Editorial Manager(tm) for Environmental Monitoring and Assessment
Manuscript Draft

Manuscript Number: EMAS8778

Title: ASSESSMENT OF THE LEVELS OF DDT AND DDE IN SOIL AND BLOOD SAMPLES FROM TABASCO MEXICO.

Article Type: Original Research

Keywords: Blood; DDT; DDE; malaria; Mexico; Soil.

Corresponding Author: IVAN NELINHO PEREZ MALDONADO, Dr.

Corresponding Author's Institution: UNIVERSIDAD AUTONOMA DE SAN LUIS POTOSI

First Author: ARTURO TORRES-DOSAL, Dr.

Order of Authors: ARTURO TORRES-DOSAL, Dr.;REBECA ISABEL MARTINEZ-SALINAS, Dr.;DIEGO HERNADEZ-BENAVIDES, BIOQUIMICO;FRANCISCO JAVIER PEREZ-VAZQUEZ, M. EN C.;CESAR ARTURO ILIZALITURRI HERNANDEZ, Dr.;IVAN NELINHO PEREZ MALDONADO, Dr.

Suggested Reviewers: Maria Athanasiadou Profesor
Departament of Environmental Chemistry, Stockholm University
mariaa@mk.su.se
She is an expert in DDT monitoring

GERARDO GOLD Dr.
PROFESOR INVESTIGADOR, UNIDAD MERIDA, CINVESTAV
gerardo.gold@gmail.com
He is an expert in analitycal chemistry

Emma Calderon Dr.
PROFESOR INVESTIGADOR, Toxicología, CINVESTAV
scalder@cinvestav.mx
She is an expert in toxic effects generated by DDT and its metabolites

Gonzalo Garcia Vargas Dr
PROFESOR INVESTIGADOR, FACULTAD DE MEDICINA, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México
ggarcia_vargas@hotmail.com
He is an expert in human biomonitoring studies

ASSESSMENT OF THE LEVELS OF DDT AND DDE IN SOIL AND BLOOD SAMPLES FROM TABASCO MEXICO.

Arturo Torres-Dosal[1], Rebeca Isabel Martinez-Salinas[2], Diego Hernandez Benavides[1], Francisco Javier Pérez-Vazquez[2], Cesar Ilizaliturri Hernández[2], and *Iván N. Pérez-Maldonado[2].

1. **El Colegio de la Frontera Sur- Unidad Villahermosa, Villahermosa, Tabasco.**
2. **Departamento Toxicología Ambiental, Facultad de Medicina, Universidad Autónoma de San Luis Potosí.**

*Corresponding author: Ivan N. Pérez Maldonado, Departamento de Toxicología Ambiental. Facultad de Medicina. Avenida Venustiano Carranza 2405, 78210, San Luis Potosí, S.L.P., México. Phone and Fax: (52-48) 262-354.
E-mail: ivan.perez@uaslp.mx

ABSTRACT

In Mexico, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) was used until the year 2000, principally in agriculture and anti-paludal program health campaigns. The southeastern region of Mexico was an important area of malaria, and from 1957 DDT was applied indoors every 6 months, with a coverage of 2 g/m^2. The current study was performed in Tabasco, a Mexican state located in the southeastern region of Mexico. 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE) were analyzed by gas chromatography/mass spectrometry. In general, low levels were found in household outdoor samples; the levels of DDT ranged from not detectable to 0.048 mg/kg, and of DDE from 0.001 mg/kg to 0.068 mg/kg. An important finding was that, in all communities where DDT in blood was analyzed, exposure to DDT was found, indicating both past and present exposure. Although the levels found in this study were lower than other studies in Mexico, there is a need to evaluate whether the people living in the study area are at risk.

Key Word: Blood, DDT, DDE, malaria, Mexico, Soil,

INTRODUCTION.

