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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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Inhabitants living near a deserted PCP factory are exposed to high PCDD/F levels.

Abstract

An abandoned pentachlorophenol plant and nearby area in southern Taiwan was heavily contaminated by dioxins, impurities formed in the PCP production process. The investigation showed that the average serum PCDD/Fs of residents living nearby area (62.5 pg WHO-TEQ/g lipid) was higher than those living in the non-polluted area (22.5 and 18.2 pg WHO-TEQ/g lipid) ($P < 0.05$). In biota samples, average PCDD/F of milkfish in sea reservoir (28.3 pg WHO-TEQ/g) was higher than those in the nearby fish farm (0.15 pg WHO-TEQ/g), and Tilapia and shrimp showed the similar trend. The average daily PCDD/Fs intake of 38% participants was higher than 4 pg WHO-TEQ/kg/day suggested by the world health organization. Serum PCDD/F was positively associated with average daily intake (ADI) after adjustment for age, sex, BMI, and smoking status. In addition, a prospective cohort study is suggested to determine the long-term health effects on the people living near factory.

Keywords: PCDD/Fs; Pentachlorophenol; Fish; Shrimp; Oyster; ADI

1. Introduction

Many reports have suggested that polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) contribute to immune deficiency, liver damage, human carcinogenesis, and neuromotor maturation in children (Becher and Flesch-Janys, 1998; Eskenazi and Kimmel, 1995; Ilsen et al., 1996; McGregor et al., 1998). 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 16 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 1981. PCDDs and PCDFs are formed as by-products in the PCP manufacturing process (Eduljee, 1999; Masunaga et al., 2001). 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) (Eduljee, 1999). In Japan, in addition to combustion processes, PCP and chlornitrofen (CNP) have been identified as the major sources of dioxins in Tokyo Bay (Yao et al., 2002). Major mass fluxes of TCDDs and OCDDs come from the impurities in CNP and

Abbreviations: PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCP, pentachlorophenol; CNP, chloronitrofen; ADI, average daily intake.

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PCP directly into the soil (Suzuki et al., 2000). Another study found that the PCDD/F abundance profiles were similar to the patterns in PCP (Muto and Sugawara, 2001). A preliminary investigation showed that the soil in the PCP factory in Tainan and sediments in the sea reservoir (14 hectares) near the deserted factory were seriously contaminated with PCDD/Fs (concentration ranges: 260–184,000 and 20–6220 pg I-TEQ/g, respectively), levels higher than in other countries (Im et al., 2002; Schuhmacher et al., 2002). The measured data provided evidence that the PCP and untreated wastewater from the factory might have spread via the wastewater and soil into the sea reservoir. Therefore, the aim of this study was to compare the serum PCDD/F levels of the inhabitants living in close proximity to the closed PCP plant and in other nearby areas. In addition, we report here the PCDD/F levels of fish and shrimp collected from a nearby fish farm and from the sea reservoir situated in the two administrative units surrounding the old factory site. We hoped that 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.

2. Materials and methods

2.1. Subject selection and serum collection

In Tainan City (population ca. 720,000), on the west coast of southern Taiwan, there is a deserted PCP factory on the boundary between Hsien-Gong Li and Lu-Erh Li, two small municipal administrative units. The reference groups lived in Tainan City and Tainan County, between approximately 5 to 8 km from the deserted PCP factory. One hundred six volunteers (age range, 18–65 years) currently living in Taiwan 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; serum samples, obtained after centrifugation, were stored at −70 °C until analysis. In addition, body fat percentage was determined for all participants by a body fat analyzer (HBF-306; OMRON Corp., Tokyo, Japan). Body mass index (BMI; weight in kg divided by the square of the height in meters (m)) was also calculated for each participant.

