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Organochlorine Pesticide Levels in Adipose Tissue of Pregnant Women in Veracruz, Mexico

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Abstract DDT and Lindane (γ-HCH) which were used until 1999 in Mexico, have provided great benefits in the combat of vectors that spread infection-borne diseases and in agriculture for crop protection. The persistence in the environment and their accumulative properties results in bioconcentration in lipid rich tissues of the human body that reflect the extent of environmental pollution. Human adipose tissue samples were taken during 2009 from abdominal cavities of 69 pregnant women by cesarean surgery and from 34 samples of control donors by autopsy in Veracruz State. The samples were analyzed by gas chromatography with ECD. The results of mean levels (mg/kg on fat basis) were higher in controls compared to pregnant women β-HCH 0.064 vs 0.027; *pp*′DDE 1.187 vs. 0.745; *op*′DDT 0.016 vs. 0.011; *pp*′DDT 0.117 vs. 0.099 and Σ-DDT 1.337 vs. 0.854. The pregnant women group was divided according to age: up to 20, 20–30, and more than 30 years, and presented an increase for the more persistent pesticides with age in terms of mean concentrations and a more pronounced higher correlation in medians levels. Pairing Body Mass Index to organochlorine pesticide mean levels revealed no correlation between these factors in pregnant women.

Keywords Organochlorine pesticides · Pregnant women

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Organochlorine pesticides, such as DDT (p,p′-dichlorodiphenyltrichloroethane) and HCH (hexachlorociclohexane) have been used for more than a half century, and have provided benefits for humans in combating vectors that transmit serious diseases and in agriculture for crop protection. From 1959 until 1999, Mexico sprayed these pesticides to combat malaria vectors. Their use throughout the decades and their biological persistence has increased the amount of DDT present in the environment. Due to its volatility, the main human exposure in areas similar to Veracruz, is from the inhalation of contaminated air (Alegria et al. 2008; Wong et al. 2008), followed by the consumption of contaminated foods of animal origin. Human exposure is influenced by the local surface soil contamination which causes pollution of air and food, the duration of this local exposure (residence time), and the capacity for elimination by metabolism and breast milk excretion (parity). The accumulation of organochlorine pesticides originates in lipophilic human body parts, particularly in lipid-rich tissues such as adipose tissue (Waliszewski et al. 2010). The monitoring of human adipose tissue, which acts as a receptor of environmental lipophilic contaminants, indicates the extent of body burden (Wolf

et al. 2007) and can reflect the extent of environmental pollution.

There are few reports available that establish the extent of DDT and HCH levels in pregnant women in areas where these pesticides were only applied in sanitary actions for malaria vectors or ectoparasites control, and which implicate these sanitary actions as a principal source of human exposure. Adipose tissue, which accumulates lipophilic compounds by virtue of physiological interaction of cellular components, allows an understanding of the biological specificity in the behavior of organochlorine pesticides in tropical environments and can be used assess the contribution to environmental pollution (Laden et al. 1999; Waliszewski et al. 2010). Variations in organochlorine pesticide concentrations between people are influenced by pollution factors that affect body deposition and by dietary habits.

The pregnant woman's exposure to persistent organic chemicals is of much interest because of exposure to the fetus and breast-fed infants that can be related to potential subsequent health effects (Eskenazi et al. 2009). During pregnancy, the behavior of chemicals is affected by physiologic changes such as: increased renal perfusion, increased volume of distribution, and increased serum lipids. During pregnancy, organochlorine pesticide residue levels in maternal and cord blood becomes a greater worry since reproductive toxicological studies have revealed that the mother and fetus are more vulnerable to their toxicity. Developing fetuses are exposed to organochlorine pesticides and its metabolites through uterine placental transfer (Wang et al. 2009). Hence, knowledge of biological levels of these contaminants in human tissues, especially in pregnant women, is necessary for the risk assessment of adverse health effects and for the identification of vulnerable groups associated with human exposure (Pathak et al. 2008).

The objective of the present study was to determine and compare the levels of organochlorine pesticides in adipose tissue of pregnant women to controls, living in state of Veracruz, exposed to organochlorine pesticide residues as a consequence of past sanitary spraying in order to combat malaria and from contaminated food consumption.

Materials and Methods

Human adipose samples (~5 g) from abdominal cavities of 69 pregnant women were taken during 2009 by cesarean surgery and 34 control samples of donors by autopsy at the Forensic Medicine Services of Veracruz. The donors originated from the state of Veracruz. All pregnant women were asked for consent to participate in the study. The samples were stored in glass jars, immediately frozen, and kept at −25°C until analyzed. The dated samples were labeled with donor origin, age and number of pregnancy.

