

Study of Some Chemical Pollutant Residues in Catfish at Sharkia Governorate, Egypt

Salah El- Dien, W.M. and Hend, A. Mahmoud*

Animal Health Research Institute, Dept. of Food Hygiene, Zagazig Provincial Lab., Egypt
*Pesticide Residue Dept., Central Pesticide Lab., Agricultural Research Center, Egypt.

ABSTRACT: Thirty samples of African catfish (*Clarias gariepinus*) were collected from the markets in Sharkia Governorate for detection and determination of 13 organochlorine pesticides (BHC, BHC, BHC, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, chlordane, endosulfan, pp DDE, pp DDD and pp DDT), 5 organophosphorus pesticides (diazinon, chlorpyrifos, chlorpyrifos methyl, profenophos and disyston) and 11 polychlorinated biphenyls (PCBs) congeners (PCB28, PCB44, PCB70, PCB101, PCB105, PCB138, PCB152, PCB153, PCB180, PCB192, and PCB194). All the tested organochlorine pesticides were detected with the frequency ranged between 30% for BHC and 76.66% for aldrin + dieldrin. Their mean concentrations varied from 1.9 ppb for aldrin to 122.2 ppb for BHC. Meanwhile all the tested PCBs were detected except PCB105 with the frequency lies between 10% for PCB28 and 53.3% for PCB152, while; the mean concentrations varied from 3.0 to 89.16 ppb for PCB194 and PCB152 respectively. All the estimated organochlorine pesticides and PCBs were below the permissible limits in all the examined samples. Meanwhile, the tested organophosphorus compounds were not detected in all the examined samples. The relatively high frequency and levels of organochlorine pesticides and PCBs may be explained by the nature of catfish habits and feeding as exhibited in this study.

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INTRODUCTION

Catfish is a type of bony fish that can be found in one form or another on every continent on the earth except Antarctica. The catfish family is extremely large and the physical size of any given catfish can range anywhere from an inch up to 3 meters long, known as the Mekong giant catfish and the African catfish (*Clarias gariepinus*) which distributed in the Nile fresh water in Egypt (*About catfish, 2009*). The African catfish tend to live in the turbid and cloudy water; furthermore, it preys on another fish, worm and insects. Thus, it exposed to different types of environmental contaminations rather than another fish types. Because of the relatively high fat contents of the African catfish meat, the fat soluble environmental pollutions as organochlorine pesticides and polychlorinated biphenyls (PCBs) are the more probable pollutant sources of the catfish meat. Furthermore, the organophosphorus pesticides could be polluting the cat fish meat via the recent agricultural using.

Pesticides are poisonous chemicals which are used to control or destroy unwanted organisms especially those which have public health significance. These compounds reach aquatic ecosystem by direct application, spray drift, aerial spraying, erosion and runoff from factories and in sewage. Organochlorine pesticides developed in 1940s for use mainly as insecticides in 2nd world war. This pesticide group is fat soluble, stable chemically and has a slow rate of bio-transformation and degradation (*Casarett and Doull,*

2001). In spite of banning of this pesticide group in Egypt since 1980s (*Dogheim et al,1996*), Organochlorine pesticides were detected in surface water and fresh water fish in previous studies in Egypt (*Al- Safy, 2000* and *Selah El Dien and Nasr, 2004*), the probable sources of this pesticide group originated from previous or illegal using. Organochlorine pesticides cause serious toxic symptoms including developmental abnormalities, growth suppression, disruption of the endocrine system, impairment of immune function, and cancer promotion (*El Nemr et al, 2003*). On the other aspect, organophosphorus pesticides are regarded as being low persistent compared with organochlorine, but some reports have indicated that residues of organophosphorus are persisting for extended period in organic soil and surrounding drainage systems (*Miles et al, 1978*).

