

Evaluation of Antemortem and Postmortem Levels of Organochlorine Pesticides in a Sample of Iraqi People

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Abstract

Background Organochlorine pesticides have long been widely used in agriculture and in public health as highly effective pest control agents. They are lipophilic and have prolonged half-lives of years to decades; as a consequence, they accumulate in human adipose tissues and can cause chronic toxicity after long-term exposure.

Objective To detect and measure the concentrations of organochlorine pesticides (trans-nonachlor and oxychlorodane) in postmortem organs and fatty tissue as well as their concentrations in antemortem serum and fatty tissue samples and study their correlation with lipids in order to reveal the need for human monitoring.

Methods The study was conducted on 40 antemortem samples of blood and fatty tissues and 41 postmortem samples of blood and different organ tissues to determine their lipid concentrations and detect metabolites of organochlorine pesticides and assess their correlations using spectrophotometer and HPLC techniques.

Results The study observed that there was normal serum concentration of triglyceride (TG) and elevated cholesterol level, which were verse correlated with elevated serum concentrations of trans-nonachlor and oxychlorodane pesticides. Serum concentrations of TG were (153.75 mg/dl) within "normal" range while mean serum of total cholesterol was (209.89 mg/dl) elevated above normal range. Percentage of concentration of serum to lipid trans-nonachlorodane was (40.28 mg/dl) higher than that of oxychlorodane was (28.42 mg/dl) in living subjects. The study observed that elevated concentrations of trans-nonachlor more than oxychlorodane in postmortem tissue organs.

Conclusion The study revealed that traces of organochlorines (trans-nonachlor and oxychlorodane) were detected in human serum, fatty tissue and postmortem organs and positively correlated with some lipid profiles indicating the presence of human contamination. Both trans-nonachlor and oxychlorodane were higher in lipid tissue than in serum and other tissues among postmortem cases.

Keywords Organochlorine, trans-nonachlor, oxychlorodane, postmortem, lipid profile

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List of abbreviations: None

Introduction

Pesticides defined as any substance or mixture of materials proposed for destroying, preventing, modifying and

resisting somewhat pest, or substances administered to animals for the controlling insects, arachnids or other pests ⁽¹⁾. A pesticide may be a biological agent, disinfectant, antimicrobial, chemical substance or device

used to combat any pest ⁽¹⁾. The major classes of pesticides include:

1. Fungicides - used against fungi.
2. Herbicides - used against weeds.
3. Rodenticides - used against rodents.
4. Bacteriocides - used against bacteria.
5. Molluscides - used against mollusk.
6. Nematocides - used against nematodes.
7. Algicides - used against algae.
8. Insecticides - used against insect ⁽¹⁾.

Organochlorine pesticides are classes of hydrocarbon compounds characterized by their cyclic structure, number and location of chlorine atoms and low volatility. They were widely used in agriculture and for pest control after they were introduced in the 1940s ⁽²⁾.

Organochlorine components have low levels in the environment. They are formed naturally. Many of their uses have been invalid or restricted since their ecological persistence and potential adverse special effects on natural surroundings and social health ⁽³⁾.

Organochlorine composites are lipophilic and expected to be bio accumulated in human body and to be found in human adipose tissue, breast milk and blood. The levels of organochlorine substances generally are almost the same at various body tissues but less in the blood ⁽⁴⁾.

Measurable levels of organochlorine pesticides have been originated in human fatty tissues, plasma and breast milk through the world ⁽⁵⁾.

Chlordane, a human-made chemical mixture structurally similar to organochlorines, was widely used on agricultural crops and as a germicide in United States until 1988. Chlordane consists of > 140 isomers; the most abundant include trans-chlordane, cis-chlordane, trans-nonachlor, beta-chlordane, and heptachlor ⁽⁶⁾.

In humans, the predominant chlordane-related contaminants detected are trans-nonachlor and oxychlordane (major metabolites of the chlordane). Chlordane's have a 10-20 years half-life in soil ⁽⁶⁾. Chlordane block inhibitory neurotransmitters and result in central nervous system toxicity and when acute high doses are taken seizures and paralysis occur ⁽⁷⁾. Chlordane perhaps carcinogenic to humans ⁽⁸⁾. A significant

trend for Non-Hodgkin's Lymphoma (NHL) was noted for in-creased levels of α -chlordane residues in dust ⁽⁹⁾. One study had reported a significantly increased risk of rectal cancer for "ever use" of chlordane ⁽⁹⁾. McGlynn et al. in 2008 found significant associations between risk of testicular germ cell tumors and serum levels of cis-nonachlor, trans-nonachlor, and total chlordanes. Similar analysis for seminoma revealed significant associations between seminoma and levels of cis-nonachlor, trans-nonachlor, oxychlordane, and to-tal chlordanes ⁽¹⁰⁾.

