PRESENCE OF ORGANOCHLORIDE COMPOUNDS IN THE BLOOD SERUM OF WOMEN IN THE SOUTHEAST MEXICO

PRESENCIA DE COMPUESTOS ORGANOCLORADOS EN SUERO SANGUÍNEO DE MUJERES EN EL SURESTE DE MÉXICO

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RESUMEN

El uso de agroquímicos es uno de los problemas más relevantes desde hace varias décadas en México; ésta práctica afecta a los ecosistemas y al ser humano. Entre los efectos de la exposición a largo plazo a los agroquímicos, se pueden mencionar diversos tipos de cáncer y efectos en el proceso de reproducción humana.

La zona de estudio fue el Distrito de Riego No. 101, Cuxtepeques, en esta área se seleccionaron ocho comunidades. El Paraíso (Nva Libertad), Guadalupe Victoria, Juan Sabines, Independencia, La Tigrilla, Nuestra Señora Benito Juárez, Amber y Diamante. El objetivo del estudio fue determinar la presencia de plaguicidas organoclorados en suero sanguíneo de mujeres. Con base en los resultados se observó que el endosulfan, el hexaclorociclohexano (HCH) y el DDT y sus metabolitos estaban presentes en la mayoría de las muestras de suero sanguíneo analizado por encima de varios trabajos realizados por otros investigadores en regiones agrícolas de la región sur-sureste de México.

Palabras clave: Plaguicidas organoclorados, suero sanguíneo, mujeres.

INTRODUCTION

One of the main problems that have compromised sustainability in recent decades are the use of agrochemicals, especially the use of pesticide (OPS, 2007). These affects agro-systems, the biodiversity, and the quality and welfare of human beings.

Agrochemicals are substances used in agriculture. Examples of these are insecticides, herbicides, and fertilizers. Their proper application is the most accepted and effective way to achieve maximum production and better quality of crops (Ferrer and Cabral, 1993; Bolognesi, 2003). The Food and Agriculture Organization of the United Nations (FAO) *defines as any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies* (CICLOPLAFEST, 1998). On the other hand, a fertilizer is defined as organic or inorganic substance that improves the quality of the substrate at nutritional level in the rooted plants that where applied with it by providing nitrogen, phosphorus or potassium (Ongley, 1997).

Pesticides can be classified by their biological activity, use, by its toxicity, chemical nature, or other factors (García, 1998; Torriggino, 2003; Farrera, 2004; Martinez and Gomez, 2007; Table 1).

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Table 1. Brief classification of pesticides

Chemical family	Uses	Toxicity
Organochlorine	insecticides	Class Ia (extremely hazardous)
Organophosphates	fungicides	Class Ib (highly hazardous)
Carbamates	herbicides	Class II (moderately hazardous)
Thiocarbamates	acaricides	Class III (slightly hazardous)
Pyrethroids	nematocides	others
Derivatives piridílicos	rodenticides	
Acid derivatives	larvicides	
Phenoxyacetic	others	
Nitrophenol-chlorine derivatives		
Triazines		
Organomercury		
Others		

In Mexico the landscape of environmental and public health impact created by the use of pesticides is critical (Bejarano, 1999; Albert 2005). There are evidences obtained from laboratory and field studies about agrochemicals. These states that they cause damage to health and the environment (González et al., 2001). Additionally, the economically active population in agriculture experience high exposure to them because they use them 85\% of the time in their daily activities (Ravelo, 1995; Cedillo, 1997; Tinoco et al., 1999; Dörner and Plagemann, 2002; Yáñez et al., 2002; Miersma et al., 2003; Altamirano et al., 2004; Rendon et al., 2004; Herrera et al., 2005).

Among the effects of long term exposure to agrochemicals, various cancers can be stated (Jeyaratnam, 1992; García, 1998; Torres et al., 1999; Waliszewski et al., 2000; Gulis et al., 2002; Waliszewski et al. 2002; Coss et al., 2003; Palacios, 2003; Waliszewski et al., 2003; Waliszewski et al., 2005; Herrera et al., 2005; Herrera et al, 2008), and they have effects on the human reproduction process(Salazar et al, 2004;.. Flores et al, 2003; Yáñez et al., 2002).

Once released into the environment, agrochemicals can contaminate rivers, groundwater, air, soil and food. The exposure of humans to these substances may occur during breathing, drinking, eating, and even through skin absorption of these products.

