

## OCCURRENCE OF DDT AND METABOLITES IN HUMAN MILK COLLECTED IN VERACRUZ CITY AND SUBURBAN AREAS (MEXICO)

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### Abstract

The contamination levels of DDT (its isomers and metabolites) were evaluated in human milk samples from surrounding areas of Veracruz City (Mexico). DDT compounds were determined by gas chromatography analyses. *p,p'*-DDE levels in analyzed breast milk samples were higher in milk samples from nursing mothers living in suburban areas of Veracruz City followed by *p,p'*-DDT, indicating direct exposure to DDT vapors applied in antimalaria control programs. The Estimated Daily Intake of an infant from this area exceeded by 2.93 times the FAO/WHO Acceptable Daily Intake limit, representing a possible health risk to the newborns.

### Introduction

Since 1990, Mexico has used approximately 3000 tons of DDT per year in anti-malaria control programs, mainly in tropic areas<sup>1)</sup>. This organochlorine pesticide has been used in the malaria-endemic areas of Veracruz, Mexico. For this purpose, a spraying regime has been followed in urban and suburban areas of Veracruz City by applying DDT every six months on indoor surfaces and dwellings at a coverage of 2g/m<sup>2</sup><sup>2)</sup>. Due to their lipophilic properties, DDT and its metabolites are primarily stored in fat-rich tissues and subsequently translocated and excreted through milk fat. Nursed babies are exposed to DDT through the fat in human milk, fact that has caused concern about the health risk to breastfed infants since a significant increase in the number of infants showing hyporeflexia, associated with and increase in the DDE concentration in breast milk, has been reported<sup>3)</sup>. More over, the evidence of organochlorine hormone-disruption activity, reduced birthweight and length has been reported as well<sup>4,5,6,7)</sup>. Nevertheless, Mexican mothers are still positively advised as to breast-feeding by a nationwide program.

The aim of this study was to determine the residue levels of DDT and metabolites in human milk samples from Veracruz City, suburban and rural areas and to estimate if the residue level findings in human milk exceed the limits recommended by FAO/WHO.

## Experimental Methods

A total of 300 (13 to 43 years of age, mean  $23.1 \pm 5.4$  years) participated in the study voluntarily and agreed with its aim. Mothers had lived in the Veracruz area during the preceding one year at least and gave birth at the General Hospital of Veracruz. The 300 human milk samples were taken from healthy donors and randomly collected over a period of 8 months from August 1996 to March 1997 on the first two months postpartum. The donors were asked to express by a breast pump about 30-100 mL of breast milk to a chemically clean glass bottle, labeled regarding the day and month of sampling and kept frozen at  $-20^{\circ}\text{C}$  until analyzed. Each mother completed a questionnaire to provide personal information such as age and place of residence.

Before analysis reagents were tested for impurities by gas chromatography. Analytical standards were purchased from Supelco, Inc. The glassware was washed with chromic mixture, rinsed with distilled water and then with distilled acetone and petroleum ether to prevent contamination of analyzed samples and to make it suitable for pesticide residues analysis. The analyses were carried out on a gas liquid chromatograph Varian model 3300 equipped with a  $^{63}\text{Ni}$  electron capture detector and a Varian model 4400 integrator. For pesticide separation according to the US EPA Method 608 <sup>8)</sup> a fused silica capillary column 30m x 0.53mm id. and 0.5  $\mu\text{m}$  film was used at the following temperature program:  $140^{\circ}\text{C}$  (for 3 min) to  $250^{\circ}\text{C}$  at  $10^{\circ}\text{C}/\text{min}$ , held 10min. Carrier gas was nitrogen at 6.7 mL/min, and direct sample injection of  $1\mu\text{L}$  was employed. The stored human milk samples were left to defrost and then centrifuged at 3000 rpm to separate the fat from milk. The fat layer was removed from the sample and processed according to Waliszewski and Szymczyński method <sup>9)</sup>. All samples were analyzed in duplicate and results represent the arithmetic mean. To determine the quality of the method, the recovery study was performed on ten replicate overspiked samples of uncontaminated cow milk fat. Statistical analysis such as frequency, means, standard deviations and ranges were estimated using Minitab 10.5 for Macintosh. Differences among DDT and metabolite levels according to the place of residence were examined using One-way analysis of variance at  $P < 0.05$ . The Tukey-Kramer Test was used to determine the difference among group means due to the place of residence (Minitab 10.5 for Macintosh)

## Results and Discussion

The fortification levels, recovery mean values, standard deviations and detection limits are presented in Table 1. The mean values ranged from 94% to 97% of recovery and the standard deviations were below 10 indicating excellent repeatability of this method. Frequency of positive samples, mean and standard deviations and ranges of obtained values are listed in Table 2. The DDT and metabolite residue levels of breast milk samples are reported on fat basis as it has been considered the most appropriate manner to express contamination with these residues, due to the effects of variations in lipid level during lactation <sup>10)</sup>.

