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PAPER

A pilot study of pesticides and PCBs in the breast milk of women residing in urban and agricultural communities of California†‡

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Currently, there is no nationally representative human milk biomonitoring program in the United States (U.S.) and no studies have reported non-persistent pesticides in the milk of U.S. women. In this pilot study we developed a multiresidue laboratory method to measure non-persistent and persistent pesticides and polychlorinated biphenyl (PCB) congeners in human milk samples from women residing in the agricultural region of Salinas, CA (n = 13) and the urban San Francisco Bay Area, CA (n = 21). Samples were collected from 2002–2007. Median concentrations in pg g^{-1} milk among urban and agricultural women, respectively were reported for: chlorpyrifos (24.5 and 28.0), *cis*-permethrin (81.9 and 103), *trans*-permethrin (93.1 and 176), hexachlorobenzene (191 and 223), β-hexachlorocyclohexane (220 and 443), *o*,*p*′-DDT (36.6 and 62.4), *p*,*p*′-DDT,(107 and 102), *o*,*p*′-DDE (5.65 and 5.17), *p*,*p*′-DDE (3170 and 3490), dacthal (2.79 and 3.43), PCB 118 (92.8 and 17.0), PCB 138 (183 and 38.2), PCB 153 (242 and 43.6) and PCB 180 (239 and 683). Among urban women, median concentrations were 4.02 and 4.32 pg g^{-1} milk for chlorpyrifos-methyl and propoxur, respectively. These results suggest that neonates and young children may be exposed to persistent and non-persistent pesticides and PCBs *via* breast milk.

Introduction

The World Health Organization[1,2] and the American Academy of Pediatrics[3] recommend exclusive breastfeeding for the first six months of an infant's life due to physiological and psychological benefits to infants and mothers.[4–7] Although maternal milk is the optimal food for infants, it can contain chemicals that reflect maternal exposures.[8–11] Several pollutants such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) and its environmental degradate, dichlorodiphenyldichloroethylene (DDE), are known to persist in the environment, bioaccumulate in fat and to be excreted in human milk.[12]

Non-persistent, contemporary-use pesticides have rarely been studied in breast milk because they typically degrade rapidly in the environment and are metabolized and excreted within hours to days in the body.[13–15] However, some of these chemicals are relatively lipophilic, and are likely excreted in human milk.[16] Recently, some non-persistent pesticides such as the organophosphate (OP) pesticides, chlorpyrifos and malathion, have been detected in human milk[17,18] at ng g^{-1} concentrations (equivalent to parts per billion). These chemicals are of concern because they have been associated with neurodevelopmental effects in children and animals.[19–23]

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Environmental impact

Breast milk is the primary source of nutrition for the majority of infants in the United States (U.S.), yet, the U.S. currently has no breast milk biomonitoring program that specifically addresses non-legacy chemicals. This pilot study is the first to report concentrations of current use pesticides such as chlorpyrifos, chlorpyrifos-methyl, permethrin and propoxur detected in the breast milk of mothers residing the U.S. These results indicate the need for further breast milk biomonitoring studies as well as studies of the health effects of lactational transfer of pesticides. While there were measurable concentrations of chemicals in all mothers' milk samples, breastfeeding remains the optimal source of nutrition for infants.

The Food Quality Protection Act (FQPA) of 1996 requires the United States (U.S.) Environmental Protection Agency (EPA) to set pesticide tolerance levels in food that reflect the vulnerability of sensitive sub-populations, particularly pregnant women and children.[24] Yet, since there are no standardized human milk biomonitoring programs in the United States, we do not know the concentrations of chemicals to which breastfed infants are exposed.

We conducted a pilot study to measure concentrations of several non-persistent and persistent pesticides and PCBs in the milk of women residing in urban and agricultural regions. The analytes, including OP, organochlorine (OC), pyrethroid and carbamate pesticides and PCBs were measured simultaneously using a new, highly sensitive extraction and a single analysis procedure.[25] We also examined the variability of these chemicals over time to determine whether they are stable biomarkers of exposure to mothers and infants.

Methods

Study populations

Participants were recruited from two California communities; one was urban (San Francisco Bay Area) and the other was agricultural (Salinas Valley). These communities were chosen to reflect the possible range of chemicals in maternal milk. All protocols were reviewed and approved by the Committee for the Protection of Human Subjects at the University of California, Berkeley and the Institutional Review Board at the Centers for Disease Control and Prevention. Written, informed consent was obtained from participants at enrollment.

The urban population was a convenience sample which consisted of women who participated in a method development and validation study, performed jointly by researchers at the Centers for Disease Control and Prevention and the University of California, Berkeley.[25] Women (N = 22) were recruited from research facilities, doctors' offices and offices for a food and nutrition program for women, infants and children (WIC). An advertisement was also placed in an electronic newsletter for parents. At the time of screening and enrollment, participants resided within 20 miles of the urban community of Berkeley, CA; were 18 or more years of age; spoke English or Spanish; and did not live near any agricultural fields. No demographic or exposure questionnaire data were collected from the urban population, but some demographic information was collected by observation and census characteristics of the neighborhood they lived in. Milk samples were collected between January 2002 and May 2004.

Women residing in the agricultural region of Salinas, CA (n = 16) were participants of a peripartum pesticide exposure study conducted in the summer of 2007 through the spring of 2008. Women were eligible to participate in this study if they were pregnant (between 24 and 34 weeks gestation), age 18 years or older, planning to deliver by Cesarean at Natividad Medical Center, eligible for poverty-based health care services, English- or Spanish-speaking, with no previous health conditions or high risk pregnancies. The Cesarean delivery criterion facilitated scheduling of our staff and collaborating physicians to attend the delivery and collect several biological samples including umbilical cord blood. A detailed demographic and exposure questionnaire was also administered to the 16 women who were enrolled in this study at approximately two weeks to four days prior to their scheduled Cesarean deliveries.

Sample collection and laboratory analyses

Urban women provided freshly pumped or previously frozen milk that they felt their infants could spare. Participants used an electric or manual pump and expressed milk samples into sample containers at home. Ten of the 22 urban women provided multiple samples resulting in a total of 121 individual samples. Of these 121 samples, 43 samples from 21 women (1 women provided insufficient volume) were selected for analysis.

Thirteen agricultural women provided one milk sample each. These samples were collected in their homes one to two weeks after delivery (mean (SD) = 9 (2) days postpartum). These women were asked to wash their hands with soap and water and to remove creams from breasts using warm water prior to sample collection. Either or both breasts were used as source of milk samples and all participants used a breast pump (Basic Nurture III, Bailey Medical Engineering, Los Osos, CA).

All freshly pumped samples from either population were collected directly into the collection bottle (from individually sealed sterile kits made for the pump). Samples were then sealed, transported in a cooler on ice packs to the laboratory and immediately transferred to glass vials with Teflon-lined tops (2 aliquots). Previously frozen samples from the urban population were transported from participants' homes on dry ice. Samples were thawed slowly on ice, mixed vigorously and aliquoted into at least two separate vials (~10–20 ml each). All final aliquots were stored frozen in the laboratory at −80 °C.

