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DDT'S, HCH AND HCB LEVELS IN BREAST ADIPOSE TISSUE IN WOMEN WITH BREAST TUMORS

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ABSTRACT

Organochlorine pesticides have been used in Mexico in agriculture, sanitation, malaria programs and livestock to combat ectoparasites. Due to their chemical stability, persistence and lipophility, their residues bioconcentrate in lipid-rich tissues, according to the equilibrium pattern of internal transport and lipid tissue content. The studies on their role as estrogenic or antiandrogenic agents have related these pesticides to breast cancer. Therefore, organochlorine pesticides were determined in breast adipose tissue of women subjected to surgery due to the diagnosis of breast abnormalities. The study pool constituted 127 women with malignant breast tumors, 127 with benign breast tumors and 127 in a control group without breast abnormalities. The pesticides determined were: HCB, β-HCH, pp'DDE, op'DDT, pp'DDT and Σ-DDT. Comparing the studied groups, the highest residue levels were determined in the benign breast tumor group. The determined levels from the control group through malignant to benign cases increased stepwise; HCB: 0.045, 0.099, 0.116 mg kg^{-1}, β-HCH: 0.163, 0.265, 0.319 mg kg^{-1}, pp'DDE: 0.782, 0.980, 1.761 mg kg^{-1}, op'DDT: 0.035, 0.094, 0.176 mg kg^{-1}, pp'DDT: 0.296, 0.351, 0.661 mg kg^{-1}, Σ-DDT: 1.112, 1.423, 2.601 mg kg^{-1}, respectively. Paired analyses of the three groups revealed divergences among them and correlation of these pesticide levels with breast tumors. Calculated relative risk (RR) values for the benign to control group and the malignant to control cases presented the following values: HCB: 2.11, 2.01, β-HCH: 1.96, 1.58, pp'DDE: 2.13, 1.17, op'DDT: 4.42, 2.27, pp'DDT: 2.33, 1.33, Σ-DDT: 2.21, 1.19 respectively. The obtained results indicate higher organochlorine pesticide residue levels in women with benign breast abnormalities and higher relative risk related to op'DDT presence.

Palabras clave: plaguicidas, tumores de seno

RESUMEN

En México, los plaguicidas organoclorados se ha utilizado en agricultura, salud pública en programas del combate a la malaria y a ectoparásitos de ganado. Debido a su estabilidad química, persistencia y lipofilidad, sus residuos se biomagnifican en tejidos ricos en grasa de acuerdo con el estado de equilibrio entre el transporte interno y el contenido de lípidos en tejidos. Los estudios sobre su papel como agentes estrogénicos o antiandrogénicos relacionan a los plaguicidas organoclorados con el cáncer mamario. Por esta razón se determinaron los niveles de plaguicidas organoclorados en mujeres sujetas a cirugía para diagnosticar anomalías mamarias. En el estudio participaron 127 pacientes con tumor maligno de seno, 127 pacientes con tumor benigno de seno y 127 pacientes como testigos sin anomalías mamarias. Los plaguicidas estudiados fueron: HCB, β-HCH, pp'DDE, op'DDT, pp'DDT y Σ-DDT. Comparando con el grupo testigo, el nivel superior se determinó en las pacientes con tumor benigno de mama. Los niveles de plaguicidas organoclorados desde el grupo testigo al grupo de casos malignos y benignos se incrementó paulatinamente: HCB: 0.045, 0.099, 0.116 mg kg^{-1}, β-HCH: 0.163, 0.265, 0.319 mg kg^{-1}, pp'DDE: 0.782, 0.980, 1.761 mg kg^{-1}, op'DDT: 0.035, 0.094, 0.176 mg kg^{-1}, pp'DDT: 0.296, 0.351, 0.661 mg kg^{-1}, Σ-DDT: 1.112, 1.423, 2.601 mg kg^{-1}, respectivamente. El análisis pareado de medias reveló divergencia entre los tres grupos y correlación de los niveles de plaguicidas organoclorados en el tumor mamario benigno. Al calcular los valores del riesgo relativo (RR) para los grupos con tumor mamario benigno y maligno, se obtuvieron los valores siguientes: HCB: 2.11, 2.01, β-HCH: 1.96, 1.58, pp'DDE: 2.13, 1.17, op'DDT: 4.42, 2.27, pp'DDT: 2.33, 1.33, Σ-DDT: 2.21, 1.19, respectivamente. Los resultados obtenidos indican mayores concentraciones de plaguicidas organoclorados en mujeres con anomalías benignas de seno y un riesgo relativo mayor relacionado con la presencia del op'DDT.

