

# Carry-over of persistent organochlorine pesticides through placenta to fetus

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**Waliszewski SM, Aguirre AA, Infanzón RM, Siliceo J. Carry-over of persistent organochlorine pesticides through placenta to fetus. Salud Publica Mex 2000;42:384-390.**

## Abstract

**Objective.** As a consequence of environmental exposure, organochlorine pesticides accumulate in lipid rich-tissues such as maternal adipose tissue and partition to maternal blood serum and umbilical blood serum. To establish their distribution in the human body, the concentration gradients of organochlorine pesticides between these compartments were determined. **Material and methods.** Maternal adipose tissue, blood serum and umbilical blood serum samples from 64 volunteers admitted for cesarean delivery at Hospital Benito Coquet Lagunes were studied in Veracruz during 1997 and 1998. The pesticide residues were determined by gas chromatography and results obtained from different sample groups were analyzed using Pearson correlation coefficients and simple lineal regression. **Results.** Significant results expressed on fat basis of organochlorine pesticides indicate that 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) levels are higher in maternal adipose tissue (4.51 mg/kg DDE and 1.27 mg/kg pp'DDT), maternal blood serum (4.45 mg/kg DDE and 0.78 mg/kg pp'DDT), and umbilical blood serum (4.70 mg/kg DDE and 0.88 mg/kg pp'DDT), due to greater affinity of DDT for lipids. **Conclusions.** The statistical evaluation of results and the pairing of samples analyzed indicate that absorbed organochlorine pesticides cross the placental barrier and reach a balanced state between mother and fetus.

Key words: xenoestrogens; placental barrier; uteroplacental circulation; pesticides; Mexico

**Waliszewski SM, Aguirre AA, Infanzón RM, Siliceo J. Paso de los plaguicidas organoclorados persistentes a través de placenta al feto. Salud Publica Mex 2000;42:384-390.**

## Resumen

**Objetivo.** Como consecuencia de la exposición ambiental a los plaguicidas organoclorados éstos se acumulan en tejidos ricos en grasa, como el adiposo materno, y se distribuyen en el suero materno y el suero del cordón umbilical. Para establecer la distribución en el organismo humano, se comparó el gradiente de concentración de los plaguicidas organoclorados entre estos compartimentos. **Material y métodos.** Se tomaron las muestras de tejido adiposo materno, suero materno y suero del cordón umbilical de 64 participantes voluntarias admitidas para cesárea en el Hospital Benito Coquet Lagunes, de la ciudad de Veracruz, Veracruz, México, durante el periodo 1997-1998. Los residuos de plaguicidas se determinaron por cromatografía de gases y sus resultados se correlacionaron entre las muestras por medio del coeficiente de correlación de Pearson y regresión lineal simple. **Resultados.** Los resultados más significativos expresados en base lipídica indican que las concentraciones del DDT fueron más altas en el tejido adiposo materno (4.51 mg/kg DDE y 1.27 mg/kg pp'DDT), suero materno (4.45 mg/kg DDE y 0.78 mg/kg pp'DDT) y suero del cordón umbilical (4.70 mg/kg DDE y 0.88 mg/kg pp'DDT), debido a su mayor afinidad a los lípidos. **Conclusiones.** La evaluación estadística de los resultados y el pareado entre las muestras indican que los plaguicidas organoclorados absorbidos atraviesan la barrera placentaria y forman un equilibrio entre el organismo materno y el feto.

