High Average Daily Intake of PCDD/Fs and serum levels in residents living near a deserted factory producing pentachlorophenol (PCP) in Taiwan: Influence of contaminated fish consumption

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Introduction

Many reports have suggested that PCDD/Fs (polychlorinated dibenzo-*p*-dioxins and dibenzofurans) contribute to immune deficiency, liver damage, human carcinogenesis, and neuromotor maturation in children[1-4]. Therefore, beginning in 1999, the Taiwan Environmental Protection Agency (EPA) conducted a survey to determine serum levels of PCDD/Fs in the general populations living around 19 incinerators in Taiwan. Relatively high average serum PCDD/F levels were unexpectedly found in Tainan city, a less industrialized area in southwestern Taiwan, than in other urban areas. We therefore reviewed the usage history of the land and found that a factory situated between Hsien-Gong Li and Lu-Erh Li, two administrative units of Tainan city, had been manufacturing pentachlorophenol (PCP) between 1967 and 1982. PCDD/Fs are formed as byproducts in the PCP manufacturing process[5-6]. Exposure to PCP and its derivatives via the food chain is the most significant intake route of PCDD/Fs in consumers in the European Union (EU) [5]. In Japan, in addition to combustion processes, PCP and chlornitrofen (CNP) have also been identified as the major sources of PCDD/Fs in Tokyo Bay[7]. A preliminary investigation showed that the soil in the PCP factory and sediments in the sea reservoir (13 hectares) near the deserted factory were seriously contaminated with PCDD/Fs (260-184,000 and 20-6220 pg I-TEQ/g, respectively), levels higher than those in other countries[8-9]. Therefore, the aim of this study was to compare the PCDD/F levels of fish meat in the sea reservoir and the serum in inhabitants living in the vicinity of the closed PCP plant and other nearby areas. The data from human and other biota samples might clarify the transmission pathway of the PCDD/F contaminants from the PCP factory to local residents, provide information about the exposure status of those living in the vicinity of the deserted PCP factory, and also lead to useful suggestions for controlling PCDD/F accumulation in those living near such facilities.

Material and Methods

Subject Selection and Serum Collection The inhabitants lived in Hsien-Gong Li and Lu-Erh Li of Tainan City, on the west coast of southern Taiwan, were selected as subjects. The reference groups of the inhabitants lived in Tainan City and Tainan County, between approximately 5 to 8 km from the deserted PCP factory. One hundred and six volunteers currently living in Tainan were recruited for our study. All had lived in the selected area for at least 5 years. After signing a consent form and the day after completing an overnight fast, each study participant provided 60 mL of venous blood. Blood samples were drawn into chemically clean tubes containing no anti-coagulants, and serum samples, obtained after centrifugation, were stored at –70°C until analysis.

Fish Collection To find the transmission pathway of the PCDD/F contamination, four categories of fishes including Milkfish (*Chanos chanos*), Tilapia (*Oreochromis mossambicus*), Borneo mullet (*Liza macrolepis*), and Bloch (*Nematalosa come*)were collected from the fish farm in Tainan and the sea reservoir located in the deserted factory.

Serum and Fish Samples Cleanups, and HRGC/HRMS Analysis of PCDD/Fs Seventeen 2,3,7,8-substituted PCDD/Fs were measured in biological samples, including human serum and fish meat samples, using isotope dilution HRGC/HRMS. Each serum sample was spiked with a mixture containing fifteen $^{13}C_{12}$-PCDD and PCDF standards as defined in USEPA Method 1613. Serum samples were enriched and fractionated by C18, SCX, silica, and highly selective adsorbent magnesium-silica gel cartridges (Florisil) before analysis. Each analytical run consisted of a method blank, a quality control, and seven unknown samples for quality assurance and quality control. The detection limit of 2,3,7,8-TCDD for the analysis was 0.03 pg/column-injection or 0.007 pg/ML-serum. All PCDD/Fs were adjusted to the lipid content analyzed from the corresponding samples and were reported as pg WHO-TEQ/g lipid. The analysis method for fish meat samples was modified according to USEPA method 1613B. A 30-50 g fish sample was homogenized in 100 ml of acetone/hexane (1:1). An internal standard mixture containing $^{13}C_{12}$-labeled 2,3,7,8-substituted PCDD/Fs was added to the tissue homogenate. The spiked sample was then mixed with an equal volume of ethanol. The sample was extracted with hexane, and fat content was determined gravimetrically. After extracting, the sample was treated with concentrated sulfuric acid and three SPF clean-up steps (acid silica, acid alumina, and florisil) were carried out. After the cleanup procedure, the instrumental analysis method for the fish sample was the same as for the serum sample.