Rural families are constantly exposed to agrochemicals due to environmental and occupational reasons. In general, the whole family works on agricultural tasks; so the exhibition to these chemicals begins at very young ages, even since the very conception. Since women and children are often the poorest, they are particularly vulnerable to agrochemicals due to their physiological characteristics and socio-cultural and economic circumstances (PAHO, 2007). The impacts on the health of women and children are serious because they can be exposed indirectly to the pesticides, and they are the least likely to

Table 2. Studies related to the use of agrochemicals and its damage to health in women and children (Table made from the literature used in this article)

Effect	Population	Parameters	Reference
Placental injury Delay intrauterine growth Delay to get pregnant	Chihuahua, Mexico Colombia	Exposure to inhibitive cholinesterase pesticides was measured. The results we classified by place of residence and activity levels of the enzyme acetylcholinesterase (EAC). Gross placental abnormalities, such as weight, diameter and surface disturbance were evaluated. A microscopic study of the placenta was performed.	Sanín LH, Olave ME, Levario M, Idrovo J. 2005
Reproductive effects Congenital malformations Spontaneous abortions Sex ratio	Mexico	Reproductive effects in male user due to its exposure to DDT during the program to control malaria Birth of children with defective parts	Salazar et al. 2004
Hypospadias	Mexico City, Mexico	DDT / DDE levels in maternal serum	Flores et al. 2003
Breast cancer	Veracruz, Mexico	Presence of DDT in breast milk, adipose tissue and serum	Waliszewski et al. 2002, 2003, 2005
Breast cancer	Buenos Aires	Presence of organochlorine pesticides: op DDE, pp 'DDE, DDD op, pp DDD, DDT po, pp DDT, HCB, HCH (alpha, beta, gamma, and delta), HXT and HXT epoxide, Aldrin, Dieldrin, Endrin, chlordane (oxy, alpha, and gamma), endosulfan (alpha and beta), methoxychlor, and mirex.	Der Persehian 2008
Cryptorchidism	Denmark	Presence of organochloride pesticides in breast milk	Damgaard I et al. 2006
	Guadalajara, Mexico	Presence of organochloride pesticides in breast milk	Martínez et al. 2006
	Guadalajara, Mexico	Presence of organochloride pesticides in breast milk	Castañeda et al. 2005
Skin cancer and penile cancer Cervix cancer and leukemia	Costa Rica	Cell analysis	PLAGSALUD 1996
Brain Tumors	Canada	Nitrogen compounds in water	Muller et al. 2004
Kidney cancer	Iowa, USA	Nitrogen compounds in water	Ward et al. 2007
Gastric cancer	Hungary	Nitrogen compounds in water	Sandor et al.2001
Methemoglobinemia	Cuba	Nitrogen compounds in water	Cañas et al. 1992

receive training about agrochemicals to reduce risks. Because the girls are incorporated into the domestic, rural and work tasks early in his life, women and girls are be exposed in different ways. I has to be noted that both homes and school zones also suffer exposure by the drift of pesticide application; therefore, the places where children learn and play are affected by the use of pesticides.

Another important way of exposure to pesticides is during the spraying campaigns to deal with Dengue, which are made by the health sector (SSA). The Irrigation District No. 101, Cuxtepeques is within the No. IV health jurisdiction, Villaflores, which was considered in the past as endemic malaria zone.

"What we want is that people come more often to spray the chemicals inside the houses, just as before. Thanks to that, everything died in the house, even the lizards (Cuija) died..." (Interview no. 5, Female resident at the locality of Independencia)

"We are happy to help you in your research, but in return we would like to ask you to tell the health sector to send their crews more often to spray insecticide to kill the mosquitos..." (Mr. Gil Jaime Ruiz Molina, Commissariat in the locality of Independencia, Com. Pers.)

Likewise, the complex processes of migration of persistent organic compounds play a major role. These substances can reach areas where they have never been used before, such as the case of their presence in ecological reserves. The importance of the problem creates the need of a study about the presence of these compounds in areas like ecological reserves. It is noticeable the lack of information about agrochemicals. User within the Irrigation District No. 101, Cuxtepeques, Chiapas don't know the toxic effects of agrochemicals, how the exposure to low-dose and long-term of them and multiple chemicals can harm their health and the environment, and the requirements that crops need of these substances (Escobar, 2008).Therefore, the results of this study will enable the design of a proper management plan to handle agrochemicals.

MATERIALS AND METHODS

Study area

Locality Santa Rita. It belongs to the municipality of Angel Albino Corzo. It is located in the buffer zone of the biosphere reserve El Triunfo (REBITRI). It has a total population of 242 inhabitants, where 115 are men and 127 are women. The economically active population is dedicated to grow organic coffee.

