2nd Sci.Congr. of Egypt. Soc. Anim. Mang., 29-31 Oct.(2007):122 - 135

ESTIMATION OF ORGANOCHLORINE, ORGANOPHOSPHORUS AND POLYCHLORINATED BIPHENYL'S (PCB's) PESTICIDES RESIDUES IN FISH AND WATER OF GIZA GOVERNORATE

By

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SUMMARY

Thirty samples of both fish and water were examined in this study for estimation of pesticide residues and polychlorinated Biphenyl's. Three samples of both fish and surrounding water were collected from ten locations at Giza Governorate. Organochlorine pesticide was the major pollutants in fish samples and detected in 30%. However, 10 of collected water samples were contained organochlorine residues with different concentrations, DDT average in water samples ranged between (min 2.1 – max 14.6 ppb), Delta BHC 0.28, 2.6, 4.6, 5.41 ppb, B.BHC 8.9 ppb, Heptachlor 2.5 ppb, endosulfan 3.7 ppb and alderine 0.48 ppb. Our results were above the permissible limits set by **World Health Organization (1984).** The observed residue levels for organophosphorus pesticide were detected in 20% of water samples and 30% of fish samples. For the examined water, two location contained ethoprophos 6.57 and 26.5 ppb and in 3 area fish samples contained Dichlorophos 2.9 ppb, methamidotos 1.63 ppb and malathion 0.7 ppb. Our results were below the permissible limit which is recorded by **FAO/WHO (2001)** which was 0.1 ppm.

Concerning polychlorinated biphenyls (PCBs), they were detected in 70% of the examined water samples PCB_{70}, PCB_{101}, PCB_{105}, PCB_{152}, PCB_{153}, PCB_{138} and PCB_{192}. Seven congeners were detected with different concentrations with an average range min 0.003- max 0.02 ppb. Only one area of collected fish samples contained PCBs congener, 44 with an average 0.006 ppb. The total PCBs were below the permissible limits in all examined samples.

INTRODUCTION

Organochlorine pesticides (OCs) are halogenated organic compounds classified as dichloro-diphenylethanes, Hexachloro-cyclohex-ans, cyclodienes and chlorinated benzenes . They are characterized by high persistence, low polarity,

low aqueous solubility and high lipid solubility (lipophilicity) and have a potential to bioaccumulate in fatty tissues **(Kasozi *et al.*, 2006)**. Fish act as non polar media that can adsorb hydrophobic organic chemicals within the water. Since birds and human conusume fish, this makes fish good biomonitors for xenobiotic pollutants **(Kasozi *et al.*, 2006)**. The ingestion of foods contaminated with persistent lipopholic pesticides can result in the accumulation of these pesticides in humans. The potential for pesticide to cross placental barriers **(Waliszewski *et al.*, 2000)** even in trace concentrations may cause serious neonatal damage and therefore the presence of the residues should be of concern **(Kasozi *et al.*, 2006)**. Already organochlorines (OCs) have been implicated in a broad range of adverse human health and environmental effects including reproductive failures and birth defects **(Edwards, 1987)**, immune system dysfunction, endocrine disruptions and cancers **(Edwards, 1987; Garabrant *et al.*, 1992** and **World Wildlife Fund., 1999)**.

Pollution with chemical pesticides is considered as one of the important environmental problems facing humans. The intensive use of pesticides to eliminate pests or to regulate crop growth has led to pesticide residues in soils, air, water, stored grains, crops, plants and rations at concentration levels which exceed the legal limits **(Susse and Muller, 1996)**. In last decade organophosphate (OP) pesticides were produced and entered the environment in greater quantities than do other pesticides **(Rossi *et al.*, 2001)**. The toxicity of organophosphorus pesticides results from the inhibition of acetylcholinesterase, produces neurotoxic effects in human and animals. Human exposure to pesticide residues can occur as a consequence of the use of a pesticide on animal or their feed if the residues transfer to the animal

commodities that humans consume. Human exposure occurs mainly as a result of agricultural or veterinary practices (**EHC 132, 1992** and **USEPA, 1999**).

