

DETERMINATION AND COMPARISON OF THE ORGANOCHLORINE PESTICIDE RESIDUE LEVELS AMONG BENNI FISH OF SHADEGAN, MAHSHAHR AND SUSANGERD CITIES, KHOZESTAN PROVINCE IN IRAN

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Received: January 2011

Accepted: June 2011

Abstract

Concentrations of hexachlorocyclohexane isomers (β, γ, δ HCH), dichlorodiphenyl trichloro ethane (pp', op' DDT) and its metabolites (pp' DDE, pp' DDD), aldrin, dieldrin, heptachlor, heptachlor epoxide, endosulfan isomers (α, β) and metoxychlor were determined in Benni fish collected from Shadegan, Mahshahr and susangerd cities at Khozestan province in Iran. All the collected fish were contaminated by 14 investigated organochlorine pesticides. The highest and lowest mean concentrations of organochlorine pesticides belonged to β - HCH (65.36 $\mu\text{g/kg}$) and op' DDT (0.13 $\mu\text{g/kg}$) and were found in Mahshahr and Shadegan Benni fish respectively.

Keywords:

Organochlorine pesticide, Contamination, Benni fish.

Introduction

Intensive use of organochlorine pesticides for agriculture and health purposes in developing countries, has led to widespread pollution of the environment (1-6). Owing to their properties, including low volatility, chemical stability, environmental resistance, lipophilicity and slow metabolic degradation, bioaccumulation and bioconcentration has occurred in birds and mammals and the food chain (2, 7, 8).

Investigation a variety of wildlife has also clearly demonstrated that extensive contamination of fish, birds and mammalian species by these compounds has emerged (1, 9-11). Contamination has

also been demonstrated in humans.

Measurable levels of organochlorine pesticides have been found in human adipose, tissues, blood and breast milk throughout the world (12-15). Nowadays an extensive attention is paid by FAO (Food and Agricultural Organization and WHO (World Health Organization) on pesticide residues in food, environment, and the possibility of harm to human following long-term of low levels exposure and consequent effects of chronic poisoning. Valuable information have been reported for organochlorine pesticide residue levels in environment and food in developing countries, but

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sufficient data about these pesticides in Iranian fish, are very rare. Benni fish (*Barbus Sharpeyi*) belonged to Cyprinidae family, Actinopterygii (ray finned fishes) class. This fish live in western Asia, endemic to Tigris Euphrate basin. Due to importance of the problem for Iranian Health Officials, the organochlorine pesticide residue levels [β , γ , δ - HCH), pp', op'-DDT and its metabolites (pp'DDE, pp'DDD), aldrin, dieldrin, heptachlor, heptachlor epoxide, endosulfan isomers (α , β) and metoxychlor] were determined in Benni fish of Shadegan, Mahshahr and Susangerd cities at Khozestan province in Iran.

Materials and Methods

Areas under study

Khozestan situated in the south-west of Iran, this province covers an area of 64236 km². It is bounded on north by Lorestan the

east by Chahar Mahal and Kohgiluyeh, in the west by Iraq and the south by Persian Gulf. (Fig. 1) The largest rivers in this province are Karoon, Karkheh, Dez, Maroon and Arvand. The reedy marshes called Hoors are numerous, the most important of which are Hoor OL-azim and those located in Susangerd and Shadegan regions. Khozestan consists of 15 cities. Ahvaz is the provincial capital and following are the other cities: Abadan, Andimeshk, Izeh, Baghmalek, Bandar-e Mahshahr, Behbahan, Dezful, Dasht-e Azadegan, Khorramshahr, Masjed-e Soleyman, Ramhormoz, Shadegan, Shush, Omidieh, Aghajari and Shooshtar. The dams of Dez, Karoon and Karkheh provide water for irrigation, Animal husbandry-particularly sheep, goat and buffalo and fishing are of high importance.



Fig. 1: The map of Khozestan province

Collection of fish

From each of three areas under study (Shadegan, Mahshahr and Susangerd), 20 Benni fish were taken with fishing net

monthly, for six consecutive months. Fish were gray color and 300 -700g weight. (Fig. 2)