Locality Emiliano Zapata. It is located in northwest part of the city of Ocozocoautla. Its north border are private properties, and its south and east border are lands that belongs to the Ingeniero Armando Zebadúa Castellanos town. Finally, the west part of the locality is delimited by the polygon number one of the core zone of the biosphere reserve Selva El Ocote. The village is geographically located at latitude N 16°57′40′′ and longitude W93°30′11′′. It occupies an area of 530 hectares of its total area of 1,915 hectares, and it has an average height above sea level of 783 m (Escalante, 2006).

Table 3. Population distribution of the localities within the Irrigation District No. 101, Cuxtepeques, Chiapas.

Locality	Area (hectares)	Population	Men	Women
El Diamante	519	1839	925	914
Juan Sabines	112	253	126	127
El Ambar	764	1797	872	925
Nueva Libertad	1262	925	475	450
Independencia	604	1796	883	913
Benito Juárez	2705	2651	1351	1300
La Tigrilla	1701	2328	1194	1134
Guadalupe V	171	778	398	380

The population of Emiliano Zapata community is mostly indigenous. They belong to the Tzotsil ethnolinguistic group, a group that migrated from the highlands of Chiapas in the year 1985 (Escalante 2006) .In the census conducted in this study, a population of 172 inhabitants was determined, where 56% were male and 44% were female. The community has no health center or medical services. At last, the economically active population is dedicated to grow organic coffee and they take care of the reserve.

The Irrigation District No. 101, Cuxtepeques, Chiapas is constituted by eight communities. El Paraiso (Nva Libertad), Guadalupe Victoria, Juan Sabines, Independence, La Tigrilla, Nuestra Señora Benito Juárez, Amber and Diamond. These localities are principally engaged in the production of corn because agriculture, in most cases, is the only source of income for the families in the area. In Table 3 the population distribution of each locality is shown.

SAMPLING AND ANALYSIS

In order to achieve the stated objective and better results, the study was developed in two stages:

During the first stage, interviews with key figures of the agricultural and health sectors were performed. In order learn about the background of pesticides in the zone, the interviews were made to the Chief of CADER SAGARPA, the chief of CONAGUA, the Doctor of the town, the representative of the locations within the study area, and the authorities of the REBITRI.

In parallel, the selection and size of the samples were made. The blood sampling were made in the rural area and the REBITRI. To define the universe of study, it should be taken into account the territorial extent of each of the towns and the amount of women in each of them that were donors. The study was conducted in three regions: Irrigation District zone (n = 175), Santa Rita (n = 24), and Emiliano Zapata (n = 46). To select the women, the variables of age (14 to 45 years) and years of residence in the town (> 15 years) were taken into account.

In the second stage, the blood sampling was made by responsible personal from SSA in the study areas. To determine persistent organic compounds in the blood serum, 3 mL blood samples were taken, and they were storaged in heparinized vacutainer tubes. The samples were transported under refrigeration to the laboratory, where they were immediately frozen at -30°C until the analysis was made. For each donor, a questionnaire was applied where features such as demographic characteristics, occupation, exposure to pesticides, use of Tabaco, habits, reproductive history, housing characteristics, and consumption of dairy products were asked (Waliszewski et al 2002, Herrera et al 2005). Immediately after the collection of the samples, the centrifugation was made to obtain blood serum, which served to determine persistent organic compounds.

ANALYSIS OF THE SAMPLES

Extraction of persistent organic compounds was performed by the method described by Atuma & Aune (Atuma and Aune, 1999). This method consists in applying poportionally acid to the blood serum. The recovery of this method is over 95% for the persistent organic compounds studied in this research. After the extraction, the amount of fat present in each sample was determined. Then, the purification of the sample was made. The determination of the analytes were made using a Varian model 3800 chromatograph equipped with a DB-5 column (5% phenyl), methylpolysiloxane of 30m x 0.25 mm x 0.32 mm, and an electron capture detector (ECD) with a source of Ni63.The analytical control and analysis of the samples was performed according to standardized methods from UNEP / IOC / IAEA (1998).

To identify the pesticides (persistent organic compounds), a mixture of standards with α,β,δ y γ-HCH, heptachlor, aldrin, heptachlor epoxide, endosulfan I, endosulfan II, endosulfan sulfate, dieldrin, pp DDE, endrin, endrin aldehyde, pp DDD, pp DDT (SUPELCO 47426-U CLP Organochloride Pesticide Mix) was used (Appendix I). The extraction was assisted by a Mars Xtraction microwave (MAE) model 907501 from Matthews CEM Corporation. The technique that was used in this study was the one described by Wentao (Wentao et al., 2007).

