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Insecticide Residuality of Mexican Populations Occupationally Exposed

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/53980>

1. Introduction

In this chapter the information generated by two research projects conducted in Sonora, Mexico (2003-2010) with the objective to assess the presence of insecticide residues (organochlorine and organophosphates) in corporal fluids of two population groups that live and work in agricultural areas (agricultural workers) are described. The presence of residues in body fluids (blood, urine, semen and breast milk) will be related to social, labor, environmental factors, health status (obtained from surveys, and clinical histories), and biochemical and biological indicators, with the purpose of elucidating the degree and persistence of the exposure to these insecticides.

In the countries in development as Mexico, the handling of toxic compounds as pesticides is inadequate; it is possible that people can be exposed to higher concentrations than that allowed by the maximum limits (LMP), as they demonstrate it studies carried out with children in San Luis Potosí state [1], in some endemic areas of malaria in Mexico like Quintana Roo [2], Chiapas and Oaxaca [3-5], and in labor exposed people of Sonora state [6,7]. This evidence suggests that the populations that work in agricultural fields, as well as those that inhabit the surrounding area could have higher exposure risk, as well as chronic contamination that the populations with a basal exposure.

The state of Sonora is amongst the regions of Mexico with more pesticide use; it is calculated that 80% of the total applied in the country is for the production of grains and export vegetables [8]. There are not reliable statistics of pesticide intoxications in rural areas, and there are

not epidemiologic studies to detect chronic effects of the pesticides; those should exist at least for the agricultural journeymen and for vulnerable groups, since they lack elementary protection, and don't have the correct information about pesticide toxicity.

The exposure doses can be small but persistent, causing chronic health problems [8]. DDT (bis[4-chlorophenyl]-1,1,1-trichloroethane, also called dichlorodiphenyl trichloroethane); was first used to protect military areas and personnel against malaria, typhus, and other vector-terminal diseases [9]. In Mesoamerica (Mexico, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) DDT was used until the year 2000; Mexico and Nicaragua being the last nations that applied the insecticide in agriculture and for the control of malaria (the amount used for Mexico is approximately 69,545 tons between 1957 and 2000) [10].

Technical-grade DDT contains 65-80% p,p'-DDT, 15-21% o,p'-DDT, and up to 4% p,p'-DDD (bis[4-chlorophenyl]-1,1,-dichloroethane)[11]. When sprayed, DDT can drift, sometimes for long distances. In the soil, the compound can evaporate or attach to wind-blown dust. In the environment, DDT breaks down to p,p'-DDE (bis[4-chlorophenyl]-1,1-dichloroethene) [12], an extremely stable compound that resists further environmental breakdown or metabolism by organism; DDE is the form usually found in human tissue in the highest concentration, especially in areas where there has been no recent use of the parent compound [9]. DDT and DDE can also be transferred from the placenta and breast milk to fetuses and infants. Although some ingested DDT is converted to DDA (bis[4-chlorophenyl]-acetic acid) and excreted, any non-metabolized DDT and any DDE produced is stored in fat, as is all absorbed DDE, which cannot be metabolized. DDT and DDE are highly soluble in lipid; their concentrations are much higher in human adipose tissues (about 65% fat) than in breast milk (2.5-4% fat), and higher in breast milk than in blood or serum (1% fat) [13]. DDT and DDE concentrations increase with age [14].

The use of DDT in Central and South America, Mexico, Africa, and some Asian countries where this has been used for vector control in the past 5-10 years, DDT concentrations in human tissues remain high. For example, in Mexico, the total DDT concentration in breast milk fat was 5.7 µg/g in 1994-95 and 4.7 µg/g in 1997-98 [15]. Others Mexican data where workers used DDT to control mosquitoes, have very high DDT concentrations. Mexican data revealed that the geometric mean of total DDT was 104.48 µg/g in adipose tissue of 40 DDT sprayers in 1996 [16]; however in Finland, USA, and Canada, the value was less than 1 µg/g in adipose tissue in the general population [17]. In another Mexican study, the serum concentration of p,p'-DDE was much higher in DDT sprayers (188 µg/L) than in children (87 µg/L); also in adults (61 µg/L) who lived in sprayed houses, but were not otherwise exposed to DDT [3].

The organophosphate insecticides have the advantage of low environmental persistence over the chlorinated pesticide compounds. However, studies carried out in individuals with exposure to insecticides in Mexico, and other countries, associate the pesticide exposure with adverse health effects, as much in the humans as in experimental animals. These damages can be evident by the presence of certain biochemical indicators in the different biologi-

cal fluids, and for the detection of morphological, histological, and molecular changes in target organs [2-4,6,7].

