',4,4',5,5'-hexachloro[13C12]biphenyl	13C PCB 153	Wellington Laboratories, Guelph, Ontario, Canada	Internal standard
4,4'-DDE (RING)-13C12, 99%	13Cp,p'-DDE	Cambridge Isotope Laboratories, Tewksbury, MA, USA	Internal standard
3,3',4,4',5,5'-hexachloro-1,1'-dimethyl(d_6)-2,2'-bipyrrole	2D3C DMBP Cl6	Scripps Institute of Oceanography, San Diego, CA, USA	Internal standard
3,3',4,4',5,5'-hexabromo-1,1'-dimethyl(d_6)-2,2'-bipyrrole	2D3C DMBP Br6	Scripps Institute of Oceanography, San Diego, CA, USA	Internal standard
3,3',4,4'-Tetrabromo-5,5'-dichloro-1,1'-dimethyl(d_6)-2,2'-bipyrrole	2D3C DMBP Br4Cl2	Scripps Institute of Oceanography, San Diego, CA, USA	Internal standard
4'-flouro-2,3',4,6-tetrabromodiphenyl ether	4 FBDE 69	AccuStandard, New Haven, CT, USA	Recovery standard
3,3',4,4',5,5'-hexachloro[13C12]biphenyl	13C PCB 169	Wellington Laboratories, Guelph, Ontario, Canada	Recovery standard

2,4,4’-tribromodiphenyl ether	BDE 28	AccuStandard, New Haven, CT, USA	Analyte
2,2’,4,4’-tetrabromodiphenyl ether	BDE 47	AccuStandard, New Haven, CT, USA	Analyte
2,2',4,4',5-Pentabromodiphenyl ether	BDE 99	AccuStandard, New Haven, CT, USA	Analyte
2,2',4,4',6-Pentabromodiphenyl ether	BDE 100	AccuStandard, New Haven, CT, USA	Analyte
2,2',4,4',5,5'-Hexabromodiphenyl ether	BDE 153	AccuStandard, New Haven, CT, USA	Analyte
2,2',4,4',5,6'-Hexabromodiphenyl ether	BDE 154	AccuStandard, New Haven, CT, USA	Analyte
2,2',3,4,4',5',6-heptabromodiphenyl ether	BDE 183	AccuStandard, New Haven, CT, USA	Analyte
1,3,5-tribromo-2-(4-methoxyphenoxy)benzene	AK 254	Scripps Institute of Oceanography, San Diego, CA, USA	Analyte
1,3,5-tribromo-2-(3-bromo-4-methoxyphenoxy)benzene	AK 256	Scripps Institute of Oceanography, San Diego, CA, USA	Analyte
1,3,5-tribromo-2-(3,5-dibromo-4-methoxyphenoxy)benzene	AK 258	Scripps Institute of Oceanography, San Diego, CA, USA	Analyte
1,5-dibromo-3-(2,4-dibromophenoxy)-2-methoxybenzene	AK 262	Scripps Institute of Oceanography, San Diego, CA, USA	Analyte
1,2,3,4-tetrabromo-5-(2,4-dibromophenoxy)-6-methoxybenzene	AK 263	Scripps Institute of Oceanography, San Diego, CA, USA	Analyte
3-Chloro-6-methoxy-2,2',4,4'-tetrabromodiphenyl ether	MOCBDE 4001	AccuStandard, New Haven, CT, USA	Analyte
6'-Methoxy-2,2',4,4',5-pentabromodiphenyl ether	MOBDE 5006S	AccuStandard, New Haven, CT, USA	Analyte

Sample Extraction and Clean-up Procedures:

Homogenization:

Defrosted breast milk samples were homogenized in a 100-200mL beaker using a Polytron PT 1300D Manual Disperser (Kinematica, Bohemia, NY, USA) for 15-30 seconds at 12,000 rpm. Homogenized breast milk was poured back into the amber collection jar, swirled, poured back into the beaker, and homogenized again at 12,000 rpm for 10-15 seconds. 10mL aliquots of breast milk were measured; one of these aliquots was spiked with known amounts of a mixture of the internal standards to be used in this analysis. The spiked sample and the remaining aliquots were returned to a -20°C freezer until sample preparation for chemical analysis.

