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Factors predicting organochlorine pesticide levels in pregnant Latina women living in a United States agricultural area

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Abstract

Organochlorine (OC) pesticide use was restricted starting in the 1970s in developed countries and the 1980s and 1990s in developing countries. Current exposure to OC pesticides – DDT, lindane (99% pure gamma-hexachlorocyclohexane (γ-HCH)), hexachlorobenzene (HCB) – occurs on a limited basis. We measured p, p' -DDE, p, p' -DDT, o, p' -DDT, HCB, beta (β)-HCH (the most persistent isomer of technical-grade HCH) and $γ$ -HCH in serum from 426 low-income pregnant Latina women living in an agricultural community in California. Detection frequencies were 94-100%. Median levels (ng/g-lipid) of p, p' -DDE (1,052), p, p' -DDT (13), β-HCH (37) and HCB (65) were significantly higher than U.S. population levels. Multivariate analyses of p, p' -DDE, p, p ′-DDT, o,p′-DDT, β-HCH and HCB indicate that time spent living outside the United States and birthplace in an area of Mexico with recent use of OC pesticides were significant predictors of exposure. Time spent living in the United States was associated with increased serum levels of p, p ′-DDE and β-HCH, but the increase for each year lived in the United States was lower than for each year lived outside the United States. There was no difference between the increase of HCB levels over time spent in or outside the United States, suggesting current and thus preventable exposure routes. However, we observed no associations between serum levels of any OC compound and current intake of saturated fat or agricultural take-home exposure risk factors. Lactation history and recent weight gain were negatively associated with serum levels of some, but not all OC compounds studied. Smoking history was borderline associated with elevated HCB levels. We observed no significant associations with body mass index. Although the weight of evidence from this study indicates that most exposure occurred prior to moving to the United States, the results for HCB indicate the possibility of ongoing exposure in this country.

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DDT/DDE; organochlorine pesticides; maternal exposure; US/Mexico; age; lactation; diet

Introduction

Organochlorine (OC) pesticides such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB) and hexachlorocyclohexane (HCH) were used worldwide in agriculture, animal husbandry and public health campaigns between the 1940s and 1970s. Concern about their persistence in the environment, bioaccumulation in the food chain and effects on wildlife and humans led to use restrictions and prohibitions, first in developed countries and later throughout the world. The United States banned all non-public health use of DDT, technical-grade HCH (an insecticide containing a mixture of 5 HCH isomers) and HCB in 1972, 1976 and 1984, respectively, whereas Mexico banned HCB in 1992 and phased out use of DDT and technical-grade HCH in 2000 (ISAT, 1998; Pardio et al., 2003).

DDT is still used in several countries for malaria control and some groups advocate expanding its use (Rosenberg, 2005). The pesticide lindane, (made of 99% pure gamma isomer of HCH (γ -HCH)), is banned in more than 50 countries as well as in California, but limited use is permitted in at least 30 countries, including Mexico, Canada and most U.S. states. Although no longer used as a fungicide, HCB is produced as a manufacturing byproduct of several chlorinated compounds, including pesticides, and is also formed during incineration of municipal waste.

Overall, levels of OC pesticides in the environment have declined worldwide since their use was banned or restricted. However, the persistence of DDT, dichlorodiphenyldichloroethene (DDE) (a breakdown product of DDT), HCB and β-HCH (5-14% of technical-grade HCH) in the environment varies by climate, with higher rates of dissipation occurring in tropical and subtropical regions. For example, most DDE and applied DDT dissipates within a year in tropical and subtropical soils, whereas the half-life in temperate region soils ranges from 2.3 to 16.7 years for DDT and more than 20 years for DDE (U.S. DHHS, 2002). In California, DDT and DDE have been detected in agricultural soils and dust more than 20 years after DDT use was discontinued (DPR, 1985; Bradman et al., 1997) and DDT, DDE, HCB and β-HCH continue to be detected in domestically-grown food (DPR, 1986-2004).

We observed a relationship between *in utero* exposure to para, para' (p, p') -DDT, ortho, para' (o, p') -DDT and p, p' -DDE and decreased mental development in children from a primarily Mexican, agricultural population in the Salinas Valley, California (Eskenazi et al., 2006). In the same population, we found in utero exposure to HCB to be associated with decreased gestational age (Fenster et al., 2006). The levels of p, p' -DDT, p, p' -DDE, β-HCH and HCB in this population of pregnant women were higher than in a national sample of Mexican American women aged 18-40 (Fenster et al., 2006). Given these findings and the possibility of ongoing exposure to OC pesticides in the California environment, we sought to identify predictors of exposure in these women, with particular attention to whether current exposure routes contributed to overall body burden.

