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Copartition Ratios of Persistent Organochlorine Pesticides Between Human Adipose Tissue and Blood Serum Lipids

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Organochlorine pesticides have been used in tropical countries in agriculture, public health programs and against ectoparasites in livestock and humans. The pesticides employed in tropical countries have provided great benefits to inhabitants, controlling the spread of vector-borne diseases and ectoparasites. Due to the persistence and lipophility, the organochlorine pesticides tend to accumulate in lipid-rich tissues of organisms, exhibiting desirable and undesirable effects (Travis et al. 1988, Charlier and Plomteux 2002, Waliszewski et al. 2003a). After entering the organism, the organochlorine pesticides come up a steady state and are bioconcentrated according to an equilibrium pattern in the lipid-rich compartment of tissues (Mussalo-Rauhamaa 1991, Waliszewski et al. 2001, 2002). Thus, the lipid-rich tissues, such as adipose tissue, act as depots or reservoirs of persistent chemical substances through the physiochemical interactions of cellular components (Mussalo-Rauhamaa 1991). The analyses of human lipid-rich compartments, such as adipose tissue and blood serum lipids, reflect the rate of environmental exposure and permit to quantification of food and human contamination associated with the health risk (Waliszewski et al. 2000).

Recently, discussions have arisen between the possible tie of the organochlorine pesticides and breast cancer (Aronson et al. 2000; Safe 2000; Woolcott et al. 2001, Waliszewski et al. 2003a). To establish the function of organochlorine pesticides in the development of breast cancer, the study was carried out analyzing human serum levels and the extent of their accumulation in the adipose tissue (Lopez-Carrillo et al. 1997, Archibeque-Engle et al. 1997, Aronson et al. 2000). In epidemiological studies, human serum has been considered an easier sample to obtain than adipose tissue. However, it is uncertain, whether the serum sample gives adequate results to those of breast adipose tissue or if it should be the appropriate medium to establish the relationships between organochlorine pesticide accumulation status and breast cancer (Waliszewski et al. 2003b). The results of a comparison study between abdominal and breast adipose tissue statistically indicates no significant differences in organochlorine pesticide concentrations (Waliszewski et al. 2003c).

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The purpose of the present study was to determine the levels of organochlorine pesticides in abdominal adipose tissue and blood serum lipids and compare these concentrations calculating copartition coefficients of these pesticides between adipose and blood lipids.

MATERIALS AND METHODS

Samples of blood (10 mL approximately) from the ventricular heart cavity and abdominal adipose tissue (5 g approximately) were taken from one hundred twenty six selected adult cadavers, submitted to autopsy at the Institute of Forensic Medicine of the University of Veracruz. The adipose samples were stored in pre-treated glass jars, immediately frozen and kept at -25°C until analyzed. The blood samples were centrifuged to separate the serum from blood cells and total serum lipid contents were determined. The remaining serum content was weighed to determine the real weight of sample and the total lipid content in the sample.

Abdominal adipose tissue and blood serum samples were analyzed according to the methods described by Waliszewski and Szymczynski (1982, 1991). The qualitative and quantitative determinations were done by gas chromatography on a Varian 3400 CX apparatus, equipped with a ^{63}Ni electron capture detector. For pesticide separation, according to the US EPA Method 608, a fused silica column SPB 608 30 m X 0.32 mm ID, 0.5 µm film was used at the following temperature program: 193°C (for 7 min) to 250°C at 6°C/min, held 20 min. The carrier gas was nitrogen at 25 cm/min, and split/splitless sample injection of 1 µL in splitless mode was employed.

All samples were analyzed for: HCB, β-HCH, pp'DDE, op'DDT and pp'DDT. The minimum detection limits expressed on fat basis for the organochlorine pesticides studied were: 0.001 mg/kg for HCB and β-HCH, 0.002 mg/kg for pp'DDE, op'DDT and pp'DDT. To determine the quality of the method, the recovery study was performed on ten overspiked replicates of a blank cow blood serum sample and a blank cow fat sample, which revealed contamination levels below detection limits. The fortification study, done at 0.010 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 89 to 96% of recovery. The standard deviation and coefficients of variation were below 9, indicating excellent repeatability of the method. The concentrated sulfuric acid used in the clean-up step of serum and adipose tissue extracts, permits quantitative fat precipitation and degrades the ubiquitous phthalate esters that interfere in the gas chromatographic identification of organochlorine pesticides (Waliszewski y Szymczynski 1990).