INTRODUCTION

Persistent organochlorine pesticides (HCB-hexachlorobenzene, HCH's- hexachlorocyclohexanes and DDT-dichlorodiphenilethane) have been used extensively in plant protection and sanitary actions throughout the world (Tomlin 2000). Their use permitted the protection of agriculture and the assurance of harvests. In sanitation, they were applied to combat the vectors that transmit malaria and typhoid, as well as ectoparasites in humans and livestock. In the 1970's, their use in agriculture for plant protection was discontinued and recently, in Mexico, since 1999, in sanitation to combat malaria. The common characteristics of these pesticides are persistence in the environment, resistance to metabolic degradation, lipophility and bioconcentration in the lipidic phase of organisms. Thus, their residues persist in the environment: soil, air, plants, and the human food chain (Waliszewski *et al.* 2003a,b), reaching elevated levels in humans (Waliszewski *et al.* 2003c). That is why the determination of the degree of body contamination serves as an indicator of exposure and permits to know the correlation between exposure rate and adverse health effects (Wolff 1995, Wolff and Toniolo 1995, Wolff and Weston 1997, Safe 2000, Snedeker 2001, Zou and Matsumura 2003).

The studies on the possible role of estrogenic or antiandrogenic pesticides in promoting breast cancer have indicated a positive relationship (Robinson and Stancel 1982, Bulger and Kupfer 1983, Bustos *et al.*1988, Kelce *et al.*1995, Golden *et al.* 1998, Woolcott *et al.* 2001, Calle *et al.* 2002, Starek 2003). In these studies, adipose tissue (Güttes *et al.* 1988, Woolcott *et al.* 2001, Waliszewski *et al.* 2003d, Muscat *et al.* 2003), serum or plasma (Schecter *et al.* 1997, Güttes *et al.* 1988, Ward *et al.* 2000, Wolff *et al.* 2000, López-Carrillo *et al.* 2002) that were convenient to particular objectives were selected. The obtained results reached conflicting conclusions indicating positive or negative relationships between organochlorine pesticide levels and the incidence of breast cancer.

There are many reports that determine the rate of exposure to persistent organochlorine pesticides on breast cancer using blood due to the easiness to obtain these samples. However, blood lipid levels may fluctuate, biasing the results. The main contributor of this bias is the transportation mechanism and partitioning of each pesticide into serum lipids, lipoproteins and various blood components according to their physical-chemical properties and blood cell composition (Petreas *et al.* 2004). Therefore, adipose tissue not blood, sample of choice for assessing the steady state of organochlorine pesticides in the body. However, the disadvantage consists in that invasive techniques are required to obtain samples.

The use of breast adipose tissue to determine the level of organochlorine pesticides in breast cancer studies limits the pool to subjects who were submitted to a biopsy after abnormal findings in the breast.

The serum/adipose tissue partition coefficient (Waliszewski *et al.* 2004) is the ratio of the concentration of a pesticide in blood (blood as the transport compartment and adipose tissue as deposit compartment at equilibrium). This indicates the degree at which the pesticide accumulates in fatty tissues of the body. Because of interindividual differences in the toxicokinetics of organochlorine pesticides, the serum/adipose tissue partition coefficients have their own ranges (Waliszewski *et al.* 2004). This makes the comparison of organochlorine pesticide concentrations in blood lipids and lipids of adipose tissue difficult. Minor variations have been observed in the distribution of organochlorine pesticides in various types of adipose tissues (Waliszewski *et al.* 2003e).