Methods

Study population

The Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) is a longitudinal birth cohort study of environmental exposures and children's health. Information on the study population is presented elsewhere (Eskenazi et al., 2003). Briefly, women were recruited through two area medical facilities and were eligible to participate in the study if they were 18 years or older, 20 weeks or less gestation, qualified for government health insurance and planning to deliver at Natividad Medical Center. We obtained written informed consent according to procedures approved by the University of California Berkeley Committee for the Protection of Human Subjects. Between September 1999 and November 2000, we enrolled 601 participants out of 1,130 eligible, a response rate (53%) that surpasses those typically obtained in prospective cohort studies (Wolff et al., 2005). Of the 538 women still enrolled at delivery, 426 had adequate serum stored for OC measurement for the aforementioned studies (Eskenazi et al., 2006; Fenster et al., 2006) as well as this analysis.

Data Collection

Participants were interviewed by bilingual, bicultural staff after enrollment (Mean \pm standard deviation (SD)=13.4 \pm 5.2 weeks of gestation) and during their third trimester (Mean \pm SD =25.9 \pm 2.6 weeks). Interviews collected information on personal and sociodemographic characteristics, residential history and occupation. The third trimester interview included a 72-item food frequency questionnaire that was based on the Spanishlanguage Block 98 Questionnaire and modified for this population (Harley et al., 2005). Information on food consumption during the second trimester was converted to average daily saturated fat intake using values from the USDA Nutrient Database for Standard Reference. Total dairy, milk and meat intake were calculated by summing consumption of individual relevant food items.

Serum collection and organochlorine pesticide measurements

Serum samples were collected by venipuncture from 393 women during routine glucose tolerance testing (Mean \pm SD=25.7 \pm 2.2 weeks gestation) and from 33 women in the hospital at delivery. Samples were frozen at -80° Celsius and shipped on dry ice to the Centers for Disease Control and Prevention (CDC).

We measured the following OC pesticides or degradates in each sample: $p, p \n\leq DDT$, $o, p \n\leq$ DDT, p, p' -DDE, HCB, β-HCH and γ-HCH. Details of the laboratory methods and quality control procedures have been published previously (Barr et al., 2003). Briefly, 1 gram (g) of serum was enriched with isotopically labeled analogues of the target analytes then lyophilized to remove all water. The OC pesticides were extracted using accelerated solvent extraction (10% dichloromethane in hexane). The extract was cleaned with Florisil® during the extraction process, purified using gel permeation chromatography and then concentrated for analysis. Extracts were analyzed using gas chromatography-high resolution mass spectrometry. Quantification was achieved using isotope dilution calibration. We included quality control materials and blank samples in each run. Levels below the limit of detection (LOD) – Mean (SD) picograms/gram-serum: 3.0 (2.0), p,p′-DDE; 1.6 (1.7), p,p′-DDT; 1.3

(2.1), o, p' -DDT; 0.8 (0.9), HCB; 1.6 (0.7), β-HCH; and 1.6 (0.8), γ -HCH – were assigned the value of the LOD/2 (Hornung and Reed, 1990; Barr et al., 1999).

We measured total cholesterol and triglycerides using standard clinical enzymatic methods (Roche Chemicals, Indianapolis, IN) and calculated total lipids using methods reported by Phillips et al. (1989){Phillips, 1989 #2021}. All laboratory methods were conducted according to guidelines set forth in the Clinical Laboratory Improvement Amendment of 1988 (U.S. DHHS, 1988). Lipid-adjusted values were used for all statistical analyses.

Statistical Analyses

We initially computed descriptive statistics for the OC pesticides and, using T-tests, compared the geometric mean (GM) of our population to the GM of all women aged 18-40, Mexican American women aged 18-40 and pregnant women aged 18-40 who participated in the 1999-2000 National Health and Nutrition Examination Survey (NHANES) (CDC, 2005). We applied sampling weights to the NHANES data for all women 18-40 years, but could not for the other groups due to insufficient sample sizes in the NHANES strata.

Our primary analysis focused on characterizing predictors of OC exposure in the CHAMACOS cohort. Body burden of OC pesticides has been negatively associated with breastfeeding (Lopez-Carrillo et al., 2001; Moysich et al., 2002) and positively associated with age (Torres-Arreola et al., 1999), dietary intake of lipid-rich foods such as meat, fish and dairy (Torres-Arreola et al., 1999; Moysich et al., 2002; Sarcinelli et al., 2003), origin from a geographic area with recent or more intense use of OC pesticides (Waliszewski et al., 2000; Wolff et al., 2005) and smoking (Lackmann et al., 2000; Deutch et al., 2003). Serum levels of OC pesticides have been both positively (Glynn et al., 2003) and negatively (Perry et al., 2005) associated with body mass index (BMI) and recent weight change (Glynn et al., 2003).

Age as a risk factor for elevated serum OC levels reflects the bioaccumulation of OC pesticides over time, assuming a constant level of exposure. Our population spent time in the United States, Mexico, Central America and a few other countries – places with varying environmental levels of OC pesticides due to differences in the duration and intensity of OC pesticide use. We therefore chose to represent a participant's age using two variables: the number of years lived outside the United States and the number of years lived inside the United States. This representation of age enabled us to compare the bioaccumulation of OC pesticides during time spent in the United States (where serum levels could be declining over time due to decreasing levels in the environment) to that during time spent in other countries (where serum levels could still be increasing over time due to more recent OC pesticide use).