The calculus of concentration in parts per million (ppm) of the analytes was executed according to the following equation:

$$Ppm = (M\ area = Std\ area) * (Std\ inyected\ vol / M\ inyected\ vol) * (Std\ concentration = 1x10\text{-}6) * (M\ vol\ in\ mL / M\ weight\ in\ grs)$$

Where:
M = Sample
Std = Standard

It has to be considered that all samples are reconstituted with 2 mL of hexane, where 1 microlitter is injected into the chromatograph.

Laboratory analysis for the determination of organochloride compounds was performed at the Centre for Ecology, Fisheries and Oceanography of the Gulf of Mexico (EPOMEX) at the Universidad Autónoma de Campeche.

The response variables were the levels of persistent organic compounds and the degradation products found in the serum. On the other hand, the exposure and control variables where the place of origin of the sample (altitudinal gradient), age, number of deeds, occupation and consumption of food in the region.

Table 4. Descriptive statistics (quantitative data) for blood serum (ppm) – HCH

Statistics	Alfa HCH	Beta HCH	Delta HCH	gamma HCH	Sum HCH
Number of observations	245	245	245	245	245
Minimum	0.015	0.004	0.000	0.017	0.000
Maximum	0.148	0.551	0.000	0.374	0.948
1st Quartile	0.022	0.050	0.000	0.028	0.000
Median	0.038	0.073	0.000	0.040	0.000
3rd Quartile	0.050	0.116	0.000	0.076	0.050
Average	0.040	0.102	0.000	0.069	0.042
Variance (n-1)	0.001	0.009	0.000	0.005	0.008
Standard deviation (n-1)	0.025	0.094	0.000	0.071	0.088

Table 5. Descriptive statistics (quantitative data) for blood serum (ppm) – Endosulfan

Statistics	Endosulfan I	Endosulfan II	Endosulfan Sulfate	Sum Endosulfan
Number of observations	245	245	245	245
Minimum	0.023	0.050	0.036	0.000
Maximum	0.222	0.619	0.522	0.744
1st Quartile	0.044	0.074	0.063	0.000
Median	0.059	0.098	0.098	0.000
3rd Quartile	0.083	0.144	0.133	0.095
Average	0.069	0.132	0.120	0.067
Variance (n-1)	0.002	0.011	0.009	0.013
Standard deviation (n-1)	0.040	0.106	0.095	0.115

RESULTS AND DISCUSSION

The values for maximum and minimum levels of HCH, endosulfan and DDT in blood serum and fat in the blood serum are shown in the tables 4, 5, and 6. The results are presented for serum and fat in the blood serum because the investigations are presented different units and they are necessary to compare results.

On the other hand, the results for fat in the blood serum can be seen in tables 7, 8, and 9.

The reported variance values states that not all samples had values above the limit of detection (LOD) of the equipment, so they are reported as Not Detected (ND).

Normality of the data was checked with the Lilliefors test using STATISTICA software version 7 (StatSoft Inc., 2001). The following values were obtained from blood serum: ∑HCH D=0.280, p<0.0001, Endosulfan D=0.280, p<0.0001, ∑DDT=0.303, p<0.0001, and from the fat in the blood serum: ∑HCH D=0.381, p<0.0001, Endosulfan D=0.434, p<0.0001.

Table 6. Descriptive statistics (quantitative data) for blood serum (ppm) – DDT

Statistics	op DDD	op DDE	op DDT	pp DDD	pp DDE	pp DDT	Sum DDT
Number of observations	245	245	245	245	245	245	245
Minimum	0.000	0.000	0.005	0.001	0.029	0.064	0.000
Maximum	0.003	0.019	0.031	0.386	3.642	0.806	3.644
1st Quartile	0.001	0.006	0.007	0.079	0.073	0.116	0.000
Median	0.001	0.007	0.009	0.108	0.140	0.177	0.077
3rd Quartile	0.001	0.009	0.012	0.158	0.315	0.277	0.234
Average	0.001	0.008	0.011	0.124	0.288	0.231	0.193
Variance (n-1)	0.000	0.000	0.000	0.005	0.205	0.024	0.140
Standard deviation (n-1)	0.001	0.005	0.006	0.074	0.453	0.155	0.374

Table 7. Descriptive statistics (quantitative data) for fat in the blood serum (ppm) – HCH