Polychlorinated biphenyl's (PCBs) are used in electrical transformers, heat exchange fluids and hydraulic systems. But since the early 1970's when the toxic effects of PCB's were established, their production and use have been drastically restricted in many countries. Studies on exposure to PCB's in the working environment suggest that they cause carcinogenic risk to humans (**GEMS, 1988**).

In Egypt, monitoring of pesticide residues in food and the environment began on as a limit program in 1985, in fish (**Dogheim *et al.*, 1988**). Similar programs were established by **Dogheim *et al.* (1991); Abu Zahw *et al.* (1993); Salama, 1993)** and **Ayoub (1994)**. Monitoring of pesticide residues also recorded in European countries (**Roos *et al.*, 2001; Yamaguchi *et al.* 2003** and **Mazet *et al.*, 2004**). The main side effect of environmental pollution by pesticides is food contamination leading to injury of non target organisms concerns the ehalth of the workers and consumers.

The present study was carried out to obtain detailed data and throw light on the levels of organophosphorus, organochlorines pesticides and PCBs pollutants in fish and surrounding water at Giza Governorate.

MATERIALS AND METHODS

Sampling:

Ten groups of water samples each group of three samples were collected in clean glass containers as well as ten groups of bolti fish (*Tilapia nilotica*) each consists of three fishes were collected randomly with surrounding water from

different villages at Giza Governorate. e.g. El-Mansorya, El-Hawamdya and El-Badrashin. Samples were taken for organochlorine, organophosphorus and polychlorinated biphenyl analysis.

1. Estimation of pesticide pollutants and PCBs in water:

Procedure:

As described in procedure of **Luke *et al.* (1981)** by extraction with methylene chloride (extract 100 ml sample with 200 ml acetone and filter), then added 100 ml petroleum ether and 100 ml methylene chloride to 80 ml filtrate. Repeat extraction with 2 additional 100 ml portions of methylene chloride.

Organic solvents were concentrated on steam bath to 1 ml. Then added 100 ml petroleum ether to reconcentrate solution to 1 ml. Repeated reconcentration with 50 ml petroleum ether then injected 2 µl into the gas chromatography detector.

2. Determination of pesticide residues and polychlorinated biphenyl in the examined fish samples:

2.1. Extraction:

Extraction of samples was carried according to the method described by **Abd El-kader (1989)**. 50 gm of muscle tissue samples were grinded, weighted and placed in high speed blinder jar. Then 8 ml n-hexane and acetone 1:1 were added then added 2 gm anhydrous sodium sulphate for each one gram. The sample and solvents were blended for 10 minutes and the extract was washed several times with distilled water in separatory funnel. The sample was dried with sodium sulphate and evaporated at 40°C in rotatory evaporator till

dryness. Portioning technique was performed to remove the dissolved fat from extract (**Leon *et al.*, 1990**).

2.2. Cleanup of samples:

Sample extracts applied to chromatography columns in 2-3 ml of hexane were eluted successively with hexane-florusil (60/100 mesh) then activated at 250°C for 12-15 hours, placed in a desiccator until cool and then used within 72 hours. Prepared columns with 6 gas deactivated floirsil (0.5%) and 100 ml hexane. Samples extracts were applied to the columns in 2-3 ml hexane, elute the column with 60 ml hexane, to elute the polychlorinated biphenyl (PCBs) and pp-DDE, collecting eluent in the 100 ml flaks as fraction 1 (F1). Next 35 ml of 30% dichloromethane in hexane, was added next 45 ml of 50% dichloromethane in hexane was added to column, to elute all organochlorine and organophosphorus in the sample. Each fraction was reduced to 0.5 ml according to **Khaled *et al.* (2004)**.

3. Preparation of blank solution:

The same volumes of solvents (n-hexane, acetone) and sodium sulphate anhydrous which used in extraction of the samples were subjected to the same extraction, partitioning and clean up procedures as the examined samples to detect any possible traces of the studied pesticides or PCBs in the solvents or distilled water.

