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ORGANOCHLORINE PESTICIDE RESIDUES IN CULTURED FISHES OF TAIWAN

SEN SHYONG JENG AND LIAN TIEN SUN

Institute of Zoology, Academia Sinica
Taipei, Taiwan 115
Republic of China

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ABSTRACT

S. S. Jeng and L. T. Sun (1974). *Organochlorine pesticide residues in cultured fishes of Taiwan.* Bull. Inst. Zool., Academia Sinica, 13(1): 37-45. The first report on organochlorine pesticide residues in cultured fishes and shellfishes on Taiwan suggests that the aquatic animals are not contaminated by pesticides. From September 1972 to September 1973, 167 fishes belonging to 5 species, 631 oysters and 20 clams were analyzed. No aldrin, endrin, heptachlor and heptachlor epoxide were detected within the sensistivity limit of the gas chromatographic method in all the fish and shellfish examined. Small residues of BHC group were present in almost all the fish and shellfish with the maximum value not higher than 0.16 ppm in fish muscle or viscera. Also present in the edible portions of the fish and oysters were small amount of DDT group with values less than 0.15 ppm. No DDT and its metabolites were detected in clams. An insignificant amount of dieldrin, less than 0.05 ppm was found in fish. In general, the results indicate that the use of organochlorine pesticides in the past 20 years did not result in significant accumulations of organochlorine pesticides in the fishes and shellfishes to render them unsuitable for human consumption.

Application of pesticides is one of the major causes which enable Taiwan to achieve high production of crops. Since palathion was first introduced in 1953, millions of pounds of pesticides were used on this island. Of these Pesticides organochlorine pesticides are the most dangerous, because they are long persistence and their great usage as compared with other classes of pesticides. It is well known that organochlorine pesticides could be biologically concantrated in an aquatic ecosystem[1]. Therefore, it is important to know the quantity of their residues in fish from the view point of food hygiene. Besides, it may also be possible to calculate the concentrations of the pesticides in water through knowledge of their concentrations in fish[2]. Of the fishes consumed in Taiwan, those caught in and around the island are most probably polluted by organochlorine pesticides. This is the first report on pesticide residues in some important fishes cultured on this island, and oysters and clams reared along the coast.

MATERIALS AND METHODS

Materials

In order to ascertain the degree of pesticide pollution on fishes, tilapia *Tilapia mossambica* Peters, the common carp *Cyprinus carpio* Linneaus, grass carp *Ctenopharyngodon idellus* (Cuvier & Valenciennes), bighead *Aristichthys nobilis*

(Richardson), and milkfish *Chanos chanos* (Forskal), were sampled from reservoir, fresh water ponds, paddy field and brackish water pond; oysters and clams were collected from the coastal area. The sampling stations, their locations and the nature of waters are shown in Table 1. These stations are in the central south regions of Taiwan, the agriculture and fish culture centers of the island.

TABLE 1

The sampling stations, types of fish ponds, the fishes and shellfishes caught for analyses.

Sampling station	Fish ponds	Fishes caught
(1) Yunlin Pref. (雲林縣三條崙)	shallow sea culture	oyster
(2) Yunlin Pref. (雲林縣金湖)	shallow sea culture	oyster
(3) Chiayi Pref. (嘉義縣東石)	shallow sea culture	clam, oyster
(4) Chiayi Pref. (嘉義縣布袋)	shallow sea culture	clam, oyster
(5) Changhua Pref. (彰化縣竹塘)	paddy field	tilapia (吳郭魚, 改良種), bighead (鰱魚), grass carp (草魚), common carp (鯉魚)
(6) Tainan Pref. (臺南縣學甲)	fresh water pond	tilapia (改良種)
(7) Tainan Pref. (烏山頭淡水養殖中心)	reservoir	tilapia (在來種), bighead, grass carp, common carp
(8) Tainan City (臺南市南興塭)	fresh water pond	tilapia (在來種), common carp
(9) Tainan City (臺南水試所)	brackish water pond	tilapia (在來種), milkfish (虱目魚)
(10) Tainan City (臺南水試所附近)	fresh water pond	bighead, grass carp, common carp