Samples were then shipped on dry ice to the Centers for Disease Control and Prevention, National Center for Environmental Health, Pesticide Laboratory in Atlanta, GA for analysis using a newly developed and validated method which employed isotope dilution (See Electronic Supplementary Information for method details).[25] Briefly, one gram of each sample was weighed and dispersed over hydromatrix. Accelerated solvent extraction with dichloromethane and hexane (80 : 20, v:v) was used to extract the analytes. The resulting eluate was concentrated to ~20 μL, then 500 μL of acetonitrile was added. Matrix interferents including sugars and fatty acids were removed using solid phase extraction cartridges packed with neutral alumina and primary and secondary amine sorbent (PSA). Analytes were eluted with acetonitrile. The eluate was again concentrated to 20 μL, then 20 μL of toluene were added and the remaining acetonitrile was allowed to evaporate. Samples were analyzed using gas chromatography/high resolution mass spectrometry. Concentrations of *p*,*p*′-DDE in some samples exceeded upper instrument detection limits; thus, these samples were diluted by a factor of 10 with toluene and re-analyzed. The resulting measured concentration was then multiplied by the dilution factor to obtain the actual concentration. All concentrations were reported in picograms/gram milk (pg g^{-1}). Individual sample limits of detection (LODs) were reported for each chemical. Amount of lipid per gram of milk was determined gravimetrically from a separate aliquot. Quality control procedures included the use of blanks and duplicate samples. The complete list of 24 analytes measured by this method is shown in Table 1 by chemical class.

Table 1 Detection frequencies and sample-specific limit of detection summary statistics (in pg g^{-1} milk) by chemical class for all chemicals measured in the milk of women residing in urban and agricultural communities of California

	Urban, N = 21			Agricultural, N = 13		
	DF[a] (%)	LOD mean ± SD	LOD Range	DF (%)	LOD mean ± SD	LOD Range
Non-persistent						
Organophosphates						
Chlorpyrifos[b]	100	$2.56 \times 10^{-1} \pm 1.22 \times 10^{-1}$	1.01×10^{-1}–5.13×10^{-1}	100	$1.51 \times 10^{-1} \pm 5.61 \times 10^{-2}$	7.00×10^{-2}–2.90×10^{-1}
Chlorpyrifos-methyl[b]	67	$2.11 \times 10^{0} \pm 1.81 \times 10^{0}$	1.83×10^{-1}–7.12×10^{0}	23	$5.09 \times 10^{-1} \pm 4.43 \times 10^{-1}$	5.00×10^{-2}–1.43×10^{0}
Fonofos	38	$4.35 \times 10^{-1} \pm 1.28 \times 10^{-1}$	2.39×10^{-1}–6.17×10^{-1}	0	$1.36 \times 10^{0} \pm 7.08 \times 10^{-1}$	6.60×10^{-1}–3.04×10^{0}
Disulfoton	10	$1.88 \times 10^{0} \pm 2.07 \times 10^{0}$	1.66×10^{-1}–7.02×10^{0}	0	$1.55 \times 10^{-1} \pm 1.20 \times 10^{-1}$	6.00×10^{-2}–4.50×10^{-1}
Diazinon	5	$3.22 \times 10^{0} \pm 1.21 \times 10^{0}$	1.44×10^{0}–5.44×10^{0}	0	$6.28 \times 10^{0} \pm 2.05 \times 10^{0}$	3.16×10^{0}–9.81×10^{0}
Pyrethroids						
cis-Permethrin[b]	100	$5.00 \times 10^{-1} \pm 2.26 \times 10^{-1}$	2.44×10^{-1}–1.02×10^{0}	100	$5.23 \times 10^{-1} \pm 1.30 \times 10^{-1}$	2.50×10^{-1}–7.20×10^{-1}
trans-Permethrin[b]	100	$5.74 \times 10^{-1} \pm 2.55 \times 10^{-1}$	2.86×10^{-1}–1.20×10^{0}	100	$6.72 \times 10^{-1} \pm 1.83 \times 10^{-1}$	2.90×10^{-1}–9.40×10^{-1}
Cyfluthrin	5	$1.43 \times 10^{1} \pm 1.03 \times 10^{1}$	3.71×10^{0}–3.96×10^{1}	38	$1.21 \times 10^{1} \pm 1.00 \times 10^{1}$	9.80×10^{-1}–2.69×10^{1}
Cypermethrin	5	$4.99 \times 10^{0} \pm 3.01 \times 10^{0}$	2.35×10^{0}–1.38×10^{1}	31	$1.21 \times 10^{1} \pm 4.34 \times 10^{0}$	4.56×10^{0}–1.85×10^{1}
Deltamethrin	0	$5.00 \times 10^{-1} \pm 3.10 \times 10^{-1}$	2.24×10^{-1}–1.42×10^{0}	23	$6.49 \times 10^{0} \pm 8.07 \times 10^{0}$	3.60×10^{-1}–2.36×10^{1}
Other						
Propoxur[b]	67	$9.55 \times 10^{-1} \pm 3.32 \times 10^{-1}$	3.61×10^{-1}–1.58×10^{0}	8	$1.65 \times 10^{0} \pm 3.14 \times 10^{-1}$	1.08×10^{0}–2.33×10^{0}
Bendiocarb	19	$7.16 \times 10^{-1} \pm 3.05 \times 10^{-1}$	3.91×10^{-1}–1.45×10^{0}	0	$5.10 \times 10^{-1} \pm 1.07 \times 10^{-1}$	3.80×10^{-1}–7.50×10^{-1}
Atrazine	43	$3.10 \times 10^{0} \pm 1.15 \times 10^{0}$	1.56×10^{0}–5.65×10^{0}	23	$3.91 \times 10^{0} \pm 1.94 \times 10^{0}$	1.79×10^{0}–8.26×10^{0}
Persistent						
Organochlorines						
Hexachlorobenzene[b]	100	$1.15 \times 10^{-1} \pm 9.24 \times 10^{-2}$	2.33×10^{-2}–3.64×10^{-1}	100	$4.46 \times 10^{-2} \pm 2.63 \times 10^{-2}$	2.00×10^{-2}–9.00×10^{-2}
p,p′-DDE[b]	100	$7.83 \times 10^{-2} \pm 4.26 \times 10^{-2}$	3.44×10^{-2}–1.63×10^{-1}	100	$3.65 \times 10^{-2} \pm 1.70 \times 10^{-2}$	2.00×10^{-2}–7.00×10-^{2}
o,p′-DDE[b]	100	$5.61 \times 10^{-2} \pm 2.75 \times 10^{-2}$	2.61×10^{-2}–1.16×10^{-1}	100	$3.04 \times 10^{-2} \pm 1.51 \times 10^{-2}$	1.00×10^{-2}–6.00×10^{-2}
β-hexachlorocyclohexane[b]	100	$6.91 \times 10^{-1} \pm 3.03 \times 10^{-1}$	3.47×10^{-1}–1.39×10^{0}	92	$1.04 \times 10^{0} \pm 6.91 \times 10^{-1}$	3.80×10^{-1}–2.49×10^{0}
p,p′-DDT[b]	95	$8.29 \times 10^{-1} \pm 3.47 \times 10^{-1}$	3.29×10^{-1}–1.47×10^{0}	100	$5.38 \times 10^{-1} \pm 1.90 \times 10^{-1}$	2.00×10^{-1}–9.00×10^{-1}
o,p′-DDT[b]	90	$5.13 \times 10^{-1} \pm 1.92 \times 10^{-1}$	2.44×10^{-1}–9.05×10^{-1}	100	$3.50 \times 10^{-1} \pm 1.17 \times 10^{-1}$	1.50×10^{-1}–5.50×10^{-1}
Dacthal[b]	100	$4.94 \times 10^{-2} \pm 2.23 \times 10^{-2}$	2.62×10^{-2}–1.15×10^{-1}	100	$2.46 \times 10^{-2} \pm 8.77 \times 10^{-3}$	1.00×10^{-2}–4.00×10^{-2}
Polychlorinated Biphenyls						
PCB 118[b]	100	$2.35 \times 10^{-1} \pm 9.16 \times 10^{-2}$	1.28×10^{-1}–4.32×10^{-1}	100	$2.92 \times 10^{-1} \pm 8.20 \times 10^{-2}$	1.60×10^{-1}–4.60×10^{-1}
PCB 138[b]	100	$4.78 \times 10^{-1} \pm 2.83 \times 10^{-1}$	1.96×10^{-1}–1.06×10^{0}	92	$3.08 \times 10^{-1} \pm 1.80 \times 10^{-1}$	1.10×10^{-1}–7.20×10^{-1}
PCB 153[b]	100	$4.16 \times 10^{-1} \pm 2.40 \times 10^{-1}$	1.89×10^{-1}–9.68×10^{-1}	100	$2.88 \times 10^{-1} \pm 1.76 \times 10^{-1}$	1.00×10^{-1}–6.90×10^{-1}
PCB 180[b,c]	81	$7.85 \times 10^{1} \pm 4.97 \times 10^{1}$	2.03×10^{1}–1.73×10^{2}	62	$3.35 \times 10^{2} \pm 9.97 \times 10^{1}$	1.85×10^{2}–4.91×10^{2}