Quechers:

The 10mL spiked aliquot of breast milk was defrosted and transferred into capped, pre-weighed 50mL glass centrifuge tubes. The capped centrifuge tubes containing breast milk were re-weighed and grams of breast milk was calculated.

10mL of a 1:1 hexane:acetone mixture was added to the samples and then vortexed for approximately 30 seconds. 4g of magnesium sulfate and 1g of sodium chloride were added. Tubes were hand-shaken and vortexed. Samples were placed on a Bellydancer (IBI Scientific, Peosta, IA, USA) and shaken at the maximum speed for 1 hour and 30 minutes. Samples were centrifuged at

1500rpm for 15 minutes using an IEC Centra CL3R (ThermoFisher Scientific, Fair Lawn, NJ, USA).

While samples were shaking and centrifuging, aluminum pans were pre-weighed using a SE2 Ultra Microbalance (Sartorius, Elk Grove, IL, USA). After centrifuging, there were three layers; the top layer was transferred to a graduated cylinder and the volume was recorded. 10% of this top layer was transferred to a pre-weighed aluminum pan using a micropipette with plastic tips and allowed to dry in the fume hood overnight to allow for measuring the amount of lipid in each sample. Aluminum pans were weighed between 24-48 hours later and recorded; the amount of lipid in each sample was calculated and recorded.

The remaining extract in the graduated cylinder was transferred to a 10 mL glass test tube. Sample extracts were evaporated in a 40°C water bath with nitrogen gas using a Zymark TurboVap (Caliper Life Sciences, Hopkinton, MA, USA) until only lipid remained. Approximately 1mL of 1:1 hexane:acetone mixture was added to the samples which were then evaporated again in a 40°C water bath with nitrogen gas using a Zymark TurboVap for approximately 5 minutes until only lipid remained.

Approximately 1mL of 1:1 hexane:acetone was added to the extract and then they were capped and shaken. 200mg of Enviro-Clean PSA (United Chemical Technologies, Bristol, PA, USA) was added to each solution and then they were hand shaken, vortexed for 30 seconds to 1 minute, and then centrifuged using an IEC Centra CL3R at 3000rpm for 5 minutes. The supernatant was transferred to a new 10mL glass test tube and evaporated in a 40°C water bath using nitrogen gas until only lipid remained. Approximately 1mL of 1:1 hexane:acetone mixture was added and samples were evaporated again in a 40°C water bath with nitrogen gas using a Zymark TurboVap for approximately 3 minutes until only lipid remained. The sample extracts were transferred to and stored in amber vials and returned to a -20°C freezer until the next step of sample extraction.

Gel Permeation Chromatography (GPC):

4.5 mL of 1:1 cyclohexane:ethylacetate mixture was added to the extract and then injected to a GPC (J2 Scientific, Columbia, MO, USA) system to remove the lipids. The GPC column, with a 2cm id and 22.5cm length, was packed with 24g of BioBeadsS-X3 in 1:1 ethylacetate:cyclohexane. The flow rate was 5 mL/minute and the mobile phase was 1:1 ethylacetate:cyclohexane. Three eluent fractions were produced and each fraction was evaporated in a 40°C water bath with nitrogen gas using a Zymark TurboVap to approximately 1-1.5mL. The sides of the test tubes were rinsed with approximately 1mL 1:1 cyclohexane:ethylacetate and evaporated again in a 40°C water bath with nitrogen gas using a Zymark TurboVap to 1-1.5mL. Fractions 1, 2, and 3 were combined into one test tube and evaporated in a 40°C water bath with nitrogen gas using a Zymark TurboVap to 1mL. 4mL of hexane was added and evaporated again in a 40°C water bath with nitrogen gas using a Zymark

TurboVap to 1mL in order to solvent exchange into hexane. Sample extracts were stored in 4mL amber vials in a -20°C freezer.