The aim of this study was to determine the organochlorine pesticides levels in breast adipose tissue of women subjected to surgical intervention resulting from the diagnosis of breast abnormalities. Moreover, the pool was divided according to the histopathological diagnosis of benign and malignant breast tumors and the results were statistically compared to determine possible differences between study groups and the control group.

MATERIALS AND METHODS

Study design. For the study, 254 patients admitted to the Mexican Institute of Social Security (IMSS) Hospital for breast adipose tissue biopsies were chosen to participate. All patients were voluntary participants, who signed agreements to participate after a clear explanation of the objectives of the study. The preliminary diagnosis of participants submitted to biopsies was breast tumor. The pooled group was divided into malignant (127 cases) and benign (127 cases) breast tumors which were confirmed by a histopathological study. Volunteers were selected from those who have lived for at least one year in Veracruz or its suburban zone and have not presented additional disorders. During biopsy surgery, surgeons' collected small amounts, of approximately 1-3 grams of breast adipose tissue adhered to the tumor.

No differences in organochlorine pesticide contents between abdominal and breast adipose have been found (Waliszewski *et al.* 2003e), so the control group was represented by 127 abdominal adipose tissue samples from women subjected to autopsies in the Institute of Forensic Medicine of the University of Veracruz as the consequence of automobile accidents.

For each participant we asked for additional data: age, place of residence, demographic characteristics, any lactation and medical history that could influence organochlorine pesticide content behavior.

Histopathology. The bioptic samples were processed and evaluated in the Pathology Laboratory of the IMSS Hospital to determine all histological breast lesions. These diagnoses were for invasive malignant diseases with metastasis and benign histological changes.

Sample Analysis

Apparatus

a) *Gas chromatograph* - Varian Model 3400CX (Palo Alto, CA) equipped with ^{63}Ni electron capture detector. Operating conditions: capillary chromatography column J & W Scientific DB-608, 30 m x 0.32 mm id. and 0.83 micron film thickness; temperature program, 193 °C (7 min) rising to 250 °C at 6 °C/min, hold 20 min; carrier gas, nitrogen at 27 cm/min; 1 µL was injected in splitless mode.
b) *Integrator* - PC computer with Star Chromatography Works Station Software Version 4.51
c) *Gas chromatograph - Mass spectrometer.* Varian Model 3800 - Saturn 2000 GC-MS-MS was used to confirm the determination of compounds corresponding to organochlorine pesticide peaks. The confirmation of peaks equivalent to organochlorine pesticides, eluted from DB-608 30 m x 0.32 mm id. and 0.83 micron film thickness capillary column, was performed comparing the obtained mass spectra of substances from adipose tissue extracts to those of standard substances, selecting the following specific ions obtained from an ion trap detector (m/z values) of HCB M+282: 249, 214, 142 of HCH isomers M+288: 254, 219, 181 of *pp'*DDT M+352 and *op'*DDT: M+352: 235, 199, 165 and of *pp'*DDE M+318: 246, 210, 176. In the analyzed samples, from the HCH isomers, only the presence of β-HCH was confirmed in the adipose tissue samples.
d) *Rotary evaporator R114* - Büchi (Flawil, Switzerland)

Reagents

(a) *Solvents* - Hexane Mallinckrodt Nanograde (Kentucky, USA; part No. 4159)
(b) *Sulfuric acid* - 95-97% for analyses (Merck, Mexico City, Mexico; part No. 1/15851).
(c) *Anhydrous sodium sulfate* - Powder (J.T. Baker, part No. 3898-32) heated at 650 °C for 16 hours before use.

(d) *Analytical standards of pesticides* - Hexachlorobenzene (HCB), β- hexachlorocyclohexane (β-HCH), *pp'*DDT, *op'*DDT, *pp'*DDE were purchased from Supelco Inc. (USA)