Statistics	Alfa HCH	Beta HCH	Delta HCH	gamma HCH	Sum HCH
Number of observations	245	245	245	245	245
Minimum	0.699	0.417	0.001	0.656	0.000
Maximum	212.401	519.227	0.495	332.597	581.223
1st Quartile	1.741	2.527	0.002	2.363	0.000
Median	3.145	5.438	0.010	5.274	0.098
3rd Quartile	50.893	14.726	0.083	49.175	6.059
Average	33.269	46.241	0.053	31.755	21.335
Variance (n-1)	2909.254	11466.067	0.008	2815.730	4957.570
Standard deviation (n-1)	53.938	107.080	0.091	53.063	70.410

Table 8. Descriptive statistics (quantitative data) for fat in the blood serum (ppm) – Endosulfan

Statistics	Endosulfan I	Endosulfan II	Endosulfan Sulfate	Sum Endosulfan
Number of observations	245	245	245	245
Minimum	1.271	1.282	2.113	0.000
Maximum	318.952	739.188	750.654	1069.606
1st Quartile	3.308	4.267	4.063	0.000
Median	6.403	9.615	12.091	0.000
3rd Quartile	17.752	156.344	149.130	4.143
Average	47.756	95.817	105.477	33.165
Variance (n-1)	7114.750	22289.337	24178.127	12678.324
Standard deviation (n-1)	84.349	149.296	155.493	112.598

Table 9. Descriptive statistics (quantitative data) for fat in the blood serum (ppm) – DDT

Statistics	op DDD	op DDE	op DDT	pp DDD	pp DDE	pp DDT	Sum DDT
Number of observations	245	245	245	245	245	245	245
Minimum	0.010	0.014	0.319	0.496	1.637	3.243	0.000
Maximum	2.984	34.463	40.075	523.161	5829.750	985.873	5832.734
1st Quartile	0.036	0.388	0.521	5.485	6.933	8.473	0.000
Median	0.081	0.747	1.454	10.142	82.119	44.007	8.429
3rd Quartile	1.049	15.241	15.481	80.213	250.075	344.833	94.606
Average	0.563	7.532	8.172	72.462	320.093	195.783	190.361
Variance (n-1)	0.537	109.683	128.633	13741.921	617690.729	62395.538	379536.799
Standard deviation (n-1)	0.733	10.473	11.342	117.226	785.933	249.791	616.066

In Annex II the values found for these compounds in the samples of blood serum and fat in the blood serum are presented.

Once that the data was verified to check that it is no normally distributed, the Spearman statistics was performed to observe if any of the independent considered variables in the study had a direct relationship with the levels of HCH, DDT and endosulfan that were found in the samples (blood serum and fat in the blood serum). Spearman's test did not report a positive correlation in the concentration of the compounds studied by using the parameters: age, number of pregnancies or fish consumption.

The results showed that the values are higher than those reported by James (James et al., 2002) in blood serum (ppt). James presented the following values: (n = 399): op DDE = 326 ± 587, ± ppDDE = 53.888 35.302, opDDT = 2,056 ± 1,566, pp DDT = 14,984 ± 8,828). On the other hand, the results are higher than those found by López in Morelos, Mexico (López et al., 2001) (ng / ml) (2001) (n = 24). Lopez reported the following values: ppDDE = 21.8 ± 2.58, 2.84 ± ppDDT = 2.9. However, the results obtaines are similar to those reported by Walisewski (Walisewski et al., 2002) (n = 112), whose values were (mg / kg): ppDDE = 3.49, ppDDT = 0.82, for women living in Veracruz, México.

On the side of HCH results, gamma isomer (Lindane) was observed in 75 samples with an average value of 0.068 ppm in blood serum, where endosulfan achieved an average value of 0.067 ppm. The presence of these compounds in the samples cannot be compared because it has not been studied properly.

DDT and its metabolites were present in most samples of blood serum or fat in the blood serum. This states that the main source of exposure to them is during health campaigns. It has to be noted that neither in the REBITRI or REBISO these campaigns have arrived. Therefore, it can be concluded that the arrival of these compounds to the body of women in the area of reserves is through drafts.

CONCLUSIONS

By the health implications that these kind of compounds represent, the little existing information about endosulfan or HCH in blood serum can lay the basis for the development of this kind of research in ecological reserves.

Regarding DDT, it was used for nearly 50 years in Mexico, and its persistence in the environment is reflected through pp DDE levels present in blood serum; however, these levels suggests a decrease over time.

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