E- Gas chromatographic analysis:

Hewlett Packered GC Model 6890 equipped with Ni^{63}- Electron capture detector and flame photometric detector were used in this study.

GC condition:

HP-5MS capillary column (30 ml length x 0.32 mm internal diameter (i.d.) x 0.25 μm film thickness). Carrier gas N_2 at a flow rate of 4 ml/min.; injector and detector temperature were 300°C and 320°C. The initial column temperature was 180°C for 2 minutes raised at 3°C/minute and then at 220°C for 1 minute, then raised at 9°C/min to 280°C for 2 minutes, until a total time of 30 minutes had elapsed. Electron capture detector was used for organochlorine and PCBs while flame photometric detector used to determine organophosphorus.

The organophosphorus and the organochlorine residues components were identified by comparing their retention times with those of the standards quantified by extrapolation of corresponding sample peak areas with those from standard curves prepared for each pesticide standard. Small variations in retention times and response factor of each compound during the experiments were corrected by obtaining fresh chromatograms of the standard mixture after every three injections.

Standard solutions of concentration ranging from 0.01 to 0.04 ppm were prepared for each pesticide standard and 1 μl was injected into the GC peak areas of standard solutions were plotted against their concentrations. The limits of detection were taken at 5 times the detector noise level.

Statistical analysis:

Statistical analysis of data of organophosphorus, organochlorine and PCBs residue was conducted according to **Petric and Watson (1999).**

Table (1): Organochlorine pesticide concentrations in the water and fish samples (ppb).

Organochlorine group	Water samples										Fish samples									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
α BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
β BHC	-	-	8.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
γ BHC	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Delta BHC	-	-	2.4	4.6	2.6	-	0.28	-	-	-	-	-	-	-	-	-	-	-	-	-
Heptachlor	-	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-	-	-	-	-
Aldrine	-	-	-	0.48	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hepta Epoxide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlordane	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Endusulfan	-	-	-	-	-	-	-	3.7	-	-	-	-	-	-	-	-	-	-	-	-
P.P. DDE	-	-	-	14.6	6.3	6.3	-	2.1	-	-	-	-	-	-	-	-	-	-	-	-
Endrin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PP DDD	10.5	-	-	-	-	5.2	-	4.3	-	-	100	-	-	58	-	-	-	-	-	-
PP DDT	2.4	-	-	10.4	5.1		-	-	-	-	74	-	-	13.5	-	1.36	-	-	-	-

Data presented as mean of three analyzed samples.

Table (2): Organophosphorus pesticide concentrations (ppb) in the examined water and fish samples.

Organophosphorus group	Water samples										Fish samples									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Dichlorophos	-	-	-	-	-	-	-	-	-	-	-	-	2.9	-	-	-	-	-	-	-
Methamidofos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.63	-	-	-	-
Ethoprophos	-	-	-	6.57	-	-	-	-	-	26.5	-	-	-	-	-	-	-	-	-	-
Diazinon	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Monochrotophos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloropyrifphos-methyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pirimyphos-methyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dimethoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chlorpyrifos-ethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Malathion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.65
Fenthoate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Profenofos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fenaninfos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Azinophos-methyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Avogan	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Azinophos-ethyl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Data presented as mean of three analyzed samples.

Table (3): Polychlorinated biphenyls (PCBs) concentrations (ppb) in the examined water and fish samples.

PCBs Congener	Water samples										Fish samples									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.63	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-	-	-	-	-	-	0.006	-	-	-	-	-	-	-
70	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-	-	-	-
101	0.007	-	-	-	-	0.021	-	0.007	-	-	-	-	-	-	-	-	-	-	-	-
152	0.021	-	0.01	-	0.029	-	0.014	-	-	-	-	-	-	-	-	-	-	-	-	-
153	-	-	-	-	-	-	0.009	-	-	-	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	0.02	-	-	-	-	-	-	-	-	-	-	-	-	-
138	-	-	0.045	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
192	0.003	-	-	0.015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
194	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Data presented as mean of three analyzed samples.