The objectives of this study were to detect and measure the concentrations of organochlorine pesticides (trans-nonachlor, and oxychlordane) in postmortem organs and fatty tissue as well as their concentrations in antemortem serum and fatty tissue samples and study their correlation with lipids in order to reveal the need for human monitoring.

Methods

A cross sectional study was conducted on 40 living individuals (antemortem group) and 41 autopsy cases (postmortem group) to study organochlorines.

Antemortem cases

Forty patients were included in the study after gotten their written consents. They were undergoing surgical operations in Al-Imamein Al-Kadhimein Medical City. Ten ml of Blood and 5-10 g of fatty tissue samples were collected from each patient and stored in the freezer at 4 °C for blood samples and -8 °C for fatty tissue samples. Each sample was sent for chemical analysis at the Department of Chemistry and Biochemistry Laboratory, College of Medicine, Al-Naharian University immediately to determine lipid profile and to measure the level of organochlorine pesticide regardless of their age and sex. Patients with chronic disease were excluded from the study. Blood samples were separated by 3000 rpm fast centrifugation for 10 minutes and the serum was collected and divided into two equal parts, first part for lipid

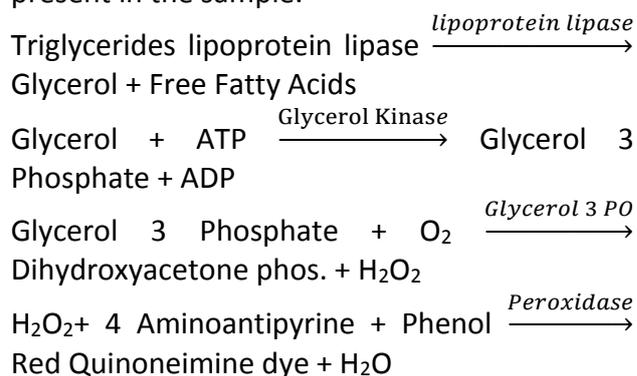
profile test and other part for levels measurement of organochlorine pesticides.

Organochlorine pesticides test in antemortem cases

Four hundred µl of serum were added to 50 µl of 25% sulfosalicylic acid to deproteinization serum and centrifugation in a centrifuge for 10 minutes, which were separated from the supernatant and leaved the precipitate that contain proteins, and added the same amount of ethanol, which to offset protein to a 1:1 ratio to make sure full protein shift (deproteinization). Finally, 20 µl was taken from prepared sample and injected to high performance liquid chromatography (HPLC).

**Serum lipid profile test of antemortem cases
Procedure of measure of cholesterol and triglyceride (TG) in antemortem samples
Procedure for TG**

Lipoprotein lipase hydrolysis TG to glycerol and free fatty acids. The glycerol formed with ATP in the presence of glycerol kinase forms glycerol 3 phosphate, which oxidized by the enzyme glycerol phosphate oxidase to form hydrogen peroxidase. The hydrogen peroxidase further reacts with phenolic compound and 4 aminoantipyrine by the catalytic action of peroxidase to form a red colored quinonemine dye complex. Intensity of the color formed is directly proportional to the amount of TG present in the sample.



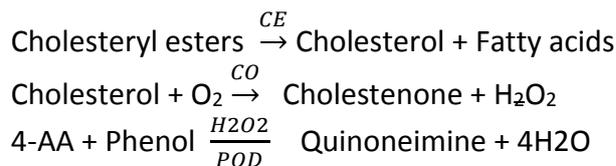
Addition Sequence	B (ml)	S (ml)	T (ml)
Working Reagent	1.0	1.0	1.0
Distilled Water	0.01	--	--
Triglycerides Standard (S)	--	0.01	--
Sample	--	--	0.01

Mix well and incubate at 37 °C for 5 minutes or at room temperature (25 °C) for 15 minutes. Measure the absorbance of the Standard (Abs. S) and Test Sample (Abs. T) against the Blank within 60 minutes at wave length 505 nm.

