

MONITORING OF POLYCHLORINATED BIPHENYLS AND ORGANOCHLORINE INSECTICIDE LEVELS IN EGYPTIAN PATIENTS

Nabil, Y. M.¹; S. H. Etaiw²; M. E. El-Houseini³ and A. A. Ghazi¹

¹ Central Lab of Residue Analysis of Pesticides and Heavy Metals in Food, Agricultural Research Center, Giza, Egypt.

² Physical and Inorganic Chemistry, Faculty of Science, Tanta University, Egypt.

³ Biochemistry, National Cancer Institute, Cairo University, Egypt.

ABSTRACT

Ninety- nine blood samples were collected in Egypt during 2003 – 2004 (85 males' and 14 female's) aged between twenty five and sixty years old from both diagnosed liver and

bladder cancer patients in addition to high-risk people to these types of cancers such as hepatitis C virus and chronic bilharzias as well as apparently healthy people as a control. The collected blood samples passed through separation of serum, extraction, evaporation, concentration and determination using gas liquid chromatography equipped with electron capture detector. The monitoring of 14 organochlorine pesticides, characterized by their long persistence in human tissues which are HCB, alpha-HCH, beta-HCH, gamma-HCH, delta-HCH, Aldrin, Dieldrin, Heptachlor and Heptachlor Epoxide, Endrin, o,p'-DDT, p,p'-DDT, p,p'-DDE and p,p'-DDD and polychlorinated biphenyls congeners (PCB's) no. 28, 52, 101, 118, 138, 153, and 180. The results showed that only DDT (mainly p,p'-DDE),HCH (mainly beta-HCH) and HCB were detected with high frequency 97% and 77.8 and 2% respectively, while polychlorinated biphenyls residues and other chlorinated pesticides were not detected in any analyzed blood samples. The findings indicate that the higher significant levels of total HCH (mainly beta-HCH) and total DDT (mainly p,p'-DDE) in bilharzias and hepatitis C virus with respect to those found in apparently healthy subjects as a control which indicate the possible role of chlorinated pesticides in neoplastic transformation of bladder and liver as well.

Keywords: Monitoring, Egyptian, Organochlorine pesticides, Polychlorinated biphenyls, Blood, Liver cancer, Bladder cancer, Hepatitis C virus and Bilharzias

INTRODUCTION

Concern about the effects of chlorinated pesticides and polychlorinated biphenyls on health has voiced in a number of reports. Efforts to prevent such effects carried out in most countries and on international level, even though a number of studies carried out on the problems of acute occupational, accidental and suicidal poisoning by pesticides.

Nearly, all populations exposed to pesticides in rural areas, residents living in or close to farming areas which may be exposed directly during routine crops spraying, while contamination in both rural and urban areas may occur through consuming sprayed vegetables and fruits and/or using contaminated water and fish. Contamination may be also occurring through out miss use of pesticides in public health program for control of disease

vectors in residential areas (WHO/UNEP 1990). Although DDT was banned in the United States in 1972 and shortly thereafter in Canada, it is ubiquitous in the food chain. Recently, new concerns regarding DDT rose because it appears to have estrogenic - like effects that may related to breast carcinoma. Therefore, the organochlorine pesticides, especially DDT, as well as polychlorinated and polychlorinated biphenyls tested extensively experimentally and have produced predominantly or exclusively liver tumors in rodents (WHO and IARC 1982). On the other hand, the organochlorine pesticides (lindane, endosulfan, methoxychlor, dieldrin, and dicofol) have shown to cause estrogenic or androgenic effect on animals and to be reproductive toxins. Furthermore, organochlorine compounds detected affect the immune system, causing increased sensitivities to allergies and resulting in more asthmatic and allergic effects including eczema (Cooper *et al.*, 2004). Organochlorine compounds such as DDT/DDE and PCB's detected have a significant correlation to transmutations found in pancreatic cancer, as well as Alzheimer disease (Porta *et al.*, 1999).

Exposures to Polychlorinated biphenyls (PCB's) for a long time cause cancer. The evidence for that summarized in recent reviews by Cogliano (1998) and the EPA. All of the Arochlor mixtures have been shown to produce liver cancer in rats (Macdonald and Metcalfe 1991) and human working in capacitors manufacturing have been reported to have elevated incidence of liver, gall bladder and biliary tract cancers (Brown *et al.*, 1987). Other specific cancers increased in exposed humans include gastrointestinal tract, malignant melanoma, lung, brain, and lymphoma (Cogliano 1998). In rats, females show a higher incidence of liver cancers than males, but actually have a lower life expectancy due to a reduced incidence of mammary cancers probably resulting from the anti-estrogen actions of coplanar congeners (Macdonald and Metcalfe 1991).

Depending on the type of pesticides exposed to, and its biotransformation in body, investigation can be done consequently, so, organochlorine pesticides and polychlorinated biphenyls are to be considered in this study, which is accumulated in body fat, and their residues can be measured for long time after exposure. The identification and quantification of organochlorine pesticides and polychlorinated biphenyls in human tissues are very important in determining the extent of exposure to these compounds and in evaluating the hazardous to human health.

Most countries have conducted initial monitoring programs to determine organochlorine pesticides and polychlorinated biphenyls in their human populations (Robinson *et al.*, 1990). Unfortunately, no information is available concerning the presence or absence of organochlorine pesticides and polychlorinated biphenyls residues in human serum of liver cancer, bladder cancer, hepatitis and bilharzias patients in Egypt. The intention of this work is to compare the results obtained with those published in other countries.

This study designed to provide baseline information on the levels of organochlorine pesticides and polychlorinated biphenyls in serum of cancer patients and high-risk people, and to compare them against apparently healthy subjects of the general population.

MATERIALS AND METHODS

The multiple residue method of analysis of the **Environment Protection Agency (1974)** for the determination of chlorinated pesticides in human body fluids found to be the most suitable for the determination of organochlorine pesticides and polychlorinated biphenyls congeners in human blood serum. Slight modifications adopted in order to validate the method in the laboratory conditions and facilities.

Sampling of Blood samples: A total of ninety-nine blood samples of newly diagnosed liver cancer patients (hepatocellular carcinoma HCC), bladder cancer patients and two high-risk groups; hepatitis C virus (HCV) and chronic bilharzias patients as well as apparent healthy group as a control collected. Eighty-five samples from males and fourteen samples from females, all volunteers were aged between twenty-five and sixty years old. The blood samples of the newly diagnosed cancer and hepatitis C patients collected from persons under medical examinations in National Cancer Institute belongs to Cairo University. Some of cancer patients had previous chemotherapy or radiotherapy and consented to participate in this study. The blood samples of the chronically diagnosed bilharzias patients collected from farmer's residents who visit Public Hospital of Desouq in Khafir El-Sheikh Governorate for medical purposes and the control samples collected from apparently healthy persons not relative to other patients. All samples collected from February 2003 to October 2004.

Table 1: Number of male and female volunteers represents different types of patients.

No.	Volunteer state	Number of male	Number of female	Total samples
1	Liver cancer patient (Hepatocellular carcinoma HCC)	14	4	18
2	Hepatitis virus (HCV)	14	3	17
3	Bladder cancer	16	4	20
4	Chronic Bilharsiasis	20	1	21
5	Control (Apparently healthy subjects)	21	2	23
Total No. of Samples		85	14	99

Blood sample Preparation: About 5 ml of blood samples withdrawn by sterile syringe from volunteers. Sterile, non-heparinized tubes used for sample collection according to the medical and blood transportation terms. The samples immediately kept in icebox during transportation to the laboratory within two hours, then centrifuged at 1300 rpm for 10 mins. We transferred at least 2 ml of clear serum from each sample into acetone cleaned glass tubes by using a disposable pasture pipettes and labeled each sample with the identification code number. All samples kept in refrigerator at -20 C° until analysis. Wearing of protective gloves and using disinfectants were necessary to avoid an injury and cross contamination.

Organochlorine Pesticides and polychlorinated biphenyls studied: The pesticides of which the residues investigated for identification and

quantification were extensively used in 1960's and 1970's and characterized by their long persistence and accumulation in human tissues. In this study, fourteen organochlorine pesticides residues and seven polychlorinated congeners of analyzed in the collected human serum samples.

Organochlorine pesticides studied:

Common name	Chemical name
α, β, δ- HCH isomers	1,2,3,4,5,6-hexachlorocyclohexane
HCB	hexachlorobenzene
γ- HCH (lindane)	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
Heptachlor	1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene
Heptachlor epoxide	1a,1b, 2a, 5ab, 6b, 6a)-2,5- methano-1H-indeno [1,2-b] oxirene
P,P'- DDE	1,1'-(2,2-dichloroethenylidene) bis (4-chlorobenzene)
Dieldrin	(1R,4S,4aS,5R,6R,7S,8S,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
Endrin	(1R,4S,4aS,5S,6S,7R,8R,8aR)-1,2,3,4,10,10-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4:5,8-dimethanonaphthalene
P,P'- DDD	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
O,P'- DDT	1,1,1-trichloro-2-(4-chlorophenyl)-2-(2-chlorophenyl)ethane
P,P'- DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane

Polychlorinated biphenyls compounds studied:

PCB' s congener no.	Chemical name	PCB' s congener no.	Chemical name
28	2,4,4-trichlorobiphenyl	138	2,2,3,4,4,5-hexachlorobiphenyl
52	2,2,5,5- tetra chlorobiphenyl	153	2,2,4,4,5,5-hexachlorobiphenyl
101	2,2,4,5,5-pentachlorobiphenyl	180	2,2,3,4,4,5,5-heptachlorobiphenyl
118	2,3,4,4,5- penta chlorobiphenyl		

Equipments:

- Centrifuge, 2,000 round per minute (r.p.m).
- Vortex, mixer.
- Analytical top balance, (0.0001/100 g).
- Terbo-vab evaporator.
- Dry stream of Nitrogen.
- Ice box.