We also evaluated current exposure factors related to contact with potentially contaminated soil: employment in agricultural field work or other agricultural work during pregnancy (versus other industries or unemployed), the number of household members who wore agricultural work clothes or shoes inside the home, whether the participant laundered agricultural work clothes and the distance between a participant's home and the closest agricultural fields (less than versus more than 60 meters). Finally, lice-treatment products

containing Lindane (99% pure γ-HCH) were another potential source of exposure to γ-HCH (use of these products was still permitted in California at the time of serum sample collection). Thus, for γ -HCH, we also considered whether a participant reported having received or given lice treatments to family members.

We evaluated the relationships between OC pesticide levels and these potential predictor variables using Spearman rho correlations (continuous variables), the Kruskal-Wallis (KW) test of medians (categorical variables) and Lowess plots. We considered lactation history (total months that previous children were breastfed), time spent inside the United States (years), time spent outside of the United States (years), dietary intake of saturated fat, meat, fish and dairy (grams per day), BMI (kilograms/meters² (kg/m²)) at the time of blood specimen collection, weight gain (kg) at the time of blood specimen collection and the number of household members who wore agricultural clothing or shoes inside the home both continuously and categorically. The remaining variables were analyzed categorically using indicator variables for each category. Birthplace categories reflected the recency and intensity of OC pesticide use: United States or other non-Latin American country (low), highland Mexico or Central America (medium) and coastal Mexico (high) (Galvan-Portillo et al., 2002; Yanez et al., 2002).

Next, we entered predictors of OC exposure into a multiple linear regression model with natural log-transformed OC pesticide levels as the outcome. We also evaluated the interaction between time spent outside of the United States and categories of birthplace because we hypothesized that, due to variation in OC pesticide use in Mexico and Central America, the effect of spending time outside of the United States might differ depending on where the time was spent. Information on where participants spent their time outside of the United States was not available, so we assumed that participants spent this time in the same place they were born. When no significant differences (Wald test, $P > 0.20$) were observed between the beta (β) coefficients for time spent outside of the United States among women born in the United States or other countries (low) and women born in highlands Mexico or Central America (medium), we collapsed these two categories of birthplace into one and examined the data as born in coastal Mexico (high) versus not born in coastal Mexico (low and medium). Interaction terms were removed when no evidence of effect modification was found $(P>0.20)$. Due to the correlation between dietary variables, we considered saturated fat, meat, dairy and fish consumption separately and retained the most strongly associated measure.

Employment in agriculture during pregnancy, the number of household members wearing agricultural work clothes inside the home and laundering of agricultural work clothes were associated with birthplace in this population (data not shown) and consequently had the potential to be acting as proxies for birthplace in the multivariate models. The risk of exposure to OC pesticides from these contemporaneous risk factors should be independent of birthplace. Thus, to determine whether these characteristics were true risk factors or were functioning as proxies for birthplace, we examined their statistical interaction with birthplace. When interaction terms for each occupational or take-home risk factor and birthplace were significant ($P(0.20)$, we concluded that the factor was serving as a proxy for birthplace and removed it from the model.

We reran final multivariate models, omitting subjects with Cook's D values (a combined measure of leverage and residual size (StataCorp, 2004)) greater than 4, to confirm that results were not influenced by outliers ($N=18$ to 29). We also examined variance inflation factors to verify that β coefficients and standard errors were not unstable due to collinearity between predictors. Lastly, to facilitate interpretation, we converted the β coefficients to measurements of the percent change in OC levels associated with a one-unit increase (continuous variables) or a yes/no difference (indicator variables) in the predictor variable using the formula: percent change=100 \times (antilog(β)-1) (Wooldridge, 2000).

Results

Demographics

Table 1 summarizes the demographic characteristics of the 426 CHAMACOS cohort participants. In addition, one-third of participants were nulliparous; parous women had a median of 2 children (range 1-6) and lactation history of 8 months. On average, women were 26.1 years old at time of enrollment, lived 7.1 years of their life inside the United States and 19.0 years outside the United States. Nearly all (86%) participants were born in Mexico; 11% were born in the United States, 1.4% in Central America (El Salvador, Honduras and Nicaragua) and 1.2% elsewhere (Philippines, Fiji and Italy). Compared to the 173 CHAMACOS participants who were not included in this analysis, participants with OC measurements lived outside the United States for 2.2 years longer, breastfed for about a month longer, ate approximately twice as much meat and had 50% more agricultural workers living in their homes (all differences $P<0.05$), but were similar with respect to all other characteristics considered in this analysis. Compared to the 529 women who were eligible to participate in the CHAMACOS study but refused, CHAMACOS participants were similar with respect to age and parity, but more likely to be born in Mexico, speak Spanish and have more agricultural fieldworkers living in their homes ($P<0.001$).