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs. S}}{\text{Abs. T}} \times 200.$$

Procedure for cholesterol

This method for the measurement of total cholesterol (1,2) in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample.



Procedure

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes.
3. Mix and incubate the tubes 10 minutes at room temperature or 5 minutes at 37 °C.
4. Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

$$\text{Total cholesterol mg/dl} = \frac{A \text{ sample}}{A \text{ standard}} \times C \text{ Standard}$$



Tubes	Blank	Sample	Calibration standard
R1. Monoreagent	1 mL	1 mL	1 mL
Sample	--	10 µL	--
Calibration Standard	--	--	10 µL

Postmortem cases

Sampling of postmortem cases

On the other hand, samples from 41 fresh and healthy autopsy cases at the Medicolegal Directory in Baghdad were included in this study during the period. These samples were weighted (5-10 g) from (liver, brain, kidney and adipose tissue) absolute in alcohol and send for chemical analysis in the same day. These samples were taken from death victims due to traumatic causes. Decomposed bodies and those with chronic diseases was also excluded from the study. All cases were matched according to their sex, age and region of residence.

Extraction of tissue

The method of chemical extraction for isolation and analysis of brain, liver, kidney and adipose tissue samples. Briefly, one gram of tissue samples was taken one ml of n-hexane was added to dissolve tissue for homogenies solution by homogenizer and add another one ml of n-hexane for operation again and add one ml of the same solution to become for 3:1 and continues until became a homogeneous solution, which then centrifuged at (3000 rpm)/min for 10 minutes, then the supernatant was taken and added it (50 µL) of 15% 5-Sulfo-salicylic acid for deproteinization and then separated by centrifuged again for another 10 minutes at (3000 rpm)/min, took the supernatant and add the same amount of ethanol with a 1:1 ratio to make sure a sample deproteinization clear again with package. After centrifugation, separated into two layers, the upper class was taken from the sample and injected a HPLC technique^(11,12).

Measured concentration in total lipid

Total lipid content was quantified gravimetrically in adipose tissue using a previously reported method. The lipid adjusted concentration of the pesticide obtained by dividing the measured pesticide residue concentration in the total tissue sample by the decimal fraction of the sample that consisted of ether-extractable lipid. The total lipid content of each specimen was estimated from its total cholesterol & triglycerides levels by using a summation method. Analytical results for OC pesticides were reported on a lipid-adjusted basis (nanograms per gram or parts per billion). The lipid-adjusted concentration of an analyte was given by:

$$C \text{ lipid adjusted} = [\text{CONC}/\text{TL}] \times 102.6$$

Where CONC is the concentration of an analyte in a sample as weight per gram of sample:

$$\text{TL (total lipid)} = (2.27 \times \text{total cholesterol mg/dL} + \text{triglycerides} + 62.3)$$

Both wet-and lipid-basis adipose tissue serum ratios were calculated by dividing each adipose tissue concentration by each serum concentration.

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Results

Antemortem group

Forty patients were engaged in the study. Females were 23 constituting 57.5% while males were 17 that were 42.5%. The mean age was 37±11.31 years.

Mean serum concentrations of triglycerides was within normal ranges while mean serum of the total cholesterol was elevated above normal range as listed in table (1).

The means for trans-nonachlor in serum and in tissue were higher than that for oxychlorodane.

There were significant differences between their concentrations in serum and tissue as listed in table (2) and shown in figure (1).

Table 1. Mean, standard deviation and range of age and lipid parameters in antemortem group

Parameters	Mean±D	Range
Age (years)	37±11.31	20-65
Serum Triglyceride (mg/dl)	153.75±41.71	72-358
Serum Cholesterol (mg/dl)	209.89±26.11	162.8-268.3
Total lipid (mg/dl)	692.49±74.54	571.96-857.34

Table 2. Comparison of serum and tissues trans-nonachlor and oxychlordan in antemortem group

Parameters	Serum mean±SD	Tissue mean±SD	P value
Transnonachlor (ng/g)	1.65±0.19	4.37±0.35	< 0.001
Oxychlordan (ng/g)	0.78±0.51	1.13±0.09	< 0.001