Gas chromatograph Electron Capture Detector (GC-ECD):

Gas chromatograph Hewelet Packared 6890 series II equipped with electron capture detector (Ni⁶³) and oven temperature program of GC-ECD as following:

level	Rate (°C/min)	Tem.(°C)	Tim (min.)
1	--	90	2
2	20	150	0
3	6	270	15

- Total run time: 40 min.
- Injector temperature : 225 °C
- Detector temperature : 80 °C
- Column head pressure : 82 kpa
- Carrier gas : Nitrogen.
- Carrier flow rate : 2 ml/min.
- Make up flow rate : 50 ml/min.
- Septum purge : 5 ml/min.
- Splitting : off (0.0 – 0.07 min.).
- Splitting ratio : (1:50)
- Chromatographic columns parameters used in GC analysis illustrated in the following:

Descriptions	Column 1	Column 2
Name	PAS – 5	PAS - 1701
Film thickness	0.52 µm	0.25 µm
Length	25 m	25 m.
Column I.D.	0.32 mm.	0.032 mm.
Phase Ratio	150	320

Glassware: Measuring flask 100 ml, Glass pipettes 0.5, 1, 2, 5, 10 ml, Culture tube of 12.5 cm long fitted to centrifuged, Graduated concentrator tube of 10 ml, Non-heparinized vacutainer tubes, Sterilized syringes, Disposable Pasteur pipettes and Small vial (2 ml). All glassware used thoroughly cleaned with hot detergent solution, and then washed with distilled water and acetone before drying. All glassware is grade A; and immediately before use, rinsed with acetone.

Reagents: N- Hexane: Pestiscan (chromatography grade 99.8%), Acetone: Pestiscan (chromatography grade 99.8%), Hexane/acetone (9:1): Put 10 ml of acetone in 100 ml measuring flask then adds to the mark by hexane, Anhydrous sodium sulphate (Riedel-deHaen).

Standard Preparation: Pesticides reference standards solution of organochlorine and polychlorinated biphenyls compounds of reference materials used in identification, quantification, and determination of different compounds. All reference standards were certified compounds from Dr. Ehrenstorfer GmbH, Augsburg, Germany of high quality over, 90 % purity. Preparation of Injection standard solution: Five ml aldrin (10 µg/ml) diluted in one-liter mixture of hexane: acetone (9:1 v/v) to give a mixture containing 0.05 µg/ml of aldrin used as internal standard calculation.

Method of analysis:

Extraction

Environment Protection Agency (EPA) (1974) Method of analysis used for the determination of chlorinated pesticides and poly-chlorinated biphenyls in human blood serum. No needs to use a florisil clean-up column due to low fat sample content.

• **Extract the kept serum samples by the following steps:**

- Serum sample (2ml) drawn into 12.5 cm long culture tube.
- The sample mixed with 6 ml hexane, screwed cap on tightly.

- The culture tube was shaken horizontally by using shaker at 50-55 r.p.m for 2 hrs, then centrifuged at 2000 r.p.m for 6 min and the upper hexane layer was transferred into a 10 ml graduated concentrator tube by using a disposable Pasteur pipette.
- Another portion of 3 ml hexane added and centrifuged for 3 min.
- The whole hexane layer was collected and evaporated to dryness at Turbo-vab at 35 - 40 C° and 0.75 ml of aldrin (0.05 ug/ml) was added as an injection standard into an extract sample in GC vial.
- The samples kept in refrigerator at -20 C° until analysis on GC-ECD system.

All reagents and solvents must check to ensure that they are free from contamination of the compounds studied by electron capturing materials at the highest extent concentration levels. Reagent blanks should run with each set of samples.

Samples were analyzed in the Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Agriculture Research Center, Ministry of Agriculture-Egypt, which accredited by FINAS according to ISO/IEC 17025.

Determination of Organochlorine Pesticides and polychlorinated biphenyls in blood samples:

The pesticide concentration and PCB's in sample Cs (mg/kg) is calculated as follows:

$$C_s = \frac{A_s / A_{is}}{A_{st} / A_{ist}} \times C_{st} \times \frac{V_f}{V_p}$$

Where,

A_s = Peak area of analyte in sample.

A_{is} = Peak area of internal standard in sample.

A_{st} = Peak area of analyte in calibration run.

A_{ist} = Peak area of the internal standard in calibration run.

C_{st} = Concentration of standard (µg/ml).

V_f = Final volume (ml).

V_p = Volume of serum taken (ml).

If V_f = 0.75 ml, V_p = 2 ml, The pesticide concentration in sample Cs (mg/kg) is calculated as follows:

C_{ca}: sample concentration calculated from calibration data.

Quality Control

An internal quality control was performed to insure our results by doing recovery test, blank samples and reagent blank. All tests performed in narrow number due to small volume of the original kept serum samples.

Recovery test: Samples of enough kept serum fortified with both organochlorine pesticides and PCB's standard solutions at one level of 10 ppb. To 1 ml serum 0.2 ml of 0.05 (mg/l) of either organochlorine pesticides or PCB's, each compound was added on individual serum sample to eliminate the overlaps of chromatographic peaks. The fortified sample extracted and analyzed with each set of samples as described earlier, and

the obtained recoveries were calculated. The average recoveries varied between 78 to 98 % and 77 to 97 with a coefficient of variation (CV %) of 7.4 and 8.6 for organochlorine pesticides and PCB's respectively. The results are summarized in table (2).

Blank sample: A free pesticides sample was analyzed more than one time and injected on GC instrument at different times to ensure if any possible cross contamination happened

Reagent blank: Reagent blank analyzed as well as a serum sample to confirm that no reagents contamination and no cross-contamination between the real samples during the analysis.

Limit of detection (LOD): The limit of detection defined as three times the standard deviation of the blank value., the limit of detection defined as the instrument detection limit and chosen to be (0.005 ug/ml) for both organochlorine pesticides and PCB's.

Statistical Analysis: For all determinations, statistical analysis carried out, according to the method of **Armitage, 1971**; for both the control (Apparently healthy subjects) and other groups of cancer patients. All 99 cases subjected to as follows.

Table 2: Mean recoveries of organochlorine pesticides and PCB's from three fortified blood samples.

No.	Compounds	Expected level (ng/ml)	Mean (ng/ml)	Mean Recovery
I- Organochlorine pesticides				
1	α- HCH	10	9.30	93
2	HCB	10	9.42	94
3	β- HCH	10	8.92	89
4	γ- HCH	10	9.57	96
5	δ- HCH	10	9.71	97
6	Heptachlor	10	9.21	92
7	Heptachlor epoxide	10	9.83	98
8	P,P' – DDE	10	9.42	94
9	Dieldrin	10	8.79	88
10	Endrin	10	9.81	98
11	P,P'- DDD	10	8.52	85
12	O,P'- DDT	10	7.88	79
13	P,P'- DDT	10	7.84	78
II- PCB's Congeners				
1	PCB 28	10	9.7	97
2	PCB 52	10	7.7	77
3	PCB 101	10	8.4	84
4	PCB 118	10	9.1	91
5	PCB 153	10	8.9	89
6	PCB 138	10	9.4	94
7	PCB 180	10	7.9	79

Analysis of Variance (F-test) “one way ANOVA”: It is a procedure used for testing the differences among the means of two or more variables. If means of subgroups are greatly different, the variance of the combined groups is

much larger than the variance of the separate groups. The analysis of variance format and the differences in means based on this fact. This fact used in circumstances when it is desirable to study several variables.

Measurements Uncertainty

The random error (U_r) estimated from spiked samples as the relative standard deviation of repeated experiments. The spiked samples run with each run of samples during several weeks. Therefore, these experiments should describe the maximum random error in the work. The error also estimated at a level close to the limit of determination but now by the same analyst and on the same day, the relative standard deviation was lower.

The systematic error (U_s) estimated from the same experiments as a random error by comparing the measured value to the theoretical value. The standard deviation of the average relative differences used as a measure of systematic error. The influence of the systematic error was small compared to random error.

The measured value of U was in all cases less than 15%. Therefore the expanded uncertainty (of 95% confidence level) expressed as $2 \times U$ of the measurements is less than $\pm 30\%$ ISO Geneva, (1993). The results showed in table 3.

Table 3: The total uncertainty of Organochlorine pesticides and PCB's expressed as random and systematic error on different serum samples.