Organochlorine Pesticide Levels

All study participants had measurable levels of p, p' -DDE, p, p' -DDT and HCB. The detection frequencies for o, p' -DDT, β-HCH and γ-HCH were 95.8%, 99.8% and 94.3%, respectively (Table 2). Median lipid-adjusted levels (ng/g-lipid) of OC pesticides were: 1,052 for *p,p*²-DDE; 12.5 for *p,p*²-DDT; 1.2 for *o,p*²-DDT; 64.9 for HCB; 36.9 for β-HCH; and 1.1 for γ -HCH. Median (10th – 90th percentile) unadjusted levels (picograms/g-serum) were: 8,660 (2,901, 71,411) for p, p' -DDE; 103 (35, 2,361) for p, p' -DDT; 10 (3, 94) for o, p ′-DDT; 536 (195, 1,551) for HCB; 306 (48, 1,080) for β-HCH; and 9 (4, 20) for γ-HCH. OC pesticide levels in 20 women with serum samples from both third trimester and delivery were highly correlated (Pearson's correlations ~ 0.98). With the exception of γ -HCH, all of the OC pesticide levels were correlated with one another, with strong associations between p, p' -DDE, p, p' -DDT and o, p' -DDT (Spearman rho (r)=0.74 to 0.87, P<0.001). γ -HCH, which is less persistent in the environment and has a half life of only 20 hours in humans (Li, 1999), was weakly correlated with the other analytes ($r=0.10$ to 0.22, $P<0.04$ to <0.001). The remaining analyte associations were moderately correlated ($r=0.13$ to 0.45, $P<0.01$).

Comparison to NHANES

Table 2 shows distributions of OC pesticide levels in the CHAMACOS cohort and three subgroups of the NHANES 1999-2000 population. CHAMACOS participants had higher levels of p, p' -DDE (geometric mean (GM)=1,392.4 ng/g-lipid) than the three NHANES comparison groups: all women aged 18-40 (GM=163.8 ng/g-lipid, P<0.05), Mexican-American women aged 18-40 (GM=624.2 ng/g-lipid) and pregnant women aged 18-40 $(GM=115.6$ ng/g-lipid).

Statistical comparisons were not made for the remaining OC compounds due to the high frequency of non-detects in the NHANES population (50-99.8%) (Table 2). However, since over 50% of the CHAMACOS population had levels of p, p' -DDT, β-HCH and HCB that were higher than the NHANES LODs (listed in Table 2), we concluded that the CHAMACOS population had higher exposures, than the U.S. population. No comparisons can be made for o, p' -DDT and γ -HCH since NHANES LODs were seven- to nine-fold higher than the CHAMACOS median levels (Table 2).

Bivariate association of maternal characteristics and organochlorine pesticides in serum

Table 1 also presents the bivariate associations between OC pesticide levels and potential exposure risk factors. OC pesticide levels decreased with longer lactation histories, although this relationship was statistically significant only for p, p' -DDE (r=-0.11), β-HCH (r=-0.18) and HCB (r=-0.15). Serum levels of OC compounds were negatively associated with the number of years lived in the United States (r=-0.07 to -0.32, P 0.01 for all but HCB and γ-HCH), but positively associated with the number of years lived outside the United States (r=0.07 to 0.37, P 0.01 for all but γ-HCH). Except for γ-HCH, serum levels were higher in women who were born in areas where OC pesticides were used more recently (KW $P(0.01)$. For example, levels of p, p' -DDE in women born in coastal Mexico were 1,903 ng/g-lipid compared to 891 ng/g-lipid in women born in highlands Mexico or Central America and 521 ng/g-lipid for women born in the United States or other countries. This trend is present for all OC pesticides. Women who consumed more fish had 24-45% higher levels of p, p' -DDT and p, p' -DDE (P 0.05), but no differences in OC levels were observed for women who ate higher quantities of saturated fat, meat or dairy. Although BMI was not strongly or consistently associated with exposure (r -0.02 to 0.09, P>0.09), weight gain was negatively associated with p, p' -DDT and o, p' -DDT (r=-0.11 and -0.19, P 0.05) but not with the other OC compounds. Lastly, higher OC levels were associated with many of the factors related to agricultural take-home exposures: women employed in agriculture had higher levels of p, p' -DDT, o, p' -DDT, p, p' -DDE and β-HCH (P 0.05) than women who did not work or who were employed in other industries; women who laundered agricultural work clothes had 2 to 42% higher levels of p, p' -DDT, o, p' -DDT and β-HCH (P 0.05); and women living in households with more agricultural workers who wear their work clothes inside the home had higher levels of all the OC pesticides except for HCB ($r=0.09$ to 0.18, P 0.07). The only characteristic marginally associated with levels of γ -HCH was the number of household members who wore agricultural work clothes inside the home ($r=0.09$, $P=0.06$); given this, we did not explore predictors of γ -HCH further.