Total serum lipids were determined colorimetrically with phosphovanillin according to the method recommended by Wiener Lab for clinical laboratories (Anonymous 1996).

To compare variability between adipose tissue and blood serum organochlorine pesticide concentrations, paired T-Test and Tukey-Kramer multiple comparison were calculated using the statistical software Minitab version 12.

RESULTS AND DISCUSSION

The deposition and concentration distribution of persistent organochlorine pesticides in the human body involve an internal transport and posterior equilibrium pattern between adipose tissue and blood serum lipids. This pharmacological model describes the internal distribution of lipophilic compounds, when it is expressed on fat basis (Parham et al. 1997). The equilibrium between both compartments is defined as chemical fugacity between adipose tissue and blood serum (Noren et al. 1999). The movement of persistent organochlorine pesticides across biological barriers and membranes occurs bidirectionally and the mode of passage is applicable in both directions (Waliszewski et al. 2001). The liposolubility is a major factor that influenced the rate of accumulation and elimination from tissues and organs (Brown and Lawton 1984). This indicates, that apparently equal concentrations can exist in compartments of the organism and the existing differences are originated by an unevenly balanced lipid content of the tissues (Henriksen et al. 1998, Russell et al. 1999).

During the study, abdominal adipose tissue and blood samples taken during autopsies were analyzed to determine the organochlorine pesticide residue concentrations. The obtained results, expressed on fat basis (mg/kg) are presented in Table 1 as mean values (X), standard deviation of means (SD), two-tailed P value that compares differences between means. Moreover, the Table 1 presents the copartition coefficients of persistent organochlorine pesticide levels between adipose tissue and blood lipids. The comparison of mean and standard deviation values for all organochlorine pesticides between both sample groups indicates the significantly higher values of serum lipids vs adipose lipids expressed as mg/kg on lipid base (HCB 0.178 vs 0.055, β-HCH 0.504 vs 0.216, pp'DDE 2.789 vs 1.063, op'DDT 0.130 vs 0.062 and Σ-DDT 3.258 vs 1.706). The comparison points out the existence of real differences between the study samples. Only pp'DDT reveals 0.340 mg/kg in serum lipids compared to 0.585 mg/kg in abdominal lipids. The inverse levels of pp'DDT are probably due to the prolonged past use of DDT in the combat of vector-transmitting diseases that cause permanent exposure of the inhabitants, who inhaled the DDT volatilized from contaminated areas. This inverse level may also be due to its higher accumulation in adipose fats. The great differences between adipose tissue and blood serum are expressed by standard deviation values, which approximate to the means and are significantly higher for β-HCH, op'DDT and pp'DDT in serum lipids group. The higher levels in blood serum lipids express that these organochlorine pesticides are inclined to the blood lipids as a body compartment and that the equilibrium pattern favors blood serum lipids. To determine the variability between means, a two-tailed comparison test was applied, showing for all pesticides P<0.0001 values and extremely significant differences between means of organochlorine pesticide concentrations in abdominal adipose tissue and blood serum lipids. Moreover, this comparison points out the major incompatibility of serum blood lipids to indicate abdominal adipose tissue accumulation.

Table 1. Comparison of organochlorine pesticide levels (mg/kg on fat basis) between abdominal adipose tissue and blood lipids, two-tailed P-value and copartition coefficients.

Pesticide	Adipose tissue (n = 126)	Blood lipids (n = 126)	Two-tailed P-value	Copartition coefficients
HCB	0.055 ± 0.051	0.178 ± 0.167	0.0001*	0.379 ± 0.212
β-HCH	0.216 ± 0.201	0.504 ± 0.529	0.0001*	0.477 ± 0.218
pp'DDE	1.063 ± 0.543	2.789 ± 1.714	0.0001*	0.487 ± 0.295
op'DDT	0.062 ± 0.079	0.130 ± 0.165	0.0001*	0.570 ± 0.332
pp'DDT	0.585 ± 0.574	0.340 ± 0.397	0.0001*	2.159 ± 0.973
Σ-DDT	1.706 ± 1.034	3.258 ± 1.997	0.0001*	0.651 ± 0.414

*extremely significant differences between means

Table 2. Mean copartition coefficient values and standard deviations depending on blood lipid level (mg/dL).