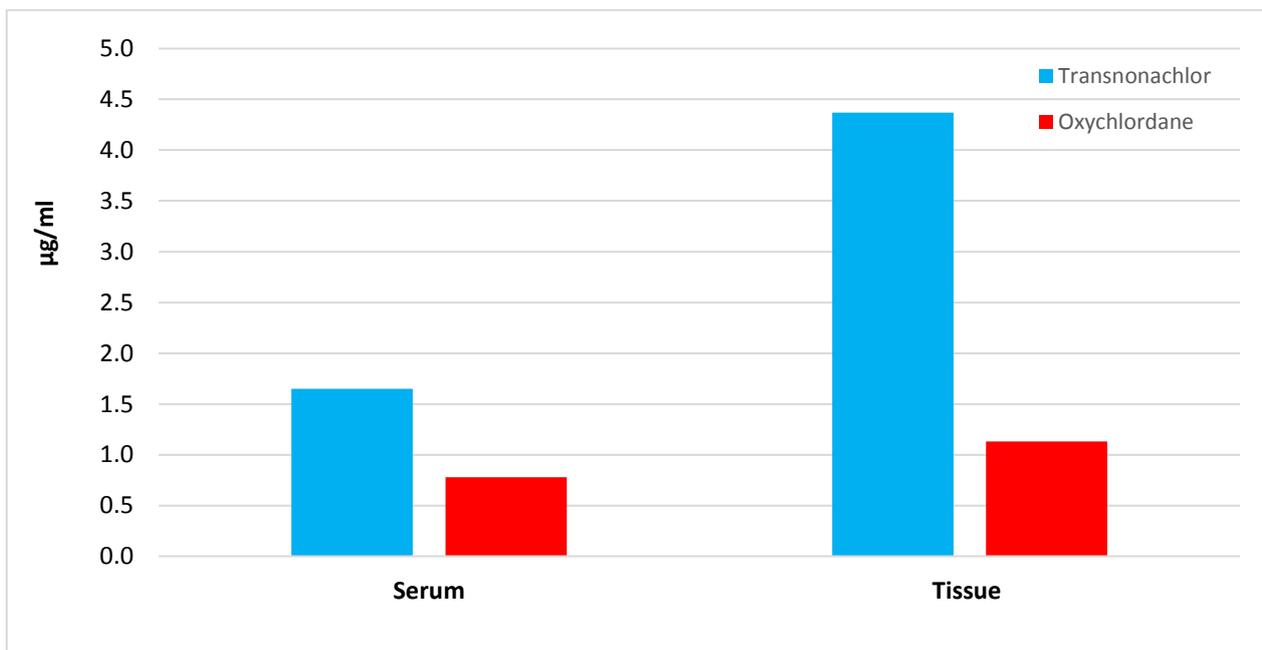


Figure 1. Serum and tissues trans-nonachlor and oxychlordan in antemortem group

Percentage of concentration of serum to lipid trans-chlordane was (40.28±7.3) higher than that of oxychlordan 28.42±11.74 as listed in table (3).

There were no significant differences between male and female regarding different compounds in the study as shown in table (4). The correlation coefficient between parameters were non-significant values as listed table (5).

There were significant positive and negative correlation between serum concentrations of trans-nonachlor and oxychlordane and their serum triglyceride and cholesterol as listed in table (6).

Table 3. Percentage of serum to lipid organochlorines

Percentage of lipid to serum	Mean	Range
Trans-nonachlor	40.28±7.3	29.48-60.89
Oxychlordane	28.42±11.74	8.24-49.14

Table 4. Comparison of parameters between females and males in antemortem group

Parameters	Females (n=23) mean±SD	Males (n=17) mean±SD	P value
Age (yrs.)	37.04±10.86	36.94±12.23	0.978
Triglyceride (mg/dL)	156.14±34.93	150.51±50.44	0.695
Cholesterol (mg/dL)	212.43±24.98	206.44±27.97	0.488
Serum Trans-nonachlor (ng/g)	1.67±0.16	1.61±0.22	0.348
Serum Oxychlordane (ng/g)	0.77±0.52	0.8±0.52	0.847
Tissue Trans-nonachlor (ng/g)	4.4±0.27	4.34±0.44	0.645
Tissue Oxychlordane (ng/g)	1.12±0.09	1.14±0.09	0.549
Total lipid	700.67±73.03	681.43±77.35	0.431
% of conc. of serum to lipid (Trans)	39.36±7.2	41.52±7.47	0.364
% of conc. of serum to lipid (oxy)	27.99±10.93	28.99±13.07	0.800