Factors predicting organochlorine pesticide levels in maternal serum

Results of multiple linear regression analyses are presented in Table 3. We observed an interaction between spending time outside of the United States and place of birth for p, p' -DDT, o, p' -DDT and p, p' -DDE (Table 3A), but not for β-HCH and HCB (Table 3B). For example, serum levels of p, p' -DDE increased 7.7% for each year lived in coastal Mexico and 5.7% for each year lived in highland Mexico or elsewhere. Similarly, serum levels of o, p $\sqrt[2]{\text{DDT}}$ and $p, p \sqrt{\text{DDT}}$ increased 7.8% and 8.0% for each year spent in coastal Mexico; levels of these two compounds did not increase significantly during time spent anywhere else outside the United States (Table 3A). Spending one year outside of the United States was associated with a 4.0% increase in HCB levels and a 7.5% increase in β-HCH levels (P<0.001), independent of birthplace (Table 3B).

Controlling for all other exposure risk factors, including time spent outside the United States, women born in coastal Mexico had 155% higher levels of p, p' -DDE, 221% higher levels of p, p' -DDT and 145% higher levels of o, p' -DDT ($P_{0.001}$) than all other women in the study combined and 220% higher levels of β-HCH and 64% higher levels of HCB (P<0.01) than women born in the United States or other countries outside Latin America. Women born in highlands Mexico or Central America had 31% and 168% higher serum levels of β-HCH and HCB, respectively, than women born in the United States or other countries, but this difference was significant only for β-HCH ($P<0.001$).

Our multivariate analysis showed that levels of p, p' -DDE, B-HCH and HCB increased about 3% per year spent in the United States, while levels of p, p' -DDT and o, p' -DDT decreased about 3% per year. Wald tests indicated that the β coefficients for time spent in the United States were different ($P<0.01$) from the β coefficients for time spent outside the United States in all models except that for HCB. Table 3B shows the β estimates for time spent in the United States and time spent outside the United States separately; when combined, the effect of time on serum levels of HCB is 3.8% increase per year of life (95% CI: 1.9-5.6).

Controlling for other maternal characteristics, lactation history was associated with lower levels of all OC pesticides, though this relationship was significant only for p, p' -DDE (2.1%) decrease per month of breastfeeding), β-HCH (2.7% decrease per month) and HCB (1.5% decrease per month) ($P<0.001$). Weight gain during pregnancy was also associated with lower levels of all OC compounds except for HCB; adjusted β coefficients were significant for p, p' -DDT (3% decrease per kg, P=0.05) and q, p' -DDT (4% decrease per kg, P=0.01). BMI at time of blood specimen collection was positively but non-significantly associated with serum OC levels. And, although current dietary intake of saturated fat had stronger associations with OC pesticide levels than intake of diary, meat or fish (data not shown), it also was not a significant predictor of exposure in a multivariate context.

Women who currently or ever smoked had 26% higher levels of HCB than women who never smoked $(P=0.08)$; this association strengthened in both magnitude and significance (to 30% higher levels, $P=0.04$ and 95% CI: 1.8, 66.6) when the 19 women missing information on BMI were included in the model. Smoking history was not associated with serum levels of the remaining OC pesticides.

Several risk factors for occupational or occupational take-home exposures were removed from the final multivariate models because evaluation of their interaction with birthplace indicated that they were acting as proxies for birthplace. Final models varied according to which of these variables were omitted. Agricultural field workers had 13% higher but not statistically significant $(P=0.24)$ levels of HCB than women who did not work or who worked in another industry. There was also no association between proximity of home to agricultural fields and p, p' -DDE, p, p' -DDT, o, p' -DDT or β-HCH levels, or between laundering of agricultural work clothes and serum HCB levels.

Final multiple linear regression models explained between 15% and 42% (R^2 =0.15 to 0.42) of the variation in OC pesticide levels (Table 3A and 3B).

Discussion

In this study of Latina, low-income pregnant women living in the Salinas Valley, California, we detected measurable levels of six organochlorine pesticides in nearly all serum samples. These levels were substantially higher than levels measured in a concurrent and representative sample of the U.S. population. Multivariate statistical analyses indicate that time spent living outside the United States and birthplace in an area of Mexico or Central America with intensive and recent use of OC pesticides were significant predictors of higher levels of p, p' -DDE, p, p' -DDT, o, p' -DDT, β-HCH and HCB in serum. Time spent living in the United States was associated with increased serum levels of p, p' -DDE, β-HCH and HCB. For p, p' -DDE and β-HCH, the increase for each year lived in the United States was lower than for each year lived outside the United States. There was no difference between the increase of HCB levels over time spent in or outside the United States. Levels of p, p' -DDE, β-HCH and HCB declined significantly with longer lactation histories. Pregnancy weight gain was inversely and significantly associated with p, p' -DDT and o, p' -DDT; although all positive, the relationships between BMI and serum OC levels were all nonsignificant. There was a borderline significant association between smoking history and elevated HCB serum levels which strengthened when women missing BMI information were included in the multivariate model, but no associations with other OC compounds were observed. There were also no associations between OC levels and any of the measures of agricultural take-home exposure pathways, including employment in agriculture, the number of agricultural workers who wear work clothes or shoe inside the home, laundering of agricultural work clothes or proximity between the home and agricultural fields. The lack of association between serum levels of γ -HCH and exposure risk factors examined in this analysis is likely due to the short half-life (∼20 hours) of this compound in the body (Li, 1999).