The degree of pesticide contamination depends on several factors, such as the formulation of the pesticide, the active ingredient, the time of exposure, the direct or indirect contact, the quantity used, the pesticides mixtures, the climate and season of the year when it's applied, and the person's age, amongst others [18, 19]. There are environmental indicators, health indicators, and other elements that help determine the exposure risk, such as the person residence and occupational history, the clinical history, as well as the presence of the pesticides studied in drinking water, in the ground, in the atmosphere, and in the fresh or processed foods in the region where the studied populations inhabit. The exposure can be increased with the daily time dedicated to the activity, as well as the years of work, the exposure form, the use of protective gear, and/or the physical proximity of the housing to agricultural fields [20-22].

Due to the previously mentioned situations it was considered important to study the degree of exposure to pesticides on workers in those agricultural areas that also reside in their proximity.

2. Materials and methods

2.1. Site description

Sonora State territory has 179,355 km² and it is located in Sierra Madre Occidental; geographically it is north 32° 29', to south 26° 18' of north latitude, to east 108° 25', to west 115° 03'. The weather in coastal of Sonora is dry. The average annual temperature is around 22°C, being the average maximum 38°C (June and July) and the average minimum temperature is 5°C (January). Only 7% of the land is appropriate for agricultural use and ninety five percent of this area is irrigated. In July and August the rain reaches 450 mm. The weather in Sonora State restricts the agricultural activities. However, in villages Yaqui and Mayo, valleys of Hermosillo, Caborca and Guaymas the major crops with irrigation are wheat, cotton, safflower, watermelon, sesame, garbanzo, sorghum, corn and vine [23]. The agriculture in the south of Sonora is based on 90% of crops such as corn, wheat, oleaginous and cotton [24].

2.2. Population study

Group 1, field workers. The municipal headboards included in this group are localized in the following coordinates: Obregon city 27° 29' north latitude and 109°39' west latitude with a height of 10 m above sea level and Navojoa 27° 05' north latitude and 109°39' west latitude with 40 m above sea level [23].

The town council Cajeme has a population of 175,177 men, from this population, 6,983 live in Yaqui town. In Huatabampo there are 38,563 men, specifically in "Jupare" (1,026 men). Navojoa has a population of 69,341 from this population, 445 men live in 5 de Ju-

nio cooperative [25]. In this study participated 37 men from Yaqui town, 19 from “Jupare” and 21 from “5 de Junio” cooperative, this is 0.53%, 1.85% and 4.7% of total population of men, respectively.

2.3. Group 2, nursing mothers

The women that participated in this study were located at Pesqueira community. The community is located between 28 and 30° parallel north latitude in Sonora, Mexico [23]. The weather in this region is dry with rain in summer [23]. The women are dedicated to cultivating and packing table grape. When this study was conducted the population in Pesqueira was 3,648 residents; 47.8% women, from this percentage only 10% were in reproductive age [25]. In the Health center there was a record of 20% women included in the breastfeeding program. However, 26% women in reproductive age (exposed or working with pesticides) participated in the present study, being 1.4% of total population. It is probably that permanent residents of agricultural areas are chronically exposed to chemical residues through wind, drinking water and even clothing from field workers.

2.4. Participation and surveys

Men and women (nursing period) that voluntarily participated in this study, signed a format according to the norms of Mexican Secretary of Health (SS). Participants filled up a survey; they provided demographic data (age, marital status, residence, residence time) and also information related to work history, pesticides exposure, clinical history, issues related to sexuality, pathology and drugs addiction (alcohol, tobacco, cocaine and marihuana, among others). People with drugs addictions or health issues that could have influence with the biochemical determinations of the body fluids were excluded from this research.

2.5. Blood, urine and semen sampling

Blood, urine and semen samples were collected from members of group 1. For hematic biometry analysis, blood chemistry and biochemical indicators, samples were taken with empty stomach. Blood samples were taken by venous puncture and collected in to vacuum tubes (Vacutainer^{MR}) with and without anticoagulant. Once blood was coagulated, it was centrifuged and supernant was transferred in to a new tube for analysis.

Urine samples were collected in a sterile container and kept at 4 °C until analysis. Semen samples were collected in sterile container (including a code, date and time when were collected) by the participant at home. Samples were analyzed no more than 24 h after sampling.