The analyses of organochlorine pesticide residues in human adipose tissue were performed by gas chromatography with ECD according to a method described by (Waliszewski et al. 2004; 2008; 2010). All of the samples were analyzed for: HCB, α, β, γ-HCH, *pp′*DDT, *op′*DDT, and *pp′DDE*. The minimum detection limits for the residues analyzed were as follows: 0.001 mg/kg for HCB, 0.002 mg/kg for the β-HCH isomer and *pp′DDE*, and 0.003 mg/kg for *pp′*DDT and *op′*DDT. To determine the quality of the method, a recovery study was performed on 10 spiked replicates of blank cow fat samples, which presented contamination levels below the detection limits. The fortification study, done at 0.01–0.03-mg/kg levels, depending on the pesticide, showed mean values from 90 to 94% recovery. The standard deviation and coefficient of variation were below 10, indicating excellent repeatability of the method.

Statistical calculations were conducted using statistical software Minitab version 12. Concentrations of organochlorine pesticide (mg/kg on fat base) were expressed as frequencies, arithmetic means and medians. The resulting concentrations were calculated determining the significance of categorical factors on pesticide levels by the variability among samples, pairing them to identify differences among means by applying the Student's *t*-test and differences among medians by applying the Mann–Whitney test at $\alpha = 0.05$.

Results and Discussion

The mean age of control participants was 38 years (range 20–65) and was 26 years for pregnant women (range 16–40). Comparing both groups, significant mean age differences ($p = 0.003$) between control and pregnants were observed. Median ages were 38 years for controls and 26 years for pregnant women, also indicating significant differences ($p = 0.002$) between the two groups.

During the monitoring study, only the presence of β-HCH, *pp′DDE*, *op′*DDT and *pp′*DDT were determined, thus only these compounds are discussed. Table 1 summarizes frequencies and ranges of organochlorine pesticides levels from 34 control samples and 69 samples of pregnant women. *pp′DDE* and *pp′*DDT were found in 100% of samples analyzed, whereas β-HCH was present in 85 and 91%, and *op′*DDT in 70 and 84% of the samples. Smaller concentrations of organochlorine pesticides corresponded to isomer β-HCH and *op′*DDT isomer of insecticide *pp′*DDT.

The evaluation of mean and median organochlorine pesticide concentrations, shown in Table 2 reveals higher mean and median levels for all organochlorine pesticides in

Table 1 Frequencies and ranges of organochlorine pesticide levels (mg/kg on fat basis) in control and pregnant women groups

Pesticide	Controls ($n = 34$)		Pregnant women ($n = 69$)	
	Frequency %	Ranges	Frequency %	Ranges
β-HCH	85	0.002–0.362	91	0.002–0.152
pp′DDE	100	0.012–6.070	100	0.034–5.218
op′DDT	70	0.003–0.036	84	0.003–0.033
pp′DDT	100	0.005–0.675	100	0.006–1.695

the control group. This observation is in accordance with differences between the ages of both groups and the possible influence of factors on organochlorine pesticide levels. The controls were older and that permitted higher and more prolonged accumulation of organochlorine pesticides in adipose tissue. To evaluate statistical differences in organochlorine pesticide concentrations between controls and pregnant women, both groups were paired and differences at $p > 0.05$ between means and medians were calculated. For β-HCH, mean and median differences were significant, indicating higher control group exposure and accumulation of persistent β-HCH isomer that relates to the consumption of meat and dairy products containing the contaminant. The *op*'DDT isomer of insecticide *pp*′DDT had lower concentrations in adipose tissue of pregnant women. Its levels are considered statistically no different between study groups. The statistically significant differences were observed for *pp*′DDT and *pp*′*DDE* mean and median levels between the controls and pregnant women group. In general, the mean concentrations of organochlorine pesticides in controls were higher compared to pregnant women. Comparing data presented in Table 2, higher values corresponded to mean concentrations compared to the median, confirming the presence of extreme contamination cases of women living in Veracruz State. The control group presented five cases with *pp*′*DDE* contamination higher levels reaching an extreme 6.070 mg/kg and three *pp*′DDT samples oscillated around a 0.675 mg/kg. The pregnant women group revealed four cases of women with *pp*′*DDE* levels reaching 5.218 mg/kg and two cases of *pp*′DDT with higher levels of 1.403 and 1.695 mg/kg, respectively. The example of extreme exposure mentioned above indicates persistence of insecticide *pp*′DDT in the environment of Veracruz State and a contamination threat to the inhabitants.

To estimate degree of temporal exposure between individuals, the pregnant women population was divided according to age into three subgroups: less than 20 years, 20 to 30 years and higher than 30 years (Table 3). To identify possible differences in the mean and median concentrations, the groups were compared and statistical tests applied. The Student *t*-test revealed no statistical significant differences ($p > 0.05$) among organochlorine pesticide mean concentrations of the three studied subgroups. However, the medians indicated statistically significant differences ($p < 0.05$) in *pp*′*DDE* concentrations between groups less than 20 years and higher than 30 years, and between the 20–30 years group and the higher than 30 years group. The same tendency can be observed among concentrations of Σ-DDT. Analyzing Table 3, it can be concluded that organochlorine pesticide mean concentrations increase with age and decrease for insecticide *pp*′DDT. The phenomenon is due to specific accumulation of these lipophilic compounds in human adipose tissue and lack of a decontamination mechanism that permits their excretion. Decrease of *pp*'DDT mean