Polychlorinated biphenyls (PCBs), originally termed "chlorinated diphenyls," were commercially produced as complex mixtures containing multiple isomers at different degrees of chlorination. In the United States, commercial production of PCBs was taken over in 1929s by Monsanto Company. PCBs were banned at 1979s, it entered the environment during their manufacture and use. Today PCBs can still be released into the environment from poorly maintained hazardous waste sites that contain PCBs; illegal or improper dumping of PCB wastes; leaks or releases from electrical transformers containing PCBs; and disposal of PCB-containing consumer products

into municipal or other landfills not designed to handle hazardous waste. PCBs may also be released into the environment by the burning of some wastes in municipal and industrial incinerators. On the other aspect, PCBs are divided into 209 congeners supplied by the commission of the European community according to their chemical structure (*U.S.E.P.A., 2010*). Some PCBs congeners elicit a diverse spectrum of toxic and biochemical response including body weight loss, immunotoxicity (*Sormo et al, 2009*) and induction of gene expression (*El Nemr et al, 2003*).

Therefore, the objectives of the current study are to detect and determine organochlorine, organophosphorus pesticides and Polychlorinated biphenyls (PCBs) in catfish samples collected from markets at Sharkia Governorate and comparing the obtained levels with the recommended permissible limits.

MATERIALS AND METHODS

Thirty samples of African catfish (*Clarias gariepinus*) were collected from markets in Sharkia Governorate for detection and determination of 13 organochlorine pesticides (BHC, BHC, BHC, heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, chlordane, endosulfan, pp DDE, pp DDD and pp DDT), 5 organophosphorus pesticides (diazinon, chlorpyrifos, chlorpyrifos methyl, profenophos and disyston) and 11 polychlorinated biphenyls (PCBs) congeners [PCB28(2,4,4), PCB44(2,2,3,5), PCB70(2,3,4,5), PCB101(2,2,4,5,5), PCB105(2,3,3,4,4), PCB138(2,2,3,4,4,5), PCB152(2,2,3,5,6,6), PCB153(2,2,4,4,5,5), PCB180(2,2,3,4,4,5,5), PCB192(2,3,3,4,5,5,6) and PCB194(2,2,3,3,4,4,5,5)].

A. Collection of samples

From each of 30 African catfish samples which collected from the markets, approximately 100 gm of the examined samples were taken and placed in polyethylene bags. The samples were identified and kept frozen till the analysis was carried out.

B. Analysis of the organochlorine compounds and PCBs residues

1- Extraction and preparation of samples

Exactly 20 gm of the each examined fish sample was homogenized with 20 gm of anhydrous sodium sulfate with tissue homogenizer till have a fine homogenate. The homogenate was extracted with 100 ml of n-hexane: acetone (2:1) and then the extract was filtered through anhydrous sodium sulphate and evaporated till dryness at 40 °C (*Amaraneni and Pillala, 2001*).

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

2-Clean up of samples

Sample extracts applied to chromatography column in 2-3 ml of hexane were eluted successively with hexane florasil (60/100 mesh) was activated at 250 °C for 12-15 hours, placed in a desiccator until cool, deactivated with 0.5% H₂O, stored in a sealed container in a desiccator overnight, and then used within 72h. Columns were rinsed with 100 ml hexane collecting eluant in beaker. Stop the flow before the top of solvent reaches the top of sodium sulfate. Discard the eluant into the waste container. Samples extracts were applied to the column in 2-3 hexane, elute the column with 60ml hexane, to elute the organochlorine compounds and polychlorinated biphenyls (PCBs) and collecting eluant in the 100 ml flask and reduce to 0.5 ml (*Khaled et al, 2004*).

3-Preparation of blank solution

The same volumes of solvents (n-hexane – acetone) and sodium sulfate anhydrous used for fish tissue extraction were subjected to the same extraction, partitioning and clean up procedures as mentioned in the examined samples to detect any possible traces of the studied organochlorine compounds or PCBs in the solvents or distilled water.