2.5.1. Breast milk sampling

Breast milk was collected either manually or with a breast milk collector in to a 50 mL conical glass tubes (wrapped with aluminum foil). Samples were kept at -20°C until analysis.

2.5.2. Blood analysis

Blood samples were analyzed in laboratory of General Hospital SS in Cd. Obregon and the laboratory of General Hospital of Navojoa. Blood analysis included hematic biometry using an analyzer Sysmex K-4500, blood chemistry test (glucose, urea, creatinine, uric acid, cholesterol and triglycerides) and total proteins (albumin and globulin) using an analyzer HITA-CHI 911 and also determination of enzymes in serum such as levels of plasma cholinesterase (Randox^{MR}), alkaline phosphatase (Roche^{MR}), transaminases, and superoxide dismutase (SOD, Randox^{MR}).

2.5.3. Urine analysis

Urine was analyzed by two types of analysis; biochemical and microscopic analysis. Biochemical analysis included glucose, proteins, bilirubin, ketotic bodies, urobilinogen (combo test-10). The macroscopic analysis included number of bacteria, erythrocytes, leucocytes, crystals, epithelial cells, etc.

2.5.4. Semen analysis

Analysis of semen was carried out by using international standardized techniques [26]. Macroscopic and microscopic analyses were included; in the first one liquefaction, aspect, viscosity, pH and volume were determined. In the microscopic analysis motility, viability, presence of leucocytes, erythrocytes, germinal cells, dendrites, agglutination (specific and unspecific), number of spermatozoa and morphology were determined.

2.5.5. Insecticides extraction

Blood, urine and semen. Samples were analyzed following the methodology proposed in [27]. Briefly, 0.5 mL of sample were taken and added 4 mL hexane; the mixture was shaken for 15 seconds and then centrifuged for 2.30 minutes at 2500 rpm. The supernatant was transferred to a tube and added 1 mL of 5% K₂CO₃ and 4 mL hexane. Centrifuged for 2.30 minutes at 2500 rpm, supernatant was transferred to a tube and evaporated to dryness. Extract was dissolved with 100 µL hexane and analyzed by gas chromatography. *Breast milk* was analyzed using a matrix solid-phase dispersion technique [28].

2.6. Insecticides residues analyses in body fluids

The analytical standards were from Chem Service (West Chester, PA). Quantitation of insecticides was by comparison of five -point calibration curve. The detection ranges used for calibration curves were 50-0.1 µg/L. The average percent recoveries for organochlorine pesticides were p,p'-DDD 95%, p,p'-DDE 98% and p,p'-DDT 105%, for organophosphates were diazinon 99%, clorpyrifos 91%, malathion 106% and parathion 92%.

Quantitative analyses were performed by gas chromatography (GC) using a Varian model CP-3800 equipped with an electron capture detector (ECD)(USA). The insecticides were separated using VA-1701 (Varian, 30 m x 0.25 mm) capillary column. The injection volume was

1 μL . Nitrogen (purity 99.999 %) was used as the carrier gas at a flow of 1.5 mL/min. The injector temperature was 180°C and the detector temperature was 300°C. The temperature program was as follows: initial temperature 220°C, increasing temperature at 9°C min⁻¹ until the final temperature of 300°C was reached. Data was analyzed using a program Star Chromatography Workstation 5.51.

2.7. Design and analysis of the studies

The design of the study 1 was of the type "Case/Control", where 77 men integrated case group and 17 the control group. Participants of both groups were the same ages (18 to 70 years old). Control group did not have evidence of pesticides exposure. The sample size for the cases group represented approximately 5% of the total male population's in the range of ages selected in the study.

The study 2 was integrated by 39 nursing mothers between 17 and 39 years old, selected randomly among those that accepted to participate. All the other characteristics were similar to study 1.

The nominal data were analyzed by group (in study 1) using contingency tables and Chi-square statistics. Continuous numeric data (age, height, weight, etc.) were reported with descriptive statistics (minimum, maximum, mean, median, and standard deviation). In study 1 there were analysis of variance comparing groups (cases *vs* controls) for the numeric and nominal variables. Several exposure indicators (reported by the literature, erythrocytes, VCM, and RDW) were analyzed by linear regression versus time of exposure, and pesticide amount on a particular body fluid. Also, multiple correlation coefficients were estimated between several biological indicators.