Adipose tissue analysis. Breast adipose tissue samples adhered to the tumor and abdominal adipose tissue were analyzed for organochlorine pesticide residues, according to the method described by Waliszewski and Szymczynski (1982). Approximately 1-3 grams from a fat sample were ground in a mortar with enough anhydrous sodium sulfate to obtain a coarse powder. The pulverized sample was transferred to a 50 x 1 cm i.d. chromatographic column with a fused-in fritted disk and Teflon stopcock. The fats were extracted with 150 mL hexane, by passing them through the column with a 3 mL/min flow rate and collecting them in a 500 mL round-bottom flask. Ten milliliters of extract were transferred to a previously weighed 50 mL round-bottom flask and the solvent was rotary evaporated to dryness for gravimetric fat determination. Because of the clean-up capacity, the fat content did not exceed 500 mg. Another 10 mL of extract were pipetted into a 15 mL tube with a Teflon stopper and 1 mL concentrated sulfuric acid was added. The tube was stoppered tightly and vigorously shaken for half a minute and then left to stand for 2-3 minutes to produce a good phase separation. The supernatant was decanted, dried by passing it through a 3-5 g layer of sodium sulfate, and washed with hexane. The cleaned-up extract, with rinses collected in another 50 mL round-bottom flask, was rotary evaporated to a few drops and quantitatively transferred to a 1 mL volumetric tube. The volume was adjusted to 1 mL with hexane and 1 μL aliquot was injected into a gas chromatograph for qualitative and quantitative analyses. The concentrated sulfuric acid, used to clean-up fat extracts, allows quantitative fat precipitation and degrades the ubiquitous phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides, permitting their accurate determination (Waliszewski and Szymczynski 1990).

All breast adipose and abdominal tissue samples were analyzed for HCB, β-HCH isomer, *pp'*DDE, *op'*DDT and *pp'*DDT. The minimum detection limits for the analyzed residues were 0.001 mg kg^{-1} for HCB and 0.002 mg kg^{-1} on a fat basis for β-HCH, *pp'*DDE, *op'*-DDT and *pp'*-DDT. To determine the quality of the method, a recovery study was performed on 10 overspiked replicates of a blank cow fat sample, which presented contamination levels below detection limits. The results of the fortification study, performed at 0.01 – 0.02 mg kg^{-1} levels for HCB and β-HCH, *pp'*DDE, *op'*DDT and *pp'*DDT, depending on the pesticide, showed mean values from 91.3 to 97.2 %. The standard deviation ranged from 6.1 to 8.2, indicating excellent method repeatability.

Statistical analysis

Organochlorine pesticide levels from control, malignant and benign groups were calculated using basic statistics, such as mean with standard deviation of means ($\bar{X}$ ± SD), standard error of means (SEM), geometric means (GM), medians and 95 % of confidence intervals (95 % CI). To calculate the variability between control and malignant breast tumor groups, control and benign tumor population and benign versus malignant breast tumor groups, the obtained results were paired applying t-tests to compare differences between mean values. To determine the magnitude of correlation between the study groups, the Pearson correlation coefficients (r), coefficients of determination (r^2), coefficients of regression (β), F-test, that determines differences between variances and differences between medians applying the Mann-Whitney confidence intervals test, were calculated. In order to determine the association between the levels of organochlorine pesticides in breast adipose tissue and breast tumor diseases, the relative risk (RR) was calculated for all organochlorine pesticides.

RESULTS AND DISCUSSION

In the study, adipose tissue samples from 127 women with malignant breast tumors, 127 with benign breast tumors and 127 in the control group without breast lesions were analyzed. The origin of the participants was the City of Veracruz and its suburban zone. The mean age of the benign breast tumor group was 48 years, for the malignant breast tumor group, it was 49 years and for the control group, it was 44 years. The age of one person, when it varies significantly, can influence organochlorine pesticide levels because of a more prolonged accumulation and exposition (Sandau *et al.* 2000, Deutch and Hansen 2000, Glavan-Portillo *et al.* 2002, Voorspoels *et al.* 2002, Waliszewski *et al.* 2002). The determined differences between ages did not vary significantly ($p>0.05$), thus the difference did not influence the organochlorine pesticide levels determined among the studied groups. The number of child births were 1.9 ± 1.6 for benign, 1.9 ± 1.6

for malignant and 2.0 ± 1.9 for the control group, indicating no significant differences ($p>0.05$) resulting from the number of children born to these groups. The lack of differences eliminates the principal factors: age and number of children that can impact the organochlorine pesticide levels in breast adipose tissue of the participants and bias the comparisons.