Preliminary treatment

Specimens caught were transported in a box containing ice to the laboratory, and frozen at −20°C until analysis. Three kinds of samples were used. *Whole body*: The entire soft part inside the shell of bivalve molluscs with 10–60 individuals grouped and treated as one sample. *Muscle*: For tilapia, the entire edible flesh of an individual was taken and treated as one sample with each station analyzing 6 individuals. For other fishes, three transverse slices of one inch thick: one slice from just back of the pectoral fins, one slice halfway between the first slice and the vent, and one slice just back of the vent, were cut to represent one fish as suggested by AOAC[3], and 3–15 fish were put together as one sample. *Viscera*: A sample comprising all pieces taken from the major organs and tissues within the body cavity of the fish. Three to fifteen viscera from the same species were combined as one sample.

Analytical procedures

Methods used were those outlined in the Pesticide Analytical Manual[4].

Representative sample (20–40 g) was lyophilized overnight, then extracted in a Soxhlet extractor with redistilled n-hexane which had been previously tested and found to be free of electron-capturing materials by gas chromatography. The n-hexane extract was subjected to partitioning with acetonitrile, then applied to 21 g column of Florisil which had been activated at 130°C overnight, slurried in n-hexane and topped with 1.5 cm anhydrous Na_2SO_4. Pesticides were eluted with 200 ml of 6% ethyl ether/n-hexane (v/v). It was found in a preliminary test that eluates of 15% ethyl ether/n-hexane (v/v) showed no peaks in gas chromatograms, hence, eluation with 15% ethyl ether/n-hexane was omitted in this experiment.

The eluates from Florisil were then concentrated to 5 ml, and analyzed by gas-liquid

chromatography on a Hitachi 023 instrument fitted with a ^{63}Ni electron capture detector for aldrin, BHC group, DDT group, dieldrin, endrin, heptachlor and heptachlor epoxide. The pesticides were identified in the basis of retention times on two glass columns: (1) 1 m × 4 mm id coiled glass column packed with 5% DC 11 on 60/80 mesh Chromosorb G (AW DMCS) operated at 200°C. Carrier gas was N_2 at 30 ml/min. (2) 2 m × 4mm id coiled glass column packed with 2% QF-1 on 60/80 mesh Chromosorb G (DMCS) operated at 180°C. Carrier gas was N_2 at 60 ml/min. The organochlorine residue levels in the samples were estimated from standard curves. Recoveries of the pesticides were above 80%, thus, as is customary in pesticide analyses, no correction has been made in the results presented here[5].

TABLE 2

Mean organochlorine pesticide residues (μg/g wet tissue) in the muscle tissues of Taiwan's cultured tilapia, *Tilapia mossambica* Peters.

Station	(5) paddy field	(6) fresh water pond	(7) reservoir	(8) fresh water pond	(9) brackish water pond
Date of catch	1972, Sep. 1973, Apr. 1973, Sep.	1972, Sep. 1973, Apr.	1972, Sep. 1973, Apr. 1973, Sep.	1972, Sep. 1973, Apr. 1973, Sep.	1972, Sep. 1973, Apr. 1973, Sep.
No. of specimen	22	12	18	18	18
Total longth (cm)	16.7 (12.0-21.4)	18.2 (14.5-22.5)	17.7 (14.5-23.2)	16.4 (13.8-19.4)	16.2 (12.1-20.2)
Total weight (g)	98.7 (38.3-229.5)	105.0 (59.9-155.9)	104.7 (50.5-186.3)	81.3 (52.3-115.6)	56.4 (32.9-116.6)
Lipid (%)	0.92 (0.18-3.31)	1.16 (0.17-2.36)	0.95 (0.38-1.83)	1.12 (0.24-2.29)	1.12 (0.34-2.13)
Aldrin	N.D.	N.D.	N.D.	N.D.	N.D.
α-BHC	0.0069	0.0059	0.0059	0.0027	0.0045
β-BHC	0.0090	0.0039	0.0055	0.0080	0.0019
γ-BHC	0.0086	0.0103	0.0147	0.0086	0.0073
δ-BHC	0.0027	0.0015	0.0009	0.0001	0.0004
Total BHC	0.0272	0.0215	0.0270	0.0194	0.0104
p, p′-DDD	0.013	0.001	0.005	0.008	0.059
p, p′-DDE	0.006	0.002	0.004	0.004	0.002
o, p′-DDT	0.013	0.004	0.007	0.002	0.020
p, p′-DDT	0.017	0.008	0.015	0.003	0.002
Total DDT	0.049	0.015	0.031	0.017	0.083
Dieldrin	0.005	0.002	0.007	0.010	0.002
Endrin	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor epoxide	N.D.	N.D.	N.D.	N.D.	N.D.