Our findings for p, p' -DDT, o, p' -DDT and p, p' -DDE are consistent with the history of DDT use in Mexico. DDT was used in malaria campaigns and domestic food production in the coastal states of Mexico until 2000. In the highland region of northern and central Mexico, however, intensive agricultural use of DDT declined in the mid-1970s as export-oriented agriculture responded to U.S. import standards for OC pesticide residues (NACEC, 2001; U.S. DHHS, 2002). In our population, women who were born in coastal Mexico showed the highest increases in p, p' -DDT, o, p' -DDT and p, p' -DDE per year lived outside of the United

States. Women born in coastal Mexico also had higher average p, p' -DDT, o, p' -DDT and p, p ′-DDE levels, possibly due to a constant (versus declining) level of environmental contamination (due to ongoing use of p, p' -DDT for malaria) or due to a time-independent factor such as differences in diet (for example, higher fish consumption).

Less is known about regional differences in HCB and β-HCH use in Mexico. However, technical-grade HCH was still being used in Mexico and Central America at the time of this study (Pardio et al., 2003) which may explain why time spent outside of the United States added more to exposure (7.5% per year) than time spent inside the United States (2.3% per year) and also why levels in Mexican and Central American-born women were higher than those in U.S.-born women, after controlling for time spent outside the United States. HCB use was discontinued in Mexico only 7 years after it was banned in the United States (compared to 20 years for DDT) and is currently produced worldwide during incineration of municipal waste and as a manufacturing by-product of several chlorinated compounds. Our observation that serum levels of HCB increased equally during time spent in the United States and time spent outside of the United States, is consistent with the more similar history of HCB use in the United States and Mexico.

Comparing the coefficients for time spent outside the United States and time spent inside the United States provides insight into whether current, and thus preventable, exposure routes are contributing to overall OC body burden. The coefficients for time spent in the United States were statistically significantly less than those for time spent outside the United States for p,p′-DDT, o,p′-DDT, p,p′-DDE and β-HCH, indicating that the rate of bioaccumulation of these compounds declines upon moving to the United States. Furthermore, the borderline significant results for p, p' -DDT, o, p' -DDT suggest that levels of these OC compounds even decline with time spent in the United States.

In contrast, our results indicate that bioaccumulation of HCB is occurring at the same rate in and outside the United States, suggesting the existence of current and therefore preventable exposure routes in this country. Current dietary and agricultural take-home exposure routes did not appear to contribute to body burden of HCB (or any other OC compound) in this analysis. However, smoking was borderline associated with 26% higher levels of serum HCB and this relationship was even stronger in a subset of 19 women in this population. Smoking has been associated with higher serum levels of HCB and other persistent pollutants in several studies (Deutch et al., 2003), as well as with higher serum levels in neonates whose mothers were exposed to tobacco smoke during pregnancy (Lackmann et al., 2000). In addition to the potential for direct exposure to HCB in tobacco, smoking may also increase body accumulation of HCB due to its effects on the enzyme systems that metabolize both nicotine and OC compounds (Deutch et al., 2003).

Lactation history was inversely associated with all OC pesticide levels; this relationship was statistically significant for p, p' -DDE, β-HCH and HCB. Previous studies have shown breastfeeding to be a primary route of elimination, with approximately 30% of body DDT and 35-60% of DDE passed to milk during lactation, depending on duration (Dorea et al., 2001).

BMI was not significantly associated with OC levels in our population. However, our study provides evidence that recent weight gain is associated with lower serum levels of OC compounds. Adjusting for all other risk factors, including BMI and consumption of saturated fat, women who gained more weight during pregnancy had significantly lower circulating levels of p, p' -DDT and o, p' -DDT. This finding is consistent with the observations reported by Glynn et al. (2003) in non-pregnant, older Swedish women. Glynn hypothesized that recent weight gain, acting as an additional reservoir for DDT and DDE, diluted serum concentrations. During pregnancy, maternal weight gain is due to increased body fat and mass associated with the growing fetus. Thus, two growing compartments may change the p, p' -DDT and o, p' -DDT equilibrium with serum, thereby explaining the inverse associations that we observed.

Lastly, our null findings for current dietary intake of saturated fat are consistent with Wolff et al. (2005) and the fact that contamination in U.S. food is declining since implementation of use restrictions (U.S. DHHS, 2002). Recent studies have reported significant associations between current dietary intake of saturated fat and other foods and OC exposure levels (Torres-Arreola et al., 1999; Moysich et al., 2002; Sarcinelli et al., 2003), but it is likely that levels of food contamination in these studies – one in Mexico, one in an older cohort of U.S. women and one in Brazil – were higher than in food consumed by our population. Our findings are limited in that they pertain only to risk of OC exposure from recent diet during pregnancy.