Palabras clave: xenoestrógenos; barrera placentaria; circulación uteroplacentaria; plaguicidas; México

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Since their introduction in agriculture and public health, persistent organochlorine pesticides, such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and hexachlorocyclohexane (HCH), have provided great benefits to humans. Their intensive use throughout decades has raised the interest in knowing the extent of their spread, leading to investigations of the magnitude of their residues in all elements of the human environment.<sup>1</sup> Investigations involving possible health and environmental hazards, have led governments of many countries to ban DDT, or to restrict the minimum accepted values of their residues in foodstuffs. In the 1980's, banned DDT reappeared, being recommended by WHO<sup>2</sup> as the pesticide of choice in the combat of malaria vectors susceptible to DDT in tropical areas.<sup>3</sup>

In tropical countries like Mexico, the extensive use of organochlorine pesticides in sanitary actions has great implications regarding their persistence in the environment and subsequent human contamination.<sup>4,5</sup> DDT is useful in sanitation as the insecticide of choice in the combat of susceptible malaria vectors.<sup>6</sup> Because of DDT's volatility and widespread propagation, the main route of human exposure in tropical areas consists of inhalation of contaminated air.<sup>7,8</sup> An alternative route of human exposure consists of consumption of contaminated food, especially food of animal origin.<sup>9,10</sup> Organochlorine pesticides, due to their lipophilic nature and high persistence, accumulate in food chains and in the human body, especially in lipid-rich tissues, such as adipose tissue. The presence of organochlorine pesticide residues in human adipose tissue has recently caused concern due to their antiandrogenic and estrogenic properties and their effects on sexual activity and development of breast cancer.<sup>1,11-13</sup> The contamination rate of tropical area inhabitants depends mainly on individual accumulation. This is influenced by local environmental pollution, diet, duration of exposure, age, capacity for elimination by metabolism, and number of nursed infants.<sup>14-20</sup> Moreover, the monitoring of human tissues could be used as an indicator for understanding the biological specificity in the accumulation of organochlorine pesticides in the tropical environment, as well as for assessing their participation in environmental pollution.<sup>21</sup>

The aims of the present study are two: a) to compare the concentrations of selected organochlorine pesticides in maternal adipose tissue, maternal blood serum, and umbilical blood serum, as a consequence of environmental exposure to these pesticides in tropical areas of Mexico, due to their spraying in the combat of vector-transmitted diseases; and b) to correlate their concentration gradients among human body matrices.

## Material and methods

### Study design

In the study, sixty-four volunteer mothers among patients admitted for cesarean delivery to Social Security Hospital Benito Coquet Lagunes in Veracruz during 1997-1998, were chosen to participate. Volunteers who signed consent, were selected from those who had lived for at least one year in Veracruz or its suburban zone and had not presented additional disorders. Approximately 5 g of adipose tissue from the abdominal cavity and umbilical blood serum samples were obtained during cesarean sections. Maternal blood samples of approximately 10 ml were collected from fasting participants by venipuncture, two days before cesarean section and the serum was separated by centrifugation. For organochlorine pesticide analysis, the sample constituted the remaining portion of blood serum taken for routine clinical analysis.

### Chemical analysis

Adipose tissue and blood serum samples were analyzed according to previously described methods.<sup>22-24</sup> The qualitative and quantitative determinations were done by gas chromatography on a Varian 3400 CX apparatus equipped with a <sup>63</sup>Ni electron capture detector. A volume of 1 µl was injected in splitless mode into a PTE-5 QTM 15 m x 0.53 mm id. 0.5 µm film capillary column, using nitrogen as carrier gas with a 6.7 ml/min flow rate and the following temperature program: 140 °C (3 min) to 250 °C at 10 °C/min, hold 10 min. The temperatures of the detector and injector were 320 °C and 220 °C, respectively.

All of the samples were analyzed for: HCB,  $\alpha,\beta,\gamma$ -HCH, Aldrin, Heptachlor, Heptachlor epoxide, pp'DDT, op'DDT, pp'DDE, pp'DDD,  $\alpha,\beta$ -Endosulfans and Endosulfan sulfate. Chlordane and its isomers, Dieldrin and Methoxychlor were degraded during clean-up of extracts with concentrated sulfuric acid. The minimum detection limits expressed on fat basis for the organochlorine pesticides studied were: 0.001 mg/kg for HCB and HCH isomers, 0.002 mg/kg for Aldrin, Heptachlor, Heptachlor epoxide and pp'DDE, and 0.003 mg/kg for pp'DDT, op'DDT, pp'DDD and Endosulfans. 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 0.005 to 0.020 mg/kg levels, depending on the pesticide, showed mean values from 89% to 95% of recovery (except  $\alpha$  and  $\beta$ -Endosulfans, caused by the par-

tial conversion of  $\alpha$ -Endosulfan to  $\beta$ -Endosulfan under the influence of concentrated sulfuric acid during the clean-up step). The standard deviation and coefficient of variation were below 10, indicating excellent repeatability of the method.