The comparison of mean organochlorine pesticide levels determined in adipose tissue of the control group and participants with breast illness are presented in **table I** and **figure 1**. The concentrations are expressed on a lipid basis (mg kg^{-1}) as mean values and standard deviation of means ($\bar{X}$ ± SD), standard error of means (SEM), geometric means (GM), median values and 95 % of confidence intervals (95 % CI). **Table I** divides the study pool into the control group, women with malignant breast diseases and the group with benign breast diseases.

The comparison indicated that all mean organochlorine pesticide (HCB, β-HCH, *pp'*DDE, *op'*DDT, *pp'*DDT and Σ-DDT) levels increased stepwise from the control group to malignant cases, finally reaching the highest values in benign breast tumor cases. The HCB levels incremented from 0.045 mgkg^{-1} in the control group to 0.099 mgkg^{-1} in malignant breast cases and to 0.116 mg kg^{-1} in benign breast cases. The β-HCH rose from 0.163 mg kg^{-1} in the control group to 0.265 mg kg^{-1} in malignant breast cases and to 0.319 mg kg^{-1} in benign breast cases. The *pp'*DDE increased from 0.782 mg kg^{-1} in the control group, to 0.980 mg kg^{-1} in malignant breast cases and to 1.761 mg kg^{-1} in benign

TABLE I. MEAN AND STANDARD DEVIATION OF MEANS ($\bar{X}$ ± SD), STANDARD ERROR OF MEAN (SEM), GEOMETRIC MEAN (GM), MEDIAN AND 95 % CI OF ORGANOCHLORINE PESTICIDE LEVELS (mg kg^{-1} on a fat basis) IN ADIPOSE TISSUE OF PATIENTS WITH BREAST TUMOR

Compound	Control (n=127)	Malignant (n=127)	Benign (n=127)
HCB	0.045 ± 0.032	0.099 ± 0.091	0.116 ± 0.158
$\bar{X}$ ± SD	0.003	0.008	0.011
SEM	0.020	0.054	0.060
GM	0.034	0.070	0.065
Median	0.039 – 0.050	0.083 – 0.115	0.089 – 0.144
95 % CI			
β-HCH			
$\bar{X}$ ± SD	0.163 ± 0.119	0.265 ± 0.210	0.319 ± 0.292
SEM	0.011	0.019	0.026
GM	0.126	0.287	0.417
Median	0.135	0.189	0.258
95 % CI	0.142 – 0.183	0.228 – 0.301	0.269 – 0.371
pp'DDE			
$\bar{X}$ ± SD	0.782 ± 0.282	0.980 ± 0.627	1.761 ± 1.090
SEM	0.025	0.056	0.097
GM	1.027	0.911	1.552
Median	0.740	0.901	1.543
95 % CI	0.733 – 0.731	0.871 – 1.089	1.571 – 1.950
op'DDT			
$\bar{X}$ ± SD	0.035 ± 0.027	0.094 ± 0.098	0.176 ± 0.170
SEM	0.002	0.009	0.015
GM	0.046	0.047	0.096
Median	0.029	0.060	0.101
95 % CI	0.031 – 0.040	0.077 – 0.112	0.147 – 0.206
pp'DDT			
$\bar{X}$ ± SD	0.296 ± 0.230	0.351 ± 0.291	0.661 ± 0.569
SEM	0.020	0.026	0.051
GM	0.306	0.370	1.149
Median	0.248	0.251	0.553
95 % CI	0.256 – 0.336	0.301 – 0.402	0.562 – 0.760
Σ-DDT			
$\bar{X}$ ± SD	1.112 ± 0.433	1.423 ± 0.856	2.601 ± 1.461
SEM	0.038	0.077	0.130
GM	1.445	1.340	2.962
Median	1.040	1.219	2.373
95 % CI	1.037 – 1.187	1.274 – 1.572	2.347 – 2.855