For the study 2 beside of the descriptive statistics, some relationships were evaluated such as the use of protective gear, age, number of years of exposure, children's number, among others. The pesticide residues in breast milk were compared with the maximum residual limits stated by international organizations.

3. Results and discussion

Based on the data obtained in the present study, it was observed that the case group had similar demographic characteristics to the control group but the last one without any pesticide exposure.

3.1. Description of the population based on the surveys and clinical history

Group 1. Field workers. 94 personal surveys were made from which 68% provided data related to medical history. Only 71% of the participants provided blood samples, 69% urine samples and 46% seminal fluid samples. A small number of medical histories and samples were obtained since the participants had the liberty to leave the study at any time.

In the case group, a total of 77 men participated; the majority maintained contact with organophosphate pesticides; the average age of this group was 40 years and 11 years of residency in the site of the study with a maximum of 45 years. The average work years with pesticide exposure was 28, with a maximum of 50 years working in agriculture. Based on data from work history a 62% of the cases had contact with pesticides; from this percentage 43% applied them, 27% works in the places where they were applied and 30% works where they are applied. Only 18% of the field workers uses protection while applying pesticides (like gloves, special clothing for welding fumes, paint fumes o foundry fumes). These results suggest that field workers are chronically exposed to pesticides due to few safety precautions are taken to handle them. There for, it is of importance that the field workers receive training to be aware of the possible health issues related to pesticides exposure.

In the case group besides being in contact with pesticides during work activities they are also in their place of residency; considering this background, the time of exposure is 16 years in average and 65% of them apply insecticides in their homes; 27% are applied with an annual frequency, 23% are applied semiannually, while 10% every 3 months and 8% monthly.

Some factors can exacerbate the toxicological effects caused by pesticide exposure, such as the consumption of alcohol and drugs. The present study found that 69% of the individuals of the case group consume alcohol with a monthly frequency, 16% consume less than 5 cigarettes daily, 8% and 6% less than 20 cigarettes and the rest only 1 cigarette, 4% consumes cocaine, none of the cases consumes marihuana, nor intravenous drugs. Erection and ejaculation problems (4%) were found in case group. Case and control groups had problems having children (6%). Unlike the control group, the case group presented sexual transmission diseases; around 4% had gonorrhea. Both groups have children with congenital health problems (approximately 6%).

Some of the reported symptoms in the case group were cramps (61%), tiredness and weakness (53%), blurred vision (45%), sweating (45%), tearing (43%), nervousness (38%), dizziness (37%) and tingling in the extremities (37%). According to literature it can be considered that pesticide intoxication is nonspecific and produce the subclinical symptoms identified in the present study in addition to anorexia, insomnia, digestive alterations and itching of skin and mucous [11]. Mostly the symptoms caused by pesticides exposure are diagnosed as common cold or flu [29]. This symptomatology is not produced at the same time because every chemical product acts in a different way and will differ in each of the persons with a chronic exposure. In the present study, during the physical auscultation, the average weight and height in the case group and control group was 82 and 81 kg and 1.72 m, respectively. The vital signs were normal for both groups; 70 and 80 pulsations/min (normal value 70-80 pulsations/min), respiratory rate 20 breaths/min (normal 12-20 breaths/min) and blood pressure 120/180 (normal 120/180).

Group 2. Nursing women, description based on surveys. A total of 51 surveys were made to nursing women, 79% of them have been living in this community for more than five years. The average age was 24 years while the median was 23. The highest age was 39 and the lowest 16. The average body weight of women was 82 kg (± 15.9), with an average height of 1.72 m (± 0.07). The average number of children was three, 98% were married (including the ones that live in free union) and only 2% were single (this includes also the ones that are divorced). The 72% of the women were housewives, but 92% of them mentioned to have worked in agriculture. The 77% of the participants were in contact with pesticides; 69% ap-

plied in more than one occasion and only 34% used protective clothing while applying (gloves, special clothing for their work and mask).

The 53% applied pesticides in their home; 38% with an annual application, 43% monthly and 19% weekly. Insecticides applied at home were pyrethroids (20%), and organophosphates (14%).

According to the literature, intoxication by pesticides is nonspecific and produce symptoms like: excitability, tremors, sweating, tiredness, dizziness, headache and convulsions; in women they can also cause a decrease in the duration of breastfeeding [11]. The symptoms present in the participants of this study were fatigue (70%), headache (62%) and perspiration (46%). Around 76% of women (39 of 51) agreed to donate breast milk, 14% (7 of 51) of the women decided to retire and not to collaborate more in the research and the remaining 10% (5 of 51), were not producing the necessary amount for analysis.