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

### Statistical analysis

Organochlorine pesticide residue mean values in blood serum and adipose tissue were calculated using basic statistics. To compare variability among body matrices, the Pearson correlation coefficients ( $r, r^2$ ) and simple linear regression coefficients were calculated using the statistical software Minitab 12.

## Results

In all analyzed samples the presence of Aldrin, Heptachlor, Heptachlor epoxide,  $\alpha, \beta$ -Endosulfan and Endosulfan sulfate was not detected and thus they are excluded from further discussion.

Samples taken from maternal adipose tissue, maternal blood serum, and umbilical cord blood serum from 64 mothers, were analyzed to confirm concentration levels in both sizes of organisms (mother and fetus). The obtained results of determinations and the

statistical calculations are presented in Tables I and II, as frequencies, arithmetic means, medians, and quartiles. The highest frequencies (100%) correspond to HCB,  $\beta$ -HCH and pp'DDE, especially in adipose tissue. A 100% frequency of pp'DDE is presented in all analyzed samples of maternal adipose tissue, maternal blood serum, and umbilical cord blood serum. Likewise, the pp'DDE is a dominant pesticide with its highest levels determined in adipose tissue at 4.51 mg/kg, in maternal blood serum at 4.45 mg/kg, and in umbilical cord blood serum at 4.70 mg/kg, indicating that lipid-rich tissues are in equilibrium among these body compartments. The observed differences among the other organochlorine pesticides depend on the lipophilicity of the pesticide and their physical-chemical properties, which govern its behavior and specific accumulation in the organism. A similar tendency is expressed by the more persistent compounds pp'DDT (1.27 mg/kg of maternal adipose tissue, 0.78 mg/kg of maternal blood serum, and 0.88 mg/kg of umbilical blood serum), and  $\beta$ -HCH (0.16 mg/kg of maternal adipose tissue, 0.20 mg/kg of maternal blood serum and 0.22 mg/kg of umbilical blood serum). Generally, the pesticides stored in adipose tissue are liberated to the blood serum, according to their partitioning coefficients and their physical-chemical properties, which are characteristic for each compound.

The lowest frequencies corresponded to isomers  $\alpha$ - and  $\gamma$ -HCH, op'DDT and pp'DDD in adipose tis-

**Table I**  
**HCB AND HCH'S COMPARISON OF FREQUENCIES, MEAN VALUES, MEDIANS, AND QUARTILES OF RESULTS (MG/KG ON FAT BASIS) FROM SAMPLES OF MATERNAL ADIPOSE TISSUE, MATERNAL BLOOD SERUM, AND UMBILICAL CORD SERUM OF 64 INHABITANTS OF VERACRUZ, MEXICO, 1997-1998**

Pesticide	Frequency (%)	Mean	Median	Q1	Q3
HCB adipose	100	0.07	0.05	0.04	0.08
HCB serum	100	0.18	0.16	0.12	0.22
HCB umbilical	98	0.29	0.25	0.16	0.38
$\alpha$ -HCH adipose	36	0.01	0	0	0.01
$\alpha$ -HCH serum	14	0.01	0	0	0
$\alpha$ -HCH umbilical	14	0.01	0	0	0
$\beta$ -HCH adipose	100	0.16	0.11	0.08	0.17
$\beta$ -HCH serum	72	0.20	0.16	0	0.32
$\beta$ -HCH umbilical	61	0.22	0.16	0	0.35
$\gamma$ -HCH adipose	63	0.01	0.01	0	0.01
$\gamma$ -HCH serum	25	0.01	0	0	0.01
$\gamma$ -HCH umbilical	14	0.01	0	0	0
$\Sigma$ -HCH adipose		0.17	0.12	0.08	0.18
$\Sigma$ -HCH serum		0.22	0.18	0	0.38
$\Sigma$ -HCH umbilical		0.24	0.16	0	0.42