[a] For urban women with multiple samples, analytes were considered detected if any of the woman's samples was >LOD. [b] Analytes with >50% detection frequency in one or both locations. [c] LODs for PCB 180 are high due to method limitations and interference with other analytes.

Statistical methods

All statistical analyses were performed using Stata 10 for Windows.[26] Although we could not collect detailed demographic information for the urban women, we estimated demographics using participants' addresses collected during screening along with the 2000 United States census data searched at the block group level for median household income and education and the block level for race and ethnicity information.[27] For agricultural women, we summarized maternal age, ethnicity, marital status, education, household income, years residing in the United States, and agricultural work during pregnancy based on data collected during a detailed maternal interview.

Detection frequencies of each analyte were calculated separately for each population. Since some urban women contributed multiple samples, a chemical was considered detected in a woman if the concentration of that chemical exceeded its LOD in any of that woman's samples.

For analytes with at least 50% detection frequency, concentrations that were below the LOD were imputed as the individual sample's LOD/$\sqrt{2}$ and summary statistics (minimum, 25th, 50th and 75th percentiles, and maximum concentrations) were calculated for each population and reported in pg g^{-1} milk.[28] We did not adjust concentrations by the lipid fraction in the milk because it is not clear whether lipid-adjustment is appropriate for all chemicals given their varying lipophilicities and the lack of information on the mechanisms by which these chemicals are transported to the milk.[29] Lipid-adjusted concentrations can be calculated by dividing reported concentrations by the average lipid concentrations measured in the same samples (0.028 g fat/g milk for urban women and by 0.013 g fat/g milk for agricultural women). For urban women, concentrations (in pg g^{-1} milk) for individuals with multiple measurements were averaged before calculating summary statistics or performing statistical tests. Wilcoxon rank sum tests were then performed to determine whether chemical concentrations differed by urban or agricultural location. Because of the small sample sizes, a Monte Carlo permutation test (10,000 repetitions) was performed to determine the exact significance level ($p_{permutation}$) and the 95% confidence interval around the p-value for the Wilcoxon rank sum test. If the exact significance level was below 0.05 and the confidence interval around it did not contain 0.05, we rejected the null hypothesis that the distributions of the chemical concentrations were the same by location.

Examination of variation in concentrations over time

Many women in the urban population provided more than one sample, which allowed us to determine whether measurements of

these chemicals in human milk were stable biomarkers of exposure over time by estimating within-woman and between-women variance. Chemical concentrations (in both pg g^{-1} milk and ng g^{-1} lipid) were log-transformed and a simple random effects model ($Y_{ij} = \mu + \alpha_i + e_{ij}$, where Y_{ij} is the *jth* observation in the *ith* group and μ is an unobserved overall mean) was used to determine standard deviations (SDs) of the between-women (α_i) and within-woman (e_{ij}) terms. The intraclass correlation coefficient (ICC), a measure of the reliability and variability of multiple measurements within the same person over time, was then calculated as follows: $ICC = (SD[\alpha_i])^2/((SD[\alpha_i])^2 + (SD[e_{ij}])^2)$. The ICC ranges from 0 to 1 and we considered an ICC > 0.75 to be a stable biomarker because of the smaller variation within a woman over time relative to between women.[30]

We selected *p,p′*-DDE, chlorpyrifos and *trans*-permethrin as representative persistent and non-persistent chemicals to illustrate patterns of excretion over time and whether patterns differed by persistence. We plotted concentrations (in pg g^{-1} milk) separately for each of these chemicals and for each woman by day of sample collection marking the sample that was collected and analyzed earliest as sample 1 and determining the days between subsequent samples and sample 1 (N = 6). For these figures, the time element describes the time between collections of each sample. Note that we were unable to record collection dates for one of three samples from woman 6 and all samples from women 7, 8 and 9; therefore, data from these women were not in the larger figures plotted over time (Fig. 1).

Results

Demographics

Urban women were predominantly Caucasian (white), approximately 30–40 years of age, and of middle- to-high income with professional careers. The observed data on race and income were supported by the census 2000 data which showed that, in the census blocks from which our women resided, 69% were white, 8% were black or African American, and 17% were Asian; 7% reported being of Hispanic ethnicity. Census block group data for the communities in which these women resided showed that on average, median household income was approximately $97,000 per year with only 7% below poverty and greater than 57% completed college or professional school. We have no parity data for urban women. Conversely, agricultural women were 22–39 years of age, born in Mexico (100%), had no education beyond primary school, and had a median household income less than $24,000 per year. Nearly half of the agricultural women had lived in the U.S. for five or fewer years and 54% worked in agriculture during pregnancy. All agricultural women were multiparous and had breastfed a mean (SD) of 2 (1) previously born children.