3.2. Blood analysis

Significant differences were observed between both groups (case and control) regarding the number of erythrocytes, mean corpuscular hemoglobin concentration (MCHC), and red blood distribution width (RDW); these values were lower for the case group (Table 1). Regarding the obtained results in the chemical blood analysis, both groups presented levels within the normal values for the measured biochemical indicators. However, it is important to mention that statistical differences were observed between the groups with respect to the concentration of total protein, albumin, alkaline phosphatase, glutamic oxaloacetic transaminase (SGOT or AST) and glutamic pyruvic transaminase (SGPT or ALT); the found levels in the case group were superior to control. In a study performed with pesticide factory workers exposed to carbamates, organophosphates and organochlorines superior values were observed in total proteins [30]. However, in studies conducted with experimental animals, total protein values were not altered by the presence of pyrethroid such as cypermethrin, but the albumin levels decreased at the fifth day of intoxication [31]. Some studies performed to determine the influence of pesticide residue on biochemical indicators have reported that glucose levels increased after expose experimental animals to malathion (20 µg/mL) and after few hours levels went back to normal [32]. On the other hand, it has been reported that cholesterol and triglycerides levels are inhibited after applying a daily dose of cypermethrin (a pyrethroid insecticide) to Wistar rats [31]. A study referring to the toxicological effect of polychlorinated biphenyls (PCB's) in fish, reported that PCB's caused lipid peroxidation, increased cholesterol levels in serum and in some species caused hepatic toxicity and hypertension [33]. Recent studies about the indiscriminate use of pesticides in Tasmania, Australia, have reported effects in the health of its habitants (obesity, hypertension and high cholesterol levels) [34]. Researchers have confirmed that acetylcholinesterase is an indicator of damage by organophosphate and carbamate pesticides [35], in this study the case group mentioned having contact with this substances and the levels of cholinesterase were below normal values. In previous research it was observed that chronic exposure to organophosphate insecticides is related to an increase of catalase, superoxide dismutase and glutathione peroxidase [36]. A study performed in the South India, related to the effect of pesticides on SOD, observed an increase in the levels of this enzyme parallel to the severity of the poisoning with organophosphates [37].

Analyses	Case group	Control group	Normal levels*
Leucocytes (cells/ μ L)	6,966.20	6,664.71	5,000 – 10,000
Erythrocytes (millions of cells/ μ L) ¹	4.96	5.13	4.6 - 6.2
Hemoglobin (g/dL)	14.83	15.24	13.5 - 18
Mean corpuscular hemoglobin concentration (MCHC) (%) ²	33.06	33.52	32 - 36
RDW (fL) ³	44.39	44.62	35 – 55

¹p<0.06, ²p<0.04, ³p<0.009.

* [50].

Table 1. Results of hematic biometry analyses conducted on men exposed to pesticides from Sonora, Mexico

Analyses	Case group	Control group	Normal levels
Serum glucose (mg/dL)	105.38	102	55 - 115
Serum urea (mg/dL)	27	27	10 - 50
Creatinin (mg/dL)	0.86	0.86	0.7 - 1.2
Cholesterol (mg/dL)	195.40	180.05	< 200
Triglycerides (mg/dL)	137	131.29	< 150
Total proteins (g/dL) ¹	7.95	7.5	6.4 - 8.3
Albumin (g/dL) ²	4.87	4.51	3.5 - 5
Globulin (g/dL)	3.09	2.98	2.3 - 3.5
Relation Albumin/Globulin	1.69	1.55	2.5
Total bilirrubin (mg/dL) ³	0.67	0.93	< 1.1
Alkaline phosphatase (U/L) ⁴	106.20	80.31	40 - 129
Cholinesterase (U/L) ⁵	968-3940	3,382-8,108	4,300-10,500
Dismutase superoxide (U/mL)	273.38	275.08	164 - 240

¹p<0.03, ²p<0.0002, ³p<0.039, ⁴p<0.0013, ⁵p<0.026.

Table 2. Levels of blood chemistry test conducted on men exposed to pesticides from Sonora, Mexico

3.3. Urine analysis

The results of the testing performed on urine for both groups were very similar and were within the normal values, it can be indicate that no abnormalities were observed in the corporal fluid that can be attributed to pesticide exposure. Besides, no statistical differences were observed amongst the study group (Table 3).