**Table II**  
**DDT'S COMPARISON OF FREQUENCIES, MEAN VALUES, MEDIANS, AND QUARTILES OF RESULTS**  
**(MG/KG ON FAT BASIS) FROM SAMPLES OF MATERNAL ADIPOSE TISSUE, MATERNAL BLOOD SERUM,**  
**AND UMBILICAL CORD SERUM OF 64 INHABITANTS OF VERACRUZ, MEXICO, 1997-1998**

Pesticide	Frequency (%)	Mean	Median	Q1	Q3
DDE adipose	100	4.51	4.08	1.68	6.40
DDE serum	100	4.45	3.66	1.55	5.97
DDE umbilical	100	4.70	4.06	1.70	6.22
DDD adipose	31	0.03	0	0	0.01
DDD serum	3	0.01	0	0	0
DDD umbilical	0	0	0	0	0
op'DDT adipose	86	0.06	0.04	0.01	0.07
op'DDT serum	13	0.04	0	0	0
op'DDT umbilical	8	0.03	0	0	0
pp'DDT adipose	98	1.27	0.34	0.17	1.28
pp'DDT serum	42	0.78	0	0	0.62
pp'DDT umbilical	31	0.88	0	0	0.72
$\Sigma$ -DDT adipose		5.86	4.63	1.94	8.03
$\Sigma$ -DDT serum		5.26	4.05	1.54	6.61
$\Sigma$ -DDT umbilical		5.59	4.73	1.81	7.30

sue and blood serum samples. These lower values are caused by less exposure to Lindane ( $\gamma$ -HCH) and diminished use of DDT in applications to combat malaria vectors. Thus, a lower level of pp'DDD was determined in the human body, which we considered an indicator of recent pp'DDT exposure, since pp'DDD is determined in the human body only for a short period immediately after DDT application.

Tables III and IV present the Pearson correlation coefficients ( $r$ ) of paired organochlorine pesticide levels among matrices studied, as well as correlation coefficients squared ( $r^2$ ) and regression coefficients ( $\beta$ ), to express the magnitude of the correlations. These coefficients indicate the degree of linear relationship between the two matrices studied; higher values express a positive correlation among adipose tissue, maternal blood, and umbilical cord blood. Higher and consistent values were found among matrices studied, which represent adipose tissue and maternal blood serum, as well as maternal blood serum and umbilical cord blood serum. Among pesticides studied, the higher values reveal that the more persistent compounds were HCB in adipose tissue - maternal blood serum ( $r= 0.97$  and  $\beta= 0.52$ ),  $\beta$ -HCH maternal blood serum - umbilical cord blood serum ( $r= 0.86$  and  $\beta= 0.68$ ), pp'DDE adipose tissue - maternal blood serum ( $r= 0.92$  and  $\beta= 0.93$ ) and pp'DDT maternal blood serum - umbilical cord blood serum ( $r= 0.84$  and  $\beta= 0.64$ ).

**Table III**  
**PAIRED PEARSON CORRELATION AND REGRESSION**  
**COEFFICIENTS OF HCB AND HCH'S RESULTS**  
**(MG/KG ON FAT BASIS) OF MATERNAL ADIPOSE TISSUE,**  
**MATERNAL BLOOD SERUM, AND UMBILICAL BLOOD**  
**SERUM FROM 64 INHABITANTS OF VERACRUZ,**  
**MEXICO, 1997-1998**