Solid Phase Extraction (SPE):

Silica (SPE) cartridges, Enviro Clean Extraction Column (UCT, Bristol, PA, USA), were loaded onto the SPE manifold and were filled with approximately 7mL of hexane which was eluted; this step was repeated once more to rinse the cartridges and the eluent was discarded. The sample extract was loaded into the SPE cartridges and eluted. The inside of the 4mL amber sample storage vial was rinsed with approximately 1mL of hexane, which was then added to the cartridge and eluted. 5mL of hexane was added to the cartridge and eluted (Fraction 1). 5mL of 40%:60% hexane:dichloromethane was added to the cartridges and eluted (Fraction 2). 5mL of dichloromethane was added and eluted (Fraction 3). 5mL of ethyl acetate was added and eluted (Fraction 4). 5mL of acetone was added and eluted (Fraction 5). Fractions 3, 4, and 5 were stored individually in 8mL amber vials in a -20°C freezer. Fractions 1 and 2 were evaporated in a 40°C water bath with nitrogen gas using a Zymark TurboVap to 1mL. Fractions 1 and 2 were then combined and evaporated in a 40°C water bath with nitrogen gas using a Zymark TurboVap to 1mL. The samples were then transferred to a GC vial and further evaporated with nitrogen gas to 200uL. Samples were then transferred to a new GC vial with a 250uL glass insert with polymer feet marked with a 100uL line. The samples were further evaporated with nitrogen gas to approximately 100uL and spiked with 100uL of recovery standards. The final sample extracts were stored in a -20°C freezer until instrumental analysis.

Instrumentation:

Breast milk samples were analyzed on an Agilent 7890B series gas chromatograph coupled with and Agilent 5977A mass spectrometer (GC/MS) with an Agilent 7693 autosampler (Agilent Santa Clara, CA, USA). Ultra-pure grade helium (99.995%: Airgas West El Cajon, CA, USA) and methane (99.999%: Airgas West El Cajon, CA, USA) were used as carrier gases. The Agilent 7693 Autosampler was used to inject 1-μL of the final extract of each sample in a splitless mode at 280°C onto an HP-5MS (30m x 250μm x .25μm thickness) capillary column (Agilent, Santa Clara, CA USA). The GC oven temperature program was the same for all compounds examined and was as follows: initially at 75°C for 2min, then ramped to 150°C at 20°C/min and held at 150°C for 2min, ramped to 260°C at 3°C/min, and ramped to 300°C at 15°C/min and held for 10 min at 300°C for a total run time of 57.083 min. The mass spectrometer was operated in electron capture negative ionization (ECNI) mode with an ion source temperature of 150°C and a single quadrupole temperature of 150°C. Selected ion monitoring (SIM) of the ions as described in the table below was used to detect seven PBDEs, seven MeOBDES, and 25 PCBs along with internal and recovery standards.

Instrumentation Quality Assurance and Quality Control

A hexane solvent blank was run prior to every sample run. A laboratory procedural blank spiked with internal and recovery standards was run with every sequence of 8 samples to determine blank levels of analytes of interest. Calibration standards were interspersed with samples during each run and quantitation methods were based on the specific set of calibration standards run with each individual sample. Three quality control criteria were used ensure the correct identification of the target analytes: GC retention times matched those of standard compounds within ± 0.4 minutes, the signal-to-noise ratio was greater than 5, and the ratio between quantification and confirmation ions was within ±25% of the theoretical value. The levels of analytes found in samples were corrected by the recovery efficiencies and the levels of the analytes found in the procedural blanks.

Appendix Table 1. Quantification and confirmation ions for analytes, internal, and recovery standards**.** Compounds in bold are internal standards and compounds underlined are recovery standards. The internal standards were used for quantification of the compounds listed below them.