RESULTS AND DISCUSSION

Samples of water and fish (*Tilapia nilotica*) were collected from different villages at Giza Governorate examined for pesticide residues organochlorine as shown in table (1). The water samples contained DDT and its metabolite with average range 2.1 – 14.6 ppb followed by BBHC 8.9 ppb and Delta BHC with average range 0.28 – 5.41 ppb then aldrin 0.48 ppb, heptachlor 2.5 ppb and endosulfan 3.7 ppb. Our results agreed with those reported by **Hassan *et al.* (1996)** they found that average levels of organochlorine residues in the water samples from near the shore were 5.09 and 2.17 µg/L also our results are inconsistence with **Dogheim *et al.* (1996)** who found that ground water had higher residues of HCHs, DDTs, alderin and heptachlor more than Nile river water and then tap water. However the organochlorine pesticide residues were found at low concentrations below the maximum permissible limits set by the **World Health Organization (1984)**. Our results were above the allowable limits for DDT, 5 ppb, alderine and dieldrine 0.03 ppb and heptachlor 0.1 ppb. Nevertheless the presence of persistent and hydrophobic OC residues in water even at low concentrations posses a risk to human health because such residues have a higher affinity for partitioning into sediment and aquatic organisms. Chemicals with long half-life values and a high solubility into lipids (fats and oils) will tend to accumulate in fatty tissue. Such lipophilic chemicals easily move into cells and are sequestered in fat when they become more persistent.

Fish samples contained higher levels of DDTs with average range 1.36 to 100 ppb. Our results disagreed with those reported by **Dogheim *et al.* (1996)** who found heptachlor epoxide in only one sample at a concentration of 0.4 ppm and DDTs were detected in all the analyzed sample with average range 4.17 to 5.866 ppm and **Abd El-Megeed *et al.* (2000)** who found organochlorine compounds in the examined fish samples in the form of total HCH with a mean of 4.09 ppb and HCB with a mean of 2.10 ppb and total DDT's with a mean of 9 ppb, while **Hassan *et al.*, (1996)** found average levels of organchlorine residues in tilapia between 4.03 and 22.42 µg/kg and lindane was most DDT metabolites were found in the majority of fish samples and this agreed with our results where the majority of fish samples contained detectable amounts of DDTs. The concentration of DDTs in this study were found below the

extraneous residue limit of 5 ppm (5000 µg/kg) set by the **Codex Alimentaroius Commission of FAO-WHO (1997)** considering the previous and present use of some chlorinated hydrocarbons in agriculture for vector control, the levels of pesticide residues found in fish in this work were very low. These low levels suggested a high degradation rate of the OCs in the tropical environment, making their use unsuitable.

As demonstrated in table (2), the residue levels in all water samples for organophosphorus pesticide were below the limit of detection except two group samples were positive for ethoprophos 6.57 and 26.5 ppb. Our results disagreed with those reported by **Dogheim *et al.* (1996)** where water samples were obtained and collected from Kafr El-Zayat as 2 from the Nile river, 3 from ground water (5 to 10 m depth) and 2 from tap water were devoid of organophosphorus pesticide residue. Organophosphorus pesticide residue was found in 3 (Bolti fish) group samples, Dichlorophos 2.9 ppb, Methamidofos 1.63 ppb, and malathion 0.65 ppb. Our results disagreed with those reported by **Santerre *et al.* (2000)** who found that chlorpyrifos was the only pesticide detected in a catfish that it exceeded its tolerance limit in fish with average range 0.072, 0.01 and 0.37 ppm, respectively while residues of heptachlor, methoxychlor, endrine, endosulfan, BHC, diazinon, malathion, methyl or ethyl parathion, cypermethrin, and fenvolerate were not measured in any of the samples. Our results were below the recorded maximum permissible limits for water and fish samples which is recorded by **FAO/WHO (2001)** as 0.1 ppm and acceptable daily intake for Dichlorophos is 0.004 mg/kg B.wt., Ethoprophos is 0.0003 mg/kg B.wt., is methamidophos 0.004 mg/kg B.wt. and malathion is 0.02 mg/kg B.wt. (Codex).