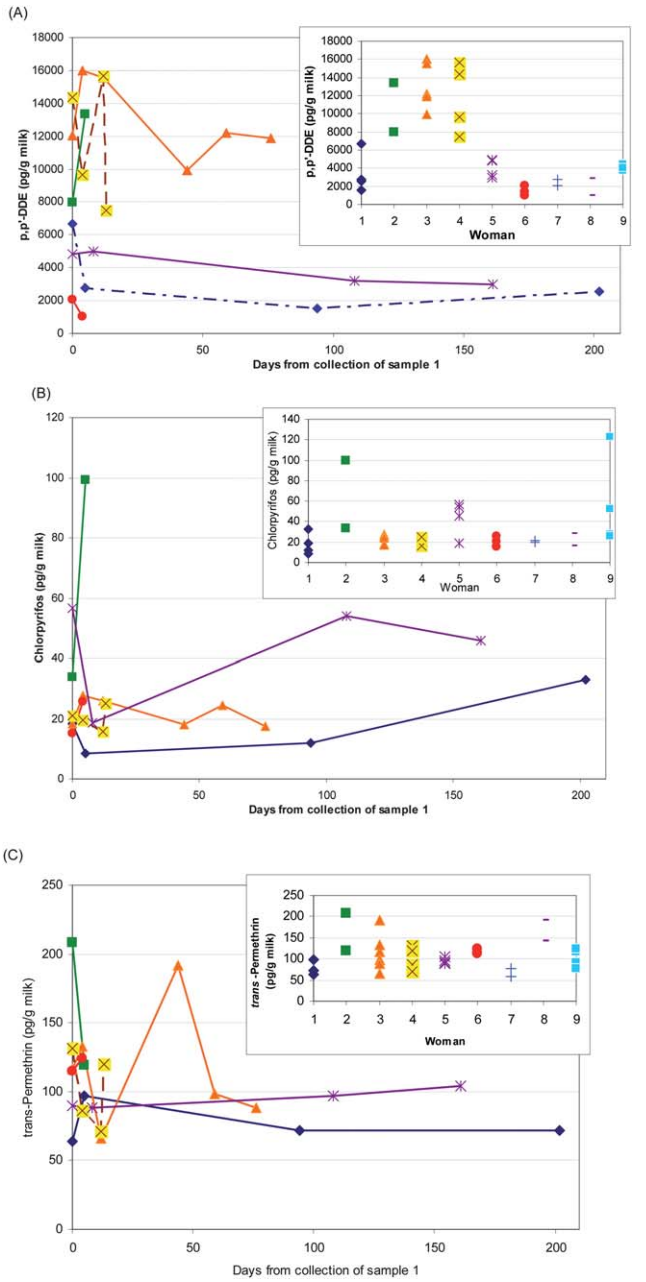

Fig. 1 Changes over time of concentrations (pg g^{-1} milk) of (A) *p,p′*-DDE, (B) Chlorpyrifos, and (C) *trans*-Permethrin in milk from six urban women who provided sample collection dates. Symbols are specific to an individual woman. The inset demonstrates within-woman and between-women variability and includes concentrations in breast milk from all women with multiple samples analyzed (N = 9) regardless of whether collection dates were provided.

Detection and chemical concentrations

As shown in Table 1, detection frequencies were ≥90% in both locations for thirteen of the 24 chemicals analyzed including the non-persistent chemicals: chlorpyrifos, *cis*- and *trans*-permethrin, as well as the persistent compounds: hexachlorobenzene, all four DDT/DDE isomers, β-hexachlorocyclohexane, dacthal, and PCBs 118, 138, and 153. Detection frequencies were between 50 and 90% in at least one location for chlorpyrifos-methyl (urban), propoxur (urban), and PCB 180 (urban and agricultural). Ten of the chemicals had detection frequencies that were <50% in both locations including: fonofos, disulfoton, diazinon, cyfluthrin, cypermethrin, deltamethrin, bendiocarb, and atrazine. Mean LODs ranged from 0.02–5 pg g^{-1} milk for most chemicals; however, diazinon, cyfluthrin, cypermethrin, deltamethrin, and PCB 180 had higher mean LODs.

Summary statistics of concentrations of chemicals with >50% detection frequency and comparisons by urban/agricultural location are shown in Table 2. Median concentrations of chlorpyrifos were 25 and 28 pg g^{-1} milk in urban and agricultural women, respectively. Among urban women, the median concentration of chlorpyrifos-methyl was 4 pg g^{-1} milk. Median concentrations (in pg g^{-1} milk) of *cis*-permethrin were 82 and 103 and of *trans*-permetrin were 93 and 176 for urban and agricultural women, respectively. Generally, the concentration of *trans*-permethrin was higher in a given sample than *cis*-permethrin. Additionally, the distributions of *cis*- and *trans*-permethrin were significantly higher in agricultural women than urban women. Concentrations of propoxur among urban women ranged from <LOD to 127 pg g^{-1} milk; the median value was 4.3 pg g^{-1} milk.

In contrast to the non-persistent pesticides, most persistent OCs were detected in milk samples from women in both urban and agricultural communities. Median concentrations of hexachlorobenzene were 191 and 223 pg g^{-1} milk for urban and agricultural women, respectively. The range of β-hexachlorocyclohexane was 45–1406 pg g^{-1} milk with a median value of 220 pg g^{-1} milk among urban women. The median value of β-hexachlorocyclohexane among agricultural women (443 pg g^{-1} milk) was twice that of urban women and the range was broader (<LOD–2438 pg g^{-1} milk). Median concentrations of the four DDT/DDE isomers measured (in pg g^{-1} milk) were 5.7 for *o*,*p*′-DDE, 37 for *o*,*p*′-DDT, 107 for *p*,*p*′-DDT and 3171 for *p*,*p*′-DDE in urban women. DDT/DDE median concentrations (in pg g^{-1} milk) among agricultural women were 5.2 for *o*,*p*′-DDE, 62 for *o*,*p*′-DDT, 102 for *p*,*p*′-DDT and 3488 for *p*,*p*′-DDE. Lastly, although dacthal was detected in all of the urban and agricultural samples, concentrations were low, ranging from 0.9 to 5.6 with a median value of 2.8 pg g^{-1} milk in urban women and ranging from 2.6 to 7.4 with a median value of 3.4 pg g^{-1} milk in agricultural women. Concentrations of persistent OCs were generally higher in agricultural women than urban women, but the differences were not statistically significant.

Table 2 Summary statistics and comparisons[a] of chemical concentrations (in pg g^{-1} milk)[b] measured with greater than 50% detection frequency[c] in the milk of women residing in urban and agricultural regions of California