Compound	Quantification Ion	Confirmation Ion
6FBDE47	78.9	80.9
BDE 77	78.9	80.9
BDE 166	78.9	80.9
BDE 28	78.9	80.9
BDE 47	78.9	80.9
<u>4FBDE69</u>	80.9	78.9
BDE 100	78.9	80.9
BDE 99	78.9	80.9
BDE 153	78.9	80.9
BDE 154	80.9	78.9

BDE 183	80.9	78.9

Compound	**Quantification Ion**	**Confirmation Ion**
6FBDE47	78.9	80.9
BDE 166	78.9	80.9
1,3,5-tribromo-2-(4-methoxyphenoxy)benzene	80.9	78.9
1,5-dibromo-3-(2,4-dibromophenoxy)-2-methoxybenzene	78.9	80.9
1,3,5-tribromo-2-(3-bromo-4-methoxyphenoxy)benzene	80.9	78.9
1,3,5-tribromo-2-(3,5-dibromo-4-methoxyphenoxy)benzene	78.9	80.9
6'-Methoxy-2,2',4,4',5-pentabromodiphenyl ether	78.9	80.9
3-Chloro-6-methoxy-2,2',4,4'-tetrabromodiphenyl ether	78.9	80.9
1,2,3,4-tetrabromo-5-(2,4-dibromophenoxy)-6-methoxybenzene	78.9	80.9

LASSO Quantile Regression Results Tables

Appendix Table 1. Lasso 0.5 quantile regression analysis, showing polychlorinated biphenyls (PCBs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 1_LASSO_50%_Qua

Appendix Table 2. Lasso 0.5 quantile regression analysis, showing polybrominated diphenyl ethers (PBDEs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 2_LASSO_50%_Qua

Appendix Table 3. Lasso 0.5 quantile regression analysis, showing organochlorine pesticides (OCPs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 3_LASSO_50%_Qua

Appendix Table 4. Lasso 0.9 quantile regression analysis, showing polychlorinated biphenyls (PCBs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 4_LASSO_90%_Qua

Appendix Table 5. Lasso 0.9 quantile regression analysis, showing polybrominated diphenyl ethers (PBDEs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 5_LASSO_90%_Qua

Appendix Table 6. Lasso 0.9 quantile regression analysis, showing organochlorine pesticides (OCPs) associated with seafood cooking methods, types of seafood consumed and maternal and infant characteristics.

Appendix_Table 6_LASSO_90%_Qua

Emory Institutional Review Board Approval

EMORY
UNIVERSITY

Institutional Review Board

October 12, 2017

RE: Determination: No IRB Review Required

Title: Levels of Persistent Organic Pollutants in the Breast Milk of Southern California Women as a Function of Diet and Environmental Exposures and the Potential Differential Accumulation of these Contaminants in Breast Milk Produced for Male versus Female Infants

PI: Catherine Rice

Dear Catherine Rice:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition of research with “human subjects” or “clinical investigation” as set forth in Emory policies and procedures and federal rules, if applicable. Specifically, in this project, you will collect de-identified information from San Diego State University. The goal is to determine if differences exist in the levels of persistent organic pollutants (POPs) in the breast milk of mothers in San Diego County produced for male versus female infants, and if such differences correlate with the dietary habits, environmental exposures and physiological and sociodemographic characteristics of the mother or infant.

Please note that this determination does not mean that you cannot publish the results. This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting

the IRB. Sincerely,

Brandy Covington

Brandy Covington
Research Protocol Analyst, Sr.

Maternal Questionnaire – Diet and Breast Milk

TODAY'S DATE: □□/□□/□□□□

mm / dd / yyyy

All of your answers are confidential and will be used solely for research purposes. For this study to succeed, it's very important for you to answer the questions as accurately as possible.