Fig. 2: Benni fish

Laboratory procedures

All glassware used in the analysis were carefully washed with water- detergent and rinsed with distilled water, ethanol, acetone and pesticide grade n- hexane respectively. The reagents and chemicals used were highly pure and obtained from various branches of Merck Company. In order to investigate the status of qualification and quantification of contamination of Benni fish of Susangerd, Mahshahr and Shadegan by selected organochlorine pesticides[(β , γ & δ -)HCH , pp', op'-DDT and its metabolites (pp'DDE ,pp'DDD), aldrin, dieldrin, heptachlor , heptachlor epoxide, (α , β) endosulfan isomers and metoxychlor)], different parts of each 20 collected fish from each of area was taken, grounded by and thoroughly mixed. 20 g of grounded fish was dried with anhydrous sodium sulfate and soxhlet extracted with n- hexane for 8h. The extract was concentrated to dryness by rotary evaporation. The lipid content of sample was dissolved in 15 ml petroleum ether and transferred into 125 ml separatory funnel. Then 30 ml CH₃CN saturated with petroleum ether was added to that separator, shaken vigorously 1 min, when layers separated, CH₃CN phase was drained into second 125 ml separator containing 15 ml petroleum ether, shaken vigorously 1 min, after layers became separated, CH₃CN phase was drained into 1 L separator containing 650 ml H₂O, 40

ml saturated NaCl solution and 100 ml petroleum ether .CH₃CN phases from each of 3 additional partitioning was passed through same 15 ml petroleum ether in 125 ml separator , each time shaken vigorously and combined CH₃CN extracts in the 1 L separator. Then aqueous layer drained into another separator, and 100 ml petroleum was added to that and shaken vigorously. When layers separated, aqueous layer discarded and petroleum ether layer was combined with that in original separator. Then, petroleum ether layer was washed with two 100 ml portions water. Petroleum layer was drawn off through sodium sulfate and concentrated about 10 ml by rotary evaporation for Florisil column chromatography cleanup. Florisil column chromatography cleanup was used for fractionation of organochlorine pesticides from fish samples by elution with solvents of increasing polarity. The chromatographic columns (50 cm \times 1.3 I.D) were slurry packed with 10 g of Florisil (activated at 675 °C). Approximately 0.5 cm anhydrous sodium sulfate was placed at the top of the column. The column was pre eluted with sufficient amount of petroleum ether. A 250 ml round bottomed flask was placed under the column. Then extracted petroleum ether was transferred into the column and allowed it pass through at 5

ml/ min , and eluted with 200 ml petroleum ether- diethyl ether (94: 6) (fraction 1) .The receiver was changed and eluted with 200 ml petroleum ether - diethyl ether (85: 15) (fraction 2). Each of elution was concentrated to dryness by rotary evaporation and residue was dissolved in 10 ml n-hexane for gas chromatography analysis. The separation and quantification of pesticide residues were performed on a Varian 6000 gas chromatograph equipped with an electron capture detector (^{63}Ni) using a capillary column of 25 m length and 0.53 mm diameter contained 3% OV17 on a 60-80 mesh chromosorb. The operating temperatures were: injector 230 °C, detector 320 °C and oven temperature was maintained at 170 °C for 8 min, then programmed at 5 °C /min to 200 °C and held at 200 °C for 14 min. Nitrogen gas was used as carrier gas at flow rate 1 ml/ min. The sample size was 1 μl . Recoveries ranged from 94 to 99% and residues levels were not corrected for recovery values. Residues of pesticides in fish were identified and quantified by comparing their retention times and area under the curves obtained for the chromatograms of samples with those obtained for the chromatograms of selected pesticides standards which were analyzed under identical conditions.

Statistical comparisons of obtained data were made by analyses of variance with the help of statistical package SPSS16. Differences were considered significant when $p < 0.05$.

Results and discussion

No one can doubt the efficacy of pesticides for protection of crops in the field, thereby providing us with abundant, inexpensive, wholesome and attractive fruits and vegetables. Suppression of a typhus epidemic in Naples, Italy by DDT

in the winter of 1943-1944(16), the control of river blindness (onchocerciasis) in the West Africa by killing the insect vector with temephos (17) and the control of malaria in Africa, the Middle East and Asia with a variety insecticides are all medical miracles accomplished by pesticides that have been documented. Despite the current dispute over pesticide use and the presence of low levels of their residues in food, groundwater, biota and air, these agents comprise integral components of our crop and health protection programs. As long as they continue to be used, accidental and/ or intentional poisoning of wildlife domestic stock, human and contamination of environment and food can be anticipated. Due to importance of the problem for Iranian Health Officials, FAO and WHO the residue levels of organochlorine pesticides were studied in this study. Tables 1, 2 and figure 3 shows the results of this research. From viewpoint of organochlorine pesticide concentrations, this survey revealed this fact that all of fish samples, in some way were contaminated by investigated pesticides (tables 1 and 2). According to results of this study, table1 shows the range of concentration values of each investigated organochlorine pesticides. The highest and lowest mean concentrations of organochlorine pesticides belonged to β -HCH (65.36 $\mu\text{g/kg}$) and op'DDT (0.13 $\mu\text{g/kg}$) which were found in Mahshahr and Shadegan Benni fish respectively (table1). Regardless to the kind of pesticide : Mahshahr and Susangerd Benni fish with 50.065 and 1.6586 $\mu\text{g/kg}$ monthly mean concentration of organochlorine pesticide were the most and least contaminated fish in Aug and May (table 2). Mahshahr Benni fish with 133.62 $\mu\text{g/kg}$ mean concentration of organochlorine was the contaminated fish (Fig. 3).