Analyses	Case group	Control group
Leucocytes (cells/field)	2	2
Uric acid crystals	53.33% (poor)	25% (moderate)
Calcium oxalate crystals	20% (abundant) 26.67% (moderate)	25% (abundant) 25% (moderate)
Amorphous salts	50% (abundant) 37.5% (poor) 12.5% (moderate)	57.14% (abundant) 28.57% (poor) 14.29% (moderate)
Epithelial cells	95% (poor) 5% (moderate)	87.5% (poor) 12.5% (moderate)
Bacteria	80.77% (poor)	54.55% (poor)
Mucine	59% (poor)	75% (moderate)

Table 3. Results of microscopic analyses of urine conducted in men exposed to pesticides from Sonora, Mexico

3.4. Semen analysis

Mostly all the differences between groups (case and control) were observed in the semen analyses. In table 4, it can be observed that the case group presented a lower volume, pH, sperm viability, fast and slow progressive motility and abnormalities in sperm morphology (spermatozoa macrocephalia, microcephalia, pyriform, band-like, pin-shaped, double head, tail coiled cytoplasmic droplets and amorphous). Additionally, this same group had a higher viscosity and immobility of spermatozoa. In previous studies [6], it was observed that liquefaction was affected by 32% in insecticide applicators, while the present study did not show abnormalities or significant differences between groups. In this study we observed that in the controls there were more live sperm (one third more than the majority of the cases). The percentage of the abnormalities detected in the present study was superior represented by a 35% that the one found in the control group, comparing this result with the study regarding the insecticide applicators in Hermosillo, Sonora, there was a similar behavior [6].

Analyses	Case group	Control group	Normal levels*
Liquefaction	6.9% (normal)	Normal	
Volume (mL) ¹	2.48	3.73	" /> 2
pH ²	8.05	8.35	7.2 – 8.0
Viscosity (Filament bigger than 2 cm)	44.83% (normal)	28.57% (normal)	
Aspect	96.55% (normal) 3.45% (anormal, yellow color)	100% (normal)	Opalescent gray color (normal)
Sperm viability	45%	75%	
Sperm fast progressive motility	42.5%	52.80%	
Sperm slow progressive motility	16.88%	21.07%	
Sperm mobility	57.13%	28.93%	
Bacteria	12%	7%	
Germinal cells (Dentritus)	30.30%	7.14%	
Specific sperm agglutination espermatozoides	24.24%	-	
Normal sperm morphology	41.08%	75.92%	

¹p<0.03, ²p<0.01, ³p<0.0201, ⁴p<0.0305.

* [26].

Table 4. Results of semen analysis conducted on men exposed to pesticides from Sonora, Mexico

Biological fluid	Diazinon	Chlorpyrifos	Malathion	Parathion
Semen ¹	17.6	32.3	53	44
Blood ²	0	9.1	0	9.1
Urine ³	0	9.1	5.5	20

¹n=11, ²n=33, ³n=55

Table 5. Organophosphate pesticide residues in biological fluids of field workers from Sonora, Mexico

Biological fluid	p,p'-DDT	p,p'-DDE	p,p'-DDD
Semen ¹	27.3	36.4	36.4
Blood ²	0	15.2	3
Urine ³	5.5	9.1	14.5

¹n=11, ²n=33, ³n=55.

Table 6. Organochlorine pesticide residues in biological fluids of field workers from Sonora, Mexico

3.5. Association of exposure indicators

In the regression analysis a relationship was observed between biochemical indicators and pesticide exposure time. The biochemical indicators involved were erythrocytes, mean corpuscular volume, red blood distribution width and urea. For every year of pesticide exposure there was a decrement of 0.082 million of erythrocytes and 0.088 fL of VCM. For every year of pesticide exposure there was an increment of 0.134 fL RDW and 0.163 mg/ dL of urea. In a research performed in Spain with workers chronically exposed to pesticides, the affected biochemical indicators were urea, TGO enzymes and lactate dehydrogenase (LDH) [38]. In the figure 1, there are the associations studied between biochemical indicators and pesticides exposure time. It is important to mention that the determination of cholinesterase in this study was carried out using butyrylcholinesterase or plasma cholinesterase. In previous studies cholinesterase was associated with other biochemical indicators. The results showed that it bound to cholesterol, triglycerides and others as transaminases [38]. This was not possible to observe in the present study, due to the difference on diet and alcohol consumption.