Pesticide	Total	< 500	500-800	> 800
HCB	0.379 ± 0.211	0.352 ± 0.210	0.376 ± 0.214	0.401 ± 0.216
β-HCH	0.477 ± 0.218	0.441 ± 0.217	0.508 ± 0.208	0.471 ± 0.228
pp'DDE	0.487 ± 0.295	0.421 ± 0.226	0.486 ± 0.358	0.533 ± 0.260
op'DDT	0.570 ± 0.332	0.604 ± 0.234	0.499 ± 0.244	0.620 ± 0.442
pp'DDT	2.159 ± 0.973	2.348 ± 0.919	2.082 ± 1.015	2.112 ± 0.968
Σ-DDT	0.651 ± 0.414	0.655 ± 0.553	0.610 ± 0.382	0.689 ± 0.336

During the study, determined blood lipid concentrations varied depending on the clinical history of each person. The studied pool included hospitalized persons, who for a few days after automobile accidents were artificially fed with infusions, presented blood lipid level below 500 mg/dL. The second group in the pool were dead persons with a normal 500-800 mg/dL blood serum lipid level. The third group constituted dead persons who had been hyperlipidemic with a blood lipid level higher then 800 mg/dL.

Table 2 shows the means and standard deviations of copartition coefficients between serum lipids and adipose tissue lipids calculated for all organochlorine pesticides studied depending on blood lipid level. For the total pool of 126 paired samples, the copartition coefficients follows: for

HCB 0.378 ± 0.211, for β-HCH 0.477 ± 0.218, for pp'DDE 0.487 ± 0.295, for op'DDT 0.570 ± 0.332, for pp'DDT 2.159 ± 0.973 and for Σ-DDT 0.651 ± 0.414. From all of them, only pp'DDT copartition coefficient was higher than one and for the remaining pesticides it was below one. The indicates that in the blood lipids, the organochlorine pesticide concentrations remain higher and that the equilibrium favors the blood compartment. Only absorbed pp'DDT is captured with a higher force to adipose tissue and is accumulated in its lipids.

To evaluate the possible differences among lipid serum levels, the total sample pool was divided in three groups depending on the blood lipid level: up to 500 mg/dL, 500-800 mg/dL and higher then 800 mg/dL. For HCB, the copartition coefficient increased from 0.352 for the persons whose lipid level was below 500 mg/dL to 0.376 for 500-800 mg/dL and to 0.401 in persons with higher then 800 mg/dL of lipid content. The β-HCH increased from 0.441 in persons with a lower blood serum lipid content to 0.508 in the group with normal blood lipid content and decreased to 0.471 in hyperlipidemic persons. The pp'DDE copartition coefficients increased from 0.421 to 0.486 in normal persons and up to 0.533 in the hyperlipidemic group. The op'DDT copartition coefficient levels decreased from 0.604 to 0.499 in persons with normal lipid levels and increased to 0.620 as the blood lipid content increased. The insecticide pp'DDT revealed a decreased tendency, diminishing its level from 2.348 to 2.082 and 2.112, depending on the blood serum level respectively. As blood lipid content increases, the pp'DDT level decreases in the blood serum lipids, indicating lower contamination of ingested lipids and dilution of pp'DDT in the blood stream by these lipids. The Σ-DDT copartition coefficient was influenced by pp'DDE, op'DDT and pp'DDT. This behavior shows a diminution from 0.655 to 0.610 and a subsequent increase to 0.689 in hyperlipidemic persons. To evaluate the possible differences of copartition coefficients among means, the Tukey-Kramer multiple comparison test was applied. This compares possible differences among means. For all pesticides and among the three lipid levels, the results for all organochlorine pesticides were $P>0.05$. This indicates no significant differences among means of the three copartition coefficients groups. Moreover, there is an individual pattern of accumulation and equilibrium of these organochlorine pesticides between blood serum lipids and lipids of adipose tissue.

In light of these results it can be assumed that there is no lineal correlation between concentrations of persistent organochlorine pesticides in adipose tissue and serum lipids. The serum sample of organochlorine pesticide concentrations varied depending on the exposure level. The copartition coefficients express that organochlorine pesticides accumulate in adipose tissue lipids where they are stored and enter in the equilibrium with blood lipids depending on their physicochemical properties. In addition, regarding the high variability expressed by standard deviation values, this make it impossible to draw an unequivocal conclusion in exactly expressing the human body burden on serum blood levels, making a blood sample a poor predictor in the evaluation of environmental and human contamination with organochlorine pesticides (Archibeque-Engle et al. 1997, Güttes et al. 1998, Strucinski et al. 2000, Waliszewski et al. 2003a).

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