In 1955, the World Health Organization (WHO) started a global malaria control program with 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT); by 1958, 75 countries had joined, and at the peak of the campaign 69,500 tons of pesticide, mainly DDT, were being applied to 100 million dwellings each year (Wijeyaratne 1993). For the control of malaria, houses were sprayed twice a year with DDT wettable powder, to kill the resting mature *Anopheles* mosquito. Later, the Stockholm Convention on Persistent Organic Pollutants, which came into force on 17 May 2004, outlawed the use of 12 chemicals, including DDT (UNEP 2011). However, one exemption clause allows malaria-endemic nations to use DDT, strictly for disease-vector control. The United Nations Environment Program (UNEP) estimates that about 25 countries will use DDT under exemptions from the DDT pesticide ban (POPs 2009). Thus, the presence of DDT around the world can be divided into three scenarios: sites where DDT is still in use; sites where its presence is the result of DDT sprayed several years ago; and sites where its presence is the result of long-range transport of the insecticide to areas where it was never used, such as the Antarctic.

The Stockholm Convention sought to determine baseline levels from environmental and biological samples; however, in developing countries the levels of these chemicals in hot spots may be an issue of public health because of their magnitude. Furthermore, taking into account the scarcity of data in any matrix,

there is an urgent need to assess the concentrations of Persistents Organic Pollutants (POPs) in environmental and biological samples.

In Mexico, DDT was used until 1999, principally in agriculture and in anti-paludal program health campaigns. The southeastern region of Mexico was an important area of malaria, where DDT was applied indoors every 6 months, with a coverage of 2 g/m^2, from 1957 (DGE SSA 1996). Therefore, the aim of this study was to assess the levels of DDT and its metabolites in the soil, and in the blood of people in living in local communities, in Tabasco, a state located in the southeastern region of Mexico. All the communities studied are malaria-endemic.

METHODS

Locations

The communities were selected from those previously identified as villages where DDT was used for malaria control from 1957 to 1999. Inclusion criteria were the age of the house (to ensure DDT spraying in the selected houses was more than 15 years old), agricultural activity, fishing activity, fish consumption by the population and location in a rural area. The geographical location and names of each community are depicted in Figure 1 and Table 1.

Population

In order to obtain a gradient of ,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDT) and 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE) exposure and study different exposure scenarios, three communities were selected: a) Centla (rural community localized in an endemic malaria zone and with fishing activity), b) Teapa (rural community localized in an endemic malaria zone and with banana agriculture activity) and c) Nacajaua (rural community localized in an endemic malaria zone and with agriculture activity). During 2009, we studied a total of 50 healthy individuals (aged 12-70 years) who were residents of Centla (15 subjects); Teapa (18 subjects) and Nacajuca (17 subjects). Subjects had similar ethnic and socioeconomic backgrounds. The children attending public schools at the sites were screened for study eligibility through personal interview with the parents. After informed consent agreements were signed by all subjects, a questionnaire was circulated and blood samples were taken. The questionnaire registered characteristics such as source of drinking water, occupational history of parents, age, weight, height, exposure to medicaments, environmental tobacco smoke exposure and infectious diseases in the last month. The study was approved by the ethical committee of the Colegio de la Frontera Sur.

Sampling areas.

The weight of sample collected in each point sampled in all communities was approximately 1000 g. Surface soil samples (1–5 cm in depth) were collected and we used a metal blade. Soil samples were transported to the laboratory in glass containers and kept under refrigeration (4°C) until analysis. Soil samples were

composed of five subsamples, in order to have greater representation in the analysis. Surface soil was collected outdoors in children's recreational areas.

DDT analysis in human blood

Quantification of DDT and DDE was performed as reported by Trejo-Acevedo et al. (2009). Briefly, a 2-mL aliquot of plasma was extracted with a mixture of ammonium sulfate/ethanol/hexane (1:1:3), then the extract concentrated and cleaned-up using florisil columns. The quantification was performed using an HP 6890 gas chromatograph coupled with an HP 5973 mass spectrometer, as described below. As internal standards α-hexachlorocyclohexane-C13, endrin-C13 and PCB-141-C13 were used.

DDT analysis in soil

The soil samples (1 g) were microwave-extracted in acetone/hexane (1:1) as described by Yañez et al. (2002). After extraction, the samples were evaporated to 0.2 mL under nitrogen and resuspended to 2.0 mL with hexane. Finally, a clean-up was carried out using florisil columns packed in 6-mL solid-phase extraction cartridges; the extraction was done with 6% ethyl ether in hexane, and the florisil eluate was concentrated to 1 mL under nitrogen. Analytical determination of the target analytes was carried out using an HP 6890 gas chromatograph coupled with an HP 5973 mass spectrometer as described below. As internal standards, PCB-141 and PCB-29 were used.