Compounds	No. of samples	Conc. (ug/l)	U Combined	U Expanded
I- Organochlorine pesticides				
α - HCH	7	10	11	22
HCB	7	10	9.5	19
β - HCH	7	10	10.5	21
γ - HCH	7	10	12	24
δ - HCH	7	10	10.5	21
Heptachlor	7	10	11	22
Heptachlor Epoxide	7	10	13	26
P,P'- DDE	7	10	15	30
Dieldrin	7	10	12	24
Endrin	7	10	13	26
P,P'- DDD	7	10	14.5	29
O,P'- DDT	7	10	15	30
P,P'- DDT	7	10	13.5	27
II- Polychlorinated Biphenyls				
PCB 28	3	10	10	20
PCB 52	3	10	12	24
PCB 101	3	10	11	22
PCB 118	3	10	13	26
PCB 153	3	10	14	28
PCB 138	3	10	12	24
PCB 180	3	10	13.5	27

RESULTS AND DISCUSSION

Monitoring of organochlorine pesticide residues and PCB's in human blood samples collected from Egyptian during 2003-2004

Organochlorine pesticides heavily used in Egypt during the 1960's and 1970's. Starting from the 1980's the use of this group was restricted until completely prohibited officially in 1987. Organochlorine pesticide residues which were investigated HCB, total HCH isomers (alpha, beta, gamma and delta-HCH), aldrin, dieldrin, heptachlor, heptachlor epoxide, endrin and DDT complexes (o, p'-DDT, p, p'-DDT, p, p'-DDE and p, p'-DDD). The mean concentration of beta-HCH, total HCH, p,p'-DDE and total DDT when compared with organochlorine pesticides detected in human blood samples of apparently healthy subjects, hepatitis C virus, hepatocellular, bilharzial and bladder cancer patients are illustrated in figure (1).

Organochlorine pesticide residues and PCB's in human blood samples of apparently healthy subjects (CONTROL)

Table (4) shows minimum, maximum, mean, median, 90th percentile values, frequency and frequency percentages of organochlorine pesticide residues (ng/ml) obtained from analysis of twenty-three blood serum samples collected from apparently healthy persons as a control during 2003 - 2004. Hexachlorobenzene (HCB), aldrin, Dieldrin, Endrin, heptachlor and its epoxide and not detected in all of the 23 blood control samples analyzed.

HCH isomers: The mean value of total HCH isomers detected in samples was 0.764 (ng/ml). The contributions of the different isomers to this figure as mean values were 0.028 and 0.735 (ng/ml) for alpha and beta isomers respectively. While no residue for delta and gamma, isomer was found. The frequency of HCH isomer detection in human blood serum samples analyzed were 2, 17 and 17 as alpha, beta and total HCH isomers respectively, with percentage of frequency 8.7, 73.9 and 73.9 % respectively. The results show that the beta isomer was found in high frequency and amount.

Total DDT's: DDT derivatives are the most common chlorinated hydrocarbon residues, detected in almost all samples analyzed. It was noticed that p,p'-DDE found in high amount. Table (4) indicates the mean values of p, p'-DDT, p,p'-DDD, p,p'-DDE as well as total DDT's is 0.03, 0.006, 2.22 and 2.25 ppb respectively. While no residue for o,p'-DDT was found. The frequency of p,p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's were 1, 1, 21 and 21 respectively with a percentage of frequency 4.3, 4.3, 91.3 and 91.3 % respectively.

PCB's congeners: The polychlorinated biphenyl (PCB) congeners investigated were 28, 52, 101, 118, 138, 153 and 180. All the investigated polychlorinated biphenyl's congeners not detected in analyzed human serum samples as shown in table (4).

Table 4: Minimum, maximum, mean, standard deviation, median, 90th % values and frequencies of organochlorine pesticides and PCB's residues (ng/ml) monitored in human blood samples of apparently healthy subjects (control)

Compounds	Min	Max	Mean ±SD	Median	90 th *	Frequency	Freq. %
HCB	-	-	-	-	-	-	-
α-HCH	N.D.	0.456	0.028±0.02	0	0	2	8.7
β-HCH	N.D.	7.09	0.735±1.64	0.092	3.3	17	73.9
δ-HCH	-	-	-	-	-	-	-
γ-HCH	-	-	-	-	-	-	-
Total HCH	N.D.	7.546	0.764±1.73	0.092	1.37	17	73.9
Endrin	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-
Heptachlor Epoxide	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-
p,p`-DDT	N.D.	0.694	0.030±0.14	0	0	1	4.3
o,p-DDT	N.D.	N.D.	N.D.	0	0	0	0
p,p`-DDD	N.D.	0.141	0.006±0.039	0	0	1	4.3
p,p`-DDE	N.D.	8.41	2.22±2.40	1.03	5.66	21	91.3
Total DDT	N.D.	9.245	2.253±2.5	1.03	5.66	21	91.3
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-
Total PCB's	-	-	-	-	-	-	-

Volunteers Number = 23

N.D. = Not detected

LOD (Lim it of detection) = 0.005 ng/ml

* 90th % value less than 10 positive results

Organochlorine pesticide residues and PCB's in human blood samples of HEPATITIS C VIRUS (HCV) patients

Table (5) describes minimum, maximum, mean, median, 90th percentile values, frequency and frequency % of organochlorine pesticide residues (ng/ml) obtained from analysis of seventeen serum samples collected from hepatitis C virus (HCV) patients during 2003-2004. Hexachlorobenzene (HCB), aldrin, dieldrin, endrin heptachlor and its epoxide, residues were not detected in any of the 17 serum samples analyzed.

HCH isomers: The mean value of total HCH isomers detected in samples was 1.57 (ng/ml). While the contributions of the different isomers to this figure as mean values were 0.097, 1.46 and 0.029 (ng/ml) for alpha, beta, and gamma isomers, respectively. While no residue for delta, isomer was found.

Total DDT's: DDT derivatives were the most common chlorinated hydrocarbon residues, detected in almost all samples analyzed. It was noticed that p,p'-DDE was found in high amount. Table (5) indicates the

mean values of p, p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's (i.e. 0.316 , 0.012 , 2.65 and 2.7 (ng/ml) respectively). While no residue for o, p'-DDT was found. The frequency of p,p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's were 6, 1, 17 and 17 respectively with a percentage of frequency 35.3, 5.9, 100 and 100 % respectively.

PCB's congeners: The polychlorinated biphenyl (PCB's) congeners investigated were 28, 52, 101, 118, 138, 153 and 180. No the investigated polychlorinated biphenyl's congeners detected in analyzed human blood samples as shown in table (5).

Table 5: Minimum, maximum, mean, standard deviation, median, 90th % values and frequencies of organochlorine pesticides and PCB's residues (ng/ml) monitored in human blood samples collected from Hepatitis C virus patients (HCV)

Compounds	Min	Max	Mean ±SD	Median	90 th *	Frequency	Freq.%
HCB	-	-	-	-	-	-	-
α-HCH	.N.D	0.53	0.097±0.149	0.032	-	9	52.9
β-HCH	.N.D	6.24	1.46±1.85	0.69	3.67	14	82.4
δ-HCH	-	-	-	-	-	-	-
γ-HCH	.N.D	0.31	0.029±0.086	0	0	2	11.8
Total HCH	.N.D	6.96	1.57±1.96	0.722	3.92	14	82.4
Endrin	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-
Heptachlor Epoxide	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-
p,p`-DDT	.N.D	1.02	0.316±0.18	0	-	6	35.3
o,p-DDT	-	-	-	-	-	-	-
p,p`-DDD	.N.D	0.186	0.012±0.046	0	0	1	5.9
p,p`-DDE	0.19	9.65	2.65±2.41	0.96	5.68	17	100
Total DDT	0.19	10.86	2.7±2.98	0.96	6.29	17	100
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-
Total PCB's	-	-	-	-	-	-	-

Volunteers no. = 17

N.D. = Not detected

LOD = 0.005 ng/ml

* No. 90th % value less than 10 positive results

Organochlorine pesticide residues and PCB's in human blood samples of HEPATOCELLULAR CARCINOMA (HCC) patients

Table (6) shows minimum, maximum, mean, median, 90th percentile values, frequency and frequency % of organochlorine pesticide residues

(ng/ml) obtained from analysis of eighteen blood samples collected from hepatocellular carcinoma (HCC) patients during 2003 - 2004.

Aldrin, dieldrin (a metabolite of aldrin), heptachlor and its epoxide and endrin pesticides were not detected in any of the 18 blood serum samples analyzed.

Hexachlorobenzene (HCB) The mean value of hexachlorobenzene (HCB) detected in samples was 0.004 ppb. The frequency of detection of HCB in samples was 1 out of 18 serum samples with a percentage of frequency 5.6 %.

Table 6: Minimum, maximum, mean, standard deviation, median, 90th % values and frequencies of organochlorine pesticides and PCB's residues (ng/ml) monitored in human blood samples collected from hepatocellular carcinoma patient (HCC).

Compounds	Min	Max	Mean \pm SD	Median	90 th *	Frequency	Freq.%
HCB	.N.D	0.075	0.004 \pm 0.018	0	0	1	5.6
α -HCH	.N.D	0.038	0.003 \pm 0.01	0	-	2	11.1
β -HCH	.N.D	6.51	0.894 \pm 1.46	0.735	1.15	16	88.9
δ -HCH	-	-	-	-	-	-	-
γ -HCH	-	-	-	-	-	-	-
Total HCH	.N.D	6.55	0.897 \pm 1.47	0.735	1.15	16	88.9
Endrin	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-
Heptachlor Epoxide	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-
p,p'-DDT	.N.D	0.338	0.019 \pm 0.08	0	0	1	5.6
o,p'-DDT	-	-	-	-	-	-	-
p,p'-DDD	.N.D	0.298	0.03 \pm 0.089	0	-	2	11.1
p,p'-DDE	0.19	15.96	3.97 \pm 3.89	3.08	7.09	18	100
Total DDT	0.19	16.596	4.02 \pm 4.02	3.08	7.15	18	100
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-
Total PCB's	-	-	-	-	-	-	-

Volunteers no. = 18

N.D.. = Not detected

LOD (Lim it of detection)=0.005 ng/ml

* : No. 90th % value for less than 10 positive results

HCH isomers: The mean value of total HCH isomers detected in samples was 0.897 (ng/ml). While the contribution of the different isomers to this figure as mean values were 0.003 and 0.894 (ng/ml) for alpha, and beta isomers respectively. The delta and gamma isomer residues not found any more. The frequency of HCH isomer detection in human blood samples

analyzed were 2, 16 and 16 as alpha, beta and total HCH isomers respectively, with percentage of frequency 11.1, 88.9 and 88.9 % respectively. The results show that the beta isomer detected in high frequency and amount.