I: Background

1.1 What day was your baby born?

□□/□□/□□□□

mm / dd / yyyy

1.2 What is the sex of your baby?

□ Girl

□ Boy

1.3 What was your baby's length at birth?

□□.□□ inches

1.4 What was your baby's weight at birth?

□□ lbs

□□ ounces

Participant ID 51 ☐☐☐

DO NOT PUT YOUR NAME ON THIS FORM

1.5 What is your date of birth?

☐☐/☐☐/☐☐☐☐

mm / dd / yyyy

1.6 What is your height?

☐ft ☐inches

1.7 What was your weight at delivery?

☐☐☐lbs

1.8 Did you have significant weight gain during your pregnancy?

☐ Yes

☐ No

1.9 What was your weight before pregnancy?

☐☐☐lbs

1.10 How many times TOTAL have you given birth? __________

1.11 How many children TOTAL have you breastfed? __________

II. Diet

Participant ID 52 ☐☐☐

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The following questions are about the types of foods you ate during pregnancy and before becoming pregnant.

2.1 Are you a vegetarian? Vegetarian is defined as someone who abstains from eating red meat, poultry, or seafood.

How long? → ☐☐years / months

☐ Yes ☐ No

2.2 Are you a vegan? Vegan is defined as someone who abstains from eating any animal product.

How long? → ☐☐years / months

☐ Yes

☐ No

2.3 Did you eat seafood *BEFORE* your pregnancy?

☐ Yes

Participant ID 53

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☐ No

2.4 These questions are about your diet *BEFORE* your pregnancy.

	HOW OFTEN DID YOU EAT THESE FOODS?								AMOUNT?
	NEVER	Less than once per month	1 - 3 times per month	1 - 2 times per week	3 - 4 times per Week	5 - 6 times per Week	1 time per Day	2 or more times per Day	Please refer to serving size handout
Canned tuna, tuna salad, tuna sandwiches and tuna casserole	◻	◻	◻	◻	◻	◻	◻	◻	S M L ◻ ◻ ◻
Fried fish, fish sticks, fish sandwich and fried	◻	◻	◻	◻	◻	◻	◻	◻	S M L ◻ ◻ ◻

DO NOT PUT YOUR NAME ON THIS FORM

shellfish (shrimp and oysters)									
White Fish (broiled or baked) such as sole, halibut, snapper and cod	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Dark fish (broiled or baked) such as salmon, mackerel, and bluefish	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Smoked fish or seafood (such as smoked salmon, lox or others)	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Sushi	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Sushi containing raw fish or seafood (including shellfish)	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐

DO NOT PUT YOUR NAME ON THIS FORM

Raw oyster, raw clams, or other raw fish (not including raw fish in sushi)	□	□	□	□	□	□	□	□	**S M L** □ □ □
Squid or Octopus	□	□	□	□	□	□	□	□	**S M L** □ □ □
Lobster or Crab	□	□	□	□	□	□	□	□	**S M L** □ □ □
Other Seafood/Fish:	□	□	□	□	□	□	□	□	**S M L** □ □ □
	HOW OFTEN DID YOU EAT THESE FOODS? (*BEFORE*)								**AMOUNT?**
	NEVER	**Less than once per month**	**1 - 3 times per month**	**1 - 2 times per**	**3 - 4 times per**	**5 - 6 times per**	**1 time per**	**2 or more times**	**Please refer to serving size handout**

DO NOT PUT YOUR NAME ON THIS FORM

				we ek	We ek	We ek	Da y	per Day	
Red meat such as beef, pork, ham, and lamb	□	□	□	□	□	□	□	□	**S M L** □ □ □
Ground meat, including hamburgers and meatloaf	□	□	□	□	□	□	□	□	**S M L** □ □ □
Hot dogs and sausage such as bratwurst and chorizo	□	□	□	□	□	□	□	□	**S M L** □ □ □
Fried chicken, including nuggets and tenders	□	□	□	□	□	□	□	□	**S M L** □ □ □
Poultry such as chicken and turkey (roasted, stewed, grilled or broiled)	□	□	□	□	□	□	□	□	**S M L** □ □ □