This study has additional limitations due to lack of available information. For instance, we may have misclassified women by assuming that all of their time outside of the United States was spent in their place of birth. We also did not have data on how OC pesticides were applied in or near our participants' former residences (e.g., agricultural application, interior residual spraying, netting) or on occupation prior to migration to the United States. This information would have enabled us to determine the magnitude of exposures that are associated with specific public health uses and to better characterize the relative contribution of occupational take-home exposure routes prior to and after migration to the United States. Our non-significant results for lactation history and p, p' -DDT and o, p' -DDT levels may be due, in part, by our inability to control for time since lactation (information that was also not available). Lastly, our ability to generalize our findings is limited due to the fact that the 426 women in this analysis differed from the 173 other participants in the CHAMACOS cohort with respect to factors significantly associated with OC levels (lactation and time spent outside the United States).

Conclusion

Our findings confirm that historical use of persistent, fat soluble OC pesticides results in exposures to pregnant women and their young children years or decades after use. In our population, women from areas in Mexico where DDT was recently used for malaria control had p, p' -DDT and p, p' -DDE levels that were significantly higher than U.S. averages in 1999-2000 and similar to historical U.S. levels when these materials were widely used (Farhang et al., 2005). In addition, our analysis indicates that exposure to HCB is ongoing despite deregistration of this pesticide in the United States in 1984 and Mexico in 1991.

HCB is formed during incineration of municipal waste (a likely exposure route for the general population), and is also an impurity in several pesticides used in the United States (Ambrus et al., 2003). None of the current risk factors we evaluated (diet, occupation) were associated with elevated HCB levels in our population. Additional research is needed to understand and reduce exposure sources. Finally, given the potential for adverse health effects at the levels of DDT and DDE observed in our population (Eskenazi et al., 2006) and the possibility that public health uses may increase in some regions (Roberts et al., 2000), additional research is needed to quantify human exposures resulting from specific application methods (e.g., interior residual spraying, mosquito nets, broadcast applications) so that effective malaria control strategies can be employed that also minimize human exposure.

Acknowledgments

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Table 1
Characteristics of CHAMACOS participants (N=426) and associated serum levels (median, ng/g-lipid) of organochlorine pesticides, Salinas *N***=426) and associated serum levels (median, ng/g-lipid) of organochlorine pesticides, Salinas Characteristics of CHAMACOS participants (** Valley, California **Valley, California**

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⁴The frequencies of detection of organochlorine pesticides did not vary significantly by participant characteristics ($\chi^2 P$ 0.99. Information on maternal characteristics was missing for subjects as follows: as follows: place of birth in Mexico (N=5); consumption of saurated fat (N=22), fish (N=11) and dairy (N=22); Body Mass Index (N=23); weight gain (N=8); number of household members who wear agricultural N=8); number of household members who wear agricultural N=23); weight gain (work clothes inside the home $(N=4)$; Lice treatments for self/family $(N=3)$; distance between home and agricultural field $(N=17)$. N=3); distance between home and agricultural field (N=22); Body Mass Index (N=11) and dairy (N=22), fish (N=4); Lice treatments for self/family (N=5); consumption of saturated fat (work clothes inside the home (place of birth in Mexico (

b Kruskal-Wallis test P-value. c Spearman rho correlation coefficient ($r_{\rm S}$, P-value). Spearman rho correlation coefficient (r_S, P-value).

Abbreviations: CHAMACOS, Center for Health Analysis of Mothers and Children of Salinas; N, number; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; HCB, Abbreviations: CHAMACOS, Center for Health Analysis of Mothers and Children of Salinas; N, number; DDT, dichlorodiphenyltichloroethane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; g, grams; kg, kilograms; m, meters. hexachlorobenzene; HCH, hexachlorocyclohexane; g, grams; kg, kilograms; m, meters.

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Table 2
Serum levels (ng/g-lipid) of organochlorine pesticides in Center for Health Analysis of Mothers and Children of Salinas (CHAMACOS) and **Serum levels (ng/g-lipid) of organochlorine pesticides in Center for Health Analysis of Mothers and Children of Salinas (CHAMACOS) and** 1999-2000 National Health and Nutrition Examination Survey (NHANES) participants **1999-2000 National Health and Nutrition Examination Survey (NHANES) participants**

 a_{Qp} -DDT, γ -HCH and β -HCH could not be measured in 2, 7 and 2 CHAMACOS subjects, respectively, due to instrument inteference. $o.p'$ -DDT, γ -HCH and β-HCH could not be measured in 2, 7 and 2 CHAMACOS subjects, respectively, due to instrument inteference.

