POLYCHLORINATED BIPHENYL CONGENERS IN FISHES INHABITED NEAR A CONTAMINATED SITE IN TAIWAN

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Introduction

Yu-Cheng incidence in 1979 in Taiwan had called attention to polychlorinated biphenyls (PCBs) poisoning. In the follow-up epidemiological study, not only Yu-Cheng women but also controls have PCB concentrations in their bloods. The ratio of PCB concentrations in serum of Yu-Cheng women to those of controls was the lowest compared to PCDDs and PCDFs[1]. The extent of possible exposure of general public in Taiwan from food products is worth investigation. Traces of PCBs have been reported in seafood, vegetables, and other food products throughout the world[2-4]. Seafood is thought to be the major source of PCB exposure to general public. Pan et al. analyzed PCBs in fishes and dairy products in Taiwan and reported that there were traces of PCBs in fish, meat and dairy products[5]. Lung et al. analyzed cultivated and ocean fishes purchased in Taiwan markets in a congener-specific basis and found that levels of 18 congeners were around 9.65~273.6ng/g lipid[6].

Most rivers in Taiwan have polluted with certain contaminants. Near a particulate river, there are several heavy metal recycling plants, which have been burning electrical parts and wires for years. Sediment was contaminated with PCBs and heavy metals. However, fishing is not prohibited in this area. Moreover, commercial fish cultivation is carried out in surrounding fishponds. This work analyzed sixteen PCB congeners in cultivated and native fishes inhabited near this known PCB-contaminated site and compare their PCB levels with previous analyzed fish samples in Taiwan.

Methods and Materials

Three kinds of popular fishes were purchased in a market near this known PCB-contaminated site. Fish samples with large (about 15-17cm in length) and medium (8-12 in length) sizes were both Tilapia (*Oreochromis hybrids*); there were nine and eleven samples, respectively. Tilapia is the most consumed fish species in Taiwan. Obtained samples were cultivated in local ponds. Fish samples with smallest size (1-3cm in length) were Si-ge (*Zacco Platypus*). It is a Taiwan native species, living in the fresh water stream. Approximately ten Si-ge fishes were pooled to become one sample; there were 15 samples for this kind of fish. According to fish vendors, those fishes were caught in local area.

Purchased fishes were refrigerated at -20ºC; they were thawed before sample preparation. Approximately 10 grams of minced Tilapia fillets was put in pear-shaped glassware and extracted with 80mL hexane/acetone (volume ratio 8:2) mixtures by Tissuemizer three times. For Si-ge, the whole fish (including fish bones) was extracted since customers usually fry and eat the whole Si-ge fish.

Kuderna-Danish (K-D) apparatus was used to concentrate the extracts to about 1 mL, which was then cleaned up by 6g of 2 % deactivated silicon gel and 3g of 3 % deactivated alumina oxides with hexane. Elutes were concentrated down to about 1 mL under a gentle stream of nitrogen and transferred to GC vials. PCB analysis was performed on a Perkin Elmer M611-9000 Gas Chromatography-Electron Capture Detector (Perkin Elmer Corporation, Norwalk, CT), equipped with a 30 m× 0.25 mm

ID× 0.25 µm Perkin Elmer PE-5 capillary column. The temperatures of the injection port and the electron capture detector were 280 ºC and 300 ºC, respectively. Quantification of GC results was conducted by internal standard method.

Lipid contents of fishes were assessed in a similar way. Approximately 10 grams of samples were put in pear-shaped glassware and extracted with 60mL hexane by Tissuemizer three times. Extracts were concentrated to about 1 mL by K-D apparatus, poured into pre-weighed aluminum cups, and baked at 80 ºC. Aluminum cups were weighed after 24, 48 and 72 hours till reaching stable weighs to obtain fat contents.

Sixteen congeners were selected, including IUPAC No. 28/31, 52, 77, 105/153, 118, 126, 138, 156, 157, 167, 169, 170, 180 and 189. Analytical standards of individual PCB congener in hexane were purchased from Ultra Scientific, North Kingston, Rhode Island. The purity of all standards was 99 % or higher.

There was one procedural blank for each batch of ten samples to assess any possible contamination. The levels of contamination of all congeners were below 1ng. One matrix spike sample per batch was conducted to assess percent recovery rates of different PCB congener. Totally, 5 matrix spike samples were analyzed; the recovery rates were from 70 % to 110 %. Surrogates (PCB IUPAC No. 103 and 112) were added to each sample to obtain percent recovery rates of each sample. They ranged from 70 % to 130 %. Internal standard PCB IUPAC No. 166 was added to each sample and used to quantify PCB congeners.