N.D., Not detected.

TABLE 3

Mean organochlorine pesticide residues (μg/g wet tissue) in the viscera of Taiwan's cultured tilapia, *Tilapia mossambica* Peters.

Station	(5) paddy field	(7) reservoir	(8) fresh water pond	(9) brackish water pond
Date of catch	1973, Sep.	1973, Sep.	1973, Sep.	1973, Sep.
No. of specimen	10	6	6	6
Total length (cm)	14.8 (12.0-18.9)	20.3 (15.7-23.3)	15.5 (14.0-18.8)	18.0 (15.3-20.2)
Total weight (g)	60.4 (38.3-110.7)	147.3 (81.8-186.3)	76.2 (52.3-115.6)	90.3 (78.8-116.3)
Lipid (%)	5.23 (0.96-7.67)	4.58	4.51	4.10
Aldrin	N.D.	N.D.	N.D.	N.D.
α-BHC	0.0322	0.0103	0.0000	0.0131
β-BHC	0.0719	0.0126	N.D.	0.0562
γ-BHC	0.0319	0.0148	0.1278	0.0234
δ-BHC	0.0209	0.0013	N.D.	0.0029
Total BHC	0.1569	0.0390	0.1278	0.0956
p, p'-DDD	0.040	0.023	0.045	0.272
p, p'-DDE	0.032	0.020	0.015	0.009
o, p'-DDT	0.065	N.D.	N.D.	0.108
p, p'-DDT	0.074	0.040	N.D.	N.D.
Total DDT	0.211	0.083	0.060	0.389
Dieldrin	0.018	0.021	N.D.	N.D.
Endrin	N.D.	N.D.	N.D.	N.D.
Heptachlor	N.D.	N.D.	N.D.	N.D.
Heptachlor epoxide	N.D.	N.D.	N.D.	N.D.

N.D., Not detected.

RESULTS AND DISCUSSION

Organochlorine residues in cultured fishes of Taiwan

From September 1972 to September 1973, 167 fishes belonging to five species, 631 oysters and 20 clams were analyzed. The mean values of these organochlorine pesticide residues in fishes, oysters and clams are given in Tables 4-6. No aldrin, endrin, heptachlor and heptachlor epoxide were detected in the fish and shellfish examined. Small residues of BHC group were present in almost all the fish and shellfish with maximum value not higher than 0.06 ppm (total BHC = α BHC + β BHC + γ BHC + δ BHC) in fish muscle, and not over 0.16 ppm in fish viscera. Oysters and clams had lower levels of BHC than fish. Also present in edible portions of the fish and oysters were small amount of DDT group with values ranging from 0.001 to 0.15 ppm (total DDT = p, p'-DDD + p, p'-DDE + p, p'-DDT + o, p'-DDT). Fish viscera had about three times more of total DDT than muscle. No DDT and its metabolites were detected in clam. An insignificant amount of dieldrin, less than 0.05 ppm was found in fish and oyster, but not in clam.

The reason why cultured fishes of Taiwan generally contained residues of BHC and DDT groups could be that these pesticides were manufactured locally, thus most commonly used.

Species and tissue variations

It is seen from Table 4 that the pesticide residues in the muscles of different fish species are virtually the same. But the concentrations of pesticides in the viscera are about 2-4 times higher than in the muscle of the same species of fish. This is consistent with the higher lipid contents in fish viscera, apparently coinciding with Sivadas' conclusion that the absorptive cells of fish intestines are primary site of uptake of pesticides[6].