	Urban (N = 21)						Agricultural (N = 13)						
	Mean (SD)	min	p25	p50	p75	max	Mean (SD)	min	p25	p50	p75	max	$p_{permuted}$
Organophosphates													
Chlorpyrifos	4.05×10^1 (4.59×10^1)	1.29×10^1	2.05×10^1	2.45×10^1	3.96×10^1	$2.2.30 \times 10^2$	1.39×10^2 (2.88×10^2)	1.28×10^1	2.38×10^1	2.80×10^1	1.38×10^2	1.07×10^3	0.417
Chlorpyrifos-methyl	7.24×10^0 (8.00×10^0)	8.92×10^{-1} [d]	2.39×10^0	4.02×10^0	8.55×10^0	3.36×10^1	– –	–	–	–	–	–	–
Pyrethroids													
cis-Permethrin	1.06×10^2 (1.35×10^2)	3.68×10^1	4.97×10^1	8.19×10^1	1.03×10^2	6.82×10^2	1.28×10^2 (9.15×10^1)	4.92×10^1	9.65×10^1	1.03×10^2	1.33×10^2	4.09×10^2	0.041[e]
trans-Permethrin	1.10×10^2 (8.11×10^1)	5.19×10^1	6.81×10^1	9.31×10^1	1.17×10^2	4.35×10^2	2.92×10^2 (3.53×10^2)	7.77×10^1	1.45×10^2	1.76×10^2	3.10×10^2	1.43×10^3	0.002[e]
Other													
Propoxur	1.49×10^1 (2.81×10^1)	5.86×10^{-1}[d]	1.05×10^0	4.32×10^0	1.09×10^1	1.27×10^2	– –	–	–	–	–	–	–
Organochlorines													
Hexachlorobenzene	2.64×10^2 ($2.14 \times 10^{2)}$	5.33×10^1	1.60×10^2	1.91×10^2	2.84×10^2	9.21×10^2	2.31×10^2 (1.20×10^2)	6.36×10^1	1.48×10^2	2.23×10^2	3.08×10^2	5.14×10^2	0.891
p,*p*′-DDE	4.54×10^3 (4.23×10^3)	4.55×10^2	1.90×10^3	3.17×10^3	3.99×10^3	1.52×10^4	1.77×10^4 (2.95×10^4)	6.72×10^2	3.07×10^3	3.49×10^3	1.35×10^4	1.04×10^5	0.189
o,*p*′-DDE	6.64×10^0 (4.27×10^0)	2.13×10^0	4.83×10^0	5.65×10^0	6.62×10^0	2.26×10^1	7.72×10^0 (5.46×10^0)	3.07×10^0	4.76×10^0	5.17×10^0	6.60×10^0	2.03×10^1	0.975
β-hexachlorocyclohexane	3.12×10^2 (3.31×10^2)	4.47×10^1	1.32×10^2	2.20×10^2	2.42×10^2	1.41×10^3	5.52×10^2 (6.11×10^2)	3.46×10^{-1}[d]	3.77×10^2	4.43×10^2	5.20×10^2	2.44×10^3	0.105
p,*p*′-DDT	1.24×10^2 (8.38×10^1)	7.85×10^{-1}[d]	6.95×10^1	1.07×10^2	1.55×10^2	3.62×10^2	3.78×10^2 (4.79×10^2)	5.69×10^1	8.92×10^1	1.02×10^2	4.05×10^2	1.65×10^3	0.183
o,*p*′-DDT	5.72×10^1 (6.04×10^1)	5.24×10^{-1}[d]	2.42×10^1	3.66×10^1	6.29×10^1	2.73×10^2	1.46×10^2 (1.99×10^2)	1.75×10^1	3.61×10^1	6.24×10^1	1.38×10^2	7.36×10^2	0.127
Dacthal	3.10×10^0 (1.13×10^0)	8.83×10^{-1}	2.38×10^0	2.79×10^0	3.52×10^0	5.55×10^0	3.81×10^0 (1.39×10^0)	2.55×10^0	2.88×10^0	3.43×10^0	4.81×10^0	7.42×10^0	0.125
Polychlorinated Biphenyls													
PCB 118	1.45×10^2 (1.47×10^2)	1.28×10^1	5.35×10^1	9.28×10^1	1.33×10^2	5.18×10^2	2.88×10^1 (2.19×10^1)	8.80×10^0	1.56×10^1	1.70×10^1	4.00×10^1	8.36×10^1	<0.0005[e]
PCB 138	2.91×10^2 (2.89×10^2)	4.03×10^1	1.54×10^2	1.83×10^2	4.15×10^2	1.25×10^3	5.39×10^1 (5.90×10^1)	1.41×10^{-1} [d]	2.62×10^1	3.82×10^1	5.53×10^1	2.39×10^2	<0.0005[e]
PCB 153	3.78×10^2 (3.87×10^2)	2.81×10^1	1.72×10^2	2.42×10^2	5.00×10^2	1.64×10^3	6.17×10^1 (6.68×10^1)	1.11×10^1	3.50×10^1	4.36×10^1	6.14×10^1	2.77×10^2	<0.0005[e]
PCB 180[f]	3.62×10^2 (4.10×10^2)	1.98×10^1 [d]	1.15×10^2	2.39×10^2	4.31×10^2	1.58×10^3	7.41×10^2 (5.28×10^2)	1.31×10^{2d}	2.70×10^2 [d]	6.83×10^2	1.11×10^3	1.74×10^3	0.022[e]

[a] Ranksum test permuted with 10,000 repetitions. [b] Values are LOD-imputed, but not adjusted for lipid content. To estimate lipid-adjusted values use a conversion factor of 0.028 g fat/g milk for Urban women and 0.013 g fat/g milk for Agricultural women. [c] Summary statistics not reported for chlorpyrifos-methyl and propoxur among agricultural women due to low detection. [d] Concentrations reported are <LOD. [e] 95% confidence interval around the permuted p-value does not contain 0.05. [f] Due to methodological issues, concentrations for PCB 180 may be less reliable.

Concentrations of three PCB congeners were significantly higher in urban women compared to agricultural women. Among urban women, median concentrations of PCBs 118, 138, and 153 were 93, 183, and 242 pg g^{-1} milk, respectively; whereas for agricultural women, median concentrations were 17, 38, and 44 pg g^{-1} milk, respectively. PCB 180 was statistically significantly lower in urban women than agricultural women with median concentrations of 239 and 683 pg g^{-1} milk, respectively.

Variability of chemical concentrations for urban women

In Table 3 we show the within- and between-women variability as well as ICCs for selected chemical concentrations measured in the milk of urban women. With the exception of dacthal and PCB 180 (ICCs of 0.531 and 0.458, respectively) we found that ICCs were highest (ranging from 0.802 to 0.940) for persistent OCs and PCBs, confirming the stability of these chemicals within women. ICCs were lower for chlorpyrifos, chlorpyrifos-methyl and propoxur (ICC = 0.537, 0.325 and 0.644, respectively). Although *cis*- and *trans*-permethrin are considered non-persistent pesticides, concentrations appeared to be almost as stable within a woman as the persistent OCs and PCBs with ICCs of 0.796 and 0.738, respectively.

Illustrations of within-woman and between-women variability and trends of concentrations over time are shown for *p*,*p*′-DDE (Fig. 1A), chlorpyrifos (Fig. 1B) and *trans*-permethrin (Fig. 1C) for each woman. Overall, most women's concentrations of *p*,*p*′-DDE generally decreased over time (Fig. 1A), although some women's concentrations increased at some points, consistent with patterns observed by Lakind *et al.*[31] Non-lipid-adjusted and lipid-adjusted random effects models clustered by woman showed statistically significant decreases in concentrations of *p*,*p*′-DDE (log_{10}-transformed) by −0.0013 pg g^{-1} milk per day (95% CI = −0.003, −0.00009) and −0.0010 ng g^{-1} lipid per day (95% CI = −0.002, −0.00002). The Fig. 1A inset shows that for many women, concentrations are tightly clustered, while some women have high variability. Within women, concentrations of *p*,*p*′-DDE ranged 641–8200 pg g^{-1} milk and the ratios of the highest to lowest concentrations within a woman ranged 1.2–4.3 with a mean (SD) ratio of 2.1 (1.0).