Interviewer-administered Questionnaire

Information obtained from the questionnaire included personal characteristics (sex, age, height, weight, occupational history, neighborhood geography, pregnancy history, etc), life style (alcohol intake and tobacco usage), and the quantity of dietary intake for the previous 1 year based on a semi-quantitative food-frequency questionnaire. Trained interviewers administered the questionnaires according to standard operating procedures prepared in advance.

Assessment of Average Daily Intake

ADI was calculated based on the following hypothesis: human lived near Hsien-Gong and Lu-Erh Li eating the fish from the sea reservoir for 10 years and 60 years from other fish farm in their whole life (70 years). Calculating equation as following:

$ADI = C_{\text{from the sea reservoir}}$ (pg WHO-TEQ/g sample) × consumption quantity (g/day) ÷ body weight (kg)×10/70+$C_{\text{from the fish farm}}$ (pg WHO-TEQ/g sample) × consumption quantity (g/day) ÷ body weight (kg)×60/70

Results and Discussions

Demographic characteristics, consumption quantities of marine products, and serum PCDD/F concentrations of the participants in the three areas are presented in Table 1. The statistical results showed that average PCDD/F concentration was higher in Hsien-Gong Li and Lu-Erh Li (62.5 pg WHO-TEQ/g lipid), lower in Tainan City (22.5 pg WHO-TEQ/g lipid), and lowest in Tainan County (18.2 pg WHO-TEQ/g lipid). Table 2 presents the distribution of age, BMI, resident areas, and consumption quantities of marine products among 4 groups of study participants with low to high quartiles of serum PCDD/F levels. Serum PCDD/F levels were higher than 30.04 pg WHO-TEQ/g lipid for 90.5% of the participants in Hsien-Gong Li and Lu-Erh Li; this was true for only 13.7% in Tainan City and for and 0% in Tainan County, however, both significantly different ($p<0.0001$). The age distribution of inhabitants and consumption quantities of sea fish and shrimp showed significant differences between the 4 groups with low to high serum PCDD/F levels. However, no elevated trend was found based on increasing serum PCDD/F concentrations. The results showed no significant differences in demographic characteristics, including age, sex, smoking habit, and BMI between the three areas. Although there were significant differences in serum PCDD/F levels based on average age and consumption of sea fish and shrimp, there were no definite trends.

Table 1 Distribution of demographic characteristics and serum PCDD/Fs levels among three areas.

Areas	**Hsien-Gong and Lu-Erh Li**	**Tainan City**	**Tainan county**	**P value**
N	21	51	34	
Age	41.9±10.9	44.0±13.1	46.8±12.9	0.307
Sex (men: %)	47.6	52.9	52.9	0.909
BMI	23.8±3.4	24.6±4.0	25.3±4.1	0.394
Smoking status[§]				0.070
Nonsmokers	9 (42.9)	9 (17.7)	4 (11.8)	
Active smokers	4 (19.1)	18 (35.3)	11 (32.4)	
Passive smokers	8(38.1)	24 (47.1)	19 (55.9)	
Consumption[†]				
Sea fish	24.3±25.6	19.9±19.6	20.2±34.1	0.184
Fresh water fish	7.3±11.2	5.9±11.1	8.1±13.9	0.877
Shellfish	1.6±1.3	1.9±3.7	1.7±1.8	0.305
Shrimp	3.2±4.1	1.6±2.9	1.8±4.6	0.053
PCDD/Fs conc.[‡]	62.5±43.1	22.5±10.1	18.2±6.6	**<0.0001****

**: $p<0.001$; [§]: Chi-Squared test, number (%); [†]: bowl per month, 200 mL per bowl;
[‡]: pg WHO-TEQ/g lipid

Table 2 Distribution of number and percentage of study subjects among the three areas from low to high serum PCDD/Fs level, age and BMI