4- Quantitative determination of organochlorine compounds and PCBs

At Pesticide Residue Department, Central Pesticide Laboratory, Hewlett Packard GC Model 6890 equipped with Ni⁶³ – electron capture detector. GC conditions: HP- 5MS capillary column (30m length X 0.32mm internal diameter (i.d.), X 0.25µm film thickness), carrier gas: N₂ at a flow rate of 4 ml/min; injector and detector temperatures were 230°C and 300°C respectively. The initial column temperature was initial oven temperature, 180°C for 2 min, raised at 3 °C/min. and then held at 220°C for 1 min., then raised at 9°C /min. to 280°C and then held to 2 minutes, until a total time of 30 minutes had elapsed, DB-17 (J & scientific) capillary column (30m length X 0.32mm initial diameter (i.d.) X25 µm film thickness). Operating temperature were column temperature was programmed 160°C to 230°C at a rate of 3°C /min. to 260°C at a rate 10°C then hold 10 minutes. Injector temperature were 280°C and detector temperature was 300°C with nitrogen carrier gas flow at 4 ml./ min was used to confirm the detected organochlorine pesticides and PCBs.

The organochlorine component and PCBs residue 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 organochlorine standard and PCBs. Small variations in retention times and response factors of each compound during the experiments were corrected for by obtaining fresh chromatograms of the standard mixture after every nine injections. Standard solutions of concentrations ranging from 0.01 to 0.04 ppm were prepared for each organochlorine and PCBs standard and 1 μ l was injected into the GC. Peak areas of standard solutions were plotted against their concentrations. A line of best fit was drawn through the point and the limits of detection were taken at 5 times the detector noise level.

5- Determination of percentage rate of recovery

The reliability of the analytical method was examined by fortifying the tested samples with known quantities of tested organochlorine pesticides and PCBs, following the same procedures of extraction, partitioning, clean up and analysis. The percentage rate of recovery of the organochlorine pesticides varied from 65.20% to 97.50% for p-p DDT and BHC respectively, while; in PCBs it ranged from 84.65% to 99.98% for PCB28 and PCB138 respectively.

C. Analysis of the organophosphorus pesticides residues

1- Extraction and preparation of samples

Extraction of each tissue sample was conducted as described by *AbdEl Kader (1989)*. Grinded and weighted tissue sample (10 gm.) was placed in high speed blender jar. Then 80 ml n-hexane – acetone (1:1) and 2 gm sodium anhydrous sulphate were added to each sample. The sample and solvent solution were blended for ten minutes, and the extract was washed several times with distilled water in separatory funnel. The sample moisture was dried with anhydrous sodium sulphate and evaporated at 40 °C in rotary evaporator till complete dryness.

Partitioning technique performed according to *Leon et al, (1990)* to remove the dissolved fat from the extract. On the other aspect, clean up of the extract was carried out according to *Mills et al, (1972)*.

2- Preparation of blank solution

Exactly 80 ml. n-hexane – acetone (1:1) solution and 2 gm sodium anhydrous sulphate was subjected to the same extraction, partitioning and clean up procedures as the examined samples to detect any possible traces of the studied pesticides in solvents or distilled water.

3- Quantitative determination of organophosphorus pesticides.

The gas chromatograph used was a Hewlett Packard GC Model 6890 equipped with a Flame Photometric Detector (FPD) with phosphorus filter. A fused silica capillary (PAS-1701), column containing 14% cyanopropylsilyloxane as stationary phase (30m length x 0.32 mm internal diameter (i.d) x 0.25 μ m film thickness), was used for the separation in the GC. CP-CIL-13CB 14% phenyl 1,86 % dimethylpolysiloxane as stationary phase (50m x 0.53 mm i.d x 1 μ m film thickness) was used to confirm the detected pesticides. GC operating conditions were as the following : Injector and detector temperatures were 240 °C and 250 °C; initial oven temperature, 170 °C for 2 min, raised at 7 °C /min. then held at 230 °C for 2 min., and raised at 10 °C /min. to 240 C and then held to 2 minutes. The carrier gas was nitrogen at 3 ml/min. and hydrogen and air were used for the combustion at 75 and 100 ml/min, respectively.

4- Determination of percentage rate of recovery

As mentioned above in organochlorine pesticides and PCBs, the reliability of the analytical method of the organophosphorus pesticides was examined by fortifying the tested samples with known quantities of tested following the same procedures of extraction, partitioning, clean up and analysis. The percentage rate of recovery of organophosphorus ranged between 78.55% to 95.66% for chlorpyrifos and diazinon respectively.