2.2. Fish and shrimp collection

To find the transmission pathway of the PCDD/F contamination, fish and shrimp were collected from the fish farm in Tainan and the sea reservoir near the deserted factory. Four species of fish were analyzed: 1. Milkfish (*Chanos chanos*); 2. Tilapia (*Oreochromis mossambicus*); 3. Borneo mullet (*Liza macrolepis*); and 4. Bloch (*Nematalosa come*). Milkfish and tilapia were the most commonly eaten and favorite seafood of the local inhabitants; we bought 12 milkfish, 4 tilapia, 3 shrimp, and 3 oysters raised in the fish farm's fishpond. We also caught shrimp and samples of the four species of fish in the sea reservoir.

2.3. Serum sample and marine sample cleanups, and HRGC/HRMS analysis of PCDD/Fs

Seventeen 2,3,7,8-substituted PCDD/Fs were measured in biological samples, including human blood samples and marine samples, using isotope dilution high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS), as reported elsewhere (Chen et al., 2003, 2004). The method included extraction, cleanup, concentration, and instrumental analysis. Quality assurance/quality control (QA/QC) protocols defined in USEPA method 1613 were followed to ensure positive identification and the quality of the measurements. The average recovery of the isotope-labeled standards was 64%.

2.3.1. Blood sample

The sample enrichment and cleanup procedures used in this study were similar to the procedures reported by Chang et al. (1993). Each serum sample was spiked with a mixture containing fifteen $^{13}C_{12}$-PCDD and PCDF standards as defined in USEPA Method 1613. The amount of each spiked congener was 0.5 ng, except that $^{13}C_{12}$-OCDD was 1.0 ng. Serum samples were enriched and fractionated by C18, SCX, silica, and highly selective adsorbent magnesium-silica gel cartridges (Florisil; U.S. Silica Company, Berkeley Springs, WV) before HRGC (8060 GC; Fison Instruments, Inc., Danvers, MA) and HRMS (AutoSpec Ultima® NT tri-sector (EBE) mass spectrometer; Waters Corp., Milford, MA) analysis. Each analytical run consisted of a method blank, a quality control, and seven unknown samples for quality assurance and quality control according to the protocols defined in USEPA method 1613. Samples were chromatographed on capillary fused-silica column (60 m, 0.25 mm ID, 0.25 μm film thickness) (Rtx-5MS; RESTEK Co., Bellefonte, Philadelphia, PA) with helium as the carrier gas at a flow rate of 0.9 mL/min. The injector temperature was 250 °C. The temperature program was (1) an initial temperature of 150 °C for two minutes; (2) programmed to 240 °C at 12 °C/min; (3) kept at 240 °C for 14 min; (4) programmed to 255 °C at 2 °C/min; (5) then kept at 255 °C for 7 min; and (6) programmed to 310 °C at 17 °C/min. Two μL of sample was injected with an autosampler in separation mode. The HRMS was operated in electron impact ionization mode. The pressure in the ionization source was about 10^{-6} torr with a source temperature of 250 °C. Isotope dilution HRGC/HRMS method was used to quantitatively determine the seventeen PCDD/PCDF congeners. Selected ion monitoring (SIM) was used to acquire M/(M + 2) or (M + 2)/(M + 4) PCDD/PCDF ions for identification. 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.

2.3.2. Marine sample

The analysis method for fish and shrimp sample was modified according to USEPA method 1613B and USEPA method 1668A (USEPA 1994; USEPA 1999). A 30–50 g marine 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 marine sample was the same as for the serum sample.

2.4. Lipid content determination

The lipid content was determined from 1-mL portions of each serum sample using an enzymatic method (Akins et al., 1989) with a commercial kit (Merck Biotrol, Nogent-sur-Marne, France).

2.5. 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. The consumption information was calculated based on estimated intake frequencies: 3 times per day, 1–2 times per day, 4–6 times per week, 1–3 times per week, 1–3 times per month, 1–11 times per year, and never (Koppen et al., 2002). Consumption quantity per meal for each type of food was also estimated based on 200-mL bowls for each food