In contrast to the negative trend observed for *p*,*p*′-DDE, Fig. 1B displays no clear trend in concentrations of chlorpyrifos among women over time. Concentrations in some women increased over time while in others concentrations decreased. The coefficient on our time variable from the random effects model of log_{10}-transformed chlorpyrifos concentrations was not statistically significant whether we used lipid-adjusted or unadjusted values (β [95% CI] = 0.0008 ng g^{-1} lipid per day [–0.0008, 0.002]; 0.001 pg g^{-1} milk per day [–0.0004, 0.003]), supporting our observations in Fig. 1B. The Fig. 1B inset shows that many women's repeat samples cluster, but the difference between the maximum and minimum concentration per woman can vary greatly (range of difference = 1.2–96.8 pg g^{-1} milk). In addition, the ratios between the highest and lowest concentrations measured within a woman ranged 1.1–4.7 with a mean (SD) ratio of 2.5 (1.2), which is somewhat higher than the mean ratios observed for *p*,*p*′-DDE.

In Fig. 1C, we observed generally smaller between-women and within-woman variation for *trans*-permethrin compared to *p*,*p*′-DDE or chlorpyrifos (note the scale difference between Fig. 1C *versus* Fig. 1A–1B). We observed no clear trend over time, as was confirmed by random effects models using either non-lipid-adjusted or lipid-adjusted concentrations (β [95% CI] = −0.00004 pg g^{-1} milk per day [−0.001, 0.001]; −0.0001 ng g^{-1} lipid per day [−0.001, 0.001]). The range of differences between the maximum and minimum concentrations per woman was 12–126 pg g^{-1} milk and the range of ratios of maximum and minimum concentrations per woman was 1.1–2.9 with a mean (SD) ratio of 1.6 (0.5).

Table 3 Within- and between-woman variability and intraclass correlation (ICC) of log_{10}-transformed concentrations (in pg g^{-1} milk) of chemicals[a] with greater than 50% detection frequency measured in milk of urban women[b]

	$SD_{between}$	SD_{within}	ICC
Chlorpyrifos	0.215	0.200	0.537
Chlorpyrifos-methyl[c]	0.365	0.527	0.325
cis-permethrin	0.247	0.125	0.796
trans-permethrin	0.185	0.110	0.738
Propoxur	0.605	0.450	0.644
o,*p*′-DDE	0.201	0.097	0.812
p,*p*′-DDE	0.361	0.156	0.842
Hexachlorobenzene	0.291	0.091	0.912
Dacthal	0.137	0.129	0.531
p,*p*′-DDT	0.518	0.131	0.940
β-hexachlorocyclohexane	0.365	0.115	0.910
o,*p*′-DDT	0.611	0.304	0.802
PCB 118	0.370	0.152	0.855
PCB 138	0.330	0.164	0.803
PCB 153	0.358	0.161	0.831
PCB 180	0.404	0.439	0.458

[a] Concentrations are log-transformed and LOD-imputed, but not lipid-adjusted. [b] N = 43 samples from 21 women; there was an average of 2 samples per woman with a range of 1–6 samples per woman. [c] Random effects model did not provide $SD_{between}$; therefore, $SD_{between}$ was estimated using concentrations averaged per woman and ICC was calculated manually as:$SD_{between}/(SD_{between}\ SD_{within})$.

Discussion

Measurements of chemicals in breast milk provide a biomarker of maternal exposure and dietary exposure to breastfeeding infants during critical developmental periods.[32] In this pilot study we detected non-persistent pesticides that are not typically measured in human milk as well as persistent organic pollutants. We also found that concentrations of persistent pesticides and PCBs were more stable over time than most non-persistent pesticides except for permethrin.

POPs, including DDT/DDE, remain important chemicals in biomonitoring studies. In comparison to demographically similar historic populations, we found that median concentrations of *p*,*p*′-DDE in our urban women (3170 pg g^{-1} milk) were much lower than concentrations in milk collected from Northern California women in ~1993 (~6000 pg g^{-1} milk, estimated)[33] and the agricultural women in this study (3490 pg g^{-1} milk) had much lower median concentrations than milk from a migrant Mexican cohort recruited from North Carolina in 1998 (~30,000 pg g^{-1} milk, estimated).[33] Worldwide policy actions since the 1970's likely led to decreases in concentrations over time for DDT/DDE

and other POPs. A recent report from a population recruited in Massachusetts in 2004 shows that after converting mean concentrations to pg g^{-1} milk, concentrations among our urban population were higher than those measured in Massachusetts for *p*,*p*′-DDE (4540 *vs.* 1170 pg g^{-1} milk), β-hexachlorocyclohexane (312 *vs.* 169 pg g^{-1} milk), and Hexachlorobenzene (264 *vs.* 50.6 pg g^{-1} milk) and were lower than theirs for: *o*,*p*′-DDE (6.64 *vs.* 28.6 pg g^{-1} milk) and *p*,*p*′-DDT (124 *vs.* 147 pg g^{-1} milk).[34] All of these chemical concentrations were higher in our agricultural population compared to the Massachusetts population except for *o*,*p*′-DDE (7.72 *vs.* 28.6 pg g^{-1} milk).[34]

PCB concentrations in our study differed by location and were lower than previously published studies. Detection for PCB 180 was poor due to method limitations and interference with other analytes; data for this congener may not be as reliable as those reported for the other three congeners (PCBs 118, 138 and 153). For congeners 118, 138 and 153, the concentration distribution for the urban population was significantly higher than the agricultural population, possibly due to diet.[35] Non-Hispanic white women have been found to eat more fish, a food in which PCBs tend to bioaccumulate and biomagnify, than Mexican Americans.[36–38] Few studies have reported concentrations of individual PCB congeners in milk, but compared to urban women in our study, concentrations of PCB 153 were approximately 13 times higher among women residing in New York in 1979.[39] A more recent study reported maternal milk concentrations of PCB 153 in North Carolina women recruited in 2004–2006. The estimated median concentration (range) from this study was 340 (40–3980) pg g^{-1} milk which was higher than the concentrations measured in either population of our study.[40]

Regarding the non-persistent pesticide, chlorpyrifos, we hypothesized that women residing in an agricultural area would have higher concentrations of chlorpyrifos in their milk compared to urban women due to the agricultural uses (~ 28,000 kg/yr in the study area). Although median levels were not significantly different, the 75th percentile and maximum values of chlorpyrifos were higher among agricultural women. Chlorpyrifos has been found to adhere to particles, such as housedust,[41] may be somewhat bioaccumulative (log octanol-water partitioning coefficient (log K_{ow}) greater than 3),[16] and may persist indoors for greater than a year.[42] Thus, historical residential applications may contribute to maternal exposures in the urban population recruited within 1–3 years of the voluntary elimination on residential use, but may not have contributed to maternal exposures in the agricultural cohort who were recruited 6–7 years after the voluntary elimination. Future studies should examine whether chlorpyrifos concentrations in breast milk are associated with agricultural pesticide use.