The obtained results showed in Table 1 revealed that the mean values of BHC, BHC and BHC were 24.56 \pm 10.85, 2.866 \pm 0.925 and 122.2 \pm 28.40 ppb respectively, the levels of BHC nearly coincided with those recorded by *Khaled et al, (2004)* in mussels in Egypt (3- 47ppb), also, our estimations of BHC agreed with those recorded in crayfish in Egypt (1.16ppb) by *Selah El Dien and Nasr, (2004)* and *Adeyemi et al (2008)* in Nigerian fish (1.2- 4.9ppb). On the other aspect, the estimated values BHC in the current study were higher than those detected by *Skarphedinsdottir et al (2010)* in fish in Iceland and *Moon et al (2009)* in sea foods in Korea. The mean levels of heptachlor and heptachlor epoxide in the present investigation were 35.83 \pm 9.217 and 2.466 \pm 0.892 ppb respectively, the detected heptachlor levels were obviously higher than those estimated by *Khaled et al, (2004)* and *Selah El Dien and Nasr, (2004)*, while; *Zidan et al (2003)* estimated higher heptachlor values (16- 957 ppb) in *Clarias Lazara* in Egypt than our estimations. Meanwhile, *Selah El Dien and Nasr, (2004)* recorded heptachlor epoxide (2.5 ppb) in levels nearly similar with those in the present study. Concerning aldrin and dieldrin, their mean values \pm S.E. were 1.90 \pm 0.605 and 42.30 \pm 11.68 ppb respectively. The aforementioned levels were parallel respectively with those recorded in fresh water crayfish (*Selah El*

Dien and Nasr, 2004), and in Red sea mussels (*Khaled et al, 2004*) in Egypt. Meanwhile, *Kasozi et al, (2006)* in fresh water fish in Uganda detected lower dieldrin levels (0.3ppb) than those recorded in the present study. On the other hand, the mean values \pm S.E. of chlordane residues was 40.733 ± 14.459 ppb, this level was obviously higher than those detected in crayfish (1.8 ppb) by *Selah El Dien and Nasr,(2004)*. Moreover, chlordane residues could not be detected by *Salem (2003)* in *Clarias Lazara* fish in Upper Egypt. The mean concentration \pm S.E. of endosulfan residues was 19.233 ± 4.411 ppb, which was higher than the mean levels (1.7 ppb) obtained in Nile Tilapia in Victoria Lake, Uganda (*Kasozi et al, 2006*). Concerning endrin levels, its mean concentration \pm S.E. in the current study was 30.56 ± 5.868 ppb, the other Egyptian study by *Salem (2003)* recorded lower endrin levels than our estimations. On the other aspect, the mean residue levels \pm S.E. of ppDDE, ppDDD and ppDDT in the present study were 5.70 ± 1.693 , 26.50 ± 6.266 and 24.33 ± 8.213 ppb respectively. These levels were higher than those obtained by *Abbasy et al (2003)* and *Khaled et al, (2004)* in *Clarias Lazara* fish and mussels in Egypt respectively, also; other recent foreign study estimated lower DDT values than our figures in cat fish fillet in USA (*Schechter et al, 2010*). On contrast, *Salem (2003)* found clearly higher DDT

levels (527, 73 and 45 ppb for ppDDE, ppDDD and ppDDT respectively) in *Clarias Lazara* muscles in Upper Egypt. Furthermore, *Storelli and Perrone,(2010)* detected higher levels of the total DDT residues (224- 799 ppb) in deep sea fish liver from Mediterranean Sea in Italy.

Regarding the frequency distribution of the estimated organochlorine pesticide residues in the examined samples, Table 2 showed that all the tested organochlorine were detected in the examined samples and their frequency were 9 (30%), 11(36.66%), 22 (73.33%), 20(66.66%), 23(76.66%),18 (60%), 18 (60%), 19 (63.3%) and 22 (73.33%) for BHC, BHC, BHC, heptachlor+ heptachlor epoxide, aldrin+ dieldrin, chlordane, endosulfan, endrin and total DDT respectively. Moreover, Table 2 exhibited that all the estimated organochlorine residues were within the permissible limits in all the examined samples. Meanwhile, the obtained results had higher incidence of organochlorine pesticides than those previously recorded in Egypt by *Salem (2003)* in *Clarias Lazara* fish.