Table 5. Correlation of age with other parameters in antemortem group

Parameters	r	P
Serum Triglyceride	-0.085	0.603
Serum cholesterol	0.170	0.296
Serum Transnonachlor (ng/g)	0.009	0.955
Serum Oxychlordane (ng/g)	0.101	0.535
Tissue Transnonachlor (ng/g)	0.178	0.272
Tissue Oxychlordane (ng/g)	-0.047	0.773
lipid equation	0.087	0.592
% of conc. Of serum to lipid (Trans)	0.001	0.994
% of conc. Of serum to lipid (Oxy)	-0.134	0.410

Table 6. Correlation of serum triglyceride and serum cholesterol with serum and tissue trans-nonachlor and oxychlordane in antemortem group

Parameters	Serum TG		Serum Chol.	
	r	P	r	P
Serum Transnonachlor (ng/g)	-0.063	0.699	0.039	0.810
Serum Oxychlordane (ng/g)	0.126	0.440	0.128	0.430
Tissue Transnonachlor (ng/g)	-0.296	0.063	-0.069	0.673
Tissue Oxychlordane (ng/g)	-0.183	0.258	-0.028	0.863

Postmortem Group

Forty-one postmortem cases were involved in the study. They included healthy victims died from traumatic injuries. Decomposed bodies were excluded from the study. Females were only 7 (%17) while males were 34 (%83). There were no significant values between male and female regarding organochlorines pesticides (trans-nonachlor, oxychlordane) in different organs and tissues as listed in table (7). There were highly significant differences between different organs and tissues regarding

trans-nonachlor concentration as listed in table (8).

There were highly significant differences between different organs and tissues regarding Oxychlordane concentration as listed in table (9) and as shown in figure (2).

The correlation coefficient values of age with other parameters were non-significant and only positive correlation with kidney oxychlordane and in tissue for these, two organochlorine as listed table (10).

Table 7. Comparison of parameters between females and males postmortem group

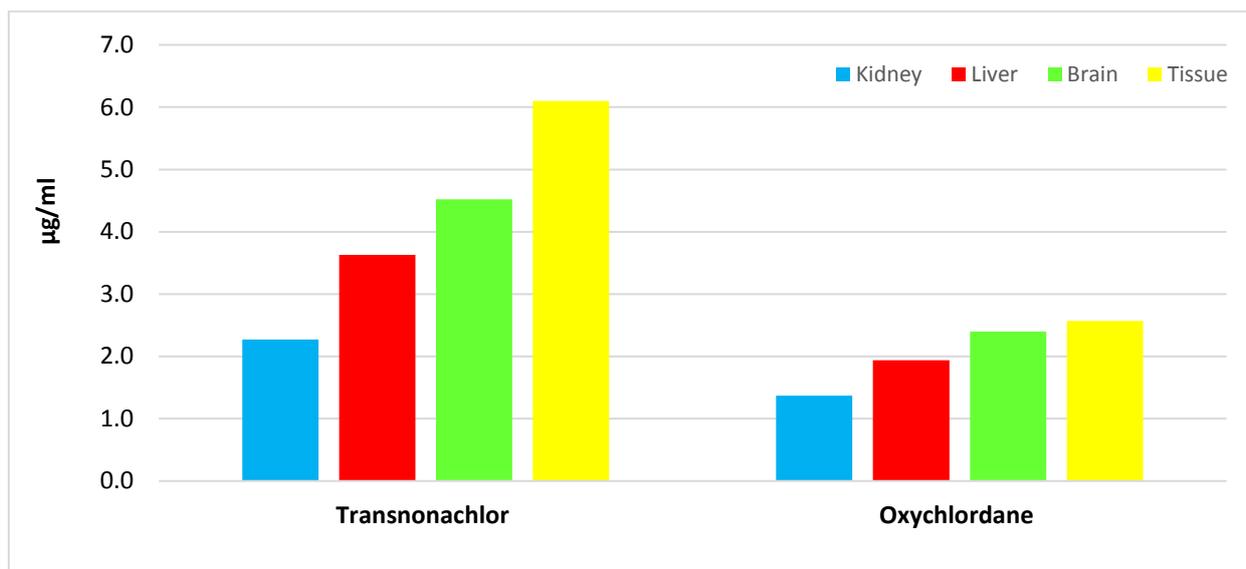
Parameters	Females	Males	P value
	(n=7) mean±SD	(n=34) mean±SD	
Age (years)	52.14±14.54	39.38±13.66	0.064
Kidney Trans-nonachlor (ng/g)	2.24±0.28	2.28±0.27	0.745
Kidney Oxychlordane (ng/g)	1.33±0.09	1.38±0.2	0.304
Liver Trans-nonachlor (ng/g)	3.69±0.45	3.62±0.33	0.717
Liver Oxychlordane (ng/g)	1.93±0.28	1.94±0.14	0.862
Brain Trans-nonachlor (ng/g)	4.37±0.26	4.56±0.25	0.128
Brain Oxychlordane (ng/g)	2.42±0.19	2.39±0.18	0.700
Tissue Trans-nonachlor (ng/g)	6.14±0.28	6.09±0.31	0.681
Tissue Oxychlordane (ng/g)	2.57±0.17	2.58±0.22	0.904