As shown in table (3), analysis of samples for contamination by total PCB's congeners were 8, 18, 28, 52, 44, 70, 101, 152, 153, 105, 138, 180, 192 and 194. The average range of contamination ranged between 0.003 to 0.02 ppb in water samples. Our results disagreed with those recorded by **Fernandez *et al.* (2000)** who concluded that PCB levels exceeded in the majority of the cases, the levels taken as the maximum (100 ng/microl) for highly polluted water.

However, analysis of fish samples for total PCBs congeners indicated in one group samples at 44 contained 0.006 ppb. Our results disagreed with **Abd El-Megeed *et al.* (2000)** where the mean of contamination with PCB's reached 88.4 ppb in the examined fish samples which were collected from local markets in great Cairo. No tolerance levels have been established internationally though some countries have drawn up National limits for PCBs in food stuffs. Fish generally contain higher levels of PCBs than any other type of food **(GEMS, 1988)** Regulatory limits for fish have been set between "500 and 5000 µg/kg" **(Abd El-Megeed *et al.*, 2000)** and thus we recorded very low level in our study.

The results in this study definitely drawn the contaminant levels between aquaculture life and fish. In general, through light in assessing the risk of human exposure to pesticides that accumulate in sediment and aquatic biota with its toxic long term effects on people and wild life.

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تقدير متبقيات المبيدات الكلورونية العضوية والفوسفورية العضوية والبولى كلورينايتد بيفينيل فى الأسماك والمياه

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أجريت هذه الدراسة لإستبيان مدى تواجد ومستويات بعض المبيدات الكلورونية العضوية والفوسفورية العضوية ومركبات الفينيل عديدة الكلور (PCBs). تم تجميع عدد 30 عينة من الأسماك البلطى و30 عينة من المياه من قرى مختلفة من محافظة الجيزة وقد أسفرت الدراسة عن النتائج التالية، بالنسبة لعينات المياه عند فحص تواجد المبيدات الكلورونية العضوية وجد أن مركبات مجموعات الدى دى دى بتركيزات تتراوح بين 201 – 1406 جزء فى المليون ومركبات دلتابنزين هكساكلوريد تتراوح بين 0.28 ، 2.6، 4.6، 2.4 جزء فى البليون ومركبات بيتا بنزين هكساكلوريد 8.9 جزء فى المليون والهيبتاكلور 2.5 جزء فى البليون ، والأندوسيلفان 3.7 جزء فى المليون بالأضافة الى الألدرين 0.48 جزء فى البليون وكانت هذه النسب أعلى من الحدود المسموح بها. بينما وجد فى عينات الأسماك متبقيات معظمها من مركبات دى دى دى بنسب تتراوح بين 1.36 – 100 جزء فى البليون وكانت هذه النسب فى الحدود المسموح بها.

وبتحليل العينات للمركبات الفوسفورية العضوية وجد أن مركبات الأيثوبروفوس بتركيز 6.57 ، 26.5 جزء فى البليون فى عينات المياه كما وجد مركب الداى كلوروفوس بتركيز 2.9 جزء فى البليون والميتاميدوفوس بتركيز 1.63 جزء فى البليون ، المالاثيون بتركيز 0.65 جزء فى البليون فى عينات الأسماك (بنسبة 30%) وكانت هذه النسب فى الحدود المسموح بها.

وبتحليل العينات للمركبات الفينيل عديدة الكلور (PCBs) وجدت فى 70% من عينات المياه أرقام 70، 101، 105، 152، 153، 138، 192 بتركيزات مختلفة تتراوح بين 0.003، 0.02 جزء فى البليون ومجموعة واحدة من الأسماك وجدت رقم 44 بتركيز 0.006 جزء فى البليون وقد كانت هذه النسب أقل من الحدود المسموح بها. ولذلك نوصى بأستمرار متابعة مستويات المبيدات ومركبات الفينيل فى مختلف الأغذية لمعرفة أى تلوث محتمل بأى منها.