Table 1: Comparison of mean and maximum concentrations (ppb) of investigated organochlorine pesticides of Benni fish of Susangerd, Mahshahr and Shadegan cities, Khozestane province, Iran

Organochlorine pesticides	Benni fish from area under study								
	Susangerd			Mahshahr			Shadegan		
	Mean	Maximum	Std. Deviation	Mean	Maximum	Std. Deviation	Mean	Maximum	Std. Deviation
β -HCH	27.3507000	131.10710	58.01933806	65.3618833	302.56450	121.27020243	15.1954833	72.62420	28.34734535
γ -HCH	3.6048400	15.71300	6.78575443	6.3042500	25.21740	9.87508216	1.8092500	4.86850	1.72029083
δ -HCH	1.0491000	3.13110	1.27832873	.2588333	1.55300	.63400960	3.3254167	15.49750	6.22365094
Dieldrin	12.6452400	20.28650	8.52266884	19.4932667	71.84960	26.77619082	10.2030500	27.30460	10.13483962
Heptachlor	6.4609200	11.56160	5.04663037	12.3461500	57.26400	22.25868654	20.9586500	46.63420	16.67510851
Aldrin	1.140840	2.9466	1.2824115	12.473967	71.5338	28.9505478	1.047383	4.0441	1.7199517
Heptachlor epoxide	15.8119000	41.02080	18.93025478	5.7866333	27.56100	10.81945487	3.9657500	22.45760	9.07006425
α -endosulfan	3.088720	9.8878	4.0711689	1.295750	4.0467	2.0098988	7.847450	37.0879	14.5161695
β -endosulfan	28.1406800	80.07790	35.29073901	2.8316500	7.66290	3.48984782	4.5716667	18.50740	7.16965119
OP'DDT	9.2257600	17.21810	8.53592887	17.6962333	67.49210	28.88873813	.1283333	.77000	.31435118
PP'DDT	14.7539200	25.89870	9.22220535	7.3358167	14.26420	6.80170666	4.0613667	17.26330	6.69905588
Metoxychlor	2.7404200	13.70210	6.12776541	11.6664833	54.84640	21.54560082	3.6468833	17.34380	6.95122660
PP'DDE	7.1391600	17.63890	7.49333369	12.3340667	35.31460	16.12722391	7.5460333	24.53340	9.59776792
PP'DDD	.4723400	2.36170	1.05618435	5.4119500	16.49460	6.19166202	6.9266333	21.25480	9.58499346
Organochlorine Total	133.6245	211.51	81.32215	180.5969	700.91	259.01353	91.2334	126.41	37.75345

Table 2: Comparison of monthly and average organochlorine total pesticide concentrations of Benni fish of Susangerd, Mahshahr and Shadegan cities, Khozestane province, Iran (ppb)

Benni fish from area under study	Month	Organochlorine total	Average of total organochlorine pesticide
Susangerd	May	211.5108	15.1079
	Jun	30.7644	2.1975
	Aug.	191.0656	13.6475
	Sep.	172.0725	12.2909
	Oct.	62.7093	4.4792
Mahshahr	May	35.0450	2.5032
	Jun	77.0443	5.5032
	July	47.6977	3.4070
	Aug.	700.9107	50.0651
	Sep.	58.6306	4.1879
	Oct.	164.2533	11.7324
Shadegan	May	23.2208	1.6586
	Jun	101.4122	7.2437
	July	113.5364	8.1097
	Aug.	109.6042	7.8289
	Sep.	73.2129	5.2295
	Oct.	126.4136	9.0295