Total DDT's: DDT derivatives were the most common chlorinated hydrocarbon residues, detected in almost all samples analyzed. It was noticed that p,p'-DDE was found in high amount. Table (6) indicates the mean values of p, p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's (i.e. 0.019 , 0.03 , 3.97 and 4.02 ppb respectively). While no residue for o, p'-DDT was found. The frequency of p,p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's were 1, 2, 18 and 18 respectively with a percentage of frequency 5.6, 11.1, 100 and 10% respectively.

PCB's congener: The polychlorinated biphenyl (PCB) congeners investigated were 28, 52, 101, 118, 138, 153 and 180. No the investigated polychlorinated biphenyl's congeners were detected in analyzed human blood samples as shown in table (6).

Organochlorine pesticide residues and PCB's in human blood samples of BILHARZIAL patients

Table (7) shows minimum, maximum, mean, median, 90th percentile values, frequency and frequency % of organochlorine pesticide residues (ng/ml) obtained from analysis of twenty one blood samples collected from bilharzias patients during 2003 - 2004. Aldrin, dieldrin (a metabolite of aldrin), heptachlor and its epoxide and endrin pesticides were not detected in any of the 21 serum samples analyzed.

Hexachlorobenzene (HCB) The mean value of hexachlorobenzene (HCB) detected in samples was 0.009 (ng/ml). The frequency of detection of HCB in samples was 1 out of samples with a percentage of frequency 4.5 %.

HCH isomers: The mean value of total HCH isomers detected in samples was 2.35 (ng/ml). While the contribution of the different isomers to this figure as mean values were 0.124 and 2.23 (ng/ml) for alpha and beta isomers respectively. While no residue for delta and gamma isomer were found. The frequency of HCH isomer detection in human blood samples analyzed were 10, 20 and 20 as alpha, beta and total HCH isomers respectively, with percentage of frequency 47.6, 95.2 and 95.2 % respectively. The results show that the beta isomer is found in high frequency and amount.

Total DDT's: DDT derivatives were the most common chlorinated hydrocarbon residues, detected in almost all samples analyzed. It was noticed that p,p'-DDE was found in high amount. Table (7) indicates the mean values of p, p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's (i.e. 0.305 , 0.15 , 5.16 and 5.68 (ng/ml) respectively). While no residue for o, p'-DDT was found. The frequency of p,p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's were 5, 7, 21 and 21 respectively with a percentage of frequency 23.8, 33.3, 100 and 100 % respectively.

PCB's congener: The polychlorinated biphenyl (PCB) congeners investigated were 28, 52, 101, 118, 138, 153 and 180. No the investigated polychlorinated biphenyl's congeners were detected in analyzed human blood samples as described in table (7).

Table 7: Minimum, maximum, mean, standard deviation, median, 90th % values and frequencies of organochlorine pesticides and PCB's residues (ng/ml) monitored in human blood samples collected from bilharzias patients.

Compounds	Min	Max	Mean ±SD	Median	90 th *	Frequency	Freq.%
HCB	N.D.**	0.203	0.009±0.04	0	0	1	4.5
α-HCH	.N.D	0.575	0.124±0.19	0	0.545	10	47.6
β-HCH	.N.D	12.58	2.23±3.18	0.844	8.66	20	95.2
δ-HCH	-	-	-	-	-	-	-
γ-HCH	-	-	-	-	-	-	-
Total HCH	.N.D	13.08	2.35±3.33	0.938	9.06	20	95.2
Endrin	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-
Heptachlor Epoxide	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-
p,p`-DDT	.N.D	4.01	0.305±0.903	0	-	5	23.8
o,p`-DDT	-	-	-	-	-	-	-
p,p`-DDD	.N.D	1.55	0.15±0.351	0	-	7	33.3
p,p`-DDE	0.398	15.94	5.16±4.92	4.01	14.76	21	100
Total DDT	0.398	17.19	5.68±5.16	2.24	16.61	21	100
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-
Total PCB's	-	-	-	-	-	-	-

Volunteers no. = 21

N.D. = Not detected

LOD = 0.005 ng/ml

* = No. 90th % value less than 10 positive results

Organochlorine pesticide residues and PCB's in human blood samples of BLADDER CANCER patients

Table (8) shows minimum, maximum, mean, median, 90th percentile values, frequency and frequency% of organochlorine pesticide residues (ng/ml) were obtained from analysis of twenty blood samples collected from bladder cancer patients during 2003 – 2004. Hexachlorobenzene (HCB), aldrin, dieldrin, heptachlor and its epoxide and endrin pesticides were not detected in any of the 23 serum samples analyzed.

HCH isomers: The mean value of total HCH isomers detected in samples was 0.237 (ng/ml). The mean contribution of the different isomers values were 0.002, 0.235 (ng/ml) for alpha and beta isomers respectively. No residue for delta and gamma isomer were detected. The frequency of HCH isomer detection in human blood samples analyzed were 1, 10 and 10 as alpha, beta and total HCH isomers respectively, with percentage of frequency

5, 50 and 50 % respectively. The results show that the beta isomer is found in high frequency and amount.

Total DDT's: DDT derivatives were detected in almost all samples analyzed with noticeably that p,p'-DDE was found in high amount. Table (8) indicates the mean values of p, p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's i.e. 0.021, 0.005, 0.762 and 0.787 (ng/ml) respectively. While no residue for o, p'-DDT was found. The frequency of p,p'-DDT, p,p'-DDD, p,p'-DDE and total DDT's were 1, 1, 19 and 19 respectively with a percentage of frequency 5, 5, 95 and 95 % respectively.

PCB's congener: The polychlorinated biphenyl (PCB) congeners investigated were 28, 52, 101, 118, 138, 153 and 180. No the investigated polychlorinated biphenyl's congeners were detected in analyzed human blood samples as described in table (8).

Table 8: Minimum, maximum, mean, standard deviation, median, 90th percentile values and frequencies of organochlorine pesticides and PCB's residues (ng/ml) monitored in human blood samples from bladder cancer patients.

Compounds	Min	Max	Mean ±SD	Median	90 th *	Frequency	Freq.%
HCB	-	-	-	-	-	-	-
α-HCH	.N.D	0.034	0.002±0.008	0	0	1	5
β-HCH	.N.D	2.64	0.235±0.584	0.31	0.3	10	50
δ-HCH	-	-	-	-	-	-	-
γ-HCH	-	-	-	-	-	-	-
Total HCH	.N.D	2.674	0.237±0.592	0.04	0.3	10	50
Endrin	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-
Heptachlor Epoxide	-	-	-	-	-	-	-
Dieldrin	-	-	-	-	-	-	-
p,p'-DDT	.N.D	0.41	0.021±0.092	0	0	1	5
o,p'-DDT	-	-	-	-	-	-	-
p,p'-DDD	.N.D	0.092	0.005±0.021	0	0	1	5
p,p'-DDE	.N.D	3.21	0.762±0.898	0.336	2.27	19	95
Total DDT	.N.D	3.712	0.787±1.06	0.336	2.27	19	95
PCB 28	-	-	-	-	-	-	-
PCB 52	-	-	-	-	-	-	-
PCB 101	-	-	-	-	-	-	-
PCB 118	-	-	-	-	-	-	-
PCB 138	-	-	-	-	-	-	-
PCB 153	-	-	-	-	-	-	-
PCB 180	-	-	-	-	-	-	-
Total PCB's	-	-	-	-	-	-	-

Volunteers no: 20

N.D. = Not detected

LOD (Lim it of detection)=0.005 ng/ml

* = No. 90th % value for less than 10 positive results

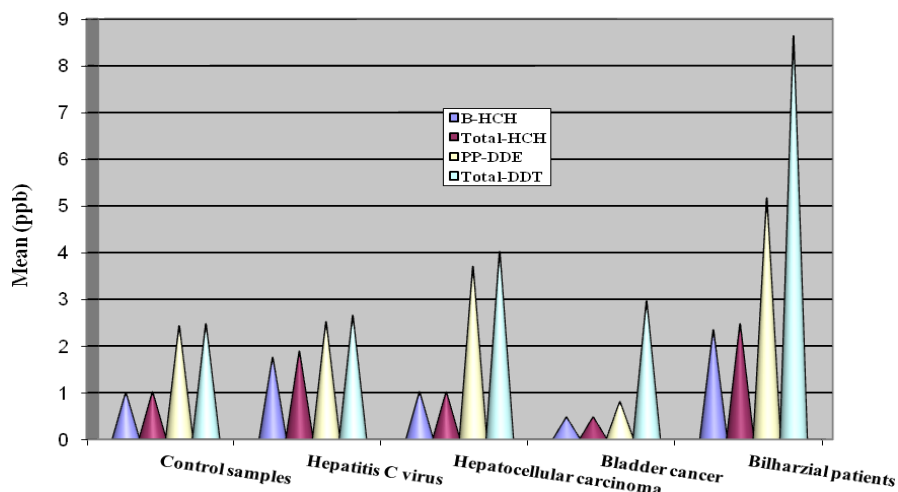


Figure 1: Mean levels of different pesticides detected in human blood samples of cancer patients

In Egypt as in other developing countries, organochlorine exposure levels might be high. The levels of such group of contaminants have been monitored and detected in medicinal plants, mother's milk, fish, soil and River Nile water samples (Dogheim *et al.*, 1986, 1988 and 1992).