TABLE 4

Mean organochlorine pesticide residues (μg/g wet tissue) in Taiwan's cultured fishes.

Station		Bighead	Common carp	Grass carp	Milffish	Tilapia
No. of specimen	M	19	22	15	21	88
	V	19	22	15	21	28
Lipid (%)	M	3.33	1.25	1.92	3.75	1.04
	V	13.08	6.61	12.00	7.10	4.69
Aldrin	M	N.D.	N.D.	N.D.	N.D.	N.D.
	V	N.D.	N.D.	N.D.	N.D.	N.D.
α-BHC	M	0.0035	0.0056	0.0064	0.0028	0.0052
	V	0.0103	0.0080	0.0102	0.0028	0.0165
β-BHC	M	0.0052	0.0118	0.0156	0.0074	0.0059
	V	0.0216	0.0153	0.0459	0.0051	0.0404
γ-BHC	M	0.0057	0.0072	0.0128	0.0023	0.0098
	V	0.0229	0.0072	0.0151	0.0062	0.0470
δ-BHC	M	0.0020	0.0018	0.0031	0.0023	0.0012
	V	0.0050	0.0051	0.0145	0.0008	0.0084
Total BHC	M	0.0164	0.0264	0.0379	0.0148	0.0221
	V	0.0598	0.0356	0.0857	0.0149	0.1123
p, p'-DDD	M	0.007	0.011	0.006	0.027	0.018
	V	0.028	0.027	0.031	0.075	0.087
p, p'-DDE	M	0.007	0.009	0.015	0.005	0.004
	V	0.017	0.019	0.035	0.007	0.021
o, p'-DDT	M	0.001	0.000	0.008	0.009	0.010
	V	N.D.	0.006	0.049	0.035	0.046
p, p'-DDT	M	0.006	0.004	0.005	0.011	0.009
	V	N.D.	0.009	0.025	0.015	0.035
Total DDT	M	0.021	0.024	0.034	0.052	0.041
	V	0.045	0.061	0.140	0.132	0.189
Dieldrin	M	0.005	0.008	0.007	0.005	0.005
	V	0.019	0.010	0.022	0.006	0.011
Endrin	M	N.D.	N.D.	N.D.	N.D.	N.D.
	V	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor	M	N.D.	N.D.	N.D.	N.D.	N.D.
	V	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor epoxide	M	N.D.	N.D.	N.D.	N.D.	N.D.
	V	N.D.	N.D.	N.D.	N.D.	N.D.

N.D., Not detected; M, Muscle; V, Viscera.

TABLE 5
Mean organochlorine pesticide residues (μg/g wet tissue) in Taiwan's cultured oyster, *Crassostrea gigas* (Thunberg)

Station	(1)a	(1)b	(2)	(3)	(4)
Date of catch*	1973, Apr., May, July, Sep.	1973, Apr., May, July, Sep.	1973 Mar., Apr., May, July, Sep.	1973 Mar., Apr., May, July, Sep.	1973, Mar., Apr., May
No. of specimen	178	135	124	146	48
Average weight (g)	0.86	1.15	1.44	2.00	2.73
Lipid (%)	1.36	1.46	1.25	1.06	0.88
Aldrin	N.D.	N.D.	N.D.	N.D.	N.D.
α-BHC	0.0022	0.0010	0.0019	0.0020	0.0015
β-BHC	0.0034	0.0038	0.0044	0.0044	0.0008
γ-BHC	0.0041	0.0035	0.0038	0.0014	0.0019
δ-BHC	0.0006	0.0004	0.0016	0.0027	0.0004
Total BHC	0.0103	0.0087	0.0117	0.0105	0.0046
p, p'-DDD	0.001	0.000	0.009	0.008	0.005
p, p'-DDE	0.001	0.000	0.003	0.002	0.007
o, p'-DDT	0.004	N.D.	N.D.	0.004	N.D.
p, p'-DDT	0.015	0.009	0.020	0.011	0.009
Total DDT	0.021	0.009	0.032	0.025	0.021
Dieldrin	0.002	0.001	0.002	0.004	N.D.
Endrin	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor	N.D.	N.D.	N.D.	N.D.	N.D.
Heptachlor epoxide	N.D.	N.D.	N.D.	N.D.	N.D.