PCDD/Fs conc.[†]	<25%	25-50%	50-75%	75-100%	P value
Areas[§]: number (%)					**<0.0001****
1.Hsien-Gong and Lu-Erh	0	1 (4.8)	1 (4.8)	19 (90.5)	
Li	13 (25.3)	17 (33.3)	14 (27.5)	7 (13.7)	
2.Tainan City	13 (38.2)	9 (26.5)	12 (35.3)	0	
3.Tainan county					
Age[‡]	34.8±11.7	46.9±12.5	50.9±9.9	45.0±10.9	**<0.0001****
BMI[‡]	24.9±5.1	24.6±3.4	25.2±3.6	23.9±3.4	0.680
Consumption[#, ‡]					
Sea fish	20.5±22.6	12.0±11.1	23.5±37.5	27.9±25.2	**0.036***
Fresh water fish	9.1±15.3	6.6±12.7	5.4±8.8	6.7±10.6	0.617
Shellfish	1.6±1.4	1.7±3.4	1.2±1.9	2.6±3.7	0.129
Shrimp	1.4±1.7	0.9±0.9	1.8±5.2	3.9±4.9	**0.012***

[†]: 1. <25%: serum PCDD/Fs level <15.95 pg WHO-TEQ/g lipid, 2. 25-50%: 15.95≤serum PCDD/Fs level <22.19 pg WHO-TEQ/g lipid, 3. 50-75%: 22.19≤serum PCDD/Fs level <30.04 pg WHO-TEQ/g lipid, 4. >75%: serum PCDD/Fs level >30.04 pg WHO-TEQ/g lipid

[§]: Chi-Squared test [‡]: Kruskal-Wallis test

[#]: bowls per month, 200 mL per bowl; *: $p<0.05$, **: $p<0.001$

The PCDD/F concentrations of the fish, shrimp and oyster bought from the Tainan fish farm and caught in the sea reservoir are given in Table 3 and table 4. The milkfish and tilapia were the popular fishes in Tainan, therefore, we just bought the two fishes from the fishing farm in Tainan. Extreme higher PCDD/Fs levels were found in fish from sea reservoir than in fishing farm, as well as for shrimp.

Table 3 Distribution of PCDD/Fs concentrations in various fishes between those from sea farm located near Hsien-Gong and Lu-Erh Li and sea reservoir

Types	Milkfish		Tilapia		Broneo mullet	Bloch
Sources	Farm	Sea reservoir	Farm	Sea reservoir	Sea reservoir	Sea reservoir
PCDD/Fs levels (pg WHO-TEQ/g sample)						
Mean	0.15	28.3	0.03	23.1	56.5	122.2
Range	0.03-0.86	11.8-58.3	0.02-0.03	7.0-49.5	39.1-72.9	103.3-134.1
PCDD/Fs levels (pg WHO-TEQ/g lipid)						
Mean	4.2	962.9	1.1	833.8	735.8	2097.9
Range	1.25-13.8	750.3-1323.6	0.82-1.3	271.2-3065.1	574.4-848.2	1738.2-2555.5

Table 4 Distribution of PCDD/Fs concentrations in various seafood between those from sea farm located near Hsien-Gong and Lu-Erh Li and sea reservoir

Seafood types	Shrimp		Oyster	
Sources	Farm	Sea reservoir	Farm	Sea reservoir
PCDD/Fs levels (pg WHO-TEQ/g sample)				
Mean	0.03	49.55	1.32	--
Range	0.21-0.05	32.57-73.15	0.21-2.95	--
PCDD/Fs levels (pg WHO-TEQ/g lipid)				
Mean	7.36	2118.14	94.02	--
Range	3.30-12.70	1238.82-3147.85	9.56-222	--

We further calculated the average daily intake (ADI) of PCDD/Fs based on the consumption quantity and PCDD/Fs levels of sea fish, shrimp and oyster. 40 persons' (38%) ADI were higher than suggested value of 1-4 pg WHO-TEQ/kg body weight/day by the world health organization (WHO). In addition, significant higher serum PCDD/Fs levels were also observed in subjects with high ADI than those with low one (Table 5). To identify the major factors contributing to serum PCDD/F concentrations, a multivariate regression model was used to examine the association between PCDD/F levels and ADI (Figure 1). Table 6 showed that after adjustment for the age, sex, BMI, and smoking status of the participants, PCDD/F levels were found to be positively associated to ADI (β=0.166, $p<0.0001$).