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Table 1. Fortification levels, mean and standard deviation and detection limits (mg/kg on fat basis) from fortification study.

PESTICIDE	FORTIFICATION LEVELS	X ± SD	DETECTION LIMITS
p,p'-DDE	0.020	96.9 ± 5.1	0.002
o,p'-DDT	0.040	97.1 ± 5.0	0.003
p,p'-DDD	0.030	94.3 ± 5.9	0.003
p,p'-DDT	0.030	95.7 ± 4.9	0.003

Table 2. DDT and metabolites residue levels (mg/kg on fat basis) in human milk samples

PESTICIDE	FREQUENCY (%)	X ± SD	RANGES
p,p'-DDE	100.0	5.302 ± 6.070	0.054 - 78.098
o,p'-DDT	91.3	0.310 ± 0.472	0.000 - 4.166
p,p'-DDD	38.3	0.088 ± 0.194	0.000 - 1.932
p,p'-DDT	99.7	2.141 ± 3.110	0.000 - 22.468
Σ-DDT		7.815 ± 8.655	0.515 - 99.338

Table 2 shows that p,p'-DDE and p,p'-DDT were found in 100% and 99.7% of samples analyzed, respectively, 91.3% contained o,p'-DDT and 38.3% p,p'-DDD. p,p'-DDE was the main contributor to ΣDDTs detected in human milk samples with a mean value of 5.302 mg/kg on fat basis and a maximum level of 78.098 mg/kg on fat basis. This metabolite was followed by p,p'-DDT with a mean value of 2.141 mg/kg on fat basis and a maximum level of 22.468 mg/kg on fat basis, indicating direct exposure to DDT vapors used in sanitary actions.

Table 3 shows the DDT and metabolite residue levels, the ratio DDE/DDT and the Estimated Daily Intake (EDI) in breast milk samples obtained from mothers living in urban and suburban areas, and rural communities around Veracruz City. The group denominated "Veracruz-suburban" refers to those mothers living in the surrounding areas of the city, which are characterized by swamplands. In these areas DDT is extensively used as an insecticide of choice against malaria vector mosquito, causing the exposure of lactating women, uptake and further excretion via breast milk, the major route of DDT elimination. Results of the ANOVA at P<0.05 confirmed significant differences among Σ-DDT mean levels and DDE/DDT ratio in human milk originating from urban and suburban areas and rural communities around Veracruz City. DDT and its metabolite levels were higher in milk samples collected from nursing mothers living in suburban areas followed by those detected in lactating women from the surrounding rural communities. DDE/DDT Ratio was higher in milk samples from the urban area, indicating previous exposures and the consumption of contaminated food with DDE. On the contrary, human

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milk samples from mothers living in suburban areas and rural communities showed lower DDE/DDT ratios, denoting recent exposure to DDT vapors.

Table 3. Mean levels of DDT and metabolites (mg/kg on fat basis), DDE/DDT ratio in human milk samples and Estimated Daily Intakes (EDI) (mg/kg body weight/day) of breastfed infants according to place of residence

PESTICIDE	PLACE OF RESIDENCE		
	VERACRUZ- URBAN	VERACRUZ- SUBURBAN	RURAL COMMUNITIES
p,p'-DDE	1.979 ± 1.341	6.941 ± 7.030*	5.597 ± 4.433
o,p'-DDT	0.127 ± 0.181	0.401 ± 0.554*	0.296 ± 0.397
p,p'-DDD	0.018 ± 0.059	0.127 ± 0.233*	0.068 ± 0.138
p,p'-DDT	0.500 ± 0.783	3.006 ± 3.511*	1.957 ± 3.010
Σ-DDT	2.578 ± 1.945	10.461 ± 9.764*	7.914 ± 6.798
DDE/DDT	6.909 ± 11.989*	4.221 ± 4.308	5.601 ± 5.565
EDI	14.437	58.582	43.318

\* Values statistically different at (p<0.05) between columns

FAO and WHO have set the acceptable daily intake (ADI) at 20 µg/kg body weight/day for Σ-DDT<sup>11)</sup>. Assuming an infant with a mean body weight of 5 kg, a mean milk fat content approximately of 3.5%, a mean milk daily intake of 0.8 L, and considering the Σ-DDT mean level of each group of mothers according to place of residence, the estimated daily Σ-DDT intake for the infants from Veracruz suburban area is 58.582 mg/kg body weight/day, 43.318 and 14.437 mg/kg body weight/day for the infants from rural communities and Veracruz City, respectively. It should be noted that the exposure of the first two groups of children is estimated to exceed the ADI of Σ-DDT by 2.93 and 2.22 times.

From a toxicological point of view, the high mean levels of DDT compounds found in this study must be considered as a possible health risk to the infant. Because of the resulting levels exceed the ADI limits, the known benefits of breast-feeding might be analyzed, outweighing possible adverse health effects. To assess future development of exposure of newborns, continued monitoring of DDT and other organochlorine pesticides in human milk is urgent.

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