Pesticide	Pearson correlation coefficient ( $r$ )	Pearson correlation coefficient squared ( $r^2$ )	Regression coefficients ( $\beta$ )
HCB adipose - HCB serum	0.97	0.94	0.34
HCB adipose - HCB umbilical	0.80	0.64	0.18
HCB serum - HCB umbilical	0.83	0.69	0.52
$\alpha$ -HCH adipose - $\alpha$ -HCH serum	0.51	0.26	0.20
$\alpha$ -HCH adipose - $\alpha$ -HCH umbilical	0.49	0.24	0.15
$\alpha$ -HCH serum - $\alpha$ -HCH umbilical	0.98	0.96	0.78
$\beta$ -HCH adipose - $\beta$ -HCH serum	0.56	0.32	0.63
$\beta$ -HCH adipose - $\beta$ -HCH umbilical	0.56	0.31	0.50
$\beta$ -HCH serum - $\beta$ -HCH umbilical	0.86	0.74	0.68
$\gamma$ -HCH adipose - $\gamma$ -HCH serum	0.33	0.11	0.15
$\gamma$ -HCH adipose - $\gamma$ -HCH umbilical	0.25	0.06	0.09
$\gamma$ -HCH serum - $\gamma$ -HCH umbilical	0.62	0.38	0.46
$\Sigma$ -HCH adipose - $\Sigma$ -HCH serum	0.55	0.30	0.58
$\Sigma$ -HCH adipose - $\Sigma$ -HCH umbilical	0.56	0.32	0.48
$\Sigma$ -HCH serum - $\Sigma$ -HCH umbilical	0.87	0.75	0.71

**Table IV**  
**PAIRED PEARSON CORRELATION AND REGRESSION**  
**COEFFICIENTS OF DDT'S RESULTS (MG/KG ON FAT BASIS)**  
**OF ADIPOSE TISSUE, BLOOD SERUM, AND UMBILICAL**  
**BLOOD SERUM, FROM 64 INHABITANTS OF VERACRUZ.**  
**MEXICO, 1997-1998**

Pesticide	Pearson correlation coefficient ( <i>r</i> )	Pearson correlation coefficient squared ( <i>r</i> <sup>2</sup> )	Regression coefficients ( $\beta$ )
DDE adipose – DDE serum	0.92	0.85	0.85
DDE adipose – DDE umbilical	0.88	0.77	0.88
DDE serum - DDE umbilical	0.85	0.73	0.93
DDD adipose – DDD serum	-	-	-
DDD adipose- DDD umbilical	-	-	-
DDD serum - DDD umbilical	-	-	-
op'DDT adipose - op'DDT serum	0.19	0.04	0.09
op'DDT adipose - op'DDT umbilical	0.13	0.02	0.08
op'DDT serum - op'DDT umbilical	0.93	0.86	1.07
pp'DDT adipose - pp'DDT serum	0.73	0.53	0.84
pp'DDT adipose - pp'DDT umbilical	0.60	0.36	0.52
pp'DDT serum - pp'DDT umbilical	0.84	0.71	0.64
$\Sigma$ -DDT adipose - $\Sigma$ -DDT serum	0.90	0.81	0.82
$\Sigma$ -DDT adipose - $\Sigma$ -DDT umbilical	0.81	0.66	0.76
$\Sigma$ -DDT serum - $\Sigma$ -DDT umbilical	0.87	0.76	0.89

## Discussion

The assumption of chemical equilibrium for deposition of persistent organochlorine pesticides in the human body during pregnancy implies chemical transport from maternal tissues to the fetus through the passive transport processes and equilibrium between pesticide concentrations in maternal tissues and fetus.<sup>25,26</sup> This equilibrium permits a rapid internal distribution of persistent organochlorine pesticides within the organism. These pesticides are quantified as uniform among maternal tissues when the determinations are expressed on fat basis.<sup>27,28</sup> Pregnancy involves the transfer of lipids and lipoproteins from maternal tissues through placenta to fetus. This process results in carry-over of persistent organochlorine pesticides through the placenta and their presence in lipid-rich tissues of the fetus. The subsequent metabolic transformation in the fetus is low, due to poor enzymatic activity in the developing organism.