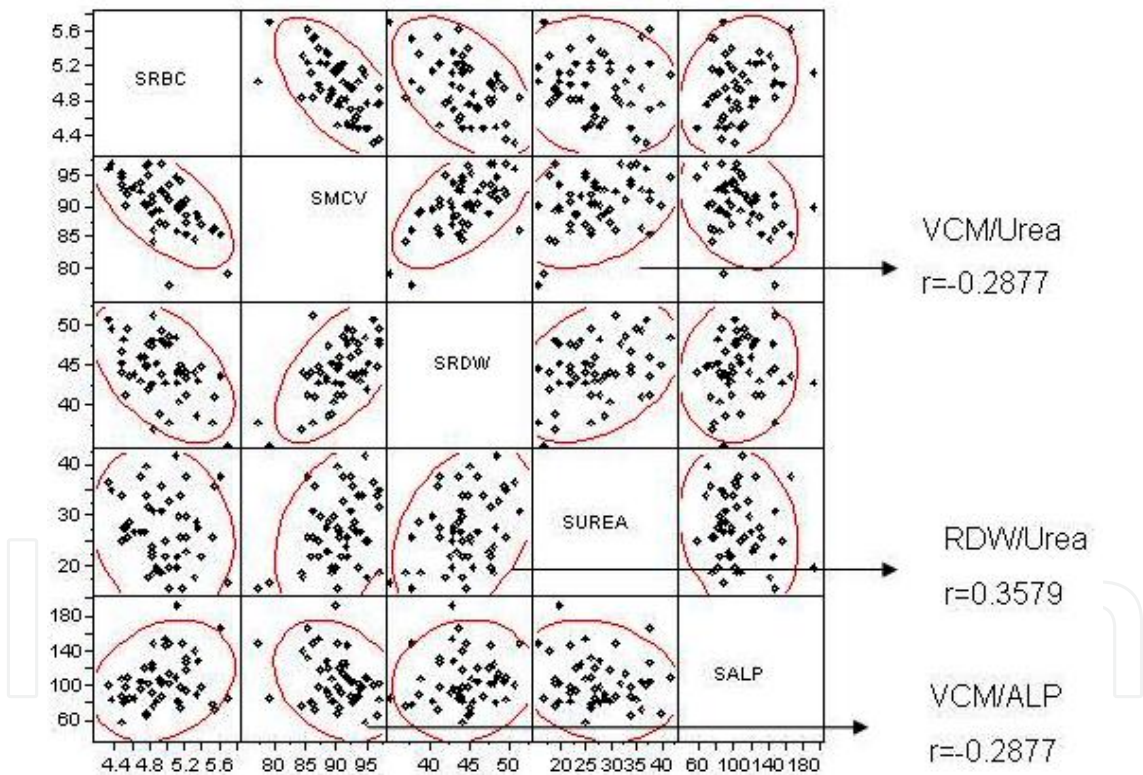


Figure 1. Correlations of blood analyses for case group

3.6. Determination of insecticides and corporal fluids

Blood, urine and semen. A total of 103 samples were analyzed: 73 (71%) were positive to some pesticide. Around 44 (60%) had organochlorine insecticides residues and 29 (40%) had organophosphorus insecticides residues. The organochlorine insecticides detected were 41%

p,p'-DDE, 39% p,p'-DDD and 20% p,p'-DDT. Regarding the organophosphate insecticides analyzed 52% had parathion, 28% chlorpyrifos, 14% malathion and 7% parathion. The highest concentration found was 7.1 µg/L of p,p'-DDE in serum and 6.4 µg/L of p,p'-DDD in urine. The highest concentration found in semen was 2.3 µg/L of p,p'-DDE. Regarding the organophosphate insecticides (chlorpyrifos, malathion and parathion) in the field workers urine the highest concentration were 3.4, 2.2 y 2.0 µg/L, respectively. This levels were considered lower in relation to other studies [39].