Quantitative analysis

For all matrices, DDT and DDE were analyzed. Quantitative analyzes were performed by gas chromatography coupled with a mass spectrometer. An HP5-MS column, 60 m × 0.25 mm ID, 0.25-µm film thickness, was used (J&W Scientific, Bellefonte, PA, USA). Column temperatures were: initial, 100°C (2 min); final, 310°C (rates 20°C/min up to 200°C, 10.0°C/min up to 245°C, 4.0°C/min up to 280°C and 30°C/min up to 310°C). The injector temperature was 270°C, operated in pulsed splitless mode. Helium was used as the carrier gas at a linear velocity of 1.0 mL/min. For quality control, organic contaminants in fortified human serum [National Institute of Standards and Technology (NIST) SRM 1958] were used; the recovery was 95 ± 5% for three isomers. For DDT in the soil, analytical reference material EC-2 (Environmental Canada, National Water Research Institute) was used. The extraction efficiency was 90–110% for all tested analytes.

Statistics

To satisfy normality criteria, the levels of DDT and DDE in all matrices were logarithm-transformed. Therefore all the results are shown as geometric means. Mean levels for DDT and DDE in all matrices were compared between communities, using one-way analysis of variance (ANOVA), followed by Tukey's test. A multivariate analysis was performed using variables such as age, sex and nutritional status as independent variables, while exposure levels to DDT and DDE were treated as dependent variables. For all statistical analyzes, Jmpin Start Statistics Software 7.0 (SAS Institute) was used.

RESULTS

Tables 2 and 3 show the DDT and DDE levels in the outdoor surface soils. Low levels were found in general household samples. The levels of DDE ranged from 0.001 mg/kg to 0.068 mg/kg, and for DDT from <LOD to 0.048, with the highest mean levels found in Centla, Teapa and Cardenas for DDT and in Teapa and Cardenas for DDE (Tables 2 and 3). Taking into account two guidelines for total DDT in residential soil, 0.7 mg/kg from Canada (Environment Canada, 2007) and 1.6 mg/kg from the state of California, USA (CALEPA, 2005), 0% of samples analyzed in our work had levels above those guidelines (Table 4).

Blood concentrations of DDT and DDE are depicted in Table 5; an important finding was that in all communities exposure to DDT or/and DDE was found, indicating a general exposure to DDT. The highest mean concentrations for DDT and DDE were recorded in people living in Teapa, a community with agriculture activity principally based on a banana crop (approximately 1043 ng/g lipid and 7600 ng/g lipid, respectively). In Centla, a community with fishing activity, the levels were approximately half (450 ng/g lipid and 3500 ng/g lipid, respectively) those found in Teapa. Individuals living in Nacajaua, a community with agriculture activity, had concentrations of DDT and DDE of approximately 300 ng/g lipid and 1800 ng/g lipid, respectively (Table 5).

It is very important to note that the quotient of DDT/DDE in the soil from all sites sampled (Table 6) was always below the unit, with the exception of Centla and Teapa, suggesting a recent use of the insecticide in those two communities. In blood samples the DDT/DDE quotient in the three communities was below the unit (Table 6), suggesting past exposure to the insecticide. It is also important to note that with the multivariate analysis no significant effects were found for variables such as age, sex and nutritional status.

DISCUSSION

Since the late 1950s, DDT has been used in Mexico for both the control of malaria and agricultural activities. Regarding the anti-malaria program, it was used until the year 2000. But as a result of its environmental persistence and because of the amounts sprayed, many individuals are still highly exposed to DDT and its metabolites. DDT and its metabolites have been found in the environment (Yañez et al. 2002) and in human tissues (Yañez et al. 2002; Pérez-Maldonado et al. 2004, 2006) in Mexico. The levels of total DDT found in the soil in this study (Table 4) are lower than those reported by Martínez-Salinas et al. (2011) in Chiapas, also in the southeastern region of Mexico, and lower than those reported by Diaz-Barriga et al. (2011) in Chihuahua, in the northern region of Mexico. The soil levels found by Martínez-Salinas et al. (2011) ranged from 0.002 mg/kg to 27 mg/kg, while the levels found by Diaz-Barriga et al. (2011) ranged from 0.001 mg/kg to 0.788 mg/kg. The levels of total DDT in the soil found in this study were also compared with