D. Statistical analysis

The statistical analysis of data was conducted using "Statistic for animal and veterinary science" (*Petric and Watson , 1999*).

RESULTS AND DISCUSSION

Table 1. Concentrations (ppb) of organochlorine pesticide residues in the examined catfish samples (n=30).

Organochlorine Pesticides	Range	Mean*	\pm S.E.
BHC	N.D.- 190	24.56	10.85
BHC	N.D.- 20	2.866	0.925
BHC	N.D.- 480	122.2	28.40
Heptachlor	N.D.- 180	35.83	9.217
Heptachlor epoxide	N.D.- 20	2.466	0.892
Aldrin	N.D.- 10	1.90	0.605
Dieldrin	N.D.- 250	42.30	11.68
Chlordane	N.D.- 270	40.733	14.459
Endosulfan	N.D.- 80	19.233	4.411
Endrin	N.D.- 90	30.56	5.868
pp DDE	N.D.- 40	5.70	1.693
pp DDD	N.D.- 110	26.50	6.266
pp DDT	N.D.- 190	24.33	8.213

*: In the mean \pm S.E calculation, non detected organochlorine pesticides were considered zero.

Table 2. Frequency distribution of the organochlorine pesticide residues in the examined catfish compared with the recommended permissible limits (n=30).

Organochlorine Pesticides	Permissible Limits (ppb)	Not Detected		Within P.L.		Exceeded P.L.	
		No.	%	No.	%	No.	%
BHC	200 ⁽¹⁾	21	70	9	30	0.0	0.0
BHC	200 ⁽¹⁾	19	63.33	11	36.66	0.0	0.0
BHC	500 ⁽²⁾	8	26.66	22	73.33	0.0	0.0
Heptachlor+ Heptachlor epoxide	300 ⁽³⁾	10	33.33	20	66.66	0.0	0.0
Aldrin+ Dieldrin	300 ⁽³⁾	7	23.33	23	76.66	0.0	0.0
Chlordane	300 ⁽³⁾	12	40	18	60	0.0	0.0
Endosulfan	100 ⁽²⁾	12	40	18	60	0.0	0.0
Endrin	100 ⁽⁴⁾	11	36.66	19	63.33	0.0	0.0
Total DDT	5000 ⁽³⁾	8	26.66	22	73.33	0.0	0.0

(1): U.S.F.D.A. (1983)

(2): German Food Law (1997).

(3): U.S.F.D.A.: (2000).

(4): Codex Alimentarius Commission (2009).

From the obtained results we can be concluded that, although all the detected organochlorine compounds were within the recommended permissible limits, it detected in the relatively higher frequency and levels comparing with the most previous studies. These results were expected because the examined African catfish (*Clarias gariepinus*) were caught and sold in agriculture environment (Sharkia Governorate, Egypt) suffered from previous using of organochlorine pesticides. In spite of prohibiting of the organochlorine pesticides in Egypt since 1980s as mentioned above, the long persistence of these compounds and their fat solubility (*Casarett and Doull, 2001*) in addition to the nature of catfish habitat which lives in cloudy water and preys on another fish, worm and insects (*About catfish, 2009*) as previously said are satisfied reasons to explain these relative high frequency and concentrations of organochlorine pesticide residues.

Reversing of the organochlorine results, all the tested organophosphorus pesticides were not detected in all the examined catfish samples.

From the results achieved in Table 3, the mean concentrations \pm S.E. of polychlorinated biphenyls (PCBs) in the examined samples were 13.166 \pm 9.073, 5.5 \pm 2.88, 21.0 \pm 9.06, 34.33 \pm 11.68, 14.533 \pm 5.388, 89.166 \pm 20.57, 53.33 \pm 27.65, 27.33 \pm 9.531, 6.00 \pm 3.0, 3.033 \pm 1.852 and 267.16 \pm 49.82 ppb for PCB28, PCB44, PCB70, PCB101, PCB138, PCB152, PCB153, PCB180, PCB192, PCB194 and total PCBs respectively. PCB105 was not detected in all the examined samples. The obtained PCBs levels were coincided with concentrations of total PCBs estimated in marine fish in Iceland (111- 377 ppb) by *Skarphedinsdottir et al (2010)*. On contrast, *Storelli and Perrone,(2010)* detected higher mean levels of PCBs (561- 1086 ppb) than those in this study in deep sea fish liver from Mediterranean Sea, Italy. Meanwhile, the most available recent investigations as those by *Cirello et al (2009)*, *Montory et al, (2010)* and *Boscher et al, (2010)* recorded lower PCBs levels than our estimations in the examined fish samples in Italy, Chile and Luxembourg respectively.