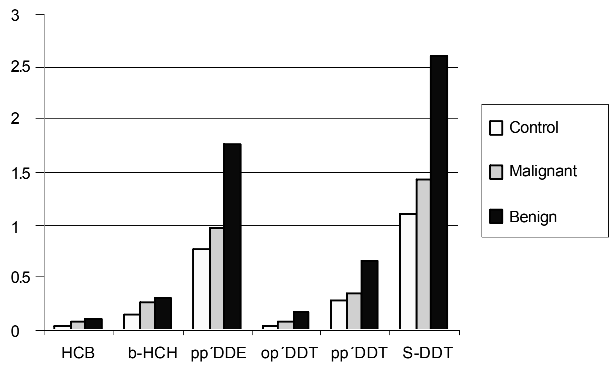

Fig. 1. Comparison of mean (mg kg^{-1} on fat basis) organochlorine pesticide levels among controls, malignant and benign breast tumor cases

breast cases. The *op'*DDT increased from 0.035 mg kg^{-1} in the control group, to 0.094 mg kg^{-1} in malignant breast tumors and to 0.176 mg kg^{-1} in benign breast cases. The insecticide *pp'*DDT augmented from 0.296 mg kg^{-1} in the control group to 0.351 mg kg^{-1} in malignant breast tumors and to 0.661 mg kg^{-1} in benign cases. The sum of DDT (Σ-DDT= *pp'*DDE + *op'*DDT + *pp'*DDT) was 1.112 mg kg^{-1} in the control group, 1.423 mg kg^{-1} in malignant breast tumors and 2.601mg kg^{-1} in benign breast tumors.

The standard deviation of means (SD) indicates how spread out the data are. The SD values for all organochlorine pesticides do not exceed the means, indicating that the data points are not scattered significantly. The standard error of means (SEM) measures how precise the population means is, indicating the extent of variability of observations. The calculated results are low and oscillated from 3 to 9 % of mean value, showing a low scattering of populations. The geometric mean (GEM) values confirm the previous conclusion on the incremented tendency of organochlorine pesticide levels from the control group to the malignant breast tumor pool pronouncing their significant increase in the benign breast cancer group. Calculated medians of organochlorine pesticide concentrations evidenced lower values than the means, expressing that the majority of the obtained results are placed within lower levels. The medians present the same tendency as arithmetic and geometric means of increasing from control to malignant and benign breast tumor groups.

To demonstrate the magnitude of differences among the studied groups (control group vs. benign breast tumor, control group vs. malignant breast tumor, benign breast tumor vs. malignant breast tumor), **table II** presents the results of statistical comparison of means and variances. For that reason, paired t-tests (that calculated the actual differences of means between two populations studied), Pearson correlation coefficients (*r*), coefficients of determination (r^2), the regression coefficient (β) (to determine the magnitude of differences) and F-test (to determine the equality or homogeneity of variances), were calculated.

The HCB results demonstrated statistically significant differences ($p<0.05$) between mean levels of control vs. benign and control vs. malignant breast tumor cases. The comparison between benign and malignant groups revealed no statistical differences (p=0.2939). Moreover, the correlation and regression tests showed lower results, indicating different origins in the three study groups. The same behavior was present in β-HCH levels, evidencing significant differences between control - benign and control - malignant breast tumor cases (p=0.0001) but no differences between benign and malignant cases (p=0.0855). The correlation and regression analyses demonstrated that the three study groups have different origins and presented bad correlations among them.

The calculation made for *pp'*DDE *op'*DDT and Σ-DDT manifested statistically significant differences among mean residue levels of these compounds in the three study groups (p=0.0001). The comparison of correlation and regression coefficients, to determine the degree of linear relationship, suggested bad correlations among study groups and different accumulation rates of organochlorine pesticides in control, benign and malignant cases. The comparison of *pp'*DDT levels between control and benign breast tumor patients demonstrated significant differences between means (p=0.0001) and a bad correlation, whereas the comparison between control and malignant breast cancer patients indicated no differences between means (p=0.0949) and a bad correlation between these groups. The calculations made for benign and malignant breast tumor patients revealed significant differences between mean levels (p=0.0001) and a bad correlation (r= -0.0306).