Since persistent organochlorine pesticides largely reside in lipids and the lipid solubility of organochlorine pesticides does not differ substantially among various lipids of the human body, the equilibrium between mother and fetus can be defined as chemical

fugacities in maternal tissues and fetus. Thus, it can be assumed that the apparently equal concentrations in both sizes of organisms, (for example, DDT total: adipose tissue 5.86mg/kg, maternal serum 5.26 mg/kg, and umbilical cord serum 5.59 mg/kg on fat basis,) depend on the lipid content of tissues.<sup>25</sup>

The analyses of paired groups of maternal adipose tissue, maternal blood serum, and umbilical cord blood serum, show that the frequency and mean values determined in maternal blood serum and umbilical cord serum are very similar. Median values reveal a greater similarity of pesticide levels between maternal adipose tissue and umbilical cord blood serum, for example, DDT total: 4.63 mg/kg in maternal adipose tissue and 4.73 mg/kg in umbilical cord serum. This finding can be explained by the equilibrium and partial retention of these pesticides in the fetus, with little possibility for them to return to the maternal bloodstream through the placenta.

To assess the relationships of organochlorine pesticide concentrations among maternal adipose tissue, maternal blood serum, and umbilical cord blood serum, values were paired and analyzed using the Pearson correlation coefficient (*r*) and the Pearson correlation factor squared (*r*<sup>2</sup>) (Tables III and IV). The correlation values are consistent with the previous observations of Kutz *et al*<sup>29</sup> and Duarte-Davidson *et al*.<sup>30,31</sup> They indicate that diversity between paired groups depends principally on the physical-chemical properties of each organochlorine pesticide and compartment composition, shown by the levels presented in the tables; such properties permit an equilibrium among compartments and their specific accumulation. Generally, the highest correlation can be observed between maternal blood serum and umbilical cord blood serum, indicating an equilibrium pattern between these body compartments.

Table V presents the comparison of selected persistent organochlorine pesticide levels in human adipose tissue reported in different countries.<sup>32-37</sup> Comparisons show that levels of pesticides determined in this study are in the same range as those from other countries, including levels determined in Greenland.<sup>37</sup> In tropical countries, like Mexico, DDT has been used by the Secretary of Health in the combat of vector-transmitted diseases, an action that causes their dispersion in the human environment. Recently, DDT use has been restricted in Mexico, leading to a decrease in DDT exposure and human contamination.

In conclusion, it can be pointed out that the organochlorine pesticides absorbed by pregnant women are distributed almost uniformly in the bloodstream of the maternal organism and pass through the placenta

**Table V**  
**COMPARISON OF PERSISTENT ORGANOCHLORINE PESTICIDE LEVELS (MG/KG ON FAT BASIS) IN HUMAN ADIPOSE TISSUE**  
**AMONG DIFFERENT COUNTRIES**

Pesticide	Kenya 1992 <sup>32</sup>	Poland 1994 <sup>33</sup>	Italy 1995 <sup>34</sup>	Spain 1995 <sup>35</sup>	Iran 1995 <sup>36</sup>	Greenland 1999 <sup>37</sup>	This study
HCB	-	0.31	-	3.37	0.06	-	0.07
β-HCH	0.03	0.23	0.21	1.53	0.73	0.11	0.16
pp'DDE	3.26	5.75	0.40	3.93	2.45	3.19	4.51
pp'DDT	2.49	0.54	0.06	0.40	0.19	0.14	1.27

to the developing fetus, where they accumulate in the lipid-rich tissues and form the first contamination source for the developing organism. Due to the estrogenic and antiandrogenic activity of DDTs, their passage through the placenta and the subsequent fetal exposure can be considered a risk factor to the fetus, which can contribute to congenital malformations of external genitalia. The obtained correlations confirm that the placenta does not retain small molecules dissolved in blood serum components, such as organochlorine pesticides, and explain our previous determination of 102 mg/kg of total DDT detected in adipose tissue obtained during the autopsy of a still-born baby.<sup>38</sup>

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