Based on the unusually high PCDD/F levels in fish from the sea reservoir, it seems clear that the sea reservoir has been contaminated with PCDD/Fs. It is possible that if the inhabitants consume fish from the sea reservoir, it will contribute to their individual accumulation of PCDD/Fs. In addition, the deserted PCP plant is surrounded by land used primarily for marine aquaculture. Furthermore, PCDD/F contaminants might have entered the local ecosystem and, through the food chain, ultimately accumulated in the local inhabitants. Our investigation may have discovered a potential hazardous area in which PCDD/F contaminants have been and continue to be easily transported via the watershed. Our finding has raised public concern about local environmental contamination and who specifically has been exposed PCDD/Fs via seafood from the nearby sea reservoir. In addition, the contamination levels of all marine species and biota samples also need to be more thoroughly evaluated. A prospective cohort study is also needed to determine the long-term health effects on the people living near the deserted PCP factory.

Table 5 Distribution of serum PCDD/Fs levels among the 4 groups with low to high calculating average daily intake (ADI) of PCDD/Fs

Group §	1	2	3	4
Number	30	35	19	21
ADI †	0.46±0.30 (0-0.99)	2.14±0.83 (1.00-3.96)	6.01±1.66 (4.03-9.10)	32.52±25.30 (10.20-121.2)
serum conc.‡	20.9±10.4	20.6±7.5	30.3±41.7	53.7±31.3**

†: mean±standard deviation (range), pg WHO-TEQ/kg body weight/day, the calculation was based on the hypothesis:

1. human living near Hsien-Gong and Lu-Erh Li eat the fish from the sea reservoir for 10 years of their whole life (70 years)
2. human lived in Tainan City and Tainan county eat fish and seafood from the fishing farm

§: Group 1, ADI <= 1 pg TEQ/kg body weight/day; group 2, 1<ADI<=4; group 3, 4<ADI<=10, group 4, ADI>10; ‡: pg WHO-TEQ/g lipid; **: p<0.0001, test by Kruskal-Wallis test

Table 6 Multivariate linear regression between serum PCDD/Fs levels and their corresponding average daily intake of PCDD/Fs (R-square=0.368)

Variables	Estimate	Prob>|t|
Intercept	2.40	**<.0001****
SEX[1]	-0.121	0.066
AGE	0.019	**<.0001****
BMI	-0.010	0.441
Active smokers	-0.045	0.620
Passive smokers	-0.049	0.543
ADI	0.166	**<.0001****

**: p<0.001, Note: PCDD/Fs levels and ADI levels were log-transformed and the regression model had adjusted for sex, age, BMI and smoking status.

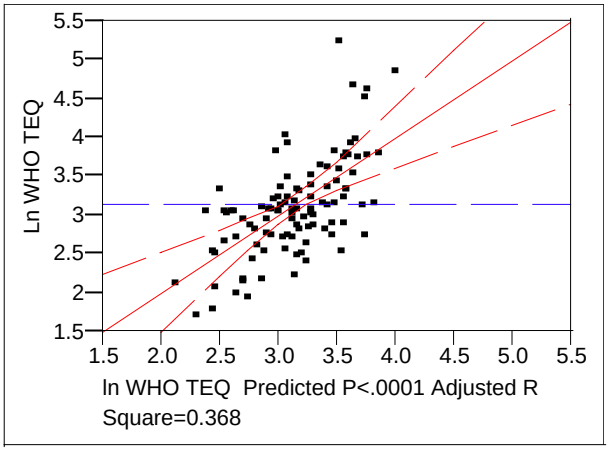

Figure 1 Multiple regression between serum PCDD/Fs levels and ADI value after adjustment for age, sex, BMI, and smoking status. PCDD/Fs levels and ADI value was natural log transformed. (R^2=0.368)

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