From the organochlorine pesticide group, dichloro diphenyl dichloromethane (DDT) and hexachlorocyclohexane (HCH) were detected in soil, water, milk and fish samples {El- Dib and Badawy, 1985; Nabawi *et al.*, 1987; El - Gendy *et al.*, 1991; Badawy *et al.*, 1995; Amr *et al.*, 1995; Soliman *et al.*, 1997}. The data from monitoring the contamination in the different matrices revealed pronounced decrease in the levels of contamination in Egypt with the lapse of time.

Monitoring of organochlorine pesticide residues in human tissue is not an easy task all over the world due to the difficulties of sample collection. The small sample size and the wide range of inter individual variability as shown by the large standard deviations limited the interpretation of some results, Also the lack of measurement of serum lipids limited in comparison with other studies where these measurements were not available.

In Egypt, this study is the first in its area of investigation where no previous database related to the chlorinated pesticide residues levels in the blood serum collected from liver cancer or Hepatocellular carcinoma (HCC), bladder cancer, Hepatitis C virus (HCV) and bilharzias patients. For this reason, no comparison carried out and the results obtained are furnishing a background for successive future work in the field of monitoring in human tissues. Several findings are of interest. First, the majority of the participants did not exhibit higher DDE or beta-HCH levels in serum samples than those reported in United States. Other countries studies {Wolff *et al.*, 1993 and Krieger *et al.*, 1994} as expected because of the high organochlorine levels in

Egypt {El- Dib and Badawy, 1985; Nabawi *et al.*, 1987; Dogheim *et al.*, 1988, 1990; El-Gendy *et al.*, 1991; Badawy *et al.*, 1995; Amr *et al.*, 1995}. This finding is in agreement with Soliman *et al.*, (2003). They found lower levels of dichloro diphenyl dichloro ethylene (DDE), total DDT, and beta-hexachlorohexane (beta-HCH) in most Egyptian women than those in other developed countries. Second, no great differences in serum organochlorine levels was observed between patients and controls (apparently healthy subjects) as reported in recent studies {Hoyer *et al.* 1999, 2000; Mendonca *et al.*, 1999; Zheng *et al.*, 2000; Wolff *et al.*, 2000; Stellman *et al.*, 2000; Laden *et al.*, 2001 a, b; Gammon *et al.*, 2002}. But there was difference in pesticide levels between hepatitis C virus and hepatocellular carcinoma patients as well as bilharzias patients and bladder cancer patients. Third, no residues of polychlorinated biphenyls were observed in any samples of patients or control (apparently healthy subjects) in opposite to developed countries due to that PCB's are industrial pollutants characterizing the developed countries but not the developing agricultural countries. Such findings are in agreement with the data showing lower levels of PCB's in human adipose tissue in Egypt.

Table 9: Correlation between mean values of pesticides residues (ng/ml) detected in human blood serum of control, cancer patients (liver and bladder) and non-cancer patients (Hepatitis C virus and Bilharzia).

Type of Cancer	β -HCH	Total- HCH	P,P'-DDE	Total- DDT
	Mean \pm SE	Mean \pm SE	Mean \pm SE	Mean \pm SE
Apparently healthy persons (Control)	0.995 ^b \pm 0.448	1.01 ^{ab} \pm 0.452	2.427 ^{bc} \pm 0.526	2.467 ^b \pm 0.526
Hepatitis C Virus Patients	1.75 ^{ab} \pm 0.489	1.88 ^{ab} \pm 0.514	2.513 ^{bc} \pm 0.642	2.649 ^b \pm 0.674
Hepatocler carcinoma Patients	1.01 ^{ab} \pm 0.379	1.01 ^{ab} \pm 0.378	3.699 ^{ab} \pm 0.917	4.0181 ^{ab} \pm 0.913
Bilharzial Patients	2.34 ^a \pm 0.721	2.467 ^a \pm 0.753	5.162 ^a \pm 1.074	5.635 ^a \pm 3.033
Bladder cancer Patients	0.76 ^b \pm 0.245	0.82 ^b \pm 0.248	2.64 ^c \pm 0.229	2.96 ^b \pm 2.294

* : Significant at the 0.05

** : Highly significant at the 0.01

N.S : Not Significant (P > 0.05)

SE : Standard Error

(a, b, ab, bc) : Means that each pesticide mean (ng/ml) takes a different letter to indicate that there is no significant difference between two means when any of two means take the same letter a or b, other than the significance was reported.

From the 14 organochlorine pesticides investigated in this study, heptachlor, heptachlor epoxide, aldrin, endrin and dieldrin residues did not show up in any of the analyzed samples means that the residues of these pesticides were diminished from blood serum, these results confirm the previous findings in the literature of no detectable residues of this group of chemical contaminants. However, DDT, HCH's and HCB have different

situation, where DDT (mainly p,p'-DDE), HCH (mainly beta-HCH) and HCB. The most persistent pesticides with high frequency 97% and 77.8 and 2% respectively, this finding is in agreement with {Lara *et al.*, 1987 ; Skaare *et al.*, 1988 ; Ataniyazova *et al.*, 2001; Soliman *et al.*, 1997; Stellman *et al.*, 1998; Rubin *et al.*, 2001; Daniel *et al.*, 2002; Schell *et al.*, 2003 and Soliman *et al.*, 2003}.

Such finding may go in a positive correlation with the monitoring data of some pesticides residues in mother's milk and environment (Dogheim *et al.*, 1991 and 1990).

Table (9) shows that bilharzias patients have higher levels of different type of pesticides in their blood samples particularly total- DDT and p, p'-DDE which recorded 5.635 ± 3.033 and 5.162 ± 1.074 respectively. On the contrary bladder cancer patients illustrated lower levels of the same pesticides in their serum which recorded as 2.96 ± 2.294 , 2.64 ± 0.229 for total- DDT and P, P'-DDE respectively. This is an alarming sign where these patients should have a follow up plan to investigate them from time to time to discover if a bladder cancer might be developed with regard to bilharzias history infection. The higher levels of pesticides of bilharzias patients may be attributed to extensive illegal use of pesticides during farming activities and pest control.

On the other hand, the hepatocellular carcinoma patients (HCC) have higher levels of total - DDT and p, p'- DDE than those of hepatitis C virus, which recorded 4.018 ± 0.913 , 3.699 ± 0.917 for total - DDT and P,P'-DDE respectively. In hepatocellular carcinoma patients (HCC) while recorded 2.649 ± 0.674 , 2.513 ± 0.642 for Total- DDT and P,P'-DDE respectively in hepatitis C virus.

In apparently healthy (Control) samples, the levels of pesticides lower than non-cancer patients (hepatitis C virus and bilharzias patients) as a high-risk group for hepatocellular carcinoma and bladder cancer patients respectively. The row data revealed lower levels of chlorinated pesticides especially DDT in control than those of cancer and non-cancer patients. This finding supports that organochlorine pesticides may be a co-factor or co-carcinogen when found in high levels in human blood. However, statistical analysis could not confirm the significance correlation between the levels of this group of pesticides in cancer and non-cancer patients with regard to control persons as illustrated in table 9.

Comparison between the mean levels of chlorinated pesticides and PCB'S in human blood in different countries for a long period

Comparing the obtained monitoring data in 2003/2004 in Egypt with those published from other countries in table(10), which classified the decremented trend in organochlorine pesticides and industrial chemicals contaminants β -HCH, total DDT, p-p'-DDE, HCB and PCB'S in Egypt compared with other countries. In Egypt, β -HCH levels decreased (2.95 ng/ml at 2002, 0.857 ng/ml at 2003).

Regarding DDT, eight data countries were reported in table (10), In India (1984-1991) total-DDT residues were decreased from 390 to 82.6 ng/ml, In Venezuela it decreases from 213 to 44 ng/ml during (1982-1987).

In Egypt the total DDT level was only 3.07 ng/ml in (2003/2004), the value obtained was slightly higher in contrast to other countries e.g. Russia, Slovak Republic and Finland, which showed 1.17, 0.27 and 1.8 ng/ml respectively.

Regarding p-p'-DDE, residues showed a significant decrease in most countries especially for the developed countries that completely prohibited the use of these chemicals officially in 10 years difference than those applied in Egypt.

In Venezuela the p-p'-DDE (the main metabolite of DDT) residues decreased from 2067 to 149 ng/ml during (1982-1987) and in USA from 9.10 to 0.37 ng/ml during (1980-2003). In Egypt the overall decreased is obvious from the level 40 to 2.92 ng/ml during (1999-2004) however these levels are still higher than those for developed countries that reflect the same misuse of these chemical contaminants as described in figure (2).