DO NOT PUT YOUR NAME ON THIS FORM

Milk (all types) as a beverage (1 cup=8oz)	◻	◻	◻	◻	◻	◻	◻	◻	_____ **Cup(s)**

2.5 Did you eat seafood DURING your pregnancy?

☐ Yes

☐ No

2.6 These questions are about your diet *DURING* your pregnancy.

	HOW OFTEN DID YOU EAT THESE FOODS?								AMOUNT?
	NEVER	**Less than once per month**	**1 - 3 times per month**	**1 - 2 times per week**	**3 - 4 times per Week**	**5 - 6 times per Week**	**1 time per Day**	**2 or more times per Day**	**Please refer to serving size handout**

DO NOT PUT YOUR NAME ON THIS FORM

Canned tuna, tuna salad, tuna sandwiches and tuna casserole	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻
Fried fish, fish sticks, fish sandwich and fried shellfish (shrimp and oysters)	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻
White Fish (broiled or baked) such as sole, halibut, snapper and cod	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻
Dark fish (broiled or baked) such as salmon, mackerel, and bluefish	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻
Smoked fish or seafood (such as smoked salmon, lox or others)	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻

DO NOT PUT YOUR NAME ON THIS FORM

	HOW OFTEN DID YOU EAT THESE FOODS? (*DURING*)								AMOUNT?
	NEVER	Less than once per month	1 - 3 times per month	1 - 2 times per week	3 - 4 times per Week	5 - 6 times per Week	1 time per Day	2 or more times per Day	Please refer to serving size handout
Sushi	◻	◻	◻	◻	◻	◻	◻	◻	S M L ◻ ◻ ◻
Sushi containing raw fish or seafood (including shellfish)	◻	◻	◻	◻	◻	◻	◻	◻	S M L ◻ ◻ ◻
Raw oyster, raw clams, or other raw fish (not including raw fish in sushi)	◻	◻	◻	◻	◻	◻	◻	◻	S M L ◻ ◻ ◻
Squid or Octopus	◻	◻	◻	◻	◻	◻	◻	◻	S M L

DO NOT PUT YOUR NAME ON THIS FORM

									☐ ☐ ☐
Lobster or Crab	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Other Seafood/Fish:	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Red meat such as beef, pork, ham and lamb	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Ground meat, including hamburgers and meatloaf	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Hot dogs and sausage such as bratwurst and chorizo	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐
Fried chicken, including nuggets and tenders	☐	☐	☐	☐	☐	☐	☐	☐	**S M L** ☐ ☐ ☐

DO NOT PUT YOUR NAME ON THIS FORM

Poultry such as chicken and turkey (roasted, stewed, grilled or broiled)	◻	◻	◻	◻	◻	◻	◻	◻	**S M L** ◻ ◻ ◻
Milk (all types) as a beverage	◻	◻	◻	◻	◻	◻	◻	◻	_____ **Cup(s)**

2.7 What part of the fish did you typically eat?

DO NOT PUT YOUR NAME ON THIS FORM

	BEFORE your pregnancy	**DURING** your pregnancy
Entire Body	◻	◻
Meat/Fillet	◻	◻
Brain	◻	◻
Liver	◻	◻
Head	◻	◻
Heart	◻	◻
Skin	◻	◻
Other:________	◻	◻
I did not eat fish	◻	◻

2.8 How was the fish typically prepared? Please mark all that apply.

	BEFORE your pregnancy	**DURING** your pregnancy
Baked	◻	◻
Fried	◻	◻
Grilled	◻	◻

DO NOT PUT YOUR NAME ON THIS FORM

Sautéed	☐	☐
Steamed	☐	☐
Crockpot	☐	☐
Broiled	☐	☐
Other:________	☐	☐
I did not eat fish	☐	☐

2.9 Where did you get your fish?

	BEFORE your pregnancy	**DURING** your pregnancy
Store	☐	☐
Fishing	☐	☐
Restaurant	☐	☐
Unknown	☐	☐
Other:________	☐	☐
I did not eat fish	☐	☐

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☐Other: __________

2.10 If the fish was from the store was it ….

☐Wild and/or

☐Farm raised

☐Unknown

2.11 If the fish was from fishing was it ….

☐Ocean and/ or

☐Lake

☐Unknown

☐Other: __________

III. Supplements / Medication

The following questions are concerning supplements or medications taken during and before pregnancy.