Breast milk. There was not detected DDT and DDT metabolites in 85.6% of breast milk samples. Although, 15.4% of samples had p,p'-DDT, p,p'-DDD and p,p'-DDE residues. The most persistent metabolite was p,p'-DDE due to its stability amongst the DDT metabolites [40-42]. The highest level found in breast milk was 9.0 µg/kg (p,p'-DDE) and the lowest level was 0.1 µg/kg (p,p'-DDT). It is important to mention that the infants fed with this contaminated breast milk were in the range of 2-6 month old and their diet was based exclusively on breast milk. Although, according to the American Academy of Pediatrics [43], from the six months onwards, milk is substituted by solid. Other author [44], mentions that breast milk is the primary route where the infants are expose to certain lipophilic toxics that are accumulated by decades in the maternal adipose tissue. If we compare the residues found in this study with the highest levels found in other studies, like the ones reported in [40] for DDE (1.06 mg/kg), and DDT (1.11 mg/kg) in breast milk. The same happened by comparing them with similar works performed in Veracruz with p,p'-DDT and p,p'-DDE (1.27 y 5.02 mg/kg, respectively) [42], and those in the peripheral zone of Mexico City [41], where 108 samples of human milk were analyzed. The content of p,p'-DDT found was 0.117 mg/kg and for p,p'-DDE 2.31 mg/kg. The decrease in the values found was associated to a possible restriction in the use of DDT, although the presence of p,p'-DDE is evident. Specifically, the studies performed in Pueblo Yaqui (Sonora) [45] in breast milk, found levels of p,p'-DDE disturbingly high (6.25 mg/kg), considering that is one of the most important agricultural areas in Mexico. At the present time, in the same zone, the authors reported the presence of p,p'-DDE in 66.66% of the samples of serum from children (between 6 and 12 years of age), with the levels of 0.1 a 443.9 µg/L [46]. These results suggest that DDT is present in the environment and the residues found in biological samples could be due to many factors such as contaminated food consumption. In the present study, the most frequent found metabolite in biological samples was p,p'-DDE due to a its degradation by enzyme system in mammals. According to literature, 50% of p,p'-DDT in the environment could be degrade in 6 years, 67% in 12 years being p,p'-DDE the only product for its degradation [11]. Therefore the contamination present in the studied breast milk can be due to an exposition for more than 12 years. The World Health Organization [47] reports maximum residue limits in foods for DDT and its metabolites of 1.25 mg/kg. While the levels of tolerance established by the FAO/WHO in 1998 [48] for the same compounds in cow milk are 0.05 mg/kg fatty base, in the present study the found levels in breast milk were below the established levels (less than 82%). It is important to mention that DDT levels and other organochlorine compounds in breast milk could be different based on the number of births, age and other factors such as diet, occupation and social status [49, 40]. It is known that levels of these compounds are higher in the breast milk from younger women. In this research, it not was possible to find a correlation

between the presence of pesticides residues in breast milk with age, number of children and occupation; this due to a this studied group did not have the necessary characteristics to determine the possible correlations.

4. Conclusions

The results found in this study indicate that the exposed group symptoms are due to pesticide exposure through agricultural activities, and residence on areas near the fields. Some serum biochemical and biometric indicators such as erythrocytes, VCM, RDW, and cholinesterase were affected by pesticide exposure. The most frequent detected pesticide found in semen on the case group was DDT; amongst the indicators affected on the exposed group were sperm mobility, viability and morphology. Although urine analysis did not show significant differences between groups, chlorpyrifos and malathion residues were higher in urine from the exposed group.

The pesticide detected in highest concentration in breast milk was p,p'-DDE (9.0 µg/kg), and the most frequent metabolite was p,p'-DDE.

There were significant differences between groups for some exposure indicators, mainly for erythrocyte count, mean corpuscular hemoglobin and RDW. Also, there was a significant association between exposure time (working or living near agricultural fields) and erythrocyte count. However, associations between pesticide residues in blood or urine with changes on the main pesticide exposure indicators were none significant.

It is important to train field workers on how to protect themselves when handling or being exposed to pesticides, and also inform them about the possible health ill effects caused by inadequate and frequent exposure to pesticides.

On the analytic part, it is recommended to use erythrocyte cholinesterase, because is a better and more specific indicator on chronic pesticide intoxications.

Acknowledgements

This study was carried out with financial support from "Fondos Mixtos-CONACyT- Sonora State Government (No. SON-2004-C01-004). The authors sincerely thank "Secretaria de Salud (SS)" for letting conduct part of the analysis in laboratories of General Hospitals in Obregon city and Navojoa as well "Centro Avanzado de Atención Primaria a la Salud (CAAPS)" in Hermosillo, Sonora. The authors express recognition to the contribution of graduate students from University of Sonora (2010-2004): Enoc Hernández Montiel, Yessica Fabiola Gómez Hernández, Lidia Sarai Güitrón Ochoa, Ana Arely Villegas Castro, Mizar Ethzalé Martínez Romero, Graciela Lizeth Pérez González, Luis Jesús Villarreal Gómez and M. Sc. Edna Delia Molina Romo.

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