As for HCB data, there were 6 countries available starting from 1988 to 1995. The high levels were from Nigeria recording 400 ng/ml at 1988, Canada 21 ng/ml at (1994 -1997) and from Slovak Republic 5.38 ng/ml at 1992. The low levels were from Finland 0.3 ng/ml at 1991, from Belgium had 0.07 ng/ml at 1999 and USA 0.03 ng/ml at 1994. While in Egypt the level becomes lower than all previous levels recording 0.003 ng/ml.

As well as for PCB's data, there were 7 countries (1980 to 2003). The highest levels were 733.1 ng/ml in Canada (1995), 3.4 ng/ml in German (2000), 2.0 ng/ml in Finland (1991), 1.79 ng/ml in Slovak Republic (1994), from 9.6 to 0.2 ng/ml were in USA (1980-2003) and the lower levels were 0.53 and 0.85 ng/ml in Russia (2003) and Belgium (1999) as shown. In Egypt the PCB's levels were decreased from 58 ng/ml at (1999-2000) to become not detected (2003 – 2004). Such findings are in agreement with the data showing low levels of contamination of PCB's in human adipose tissue and mother' s milk in Egypt.

There is 10 years (decade) difference in prohibition of DDT in the United State and Egypt. So, the DDT residues are slightly comparable if we consider this time period of starting the prohibition as shown in figure (2). The data figure (2) also revealed the disappearance trend in DDT accumulated in human tissue with time lapse overall the world as reported in India, Venezuela, United States and Egypt. This means that Egypt is following other countries i.e. by 10 years in the level of residues where these countries banded the use of such group of contaminants 10 years earlier.

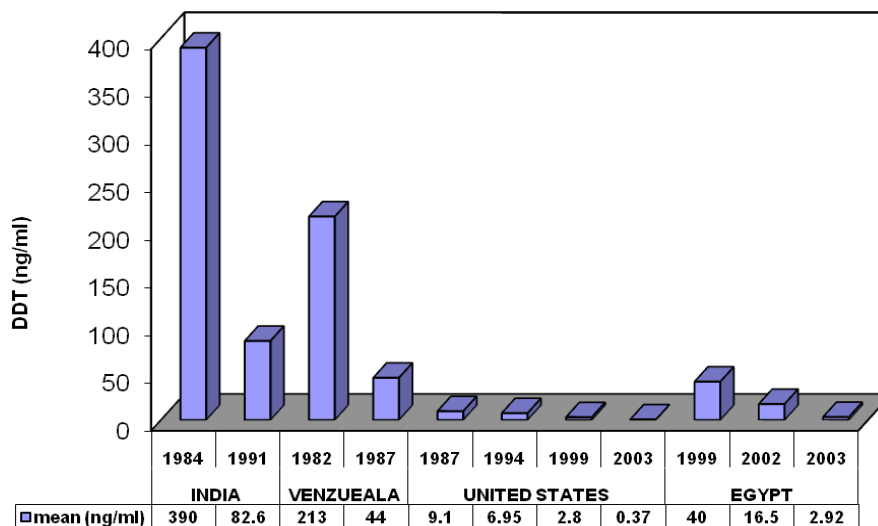


Figure 2: Disappearance of DDT residues in some countries in certain period

Conclusion

Only the residues of DDT's (p,p'-DDE mainly) and HCH,s (beta -HCH mainly) are the contaminants from the organochlorine group of pesticides that are still detected in human blood serum in Egypt. Preventing any new addition or release of organochlorine pesticides and/or polychlorinated biphenyls to the environment as well as other contaminants e.g. heavy metals, dioxin and dibenzofurans, which might lead to exposure risk to humans. The government should support and control the development of clear technologies and disposal techniques. Since, exposure to PCB's might increase the health risks such as non-Hodgkin's lymphoma, diabetes and liver disease, However, PCB's were absent from the serum of any of the samples analyzed. With respect to the keeping of lower risk hazards for food contaminants, we should control foods imported from the industrial countries that might have those contaminants especially PCB's. Start studies on the risk assessment of those food contaminants in Egypt. Report cancer cases and set cancer map to facilitate investigations on the root cause of cancer and correlate to the monitoring programs of chemical contaminants. The higher significant levels of different organochlorine pesticides namely beta-HCH, total HCH, p,p'-DDE and total DDT in bilharzias and hepatitis C virus with respect to apparently healthy subjects as a control could indicate the possible role of these agents in neoplastic transformation of bladder and liver as well. Further research work should be done to investigate the possible role of chlorinated pesticides and the incidence of cancer. Continuous monitoring with organochlorine pesticides and polychlorinated biphenyls residues should be carried out to evaluate the situation of contamination of human tissues in Egypt periodically.

REFERENCES

- Ahmed, M.T.; N. Loutfy and E. El Shiekh (2002) Residue levels of DDE and PCBs in the blood serum of women in the Port Said region of Egypt, *J. Hazard Mater*, 4 ; 89(1): p41-8.
- Amr, M.M.; A.W. Moursy; R.S. Hafez; S.M. Dogheim and A.M. Abozeid (1995) Pesticide residues in milk and dairy products in Egypt. *Egypt. J. Occup. Med.* 19, 147–168.
- Armitage p. (1971) *Statistical methods in medical research*, Blackwell science Publication London.
- Ataniyazova, O.A; R.A. Baumann; A.K.D. Liem; U.A Mukhopadhyay; E.F. Vogelaar and E.R. Boersma (2001) Levels of certain metals, organochlorine pesticides and dioxins in cord blood, maternal blood, human milk and some commonly used nutrients in the surroundings of the Aral Sea (Karakalpakstan, Republic of Uzbekistan), *Acta Paediatrica*, 90:(7), p 801-808,37 ref.
- Badawy, M.I.; R.A. Wahaab and H.F. Abou Waly (1995) Petroleum and chlorinated hydrocarbons in water from Lake Manzala and associated canals. *Bull. Environ. Contam. Toxicol.* 55, 258–263.
- Baris, D.; L.W. Kwak; N. Rothman; W. Wilson; A. Manns; R.E. Tarone; P. Hartge (2000) Blood levels of organochlorines before and after chemotherapy among non Hodgkin's lymphoma patients, *Cancer Epidemiology Biomarkers and Prevention*,9: (2), p193-197, 21 ref.
- Brown JF; DL Bedard ; MJ Breanan; JC Carnahan; H. Feng and RE. Wagner (1987) Polychlorinated Biphenyls dechlorination in aquatic sediments. *Science*, 236: p 709- 711.
- Butler. W. J.; L. Seddon; E. McMullen; J. Houseman; K. Tofflemire; A. Corriveau; J.P. Weber; C. Mills; S. Smith and J. Van Oostdam (2003) Organochlorine levels in maternal and umbilical cord blood plasma in Arctic Canada. *Sci. Total Environ.*, 302 (1-3): p 27-52.
- Chand, B.; T. Sankaranarayan; R.L. Yadava and M.V.V.L. Narasimham (1991). Residues of DDT and its metabolite in blood of exposed factory workers and their correlation with ill health symptoms, *Journal of Communicable Diseases*, 23:(4), P 245-247, 8 ref.
- Cogliano VJ. (1998) Assessing the cancer ask from environment PCB's *Environ Health prospect*, 106: p 317-323.
- Cooper, G.S., S.A. Martin; M.P. Longnecker; D.P. Sandler and D.R. Germolec (2004) Associations between plasma DDE levels and immunologic measures in African-American farmers in North Carolina, *Environ. Health Perspect.*,112 (10): p 1080-4.
- Covaci, A.; P. Jorens; Y. Jacquemyn and P. Schepens (2002) Distribution of PCBs and organochlorine pesticides in umbilical cord and maternal serum, *Science of the Total Environment*, 298: (1-3), p 45-53.
- Daniel, V., W. Huber; K. Bauer; C. Suesal; C. Conradt and G. Opelz (2002) Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyl-dichloroethylene (DDE) 4.4 blood levels with plasma IL-4, *Arch. Environ. Health*, 57 (6): p 541-7.