* Samples collected at different time had same levels of residues.
N.D., Not detected. Station (1)a, cultured area near shore; (1)b, off shore.

As appeared in Tables 5 and 6, the residues of organochlorine pesticides in oyster were about half of those in fish muscles, and no residues were detected in clams except BHC group in extremely low levels. Oysters in Taiwan are reared in the south-west coast facing open sea, this situation may make it difficult for the oyster to accumulate organochlorine pesticides. The clams investigated here were obtained from tidal flat some distance away from rivers. This may account for trace contamination.

Water source and organochlorine residues in fish

Tilapia is one euryhaline fish which can live in a wide range of salinities. Hence, it was chosen for use as an indicator in investigating the relation of water source and organochlorine residues. As shown in Table 2, there does not appear to be any relationship between organochlorine pesticide residues and muscles of tilapia collected from different locations. Station 7 (Table 1) is a reservoir high in the mountain where there should be little pesticide contamination. But tilapia collected from this reservoir also contained BHC, DDT and dieldrin in its muscles with values not any lower than those collected from paddy fields. Since small amount of pesticides were generally present in tilapia

TABLE 6

Mean organochlorine pesticide residues (μg/g wet tissue) in Taiwan's cultured clam, *Meretrix lusoria* (Roding).

Station	(3)	(4)
Date of catch	1973, May	1973, May
No. of specimen	10	10
Lipid (%)	0.56	0.68
Aldrin	N.D.	N.D.
α-BHC	0.0005	0.0025
β-BHC	N.D.	N.D.
γ-BHC	0.0000	0.0032
δ-BHC	N.D.	0.0002
Total BHC	0.0005	0.0059
p, p'-DDD	N.D.	N.D.
p, p'-DDE	0.000	N.D.
o, p'-DDT	N.D.	N.D.
p, p'-DDT	N.D.	N.D.
Total DDT	0.000	N.D.
Dieldrin	0.000	N.D.
Endrin	N.D.	N.D.
Heptachlor	N.D.	N.D.
Heptachlor epoxide	N.D.	N.D.

N.D., Not detected.

muscles, it is presumed that contamination could have been mediated by air.

A comparison of the pesticide residues in viscera of tilapia collected from different water sources indicated that tilapia from paddy fields had 3–4 times higher concentrations of total BHC and DDT than those from reservoir (Table 3). The higher residues in viscera of tilapia from paddy fields may come from agricultural applications of pesticides. The value of BHC in tilapia from brackish water pond was in between those from paddy fields and reservoir. But the residue of total DDT was greater than in samples from the other four locations, and DDD represented the bulk metabolite of the DDT group. The brackish fish pond receives family sewage from the Tainan Canal which may be the sources of the DDT group. Beside tilapia, the common carp and the grass carp from paddy fields also had 3–10 times higher concentrations of total BHC and total DDT than those from reservoir.

In Taiwan, dieldrin is mostly used for the protection of bananas and oranges. The small amount of dieldrin in cultured fishes of Taiwan could have come about through air contamination.

Biological significance for the fishes and shellfishes

Based on the levels of pesticide residues determined here, there seems no hazard for the survival of the aquatic animals. Sprague *et al.*[7] concluded that mortality in fish was generally associated with a DDT content of 4–7 ppm in the whole body, or 3–12 ppm in the muscle. The cultured fishes studied contained total DDT of less than 0.3 ppm in muscle or viscera (Table 4) which was far below the dangerous threshold. Butler[8] considered that 0.01–0.12 ppm of DDT residue in oyster as "of negligible significance". Oysters and clams studied in this investigation had total DDT concentrations of less than 0.03 ppm (Tables 5 and 6). The toxicity of BHC to fish is much less than DDT[9]. Cultured fish of Taiwan contained very low BHC and its isomers (0.01–0.1 ppm). Dieldrin, as previous mentioned, showed only in trace. Therefore, the pesticides do not seem to threaten fish survival.