3.1 Have you taken a prenatal vitamin?

☐ Yes → **What is the name of prenatal vitamin you took?**

☐ No

____

☐ Do not know ☐ Do not know

↓

DO NOT PUT YOUR NAME ON THIS FORM

3.2 How often did you take omega-3 supplement (that was separate from your prenatal vitamin)?

	BEFORE your pregnancy	**DURING** your pregnancy
Less than once per month	◻	◻
1-3 times per month	◻	◻
1-3 times per week	◻	◻
4-6 times per week	◻	◻
1 time per day	◻	◻
2 or more times per day	◻	◻
I did not take	◻	◻

3.3 What Type of omega-3 supplement did you take?

	BEFORE your pregnancy	**DURING** your pregnancy

DO NOT PUT YOUR NAME ON THIS FORM

Fish Oil	◻	◻
Flaxseed	◻	◻
Combined	◻	◻
Other: ________________	◻	◻
Do not know	◻	◻
I did not take	◻	◻

3.4 What form of omega-3 supplement did you take?

	BEFORE your pregnancy	**DURING** your pregnancy

DO NOT PUT YOUR NAME ON THIS FORM

Capsule	◻	◻
mg/capsule	◻	◻
teaspoon	◻	◻
tablespoon	◻	◻
Do not know	◻	◻
I did not take	◻	◻

3.5 Do you take any other supplements on a regular basis other than your prenatal vitamin or omega-3?

☐ Yes → **What is the name of the supplement?** ______________________

☐ No

☐ Don't know

3.6 Did you take any medications?

	BEFORE your pregnancy	**DURING** your pregnancy	**CURRENTLY**

DO NOT PUT YOUR NAME ON THIS FORM

High blood pressure	☐	☐	☐
Heart disease	☐	☐	☐
Asthma	☐	☐	☐
Arthritis	☐	☐	☐
Cancer	☐	☐	☐
Other: __________	☐	☐	☐
Do not know	☐	☐	☐
None	☐	☐	☐

IV: Smoking

The following questions are about your smoking status and exposure.

4.1 Have you ever smoked about 100 cigarettes in your lifetime?

☐ Yes → **Have you ever smoked daily for 6 months in your lifetime?**

☐ No

☐ Do not know

☐ Yes

☐ No

☐ Don't know

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When did you last smoke on a daily basis?

□□/□□/□□□□

mm / dd / yyyy □ Don't know

4.2 Do you currently smoke cigarettes every day, some days, or not at all?

□ Everyday

□ A few times per week

□ A few times per month

□ Never

4.3 These questions are about how often you are exposed to smoke or burn candles and/or incense.

HOW OFTEN HAVE YOU?

NEVER	Less than once	1 – 3 time	1 – 2 tim	3 – 4 tim	5 – 6 tim	1 time	2 or more

DO NOT PUT YOUR NAME ON THIS FORM

		per mon th	s per mon th	es per we ek	es per We ek	es per We ek	pe r Da y	time s per Day
been exposed to second hand smoke at home in the past year	☐	☐	☐	☐	☐	☐	☐	☐
been exposed to second hand smoke outside of your home in the past year	☐	☐	☐	☐	☐	☐	☐	☐
burned candles inside your home in the past year	☐	☐	☐	☐	☐	☐	☐	☐
burned incense inside your home in the past year	☐	☐	☐	☐	☐	☐	☐	☐

V: Miscellaneous

Participant ID 71

DO NOT PUT YOUR NAME ON THIS FORM

The following questions are about personal care products you may use and exposures you may have encountered before and during pregnancy.