To specify the behavior of organochlorine pesticide levels between control, benign and malignant groups, the F-test was applied to determine equality of variances. When the F-value is higher than 1, it indicates that variances are unequal. For the study groups, only β-HCH (benign vs. malignant, F=0.0003) and *pp'*DDT (control vs. malignant, F=0.0089) variances values were not different, reaffirming the previous results of mean comparison (p=0.0855 and p=0.0949) and lack of differences

TABLE II. STATISTICAL COMPARISON: *t*-TEST (p), PEARSON CORRELATION COEFFICIENT (r), COEFFICIENT OF DETERMINATION (r^2), REGRESSION COEFFICIENT (β) AND F-TEST (F) AMONG CONTROLS, MALIGNANT AND BENIGN BREAST TUMOR GROUPS

Compound	Control vs. benign	Control vs. malignant	Benign vs. malignant
HCB	0.045 vs 0.116	0.045 vs 0.099	0.116 vs 0.099
p	0.0001	0.0001	0.2939*
r	-0.0130	0.0983	0.1172
r^2	0.0002	0.0097	0.0137
β	-0.0026	0.0341	0.2033
F	4.471	5.960	1.705
β-HCH	0.163 vs 0.319	0.163 vs 0.265	0.319 vs 0.265
p	0.0001	0.0001	0.0855*
r	-0.0623	0.1599	0.1672
r^2	0.0039	0.0256	0.0279
β	-0.0254	0.0882	0.2331
F	2.687	5.676	0.0003
op'DDE	0.782 vs 1.761	0.782 vs 0.980	1.761 vs 0.980
p	0.0001	0.0013	0.0001
r	-0.2953	0.0299	-0.0321
r^2	0.0381	0.0009	0.0011
β	-0.0505	0.0135	-0.0558
F	1.788	1.332	1.426
op'DDT	0.035 vs 0.176	0.035 vs 0.094	0.176 vs 0.094
p	0.0001	0.0001	0.0001
r	-0.1602	0.1513	0.0859
r^2	0.0256	0.0229	0.0074
β	-0.0259	0.0429	0.1503
F	4.050	1.267	3.223
pp'DDT	0.296 vs 0.661	0.296 vs 0.351	0.661 vs 0.351
p	0.0001	0.0949*	0.0001
r	-0.1308	0.0736	-0.0306
r^2	0.0171	0.0054	0.0009
β	-0.0528	0.0582	-0.0598
F	1.097	0.0089	3.556
Σ-DDT	1.112 vs 2.601	1.112 vs 1.423	2.601 vs 1.423
p	0.0001	0.0003	0.0001
r	-0.1897	0.1056	-0.1099
r^2	0.0359	0.0111	0.0121
β	-0.0562	0.0534	-0.1877
F	4.976	1.839	4.529

* no significant differences at $p<0.05$

between means. For all other pesticides and comparison groups, the F-values were higher than one, indicating that the standard deviations are not equal, reaffirming the existence of differences among means.

In general, the organochlorine pesticide levels determined in adipose tissue of the three patient groups presented differences. From the control group, the levels increased significantly in benign breast tumors that constituted non invasive breast tumors. In malignant breast tumors, the increase of organochlorine pesticide levels was significant (except *pp'*DDT) but the increase was not so clearly pronounced as in benign cases. The comparison of obtained results between benign and malignant cases, presented only significant differences among the DDT family, indicating higher values in benign than in malignant cases and the correlation of these diseases to organochlorine pesticide levels determined in breast adipose tissue (Siddiqui *et al.* 2005).