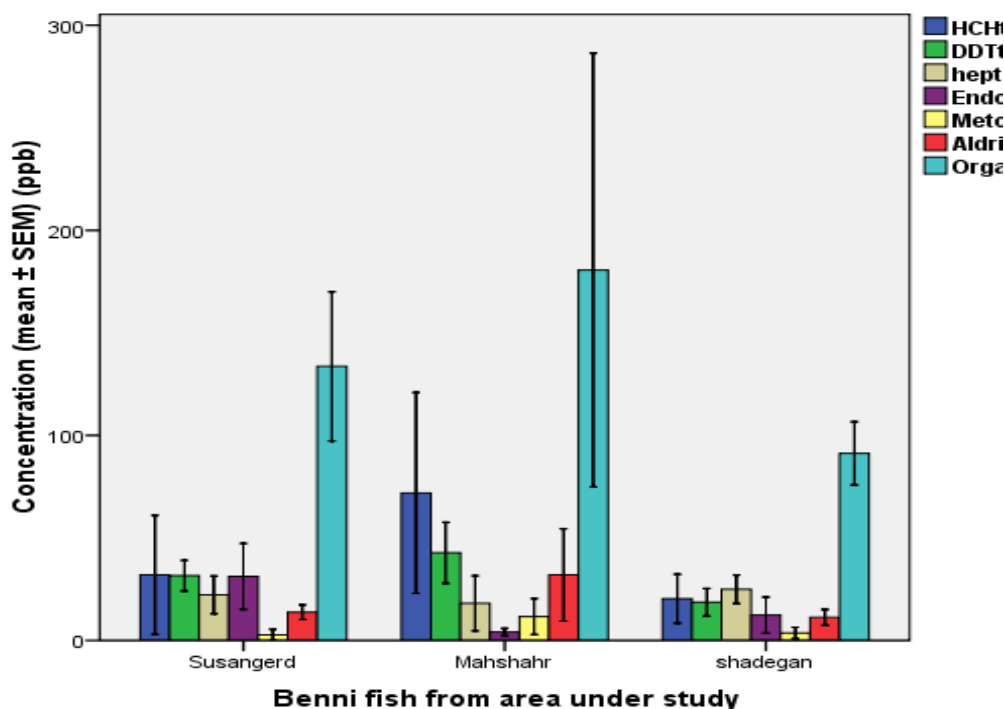


Fig. 3: Comparison of HCH total, DDT total, Aldrin total, Heptachlor total, Endosulfan total, metoxychlor and organochlorine total of Benni fish of Susangerd, Mahshahr and Shadegan cities, Khuzestane province Iran.

HCH total = β -HCH + γ -HCH + δ -HCH

DDT total = op'DDT + pp'DDT + pp'DDE + pp'DDD

Aldrin total= aldrin + dieldrin

Heptachlor total= heptachlor +heptachlor epoxide

Endosulfan total= α -endosulfan + β - endosulfan

Ebadi and his coworker measured the residues levels of lindane (organochlorine pesticides) in four species of the most consumed fish(sefid, koli, kilca and kafal fish) at four sites (Chalous and Babolsar cities, Khazar Abad and Miankaleh regions) in the Mazandaran provinces of Iran in the Caspian Sea. The results of this study showed that investigated fish Samples contained detectable concentrations of lindane, but at concentrations below the maximum residue limit (MRL). No differences were found in the lindane concentrations between the types of fish at each site(18). Davodi et al determined the

concentrations of polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), such as dichlorodiphenyltrichloroethane (DDT) and analogs, hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB), in 8 fish species collected during October and November 2007 from the largest Iranian wetland, the Shadegan Marshes. The results of this research indicated that Common carp (*Cyprinus carpio*) had the highest OCs (1680 ng/g lipid wt.), with DDTs being most prevalent and p,p'-DDE contributing by 53-88% to the total DDTs. In all samples, OCs were found in order of DDTs>HCHs>PCBs>HCB. The highest

concentration of HCHs was found in common carp ($410 \pm 180 \text{ ng/g}$ lipid wt.) and [α]-HCH was the predominant compound among HCH isomers (range 70-90%, mean 82%) in all species. OC levels in fish were relatively low, but the levels of several OCs in some of their specimens exceed the guidelines for food safety issued by the European Union (EU) and US Food and Drug Administration (FDA) (19).

Use of pesticides is inevitable for prevention of pest damage and increase agricultural products. The main concern is training of farmers for correct and safe handling of pesticides and for production of safe and pesticide free yields. To achieve this objective the role of governments, organizations related bodies is vital and of great importance. As mentioned previously, the information regarding to the occurrence of organochlorine pesticides in Iranian fish is very limited, thus continued periodic monitoring of organochlorine pesticide residues levels in these fish is recommended.

Acknowledgement

This article is a part of research project carried out in the school of pharmacy. It was financially supported by vice chancellor of research Ahvaz, Jundishpur University of Medical Sciences.

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