For another non-persistent pesticide, permethrin, the agricultural population had significantly higher concentrations compared to the urban population for both isomers. In addition to agricultural use of permethrin (~20,000 kg/yr),[43] low SES families may reside in poorer quality housing possibly leading to increased use of home pesticides containing permethrin.[44] Reported home pesticide use of permethrin in agricultural homes was minimal during our study, but historically used permethrin may persist indoors as evidenced by the high detection of permethrin in household dust from similar homes in the study area.[41,45]

Few studies have reported concentrations of non-persistent pesticides in human milk; one report from India showed extremely high concentrations of chlorpyrifos (estimated 230,000 pg g^{-1} milk compared to 28 pg g^{-1} milk in agricultural women in this study).[18] The authors attributed these concentrations to non-compliance of recommended re-entry waiting periods after application of chlorpyrifos. Reported concentrations of permethrin in the milk of South African women (estimated 8000 pg g^{-1} milk) were much higher than women in our populations (maximum values of 93 and 176 pg g^{-1} milk in urban and agricultural women, respectively).[46] In South Africa, permethrin is used indoors for malaria vector control. Thus, regulations and use patterns in different countries may explain why concentrations were lower in our study.

Chlorpyrifos-methyl and propoxur were frequently detected in the urban population, but not in the agricultural population. For chlorpyrifos-methyl the discrepancy may be due to the timing of sample collection for each population and government and industry policies that resulted in discontinued use in 2004.[47] Propoxur is an indoor and outdoor home-use insecticide that is not used in agriculture.[48] We have no explanation for the discrepancy in detection for propoxur.

Pesticide degradation in the environment or during storage and relatively higher LODs may explain why some non-persistent pesticides widely used in California, including diazinon, were not detected. Other chemical properties may determine whether some chemicals persist in the environment or in the body. For example, we observed that chemicals with higher log K_{ow}s had higher ICCs. The log K_{ow} values for permethrin and *p*,*p*′-DDE are 6.10 and 6.51, respectively; whereas log K_{ow}s for chlorpyrifos and diazinon are lower at 4.96 and 3.81, respectively, indicating less potential to partition into lipids. These characteristics may in part explain why concentrations for persistent chemicals and permethrin had lower within-woman variability. Our study showed that while non-persistent pesticides such as chlorpyrifos, chlorpyrifos-methyl, and propoxur may be important chemicals to biomonitor, based on the ICCs and the high within-woman variability these chemicals may require multiple samples to assess an individual's exposure. In addition, as shown in Fig. 1 A–C, our study found some evidence of depuration for *p*,*p*′-DDE, but no clear pattern over time for chlorpyrifos or *trans*-permethrin. More research in larger populations is needed to assess within- and between-person variability, and long-term trends of non-persistent compounds in breast milk as well as their validity for use in exposure, risk assessment, and epidemiologic studies.

This research was intended to be a pilot study, thus there are several limitations. The small sample size limited the power of statistical tests. Also, milk samples collected from urban women were likely mature milk samples, collected more than 14 days postpartum (based on observation of the child's age at the time of enrollment), whereas the majority of samples from agricultural women were colostrum or transitional milk. The composition of the milk differs by lactation stage, with colostrum having lower lipid content. Maternal diet or other behaviors may also be different closer to parturition compared to women with older breastfeeding children. For persistent lipophilic chemicals that may decrease over the course of lactation,[49] concentrations measured in the milk of women who have lactated longer may

underestimate infant exposures closer to parturition. Another limitation for non-persistent pesticides may be storage stability. Degradation during storage may lead to underestimates of actual concentrations and exposures experienced by our mothers and infants

Many advocates support human milk biomonitoring in the U.S.[50] Researchers who study early childhood exposures should consider both persistent and non-persistent chemicals in breast milk. Under FQPA,[24] the U.S. EPA is required to conduct dietary exposure assessments for current-use non persistent pesticides, but the primary food for the youngest and most vulnerable population, breast milk, has not been adequately studied and the potential health effects in infants and young children from lactational exposure are not known. The lack of a comprehensive breast milk biomonitoring program is a shortcoming of FQPA.

In summary, to our knowledge this is the first pilot study that reports concentrations and variability of several current-use, non-persistent pesticides in breast milk from U.S. mothers, including chlorpyrifos, chlorpyrifos-methyl, permethrin and propoxur. For persistent chemicals, most OC and PCB concentrations were lower than in studies from at least a decade ago. In general, OC concentrations were higher in the agricultural populations than the urban population; the reverse was true of the PCBs. Lastly, some non-persistent chemicals such as chlorpyrifos may not be stable biomarkers of exposure in human milk given the wide variability of concentrations observed within women. While there were measurable concentrations of chemicals in all mothers' milk samples, breastfeeding is still the optimal source of nutrition for infants. More research is needed in larger populations of women to identify sources of exposure to mothers and reduce exposures to women of childbearing age and their breastfeeding infants.

Abbreviations

95%CI	95% Confidence Interval
β	beta
DDE	Dichlorodiphenyl trichloroethylene
DDT	Dichlorodiphenyl trichloroethane
DF	Detection frequency
EPA	Environmental Protection Agency
FQPA	Food Quality Protection Act
g	grams
ICC	Intraclass Correlation
K_{ow}	octanol:water coefficient
LOD	Limit of Detection
N	Sample size
ng g^{-1}	Nanogram per gram
OC	Organochlorine
OP	Organophosphate
PCB	Polychlorinated biphenyl
pg g^{-1}	Picogram per gram
POP	Persistent Organic Pollutant
ppb	part per billion
PSA	primary and secondary amine
SD	Standard Deviation
SES	Socioeconomic status
US	United States
WIC	The Special Supplemental Nutrition Program for Women, Infants, and Children