For the comparison of two population medians, a two-sample Mann-Whitney test was applied (**Table III**). The test computes the equality of two population medians, corresponding point estimate and confidence intervals. The calculation, made for all study groups, asseverated the previous statements on differences (α values smaller then 0.05) and divergences between the population medians in the three

TABLE III. RESULTS OF MANN-WHITNEY CONFIDENCE INTERVAL TESTS TO DETERMINE DIFFERENCES AMONG STUDY GROUPS (CONTROL, BENIGN AND MALIGNANT BREAST TUMOR PATIENTS)

Pesticide		Medians	difference	W	95 % CI		α=0.05
HCB	b/c	0.065/0.034	0.028	20273	0.020,	0.037	0.000
	m/c	0.070/0.034	0.031	20173	0.021,	0.042	0.000
HCH	b/c	0.258/0.135	0.106	20158	0.076,	0.141	0.000
	m/c	0.189/0.135	0.062	18835	0.036,	0.095	0.000
pp'DDE	b/c	1.543/0.740	0.785	22446	0.632,	0.943	0.000
	m/c	0.901/0.740	0.122	17535	0.019,	0.229	0.022
op'DDT	b/c	0.101/0.029	0.074	22754	0.057,	0.102	0.000
	m/c	0.060/0.029	0.031	20591	0.023,	0.040	0.000
pp'DDT	b/c	0.553/0.248	0.252	19899	0.161,	0.366	0.000
	m/c	0.251/0.248	0.032	17033	- 0.014,	0.079	0.151*
Σ-DDT	b/c	2.373/1.040	1.269	22138	1.019,	1.518	0.000
	m/c	1.219/1.040	0.173	17635	0.036,	0.317	0.014

c= controls, b= benign breast tumors, m= malignant breast tumors
*difference not significant at α=0.05

study groups with the exception of *pp'*DDT. The Mann-Whitney medians comparison test indicated α=0.151 between control and malignant breast tumor cases for *pp'*DDT, stating the previous conclusions of no differences between medians, means (p= 0.0949) and standard deviations (F= 0.089).

To determine the relationship between environmental exposure to organochlorine pesticides and its consequences for human health, the relative risk (RR) and its 95 % confidence intervals (CI) were calculated (**Table IV**). The values obtained for benign cases indicated higher relative risk than those determined for malignant cases, suggesting that their development is correlated to organochlorine pesticide exposure. From all compounds studied, the highest RR values were those pertaining to *op'*DDT in benign breast tumor cases (4.42; 95 % CI 4.22 – 4.62). The compound is known as a highly estrogenic agent that can interfere in the hormonal balance in women's bodies (Bulger *et al.* 1983, Bustos *et al.* 1988, Robinson *et al.* 1982). The second place corresponded to *pp'*DDT (2.33; 95 % CI 2.23 – 2.43), Σ-DDT (2.21; 95 % CI 2.15 – 2.28), *pp'*DDE (2.13; 95 % CI 2.06 – 2.21) and HCB (2.11; 95 % CI 1.98 - 2.25) in benign breast tumor cases. The values of relative risk (RR) indicated strong relationships between the organochlorine pesticide presence in human breast adipose tissue and a higher risk of developing benign breast tumors and in consequence a stronger relationship to active hormonal compounds. The obtained RR results are in concordance with those calculated by Muscat *et al.* (2003) and Siddiqui *et al.* (2005), expressing the existence of an elevated risk from these compounds on human health.

TABLE IV. RELATIVE RISK (RR) VALUES CALCULATED FOR BENIGN AND MALIGNANT BREAST TUMOR PATIENTS

Compound	Relative risk (RR)	95 % CI
HCB benign	2.11	1.98 – 2.25
HCB malignant	2.01	1.94 – 2.07
β-HCH benign	1.96	1.90 – 2.01
β-HCH malignant	1.58	1.54 – 1.62
pp'DDE benign	2.13	2.06 – 2.21
pp'DDE malignant	1.17	1.11 – 1.23
op'DDT benign	4.42	4.22 – 4.62
op'DDT malignant	2.27	2.18 – 2.37
pp'DDT benign	2.33	2.23 – 2.43
pp'DDT malignant	1.33	1.25 – 1.41
Σ-DDT benign	2.21	2.15 – 2.28
Σ-DDT malignant	1.19	1.15 – 1.23

In conclusion, our study, carried out in the tropical region of Mexico, considered as contaminated with organochlorine pesticide residues applied principally to combat malaria, produced results that indicate the correlation of their presence and levels in breast adipose tissue to breast diseases. This level correlates principally to benign breast diseases in women living in Veracruz, Mexico.

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