 b Mean (SD) LODs (ng/g-lipid) for CHAMACOS: $\rho_{x}\rho'$ -DDE=0.4 (0.3), $\rho_{x}\rho'$ -DDT=0.2 (0.2), $o_{x}\rho$ -DDT=0.2 (0.3), HCB=0.1 (0.1), B-HCH=0.2 (0.1), γ -HCH=0.2 (0.1). Mean (SD) (ng/g-lipid) for Mean (SD) LODs (ng/g-lipid) for CHAMACOS: p p[/]-DDE=0.4 (0.3), p _p/-DDT=0.2 (0.2), o,p[/]-DDT=0.2 (0.3), HCB=0.1 (0.1), β-HCH=0.2 (0.1), γ-HCH=0.2 (0.1). Mean (SD) (ng/g-lipid) for NHANES: pp '-DDE=not available, pp '-DDT=10.6 (3.4), o.p '-DDT=10.6 (3.3), HCB=60.5 (19.3), B-HCH=4.8 (1.7), y-HCH=7.5 (2.4). NHANES: p,p′-DDE=not available, p,p′-DDT=10.6 (3.4), o,p′-DDT=10.6 (3.3), HCB=60.5 (19.3), β-HCH=4.8 (1.7), γ-HCH=7.5 (2.4).

Statistics for NHANES Mexican American and pregnant women are not weighted due to insufficient sample size. Statistics for NHANES Mexican American and pregnant women are not weighted due to insufficient sample size.

 d satistical comarisons for $p.p'$ -DDT, $p.p$ -HCH, p -HCH and HCB were not possible due to the high non-detect frequency in NHANES and differences in analytic detection limits (see note b). Statistical comarisons for p_p ^{-DDT}, α_p , ¹DDT, γ -HCH, β-HCH and HCB were not possible due to the high non-detect frequency in NHANES and differences in analytic detection limits (see note b).

 $e_{\cdot \cdot = \triangle}$ OD. $- = 100$.

Abbreviations: DDT, dichlorodiphenyltrichloro-ethane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; LOD, limit of detection; N, number. **Abbreviations:** DDT, dichlorodiphenyltrichloro-ethane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; LOD, limit of detection; N, number. Author Manuscript

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Multiple linear regression results for predictors of organochlorine pesticide levels in serum (ng/g-lipid) in CHAMACOS participants, Salinas Multiple linear regression results for predictors of organochlorine pesticide levels in serum (ng/g-lipid) in CHAMACOS participants, Salinas Valley, California, 1999-2000 **Valley, California, 1999-2000**

J Expo Sci Environ Epidemiol. Author manuscript; available in PMC 2018 January 12.

 Coastal Mexico 63.6 (10.3, 142.7) 0.49 0.01 219.7 (99.8, 411.5) 1.162 * Y_2 ears spent in the United States 3.3 (1.1 , 5.5) 3.3 Lactation (months) -1.5 (-3.1, -0.9) -0.02 = -2.7 (-2.1, -2.7 * -2.7 * -2.7 * -2.7 * -2.7 * -2.7 * -2.7 * -2.7 $\begin{array}{cccccccccc} \text{S31} & \text{$

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63.6

 0.08

×

1.162 0.023 -0.027 0.004

 $(99.8, 411.5)$ $(-0.3, 5.0)$ $(-3.4, -2.0)$ $(-0.4, 1.2)$ $(-1.4, 3.4)$

219.7

 0.01

 0.49

 2.3 -2.7 0.4 \overline{a}

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0.03

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 0.31 0.42

 0.01

 BMI (kg/m²)

 (4) (6) $(1,4)$ (5) $(1,4)$ $(1$

 0.13 0.52

 0.01 0.01

 -0.02

 -1.5 3.3

 0.5 0.6

Saturated fat intake (grams/day)

Lactation (months)

Years spent in the United States

Coastal Mexico

Table 3B

saturated fat ($N=22$); BMI ($N=19$); weight gain ($N=8$); number of household N=8); number of household N=19); weight gain (N=22); BMI ($N=5$); consumption of saturated fat (members who wear agricultural work clothes inside the home $(\lambda=4)$; distance between home and agricultural field $(\lambda=17)$. $N=4$); distance between home and agricultural field (members who wear agricultural work clothes inside the home (

 b percent change in organochlorine pesticide level associated with a 1-unit increase in participant characteristic. percent change in organochlorine pesticide level associated with a 1-unit increase in participant characteristic.

other includes highlands Mexico, Central America, Fiji, Italy and Philippines other includes highlands Mexico, Central America, Fiji, Italy and Philippines.

 d other includes Fiji, Italy and Philippines. other includes Fiji, Italy and Philippines.

Abbreviations: CHAMACOS, Center for Health Analysis of Mothers and Children of Salinas; B, beta coefficient; CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; DDE, **Abbreviations:** CHAMACOS, Center for Health Analysis of Mothers and Children of Salinas; β, beta coefficient; CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; BMI, body mass index; kg, kilograms; m, meter. dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; BMI, body mass index; kg, kilograms; m, meter.