Table 8. Comparison of different tissues trans-nonachlor in postmortem group by ANOVA

Type of tissue	mean±SD	P value
Kidney	2.27±0.27	< 0.001
Liver	3.63±0.35	
Brain	4.52±0.26	
Tissue	6.1±0.3	

Table 9. Comparison of different tissues oxychlordane in postmortem group by ANOVA

Type of tissue	mean±SD	P value
Kidney	1.37±0.19	< 0.001
Liver	1.94±0.17	
Brain	2.4±0.18	
Tissue	2.57±0.21	

**Figure 2. Different tissues trans-nonachlor and oxychlordane in Postmortem group****Table 10. Correlation of age with other parameters in postmortem group**

Parameters	r	P
Kidney Trans-nonachlor (ng/g)	-0.025	0.877
Kidney Oxychlordane (ng/g)	-0.137	0.394
Liver Trans-nonachlor (ng/g)	-0.235	0.139
Liver Oxychlordane (ng/g)	-0.156	0.329
Brain Trans-nonachlor (ng/g)	-0.175	0.275
Brain Oxychlordane (ng/g)	-0.039	0.810
Tissue Trans-nonachlor (ng/g)	0.197	0.216
Tissue Oxychlordane (ng/g)	0.206	0.197

Discussion

The aim of this study was to determine concentrations of pesticides in human adipose tissue and serum samples from individual's adipose tissue in Iraq and evaluate some rationale for their occurrence and potential health risks based on the results; and up to our knowledge, this is the first comprehensive study of human adipose tissue and organs in the Iraq.

The study was to verify the hypothesis that levels of the various lipid components (total cholesterol and tri-glycerides) are differentially associated with concentrations of trans-nonachlor and oxychlordane and do not have identical associations in serum samples obtained from post-mortem and antemortem cases in this cross-sectional study. Since levels of chlorinated pesticides change in direct

proportion to blood lipid levels, improper test interpretations can result from examining only the concentrations in blood. Measurement of cholesterol and tri-glycerides in the serum from the same specimen used to perform the testing allows calculation of total lipid level. The chlorinated pesticides concentrations can then be expressed as nano-gram per gram (ng/g) lipid⁽¹⁵⁾. Serum and different tissue organ samples were obtained from 41 post-mortem cases and serum and fatty tissue samples were also obtained from 40 antemortem cases who were not on any lipid-lowering medication and were analyzed for trans-nonachlor, oxychlorodane, total cholesterol and triglyceride concentrations. Associations between toxicant concentrations and lipid levels were determined using multiple linear regression analysis. The study observed that elevated serum concentrations of lipids were positively associated with elevated serum concentrations of trans-nonachlor and oxychlorodane pesticides in analyses adjusted for age and gender. The mean serum concentrations of triglycerides (153.75) was within "normal" ranges (triglycerides <200 mg/ml) while mean serum of the total cholesterol (209.89), which was elevated above normal range (120-200 mg/ml). Elevations in levels of trans-nonachlor, oxychlorodane were associated with elevated levels of serum lipids. Since elevated serum lipids are a major risk factor for cardiovascular diseases, the previous association, if causal, may have significant effects on human health⁽¹⁶⁾. The strongest associations in antemortem cases were seen for trans-nonachlor compared with oxychlorodane. There were significant positive and negative correlation between serum concentrations of trans-nonachlor and oxychlorodane and their serum triglyceride and cholesterol. Positive and statistically significant correlations were observed between adipose tissue and serum concentrations of trans-nonachlor and oxychlorodane but not of the remaining persistent organic pollutants, confirming reports that serum or plasma concentrations may not provide an accurate representation of concentrations in adipose