5.1 These questions are about how often you have used certain personal care products.

HOW OFTEN HAVE YOU USED?

	NEVER	Do not know	A few times per month	A few times per week	Everyday
Antibacterial soap	☐	☐	☐	☐	☐
Perfume	☐	☐	☐	☐	☐
Sunscreen lotion or cream	☐	☐	☐	☐	☐
Makeup	☐	☐	☐	☐	☐

DO NOT PUT YOUR NAME ON THIS FORM

5.2 How often were pesticides for pest control used at your house?

	BEFORE your pregnancy	**DURING** your pregnancy
Weekly	◻	◻
Monthly-Quarterly	◻	◻
Rarely	◻	◻
Never	◻	◻
Do not know	◻	◻

5.3 What pesticide(s) was used?

☐Raid

☐Spectracide

☐Ortho Home Defense

☐Real Kill

☐ Other: ________

☐Don’t know

☐Did not use

5.4 How often were herbicides used in your yard at home?

DO NOT PUT YOUR NAME ON THIS FORM

	BEFORE your pregnancy	**DURING** your pregnancy
Weekly	◻	◻
Monthly-Quarterly	◻	◻
Rarely	◻	◻
Never	◻	◻
Do not know	◻	◻

5.5 What herbicide(s) was used?

☐Organocide
☐Daconil
☐Safer Brand
☐Round-up
☐IMAGE
☐Monterey
☐ Other: _________
☐Don't know
☐Did not use

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VI: Demographics

6.1 What is your ethnicity?

☐Latino or Hispanic

☐Not Latino or Hispanic

☐Decline to answer

6.1 Which of the following would you use to describe yourself?

☐Black or African American

☐White or Caucasian

☐ American Indian or Alaskan Native

☐Asian

☐Pacific Islander

☐Bi- or Multiracial (please describe):____________

☐Other (please describe):____________________

☐Decline to answer

6.2 What is your family income level?

☐< $25,000

☐ $25,000- $39,000

☐ $40,000 - $49,999

☐ $50,000 - $75,000

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☐> $75,000

☐ Don’t know

☐ Decline to answer

6.3 In what country were you born?

☐ United States

☐ Mexico

☐ Other:____________________

☐ Don't know

6.4 How many total years have you lived in the United States?

☐☐ Years [Round to whole; if **less than 1** yr. then months]

☐☐Months

6.5 How many total years have you lived in California?

☐☐ Years [Round to whole; if **less than 1** yr. then months]

☐☐Months

6.6 What languages can you speak?

6.7 Which language do you prefer to speak?

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6.8 When reading, which languages do you prefer?

6.9 What was the ethnic origin of the friends and peers you had, as a child from 6 to 18?

☐ Latino or Hispanic

☐ Black or African American

☐ White or Caucasian

☐ American Indian or Alaskan Native

☐ Asian

☐ Pacific Islander

☐ Bi- or Multiracial (please describe):____________

☐ Other (please describe):____________________

6.10 What is your marital status?

☐ Single

☐ Married

☐ Divorced

☐ Widowed

☐ Other

☐ Decline to answer

6.11 What is the highest level of education you have completed?

Participant ID 77 ☐☐☐

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☐ None

☐ 1st grade

☐ 2nd grade

☐ 3rd grade

☐ 4th grade

☐ 5th grade

☐ 6th grade

☐ 7th grade

☐ 8th grade

☐ 9th grade

☐ 10th grade

☐ 11th grade

☐ 12th grade

☐ GED

☐ High school graduate

☐ Trade, vocational training

☐ Some college

☐ 2-year Associate degree

☐ 4-year college degree

☐ Post-graduate study

☐ Graduate degree (Masters, Doctorate)

☐ Other: ______________________________

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☐ Decline to answer