Concentrations of PCB congeners were expressed as wet weight concentrations (ng/g wet) and lipid concentrations (ng/g lipid). The reported data were all blank-corrected, but not adjusted by percent recovery rates. Those concentrations below method detection limits (MDLs) were taken as zero. Toxic equivalent quantities (TEQs) were calculated by multiplying concentrations of each congener by its toxic equivalent factor, which was from WHO (WHO-TEFs). Krustal-Wallis test and Scheffe test in Statistical Analysis Software (SAS version 6.12, SAS Institute Inc., Cary, NC) were used to compare PCB concentrations with different classification.

Results and Discussion

One or more PCB congeners were found in every fish sample analyzed. In a previous study, only 37 % of Tilapia obtained from Taiwan market was found to have PCB contamination. Market fishes inhabited near this contamination site apparently had higher percentage of PCB contamination. The lipid content of analyzed samples were about 1.5 %, 5.2 %, and 9.5 % for large Tilapia, medium-size Tilapia, and Si-ge samples, respectively. The measured lipid contents were decreased with fish sizes.

Total concentrations of 16 PCB congeners are presented in Table 1. Medium-size Tilapia had the highest PCB concentrations both in wet weight and lipid basis; they were statistically significantly higher than PCB levels in large Tilapia and Si-ge samples ($p=0.0001$). Medium-size Tilapia samples also had the highest TEQ values.

Tilapia fishes are usually cultivated in fishponds about seven months before being sold on the market. Medium size Tilapia is a slightly different kind of Tilapia compared to larger one; however, they were both cultivated with a similar period of time. Inhabited in a similarly polluted fishpond area and surrounded by probably similar PCB levels in water environment, Tilapia fishes may be accumulated to a similar amount of PCBs. Medium size Tilapia had higher PCB concentrations because they had lighter body weights. Si-ge fishes with only 1-3cm in length are actually juvenile fishes. Their life span would probably be only a couple of months. Therefore, they had the lowest PCB lipid concentrations among those three kinds of fishes.

Lung et al. (2001) reported that mean values of 18 PCB congeners in 30 Tilapia fish samples were about 2.06 ± 0.30 ng/g wet weight and 14.4 ± 22.5 ng/g lipid[6]. Those values were comparable to PCB levels in large Tilapia in this work in terms of wet weight concentrations. However, large Tilapia samples in this study had higher lipid PCB concentrations than those previously reported. And medium size Tilapia had much higher PCB concentrations both in wet weight and lipid basis. Furthermore, TEQ values were zero in previously analyzed 30 Tilapia samples, while currently presented data showed much higher TEQs. The TEQ levels were also higher than levels in market fishes in other countries[7,8]. Therefore, people consumed those Tilapia fishes cultivated in this known PCB contaminated area would be exposed to more toxic PCB congeners than otherwise.

Congener profiles of studied three fish species are in Table 2. PCB 52 was the major congener in both Tilapia species, while PCB 138 accounted for the majority of PCBs in previously analyzed Tilapia samples. The congener profiles of those fish cultivated near this known PCB contamination area were different from Tilapia cultured in general environment in Taiwan. For Si-ge, PCB 138, 118 and 77 were the main congeners.

Cultivated and native fishes inhabited near this PCB contamination site had higher percentage and higher concentrations of PCB contamination and more toxic PCB congeners. Their TEQ values were much higher than fish cultivated in general environment in Taiwan. Our government ought to consider restriction of fishing and cultivation in known PCB contamination areas in order to protect public health.

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Table 1. Concentrations and WHO-TEQs of 18 PCB congeners in five fish species (mean±SD)

Species	Tilapia (large)	Tilapia (medium)	Si-ge
(ng/g wet)	1.22±0.73	19.6±10.2	1.46±1.03
(ng/g lipid)	82.6±47.5	379 ±198	19.9±17.7
(pg-WHO-TEQ/g wet)	2.03±2.74	23.5±16.4	10.9±18.9
(pg-WHO-TEQ/g lipid)	143±194	454±318	111±100

Table 2. Percentage of different PCB Congeners in Fish Samples

PCB NO	Tilapia (large)	Tilapia (medium)	Si-ge	Tilapia (Lung *et al.* 2001)[6]
31/28	12.4 %	13.3 %	4.25 %	28.2 %
52	26.5 %	36.6 %	8.43 %	0.00 %
77	17.1 %	4.62 %	11.5 %	0.00 %
118	17.4 %	14.4 %	15.1 %	0.00 %
105/153	14.4 %	9.81 %	9.59 %	4.30 %
138	8.67 %	11.9 %	26.7 %	50.0 %
126	1.05 %	1.10 %	6.48 %	0.00 %
167	0.07 %	1.35 %	0.92 %	0.00 %
156	0.01 %	0.90 %	0.66 %	9.90 %
157	0.00 %	0.20 %	0.96 %	5.00 %
180	2.15 %	3.15 %	9.07 %	2.60 %
169	0.00 %	0.00 %	2.71 %	0.00 %
170	0.22 %	1.84 %	2.93 %	0.00 %
189	0.00 %	0.83 %	0.69 %	0.00 %