In recent years, oysters reared in the southwest coastal area of Taiwan suffered high mortality. Organochlorine pesticides was one of the suspects for this high mortality. However, based on the data presented, it is reasonable to assume that organochlorine pesticides are not directly responsible for oyster death.

Significance as human food

There are no official seafood tolerance levels for organochlorine pesticides set in Taiwan now. Campbell *et al.*[10] estimated 0.20 ppm DDT+DDE as average for meat, seafood and egg in human diet in the USA. Dugal[11] analyzed Canadian freshwater fish in 1968, and found the average residue level of total DDT to be 0.12

ppm, dieldrin less than 0.01 ppm. Jensen *et al.*[12] reported DDT group residue in Swedish fish flesh at about 0.032 ppm. It is shown in Tables 4–6 that the DDT group cocentrations in Taiwan fishes and shellfishes do not seem to be higher than the values in marine life reported elsewhere.

There are few reports on BHC content in fish muscle in American and European countries. But Japanese food usually contains BHC group. For example, milk in Japan has been found to have BHC concentration averaging 0.294 ppm (0.02–0.939 ppm)[13]. Cultured fishes in Taiwan are low in BHC residues compared with concentrations in Japanese food.

In general, our results indicate that the use of organochlorine pesticides in the last 20 years did not result in significant accumulations of organochlorine pesticides in the edible portions of fishes and shellfishes that could render their flesh unsuitable as food for humans.

Evaluation of organochlorine pesticide pollution of Taiwan waters from residues in fish

There is now a considerable body of evidence to suggest that some form of equilibrium exists between the level of organochlorine pollutants in water and that in marine animals[2]. Therefore, it is suggested to calculate the concentrations in the water indirectly from the concentrations in fish. The small residues of organochlorine in fishes and shellfishes showed that the use of the pesticides in Taiwan did not generally result in significant pollution of Taiwan waters. The principal sources of water pollution by pesticides are runoff from the land and discharges of industrial wastes[14]. Since fishes and shellfisher collected from general fish ponds or coastal area contained little or no organochlorine pesticides, it seemed that there was little pollution of pesticides from the land. For Taiwan, what should be corrected is regional discharge of industrial waste, either from industries that manufacture or formulate pesticides or from those that use these compounds in their manufacturing processes.

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臺灣養殖魚貝類之有機氯劑農藥含量

鄭森雄　孫藍天

1. 分析 1972 年 9 月至 1973 年 9 月，自臺灣各地捕獲之 165 尾魚（包括吳郭魚、草魚、鰱魚、鯉魚及虱目魚），631 隻牡蠣及 20 隻文蛤，發現任何可食部份或內臟皆不含 aldrin, endrin, heptachlor 與 heptachlor epoxide。但是，所測魚貝類中皆含有 BHC 類 (BHC 類在 0.01 至 0.16 ppm 之間)。除文蛤外，所有魚貝類皆含有微量之 DDT 及其代謝產物 (DDT 類在 0.001 至 0.15 ppm 左右)。與極微量之 dieldrin (0.006 ppm 左右)。

2. 不同魚類（吳郭魚、草魚、鰱魚、鯉魚及虱目魚）肌肉中有機氯劑殘餘量沒有特別不同，但內臟之殘餘量却有差異。一般說來，同一魚體中，內臟農藥含量約爲肌肉之 2～4 倍，此可能與內臟含有較高之脂質及小腸吸收細胞之吸收農藥有關。

3. 稻田混養之吳郭魚、草魚及鯉魚內臟中 BHC 類與 DDT 類比高山水庫養殖之魚多 3～10 倍，此可能與稻田使用農藥有關。

4. 臺灣養殖魚貝類農藥含量甚低，似乎不影響魚貝類之生存。BHC 及 dieldrin 含量與世界其他地區之魚貝類相較，亦低於甚多，此濃度之殘餘量似乎不足以威脅消費者之健康。

5. 由養殖魚貝類之有機氯劑殘餘量，似可判斷臺灣水域尚未受到農藥嚴重之污染。但需注意因工業廢水所引起之局部農藥污染。