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References

1 WHO, *Global strategy for infant and young child feeding*, World Health Organization, Geneva, 2003.
2 M. S. Kramer and R. Kakuma, *The optimal duration of exclusive breastfeeding: A systematic review*, World Health Organization, Geneva, 2002.
3 L. M. Gartner, J. Morton, R. A. Lawrence, A. J. Naylor, D. O'Hare, R. J. Schanler and A. I. Eidelman, *Pediatrics*, 2005, **115**, 496–506.
4 L. A. Hanson, M. Korotkova and E. Telemo, *Ann. Allergy, Asthma Immunol.*, 2003, **90**, 59–63.
5 L. A. Hanson, M. Korotkova, S. Lundin, L. Haversen, S. A. Silfverdal, I. Mattsby-Baltzer, B. Strandvik and E. Telemo, *Ann. N. Y. Acad. Sci.*, 2003, **987**, 199–206.
6 J. W. Anderson, B. M. Johnstone and D. T. Remley, *Am J Clin Nutr*, 1999, **70**, 525–535.
7 A. K. Leung and R. S. Sauve, *J Natl Med Assoc*, 2005, **97**, 1010–1019.
8 D. Smith, *Int. J. Epidemiol.*, 1999, **28**, 179–188.
9 W. J. Rogan and B. C. Gladen, *Environ. Health Perspect.*, 1985, **60**, 215–221.
10 M. N. Bates, B. Thomson and N. Garrett, *Arch. Environ. Health*, 2002, **57**, 591–597.
11 S. M. Waliszewski, A. A. Aguirre, R. M. Infanzon and J. Siliceo, *Bull. Environ. Contam. Toxicol.*, 2002, **68**, 324–331.
12 S. M. Waliszewski, G. Melo-Santiesteban, R. Villalobos-Pietrini, S. Gomez-Arroyo, O. Amador-Munoz, M. Herrero-Mercado and O. Carvajal, *Bull Environ Contam Toxicol*, 2009.
13 D. B. Barr, *J. Chem. Health Saf.*, 2008, **15**, 20–29.
14 J. D. Racke, *Degradation of organophosphorous insecticides in environmental matrices*, Academic Press, Inc, San Diego, CA, 1992.
15 J. Starr, S. Graham, D. Stout2nd, K. Andrews and M. Nishioka, *Environ. Res.*, 2008, **108**, 271–279.
16 R. V. Thomann, *Environ Health Perspect*, 1995, **103**(Suppl. 5), 53–57.
17 S. A. Lederman, *Reprod. Toxicol.*, 1996, **10**, 93–104.
18 R. Sanghi, M. K. Pillai, T. R. Jayalekshmi and A. Nair, *Hum. Exp. Toxicol.*, 2003, **22**, 73–76.
19 A. P. Silva, F. C. Meotti, A. R. Santos and M. Farina, *Neurotoxicology*, 2006.
20 B. Eskenazi, A. R. Marks, A. Bradman, K. Harley, D. B. Barr, C. Johnson, N. Morga and N. P. Jewell, *Environ. Health Perspect.*, 2007, **115**, 792–798.
21 B. Eskenazi, J. Chevrier, L. G. Rosas, H. A. Anderson, M. S. Bornman, H. Bouwman, A. Chen, B. A. Cohn, C. de Jager, D. S. Henshel, F. Leipzig, J. S. Leipzig, E. C. Lorenz, S. M. Snedeker and D. Stapleton, *Environ Health Perspect*, 2009, **117**, 1359–1367.
22 V. A. Rauh, R. Garfinkel, F. P. Perera, H. F. Andrews, L. Hoepner, D. B. Barr, R. Whitehead, D. Tang and R. W. Whyatt, *Pediatrics*, 2006, **118**, e1845–1859.

23 S. M. Engel, G. S. Berkowitz, D. B. Barr, S. L. Teitelbaum, J. Siskind, S. J. Meisel, J. G. Wetmur and M. S. Wolff, *Am. J. Epidemiol.*, 2007, **165**, 1397–1404.
24 Food Quality Protection Act, 104–170, 104th Congress. (1996).
25 M. Davis, R. H. Weldon, G. Morales-Agudelo, W. Roman, A. Bradman, N. Holland, B. Eskenazi and D. Barr, *Epidemiology*, 2008, **19**, S335–S335.
26 StataCorp, *Stata Statistical Software: Release 10*, 2007.
27 U.S. Bureau of the Census, *American Factfinder*, U.S. Census Bureau, Washington, DC, 2000.
28 R. W. Hornung and L. D. Reed, *Appl. Occup. Environ. Hyg.*, 1990, **5**, 46–51.
29 E. F. Schisterman, B. W. Whitcomb, G. M. Louis and T. A. Louis, *Environ. Health Perspect.*, 2005, **113**, 853–857.
30 B. Rosner, *Fundamentals of Biostatistics*, Seventh edn, Brooks/Cole, Boston, 2011.
31 J. S. LaKind, C. M. BerlinJr., A. Sjodin, W. Turner, R. Y. Wang, L. L. Needham, I. M. Paul, J. L. Stokes, D. Q. Naiman and D. G. PattersonJr., *Environ Health Perspect*, 2009, **117**, 1625–1631.
32 National Research Council, *Pesticides in the Diets of Infants and Children*, National Academy Press, Washington, D.C., 1993.
33 C. A. Lovelady, R. A. Whitehead, M. A. McCrory, L. A. Nommsen-Rivers, S. Mabury and K. G. Dewey, *J. Hum. Lactation*, 1999, **15**, 307–315.
34 B. Johnson-Restrepo, R. Addink, C. Wong, K. Arcaro and K. Kannan, *J. Environ. Monit.*, 2007, **9**, 1205–1212.
35 A. Beyer and M. Biziuk, *Rev. Environ. Contam. Toxicol.*, 2009, **201**, 137–158.
36 J. M. Hightower, A. O'Hare and G. T. Hernandez, *Environ. Health Perspect.*, 2006, **114**, 173–175.
37 P. M. Schwartz, S. W. Jacobson, G. Fein, J. L. Jacobson and H. A. Price, *Am. J. Public Health*, 1983, **73**, 293–296.
38 S. Corsolini, N. Ademollo, T. Romeo, S. Greco and S. Focardi, *Microchem. J.*, 2005, **79**, 115–123.
39 B. Bush, J. Snow, S. Connor and R. Koblintz, *Arch. Environ. Contam. Toxicol.*, 1985, **14**, 443–450.
40 I. J. Pan, J. L. Daniels, A. H. Herring, W. J. Rogan, A. M. Siega-Riz, B. D. Goldman and A. Sjodin, *Paediatr Perinat Epidemiol*, **24**, pp. 262–271.
41 M. E. Harnly, A. Bradman, M. Nishioka, T. E. McKone, D. Smith, R. McLaughlin, G. Kavanagh-Baird, R. Castorina and B. Eskenazi, *Environ. Sci. Technol.*, 2009, **43**, 8767–8774.
42 R. J. Buck, H. Ozkaynak, J. Xue, V. G. Zartarian and K. Hammerstrom, *J. Exposure Anal. Environ. Epidemiol.*, 2001, **11**, 253–268.
43 DPR, *Pesticide Use Report, Annual 2007, Indexed by Chemical and by Crop*, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA, 2007.
44 A. Bradman, J. Chevrier, I. Tager, M. Lipsett, J. Sedgwick, J. Macher, A. B. Vargas, E. B. Cabrera, J. M. Camacho, R. Weldon, K. Kogut, N. P. Jewell and B. Eskenazi, *Environ. Health Perspect.*, 2005, **113**, 1795–1801.
45 L. Quirós-Alcalá, A. Bradman, M. Nishioka, M. E. Harnly, A. Hubbard, T. E. McKone, J. Ferber and B. Eskenazi, *Environ. Health*, 2011, **10**, 1–15.
46 H. Bouwman, B. Sereda and H. M. Meinhardt, *Environ. Pollut.*, 2006, **144**, 902–917.
47 U.S. EPA, *Report on FQPA Tolerance Reassessment and Risk Management Decision for Chlorpyrifos methyl*, Office of Prevention, Pesticides and Toxic Substances, Washington, D.C., 2000.
48 U.S. EPA, *Reregistration Eligibility Decision (RED): Propoxur*, United States Environmental Protection Agency; Office of Prevention, Pesticides and Toxic Substances, Washington, D.C., 1997.
49 J. S. LaKind, C. M. Berlin and D. Q. Naiman, *Environ. Health Perspect.*, 2001, **109**, 75–88.
50 J. S. LaKind, C. M. Berlin and M. N. Bates, *J. Toxicol. Environ. Health, Part A*, 2005, **68**, 1683–1689.