- Delgado, I.F.; H.H. Barretto; T.A. Kussumi; I.B. Alleluia; C.A. Baggio and F.J. Paumgarten (2002) Serum levels of organochlorine pesticides and polychlorinated biphenyls among inhabitants of Greater Metropolitan Rio de Janeiro, Brazil, *Cad. Saude Publica.*, 18 (2): p 519-24.
- Dogheim, S.M. ; M.M Almaz; N. Sh. Takla and R. A. Yousef (1986) Multiple analysis of pesticides residues in certain plants of medicinal importance, *Bull. Ent. Soc. Egtpt. Econ. Ser.* 15: 157- 163.
- Dogheim, S.M.; M.M.Almaz; S.N. Kostandi and M.E. Hegazy(1988) Pesticide residues in milk and fish samples from upper Egypt. *J. Assoc. Off. Anal. Chem.* 71, 874–876.
- Dogheim, S.M.; E.N. Nasr; M.M. Almaz and M.M. El-tohamy (1990) Pesticide residues in milk and fish samples collected from two Egyptian governorates. *J. Assoc. Off. Anal. Chem.* 73, 19–21.
- Dogheim, S.M.; M. A. El- Shafeey; A. M. H. Afify and F.E. Abdel – Aleem (1991). Levels of pesticide residues in Egyptian human milk samples and infant dietary intake, *J. Assoc. Off. Anal. Chem.* V. 74 (1), 89 – 91.
- Dogheim, S.M.; M. El-moattassem; F. Mackhlad; N. Shaker and S. Hassan (1992). Pesticide residues in the rosetta branch of the river Nile during the winter closure period (Dec. 1990- May 1991). *International conference on protection and development of the Nile and other major rivers, Cairo Vol. 1.*
- El-Dib, M.A. and M.I. Badawy (1985) Organo-chlorine insecticides and PCB's in river Nile water, Egypt, *Bull. Environ. Contam. Toxicol.* 34, 126–133.
- El-Gendy, K.S.; A.A. Abdalla and A.A. Aly (1991) Residue levels of chlorinated hydrocarbon compounds in water and sediment samples from Nile ranches in the delta, Egypt, *J. Environ. Sci. Health B* 26 (1), 15–36.
- Environment Protection Agency (EPA) (1974) Analysis of pesticide residues in human and environmental samples. U.S. Environmental Protection Agency (EPA) and Research triangle park, N.C.
- Gammon, M.D.; M.S. Wolff; A.I. Neugut; S.M. Eng; S.L. Teitelbaum; J.A. Britton; M.B. Terry; B. Levin; S.D. Stellman; G.C. Kabat; M. Hatch; R. Senie; G. Berkowitz; H.L. Bradlow; G. Garbowski; C. Maffeo; P. Montalvan; M. Kemeny; M. Citron; F. Schnabel; A. Schuss; S. Hajdu; V. Vinceguerra; N. Niguidula; K. Ireland and R.M. Santella(2002) Environmental toxins and breast cancer on Long Island. II.Organochlorine compound levels in blood, *Cancer Epidemiol Biomarkers Prev.*, 11(8): p 686-97.
- Hoyer, A.P.; P. Grandjean; T. Jorgensen; J.W. Brock and H.B. Hartvig (1999). Organochlorine exposure and risk of breast cancer. *Lancet* 352, 1816–1820.
- Hoyer, A.P., P. Grandjean; T. Jorgensen; J.W. Brock and H.B. Hartvig (2000) Organochlorine compounds and breast cancer is there a connection between environmental pollution and breast cancer, *Ugeskr Laeger.*, 14 ; 162 (7) : p 922-6.

- ISO, Geneva (1993) International vocabulary of basic and general terms in Meteorology, Government chemist, London (1995), ISBN 0948926-08-2948.
- Kocan, A.; J. Petrik; B. Drobna and J. Chovancova (1994) Levels of PCBs and some organochlorine pesticides in the human population of selected areas of the Slovak Republic. I. Blood, *Chemosphere*, 29 (9-11): p 2315-25.
- Krieger, N.; M.S. Wolff; R.A. Hiatt; M. Rivera; J. Vogelmann and N. Orentreich (1994) Breast cancer and serum organochlorines: a prospective study among white, black, and Asian women, *J. Natl. Cancer Inst.*, 86 (8): p 589-99.
- Laden, F.; G. Collman; K.I. Wamoto; A.J. Alberg; G.S. Berkowitz; J.L. Freudenheim; S.E. Hankinson; K.J. Helzlsouer; T.R. Halford; H.Y. Huang; K.B. Moysich; J.D. Tessari; M.S. Wolff; T. Zheng and D.J. Hunter (2001a) 1,1-dichloro-2-bis(p-chlorophenyl)ethylene and polychlorinated biphenyl and breast cancer: combined analysis of five US studies, *J. Natl. Cancer Inst.* 91, 568-574.
- Laden, F.; S.E. Hankinson; M.S. Wolff; G.A. Colditz; W.C. Willett; F.E. Speizer and D.J. Hunter (2001b) Plasma organochlorine levels and the risk of breast cancer: an extended follow-up in the nurses' health study, *Int. J. Cancer* 91, 568-574.
- Lara, W.H.; H.H.C. Barreto and O.N.K. Inomata (1987) Levels of organochlorine pesticides in blood serum from exposed people in a Chagas' disease area in Brazil, *Revista do Instituto Adolfo Lutz Brazil*, 47: (1-2), p 19-24.
- Macdonald C.R. and Metcalfe C.D. (1991) Concentration and distribution of PCB congeners in isolated Ontario Lakes contaminated by atmospheric deposition. *Can J Fish Aquatic Sci.* 48: p 371- 381.
- Mazzari, B.H.; D.L.M. Mazzari and M. M.D. Lauschner (1987) Blood levels of chlorinated insecticides in persons occupationally exposed to these toxins in Venezuela 1984, *Boletin de la Direccion de Malariologia y Saneamiento Ambiental*, 27: (1- 4), P 8-20, 9 ref.
- Mazzari, B.H.; D.L.M. Mazzari and M. M.D. Lauschner (1989) Blood concentrations of chlorinated pesticides in spraymen. Venezuela 1984, *Boletin de la Oficina Sanitaria Panamericana*, 106:(3), P 224-234, 9 ref.
- Mendonca, G.A.S.; N.J. Eluf; S.M.J. Andrada; P.A.O. Carmo; H.H.C. Barreto; O.N.K. Inomata and T.A. Kussumi (1999) Organochlorines and breast cancer: a case control study in Brazil, *International Journal of Cancer*, 83: (5), p 596-600, 18 ref.
- Mpofu, M. (1986) Human levels of DDT residues in selected Zimbabwe communities, *Central African Journal of Medicine*, 32 :(12), P 285-289, 15 ref.
- Mussalo, R.H. (1991) Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue, *Sci. Total Environ.*, 15;103 (2-3): p 159-75.

- Nabawi, E.; B. Heinzow and H. Kruse (1987) Residue levels of organochlorine chemicals and polychlorinated biphenyls in fish from Alexandria region, Egypt, Arch. Environ. Contam. Toxicol. 16, 689–696.
- Okor, D.I. and S.S. Atuma (1988) Determination of organochlorine residues in maternal and cord blood plasma, International Journal of Environmental Analytical Chemistry, 33 : (2), p 141-147, 12 ref.
- Porta, M., N. Malats; M. Jarrod; J.O. Grimalt; J. Rifa; A. Carrato; L. Guarner; A. Salas; S.M. Santiago; J.M. Corominas; M. Andreu and F.X. Real (1999) Serum concentrations of organochlorine compounds and K-ras mutations in exocrine pancreatic cancer. PANKRAS II Study Group, Lancet, 18-25; 354 (9196): p 2125-9.
- Puerto, K. D.; F.A.M. Dias; K.M.D. Armas; F.L. Mantil; P.K. Barselo; V.K.A. Arias; A.M.D. Fernandes; M. D. A. Khimenes and L. M. Fernandes (1990) Levels of DDT and its metabolites in humans, Gigenai Sanitariya, 10: p 73-75, 6 ref.
- Ramachandran, M.; B.D. Banerjee; M. Gulati ; A. Grover ; S.S.A. Ritchie, J.M.; S..L Vial; L.J. Fuortes.; H. Guo; V.E. Reedy and E.M. Smith (2003) Organochlorines and risk of prostate cancer, J. Occup. Environ. Med., 45 (7): p 692-702.
- Robinson, P. E.; G. A. Mack; J. Remmers; R. Levy and L. Mohadjer (1990) Trends of PCB, hexachlorobenzene and Benzene hexachloride levels in adipose tissue of the U.S. populations. Environ. Res. 53: 175 – 192.
- Romieu, I.; A.M. Hernandez; P.E. Lazcano; J.P. Weber and E. Dewailly (2000) Breast cancer, lactation history, and serum organochlorines, Am.J. Epidemiol., 152(4): p 363-70.
- Rubin C.H; A. Lanier; M. Socha; J.W. Brock; S. Kieszak and S. Zahm (2001) Exposure to persistent organochlorines among Alaska Native women, Int. J. Circumpolar Health, 60(2): p 157-69.
- Sandanger, T.M.; J.O. Odland; A. Tkachev and I.C. Burkow (2003) Persistent organic pollutants in plasma of delivering women from Arkhangelsk, Sci. Total Environ. , 1; 306 (1-3): p 171-8.
- Schafer, M.; G. Petzold; G. Ostendorp; G. Schade; S. Mohr and B. Heinzow (2000) Dietary exposure and human body burden to organochlorine pesticides and PCBs in women, Umweltmedizin in Forschung und Praxis, 5: (3), p 154-160.
- Schantz, S.L.; J.L. Jacobson; H.E. Humphrey; S.W. Jacobson; R. Welch and D. Gasior (1994) Determinants of polychlorinated biphenyls (PCBs) in the sera of mothers and children from Michigan farms with PCB contaminated silos, Arch. Environ. Health, 49 (6) : p 452-8.
- Schell, L.M.; L.A. Hubicki; A.P. DeCaprio; M.V. Gallo; J. Ravenscroft; A. Tarbell; A. Jacobs; D. David and P. Worswick (2003) Organochlorines, lead, and mercury in Akwesasne Mohawk youth, Environ. Health Perspect., 111(7): p 954-61.