tissue in all situations⁽¹⁷⁾. Reports on correlations between serum and adipose tissue concentrations range from negative values to coefficients above 0.8⁽¹⁸⁾. Knowledge remains limited on relationships between serum and adipose tissue concentrations of persistent organic pollutants⁽¹⁹⁾. Concentrations in the two matrixes have different biological meanings: adipose tissue levels have been proven to be a good indicator of cumulated long-term exposure, whereas serum levels are considered a measure of current exposure and the mobilization of persistent organic pollutants from fatty tissues⁽²⁰⁾. The study observed that elevated concentrations of trans-nonachlor more than oxychlorodane in post-mortem tissue organs as well. The results reported in this study were consistent with conclusions made in study of a Native American population in which pesticide levels were found to be positively correlated with serum total cholesterol and triglyceride concentrations. The study showed that elevated concentrations of Trans-nonachlor and oxychlorodane in postmortem tissue organs and antemortem tissue samples more than in serum. Since these chemicals are fat-soluble and tend to bioaccumulation in humans' tissues, they can cause a variety of health problems that often begin slowly. All fat-soluble toxins are carried in the lipid fraction of the serum, mostly in low-density lipoprotein particles (LDL). Since levels of chlorinated pesticides change in direct proportion to blood lipid levels, improper test interpretations can result from examining only the concentrations in blood. We have found significant positive associations between serum concentrations of (trans-nonachlor and oxychlorodane) and their prevalence in a sample of Iraqi population after adjustment for age, sex and total concentrations of serum lipids. While these results do not prove cause and effect, they are consistent with the findings and conclusions of other studies. Serum concentrations of trans-nonachlor and oxychlorodane were found to have strong associations with lipid components. The result showed elevated serum concentrations of lipids were significantly

associated with serum concentrations of organochlorines pesticides (trans-nonachlor and oxychlorodane). Increased concentrations of organochlorine pesticides were associated with elevations in total serum lipids, total cholesterol and triglycerides. These observations showed selective effects of different organochlorines on serum concentrations of different groups of lipids. This elevation in concentrations of serum lipids may be attributed for the increased incidence of cardiovascular diseases found in persons with elevated exposures to chlorinated pesticides ⁽²¹⁾. The study also showed value of total lipid in serum and elevated percentage of concentration of lipid to serum trans-nonachlor more than that of oxychlorodane. The study revealed that levels of (trans-nonachlor and oxychlorodane) in adipose tissue were more than levels in serum, because of half-life of organochlorines pesticides (trans-nonachlor and oxychlorodane) in serum is few hours less than that for organochlorines pesticides (trans-nonachlor and oxychlorodane), which are accumulated mainly in adipose tissue and their half-life continue for many years. There were elevated concentrations of trans-nonachlor in liver, brain and tissue more than in kidney and there were significant differences between them. There was elevated concentration of trans-nonachlor in brain more than other organs. There were also significant differences in the concentration of oxychlorodane in liver, brain, kidney and fatty tissue. The study observed elevation of oxychlorodane in adipose tissue more than in liver, brain and kidney and elevated concentration of oxychlorodane in brain more than liver. The amounts stored in adipose tissue were the result of bioaccumulation of these toxins over a lifetime. However, since these chemicals are fat-soluble and tend to bioaccumulate in animals and humans, they can cause a variety of health problems that often begin slowly. The effects of these compounds are most often seen secondary to mitochondrial toxicity in neurological, immunological, and endocrinological systems; moreover, they can also affect the cardiovascular, respiratory, gastrointestinal, and other

systems in the body. Relation of these two organochlorodane with age in each organ give negative correlation only in adipose tissue give positive correlation these mean the accumulation of organochlorines increase with increased of age ⁽²²⁾.

This study concluded:

1. Traces of organochlorines (trans-nonachlor and oxychlorodane) were detected in human serum and tissue indicating the presence of human contamination.
2. There was positive correlation between both trans-nonachlor and oxychlorodane with some lipid profile (Cholesterol and triglyceride).
3. Both trans-nonachlor and oxychlorodane were higher in lipid tissue than serum and other tissue among postmortem cases.
4. Trans-nonachlor concentrations were higher than oxychlorodane in all samples of the study.

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Authors Contribution:

Hmood: Collection of samples. Dr. Ali: Analysis and interpretation. Dr. Al-Qazzaz: Discussion.

Conflict of interest

The authors declare no conflict of interest.

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