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Persistent Organochlorine Pesticide Levels in Breast Adipose Tissue in Women with Malignant and Benign Breast Tumors

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Persistent organochlorine pesticides (HCB- hexachlorobenzene, HCH's-hexachlorocyclohexanes and DDT-dichlorodiphenilethane) have been used extensively since the early 1940's in plant protection and sanitary actions throughout the world. The use permitted the protection of agriculture efforts and the assurance of harvests. In sanitation, they were applied to combat the vectors that transmit malaria, typhoid and ectoparasites in humans and livestock. In the 1970's, their were discontinued in agriculture for plant protection and since 1999 in Mexico, 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, the residues persist in the environment: soil, air, plants, and the human food chain (Waliszewski et al. 2003a, 2003b), 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 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) that were convenient to particular objectives were selected. The obtained results reached conflicting conclusions that indicated positive or negative relationships between organochlorine pesticide levels and the incidence of breast cancer.

A blood sample has been used to determine the rate of exposure to persistent organochlorine pesticides in many breast cancer studies. The concentrations determined in adipose tissue reflect a steady state of organochlorine pesticides compared to the blood samples, whereas blood

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lipids levels may fluctuate, biasing the results. The principal contributor of this bias is the transport 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). 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).

Adipose tissue is the sample of choice for assessing the steady state of organochlorine pesticides in the body. The disadvantage consists, 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 aim of the 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

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 histopathological study. Volunteers were selected from those who have lived for a 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.

The control pool, 127 abdominal adipose tissue samples from women subjected to autopsies as the consequence of automobile accidents, were collected. Because no differences in organochlorine pesticide contents between abdominal and breast adipose tissue were determined (Waliszewski et al. 2003e), the control group needed to be abdominal

adipose tissue samples from women subjected to autopsy in the Institute of Forensic Medicine of the University of Veracruz.

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.

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

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). 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 for HCB and 0.002 $mgkg^{-1}$ 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 $mgkg^{-1}$ 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.

Organochlorine pesticide levels from control, malignant and benign groups were calculated using basic statistics, such as mean with standard deviation of means ($x \pm SD$). 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. 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 age and number of children as factors that can impact the organochlorine pesticide levels in breast adipose tissue of the participants.

Table 1. Mean and standard deviation of means (X±SD) of organochlorine pesticide levels ($mgkg^{-1}$ 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
β-HCH	0.163 ± 0.119	0.265 ± 0.210	0.319 ± 0.292
pp'DDE	0.782 ± 0.282	0.980 ± 0.627	1.761 ± 1.090
op'DDT	0.035 ± 0.027	0.094 ± 0.098	0.176 ± 0.170
pp'DDT	0.296 ± 0.230	0.351 ± 0.291	0.661 ± 0.569
Σ-DDT	1.112 ± 0.433	1.423 ± 0.856	2.601 ± 1.461

The comparison of mean organochlorine pesticide levels determined in adipose tissue of the control group and participants with breast illness are presented in table 1. The results are divided into the control group, women with malignant breast diseases and the group with benign breast diseases. The concentrations are expressed on lipid base ($mgkg^{-1}$) as mean values and standard deviation of means (x ± SD).

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 $mgkg^{-1}$ in benign breast cases. The β-HCH rose from 0.163 $mgkg^{-1}$ in the control group to 0.265 $mgkg^{-1}$ in malignant breast cases and to 0.319 $mgkg^{-1}$ in benign breast cases. The *pp*'DDE increased from 0.782 $mgkg^{-1}$ in the control group, to 0.980 $mgkg^{-1}$ in malignant breast cases and to 1.761 $mgkg^{-1}$ in benign breast cases. The *op*'DDT incremented from 0.035 $mgkg^{-1}$ in the control group, to 0.094 $mgkg^{-1}$ in malignant breast tumors and to 0.176 $mgkg^{-1}$ in benign breast cases. The insecticide *pp*'DDT augmented from 0.296 $mgkg^{-1}$ in the control group to 0.351 $mgkg^{-1}$ in malignant breast tumors and to 0.661 $mgkg^{-1}$ in benign cases. The sum of DDT (Σ-DDT= *pp*'DDE + *op*'DDT + *pp*'DDT) was 1.112 $mgkg^{-1}$ in the control group, 1.423 $mgkg^{-1}$ in malignant breast tumors and 2.601$mgkg^{-1}$ in benign breast tumors.

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), the results were

paired to calculate t-tests, determining real differences of means between two populations studied. The results demonstrated statistically significant differences at p=0.05 between mean levels of control vs. benign and control vs. malignant breast tumor cases, especially for *op*'DDT, Σ-DDT, *pp*'DDE and β-HCH. Moreover, the correlation and regression tests confirmed results that indicating different origins of the three study groups and bat correlation among them.

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 at p=0.05 (except *pp*'DDT) but the increase was not as 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 benign disease to organochlorine pesticide levels determined in breast adipose tissue, observation that correlate with Siddiqui et al. 2005.

Table 2. Relative risk (RR) values calculated to 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

To determine the relationship between environmental exposure to organochlorine pesticides and its consequences for human health, the relative risk (RR) and its 95% CI were calculated (table 2). The values obtained for benign cases indicated higher relative risk values 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 (4.26; 95%CI 2.81 – 5.73). 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 Σ-DDT in benign breast tumor cases (2.57; 95% CI 1.42 – 3.24). The values of relative risk (RR) indicated strong

relationships between the organochlorine pesticide family 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 relationship, expressed in relative risk values (RR) higher than two, was noted also for *pp*'DDE and HCB. 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 to develop benign breast lesion.

The residues of organochlorine pesticides still persist in the environment and drift from contaminated areas, propagated by the wind around the world, exposing people and all living organisms.

Our study indicated the correlation of organochlorine pesticides in breast adipose tissue to benign breast diseases in women.

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