- Skaare, J.U.; J.M. Tuveng and H.A. Sande (1988) Organochlorine pesticides and polychlorinated biphenyls in maternal adipose tissue, blood, milk, and cord blood from mothers and their infants living in Norway, *Archives of Environmental Contamination and Toxicology*, 17: (1), p 55-63, 46 ref.
- Soliman, A.S.; M.A. Smith; S.P. Cooper; K. Ismail; H. Khaled; S. Ismail; R.S. McPherson; I.A. Seifeldin and M.L. Bondy (1997) Serum organochlorine pesticide levels in patients with colorectal cancer in Egypt, *Arch. Environ. Health*, 52(6): p 409-15.
- Soliman, A.S.; X. Wang; J. DiGiovanni; S. Eissa; M. Morad; S. Vulimiri; K.G. Mahgoub; D.A. Johnston; K.A. Do; I.A. Seifeldin; P. Boffetta and M.L. Bondy (2003). Serum organochlorine levels and history of lactation in Egypt, *Environ. Res.*, 92(2): p 110-7.
- Stellman, S.D.; M.V. Djordjevic; J.A. Britton; J.E. Muscat; M.L. Citron and M. Kemeny (2000) Breast cancer risk in relation to adipose concentrations of organochlorine pesticides and polychlorinated biphenyls in Long Island, New York. *Cancer Epidemiol. Biomark. Prev.* 9, 1241–1249.
- Stellman, S.D.; M.V. Djordjevic; J.E. Muscat; L. Gong; D. Bernstein; M.L. Citron; A. White; M. Kemeny; E. Busch and A.N. Nafziger (1998). Relative abundance of organochlorine pesticides and polychlorinated biphenyls in adipose tissue and serum of women in Long Island, New York, *Cancer Epidemiology Biomarkers & Prevention*, 7(6) : p 489-496.
- World Health Organization and International Agency for research on cancer IARC (1982) Monograph on the evaluation of carcinogenic risks of chemicals to humans: Chemicals, industrial processes and industries associated with cancer in human Volume 1-29. Supplement 4. Lyon.
- WHO / UNEP (1990) Public health impact of pesticides used in agriculture, Geneva World Health Organization.
- Wolff, M.S.; J.A. Zeleniuch; N. Dubin and P. Toniolo (2000) Risk of breast cancer and organochlorine exposure, *Cancer Epidemiol. Biomarkers Prev.*, 9 (3): p 271-7.
- Wolff, M.S.; P.G. Toniolo; E.W. Lee; M. Rivera and N. Dubin (1993) Blood levels of organochlorine residues and risk of breast cancer, *J. Natl. Cancer Inst.*, 21; 85 (8): p 648-52.
- Zheng, T.; T.R. Holford; S.T. Mayne; J. Tessari; B. Ward; D. Carter; P.H. Owens; P. Boyle; R. Dubrow; E.S. Archibeque; O. Dawood and S.H. Zahm (2000). Risk of female breast cancer associated with serum polychlorinated biphenyls and 1,1-dichloro-2,2'-bis(p-chlorophenyl)ethylene, *Cancer Epidemiol. Biomarkers Prev.*, 9(2): p 167-74.
- Zheng, T.; T.R. Holford; S.T. Mayne; J. Tessari; P.H. Owens; S.H. Zahm; B. Zhang; R. Dubrow; B. Ward; D. Carter and P. Boyle (1999) Environmental Exposure to hexachlorobenzene (HCB) and risk of breast cancer in Connecticut, *Cancer Epidemiol Biomarkers Prev.*, 8(5):407-11.

مراقبة مستويات ثنائي الفينيل متعدد الكلور والمبيدات الحشرية العضوية الكلورينية في المرضى المصريين

ياسر محمد نبيل^١، صفاء الدين حسن عطيو^٢، مطاوع عيسى الحسيني^٣ و أحمد عبد المقصود غازي^١

^١ المعمل المركزي لتحليل متبقيات المبيدات والعناصر الثقيلة في الأغذية، مركز البحوث الزراعية بوزارة الزراعة، الجيزة - مصر.

^٢ قسم الكيمياء الفيزيائية والغير عضوية، كلية العلوم، جامعة طنطا - مصر.

^٣ قسم الكيمياء الحيوية، المعهد القومي للأورام، جامعة القاهرة - مصر.

تم تجميع ٩٩ عينة دم في مصر بين عامي ٢٠٠٣-٢٠٠٤ باجمالى ٨٥ عينة للذكور و ١٤ للاثان بأعمار تتراوح بين ٢٥ و ٦٠ سنة من أناس متبرعين و مصابين بسرطان الكبد و المثانة وكذلك آخرون أكثر عرضة للأصابة بتلك السرطانات مثل مرضى التهاب الكبدى الوبائى س و البلهارسيا المزمنة بالإضافة الى أناس يبدون ظاهريا أصحاء كمجموعة أساس للتقييم. العينات المجمعة يتم مرورها على فصل السيرم ثم من خلال خطوة استخلاص ثم التبخير والتركيز لتكون جاهزة لتقدير المبيدات وثنائي الفينيل متعدد الكلور بواسطة عن طريق استخدام جهاز كروماتوجرافي الغاز المزود بكشاف الاصطياد الالكتروني وذلك لتقصى ١٤ مبيد من المبيدات العضوية الكلورينية والتي تتميز باستمرار تراكمها لفترات طويلة داخل انسجة جسم الإنسان وهى: بنزين سداسى الكلور، ألفا-هكسان سداسى الكلور، بيتا-هكسان سداسى الكلور، جاما-هكسان سداسى الكلور، دلتا-هكسان سداسى الكلور، سباعى الكلور، سباعى الكلور ايبوكسيد، الإندرين، الدرين، دالدين، بارا بارا د. د. ت، أورثو بارا د. د. ت، بارا بارا د. د. د، بارا بارا د. د. اى وقد تم ايضا تقصى مشابهات ثنائى الفينيل عديدة الكلور أرقام (١٨٠، ١٥٣، ١٣٨، ١١٨، ١٠١، ٥٢، ٢٨).

وقد أظهرت النتائج تواجد فقط كل من متبقيات مبيدات د. د. ت. (خاصة بارا بارا د. د. اى) و متبقيات هكساكلورو سيكلو هكسان (خاصة بيتا هكساكلورو سيكلو هكسان) والبنزين سداسى الكلور فى السيرم بنكرارية بلغت ٩٧% ، ٧٧.٨% ، ٢% على التوالى. بينما لم يتم التعرف على ثنائى الفينيل عديدة الكلور فى اى من العينات فضلا عن باقى المبيدات الحشرية المكورة الأخرى فى أى من عينات المصل التى تم تحليلها. وقد أوضحت الدراسة تواجد مستويات عالية من علاقة الارتباط لمجموع مبيدات هكساكلوروسيكلو هكسان (خاصة بيتا هكساكلوروسيكلو هكسان ومجموع مبيدات د. د. ت. (خاصة بارا بارا د. د. اى) فى دم مرضى البلهارسيا والالتهاب الكبدى الوبائى س بالمقارنة بتلك الموجودة فى أناس يبدون ظاهريا أصحاء والتي تشير الى امكانية الدور الذى قد تقوم به المبيدات المكورة كمسببات قد تؤدى الى تحول خلايا الكبد والمثانة الى خلايا سرطانية أيضا.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة
مركز البحوث الزراعية

أ.د / علي عبد الهادي
أ.د / أشرف محمود المرصفي

Table 10: Mean levels of organochlorine pesticide residues and total PCB's in human blood in different countries for a long period.

Year of work	Organochlorine Pesticides Residues and Total-PCB's as ng/ml					Country	Reference
	β -HCH	Total-DDT	P,P'-DDE	HCB	Total- PCB's		
1984	280	390				India	Ramachandran <i>et al.</i> , (2003)
1991		82.6	27.8				Chand <i>et al.</i> , (1991)
1986		828				Zimbabwe	Mpofu <i>et al.</i> , (1986)
1988	1500	7600		400		Nigeria	Okor and Atuma (1988)
1982-83		213	2067			Venezuela	Mazzari <i>et al.</i> , (1989)
1987		44	149				Mazzari <i>et al.</i> , (1987)
1983-85		53				Cuba	Puerto <i>et al.</i> , (1990)
1991		1.8		0.3	2.0	Finland	Mussalo R.H. (1991)
1992		0.27	6.05	5.38	1.79	Slovak Republic	Kocan <i>et al.</i> , (1994)
1995-1996			5.1			Brazil	Mendonca <i>et al.</i> , (1999)
1994-1997				21.0		Canada	Zheng <i>et al.</i> , (1999)
1995-1997			460.1		733.1		Zheng <i>et al.</i> , (2000)
1990-1995			3.84			Mexico	Romieu <i>et al.</i> , (2000)
1997			1.3		3.4	Germany	Schafer <i>et al.</i> , (2000)
1999			0.49	0.07	0.85	Belgium	Covaci <i>et al.</i> , (2002)
1999			4.9			Brazil	Delgado <i>et al.</i> , (2002)
2003	3.59	1.17	5.42		0.53	Russia	Sandanger <i>et al.</i> , (2003)
1980- 1987					7.56	United States	Rubin <i>et al.</i> , (2001)
1987-1992			9.10		5.04		Wolff <i>et al.</i> , (2000)
1994			6.95		9.6		Schantz <i>et al.</i> , (1994)
1994-1995				0.03	0.78-0.93		Baris <i>et al.</i> , (2000)
1994-1999			2.8		4.42		Butler <i>et al.</i> , (2003)
2003			0.37		0.2		Schell <i>et al.</i> , (2003)
1999 -2000			40		58	Egypt	Ahmed <i>et al.</i> , (2002)
2002	2.95		16.5				Soliman <i>et al.</i> , (2003)
2003-2004	0.857	3.07	2.92	0.003	N.D.		This Study (2007)

N.D. : Not detected