Table 3 Organochlorine pesticide levels (mg/kg on fat basis) in pregnant women group divided according to age

Pesticide	Less than 20 ($n = 15$)		20–30 ($n = 36$)		Higher 30 ($n = 18$)	
	Mean	Median	Mean	Median	Mean	Median
β-HCH	0.023	0.014	0.026	0.012	0.034	0.023
pp′DDE	0.532	0.249*	0.722	0.348*	0.988	0.720*
op′DDT	0.011	0.008	0.010	0.010	0.014	0.014
pp′DDT	0.136	0.036	0.097	0.040	0.074	0.057
Σ-DDT	0.678	0.296*	0.827	0.402*	1.075	0.762*

* significant differences at $p < 0.05$

Table 2 Mean, median levels (mg/kg on fat basis) of organochlorine pesticides in adipose tissue of women from Veracruz State

Pesticide	Mean		Median		Differences	
	Control	Pregnant	Control	Pregnant	*p*-value for means	*p*- value for medians
β-HCH	0.064	0.027	0.026	0.015	0.03*	0.03*
pp′DDE	1.187	0.745	0.695	0.421	0.05*	0.03*
op′DDT	0.016	0.011	0.016	0.010	0.18	0.12
pp′DDT	0.117	0.099	0.067	0.042	0.05*	0.04*
Σ-DDT	1.337	0.854	0.873	0.474	0.05*	0.05*

* significant differences among means and medians at $p < 0.05$

Table 4 Organochlorine pesticide levels (mg/kg on fat basis) in pregnant women divided according to number of parities

Pesticide	One ($n = 36$)		Two ($n = 19$)		Three and more ($n = 14$)	
	Mean	Median	Mean	Median	Mean	Median
β-HCH	0.025	0.013	0.030	0.020	0.032	0.016
pp′DDE	0.619	0.402	1.070	0.691	0.628	0.367
op′DDT	0.010	0.010	0.014	0.012	0.010	0.007
pp′DDT	0.092	0.042	0.150	0.044	0.050	0.038
Σ-DDT	0.720	0.456	1.231	0.738	0.686	0.406

concentrations indicates a decrease of exposure to the insecticide due to its ban since 1999 in Mexico.

The total pregnant women group has mean 1.8 ± 1.4 (1–11) parities. To evaluate whether number of parities influences organochlorine pesticide levels, the group was divided according to number of parities into: one (mean old 23.9 years), two (mean old 26.8 years) and three or more parities (mean old 30.4 years) (Table 4). The comparison of samples revealed no statistically significant ($p > 0.05$) differences in mean and median concentrations among groups and no correlation between number of children and organochlorine pesticide levels accumulated in adipose tissues of pregnant women. However, the mean and median concentrations increase from one to two parity, but calculated differences are not statistically significant. The number of children correlated to ages of participants, increasing from one to three. The results indicate that the number of children was not a significant influence in the study population as a decontamination factor. The present environmental conditions in Veracruz, results continuous contamination of its inhabitants and this does not permit time to develop a decontamination mechanism, resulting in permanent accumulation and increase of levels with age.

The apparent decrease in concentrations of the organochlorine pesticides with increased parity may be attributed to the depuration of these chemicals from successive breast-feedings (Waliszewski et al. 2009) because the amount of these pesticides are eliminated by the transplacental route and by lactation. The smaller concentrations for pesticides associated with primiparity participants might be attributable to the smaller diffusion of these pesticides across the environment and the visible decrease in their concentrations in the environment of Veracruz State. The effects of parity and age evaluated in the study on the organochlorine pesticide concentrations support the selection of primiparas as an index of exposure to these chemicals among pregnant women.

The results of organochlorine pesticide levels from pregnant women were grouped according to their Body Mass Index: less than 29, 29–34 and 34–44.5 to determine a possible correlation between Body Mass Index and organochlorine pesticide levels. Results presented in Table 5, show an increase in the levels in the 29–34 group, but the differences were not statistically significant ($p > 0.05$) among mean concentrations, showing a negative correlation between Body Mass Index and organochlorine pesticide levels in pregnant women from Veracruz.

Table 5 Organochlorine pesticide levels (mg/kg on fat basis) in pregnant women divided according to the Body Mass Index (BMI)

Pesticide	Less than 29 mean = 26.3	29–34 mean = 31.0	34–44.5 mean = 37.8
β-HCH	0.026	0.032	0.021
pp′DDE	0.621	0.846	0.716
op′DDT	0.012	0.010	0.011
pp′DDT	0.067	0.107	0.136
Σ-DDT	0.698	0.962	0.863
Age	28.1	24.9	25.1
Parities	2.3	1.6	1.6

In conclusion, the pregnant women living in Veracruz State presented lower rate of exposure compared to control group. Their contamination levels depended principally on the time of exposure, expressed in the age of a person. Parity and Body Mass Index were not found to be significant contributors to organochlorine pesticide levels accumulated in adipose tissue. The comparison of results obtained in this study to a previous one (Waliszewski et al. 2010) show a decrease in organochlorine pesticide concentrations in women living in Veracruz and diminishing trends in their levels.

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