Table 3 showed that the estimated PCB28, PCB44, PCB70, PCB101, PCB138, PCB152, PCB153, PCB180, PCB192, PCB194 and total PCBs were detected in 3 (10%), 4 (13.3%), 8 (26.6%), 9 (30%), 8 (26.3%), 16 (53.3%), 7 (23.3%), 8 (26.6%), 4 (13.3%), 4 (13.3%) and 22 (37.3%) respectively. Moreover, all the detected PCBs were below the permissible limits (2000 ppb) recommended by *U.S.F.D.A.(2007)* in all the examined samples. On the other hand, the number of the detected PCBs among the tested congeners and the frequency of the each detected PCB among the examined samples were higher in the present study than those obtained in the edible fish species in Brazil by *Da Silva et al, (2003)*. Meanwhile *Khaled et al, (2004)* detected high frequency of PCBs - similar with our

figures - within the mussel samples collected from Red Sea, Egypt, although they estimated lower PCBs levels than those in this study.

As the obtained results of the organochlorine compounds, the examined African catfish (*Clarias gariepinus*) had high frequency and considerable levels of PCBs, this result may be attributed to the lipophilic nature of PCBs in addition the high fat contents of catfish meat. Moreover, many of the sold catfish in rural markets caught from the drain water channels which may carried the industrial effluents contained PCBs, especially; this examined fish type grow abundantly in the drain water because of their previously described habits.

Generally, the results of the current study indicated considerable levels of both organochlorine compounds and PCBs, which were prohibited since a long period. Meanwhile, the permitted organophosphorus compounds were not detected. This result declared the serious degree of organochlorine and PCBs compounds, and exhibited the importance of their banning. Moreover, the present investigation revealed that all the examined samples were fit for the human consumption regarding the estimated chemical pollutants although their relative slightly high levels. The detected organochlorine and PCBs were not indicate to the illegal use or environmental pollution in the Egyptian ecosystem, other recent studies in more advanced countries as Iceland (*Skarphedinsdottir et al, 2010*) and Italy (*Storelli and Perrone, 2010*) recorded high levels of the tested chemical pollutants in the examined fish. We could be concluded that the continuous censorship on the catfish fishing from the drainage channels, continuous monitoring of the chemical pollutant residues in the marketed fish and more scientific attention about catfish feeding and breeding were highly recommended.

Table 3. Frequency and concentrations (ppb) of polychlorinated biphenyl (PCBs) residues in the examined catfish samples (n = 30).

PCBs Congeners	Positive Samples		Range	Mean*	±S.E.
	No.	%			
PCB28	3	10	N.D. - 260	13.166	9.073
PCB44	4	13.3	N.D. - 70	5.5	2.88
PCB70	8	26.6	N.D. - 210	21.0	9.06
PCB101	9	30	N.D. - 240	34.33	11.68
PCB138	8	26.3	N.D. - 130	14.533	5.388
PCB152	16	53.3	N.D. - 380	89.166	20.57
PCB153	7	23.3	N.D. - 750	53.33	27.65
PCB180	8	26.6	N.D. - 180	27.33	9.531
PCB192	4	13.3	N.D. - 60	6.00	3.0
PCB194	4	13.3	N.D. - 50	3.033	1.852
Total PCBs	22	73.3	N.D. - 1050	267.16	49.82

*: In the mean ±S.E calculation, non detected polychlorinated biphenyl pesticides were considered zero.

N.B.: All the examined samples were below the permissible limit of total PCBs in fish (2000 ppb) recommended by *U.S.F.D.A. (2007)*.

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