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Concentrations of organochlorine pesticides (OCPs) in human blood plasma from Hong Kong: Markers of exposure and sources from fish

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ABSTRACT

Previous studies revealed that food, particularly fish products, is the major source for human exposure to organochlorine pesticides (OCPs). Our previous studies revealed that contamination of Hong Kong market fish with DDT was 0.74–131 with a mean of 12.2 ng g^{-1}, ww, a result suggested that local people might be exposed to hazardous concentrations of OCPs. Therefore, the present systematic study was conducted to determine concentrations of OCPs in blood plasma of Hong Kong residents, develop marker substances and evaluate sources of 19 individual OCPs from fish. Concentrations of $\sum$OCPs, $\sum$DDTs and $\sum$HCHs ranged from 294 to 9732, 172 to 8842, and 115 to 1616 ng g^{-1} lipid weight (lw), respectively. These concentrations were greater than those in blood of people from most developed countries but lower than those from most developing countries. The upper age group (>50 years) had significant ($p<0.05$) greater concentrations of OCPs than other groups. Furthermore, concentrations of OCPs in males were significantly ($p<0.05$) greater than those in females. *p, p′*-DDE was the predominant congener and marker substance of DDTs, while *β*-HCH was the predominant congener and marker substance of HCHs. *p, p′*-DDE was more correlated with $\sum$OCPs ($r^2=0.830$, $p<0.05$) than other individual OCPs, which suggested that *p, p′*-DDE is a good marker for accumulation of OCPs in blood plasma. Concentrations of individual OCPs were significantly correlated with not only their corresponding total concentrations in fishes from Hong Kong markets ($r^2=0.391$, $p=0.024$), but also their bioaccessible fractions, which were estimated by an *in vitro* digestion method ($r^2=0.784$, $p=0.000$). These results suggested that the *in vitro* gastrointestinal model is a more accurate method to evaluate accumulation of and health risks caused by dietary intake of OCPs. This study, which was the first systematic study to investigate concentrations of OCPs in blood of Hong Kong people, provides a baseline to which future measurements can be compared.

1. Introduction

Since the second world war, organochlorine pesticides (OCPs) have been used all over the world (Wong et al., 2005). The organochlorine (OC) insecticides aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT) categorized as "persistent organic pollutants (POPs)" have been selected as the "dirty dozen" under the Stockholm Convention (UNEP, 2005). Some OCPs, such as DDT and its metabolites, can disrupt normal functions of endocrine and reproductive systems of humans (Li et al., 2008; Soto et al., 1995). Although usage of DDT and hexachlorocyclohexane (HCH) in mainland China has been banned since 1983, these OCPs were the leading pesticide products from the 1950s to 1980s and therefore large amounts of these insecticides had been used before they were prohibited.

Generally, the greatest source for human exposure to OCPs is daily intake *via* food consumption. Results of previous studies indicated that over 90% of the body burden of DDTs in the general population are derived from food, particularly fish and other fatty foods of animal origin (Wang et al., 2011a, 2011b). Concentrations of $\sum$HCHs ranging from 0.33 to 9.88 with a mean of 1.57 ng g^{-1}, wet weight, ww and concentrations of $\sum$DDTs ranging from 0.74 to 131 with a mean of 12.2 ng g^{-1}, ww were measured in fish from markets in Hong Kong (Wang et al., 2011b). Furthermore, samples of muscle from 45% freshwater and 70% marine fishes exceeded the screening value for

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DDTs set by USEPA (Cheung et al., 2007). Considering that fish contributed the largest portion of the dietary intake of DDTs (57%) for local residents (Guo et al., 2010), attention should be paid to accumulation of OCPs and associated risks to the health of residents of Hong Kong.

Blood, milk and adipose can be good biomarkers to assess human exposure to organic pollutants (Mueller et al., 2008; Wang et al., 2011c). Compared with milk which can only be obtained from the female population and only during lactation, blood is more easily collected. Furthermore, blood can cover a wide range of ages of both genders. Therefore, blood is considered to be a good matrix in which to assess concentrations of pollutants in the general population. The objectives of the present study were to:1) determine concentrations of OCPs in 117 blood plasma samples representative of the local Hong Kong population; 2) determine factors affecting accumulation of OCPs; 3) identify marker substances for DDTs, HCHs and OCPs in blood plasma; and 4) evaluate contribution of fish consumption for body OCP accumulation based on our published total and bioaccessible concentrations of OCP in Hong Kong market fish (Wang et al., 2011b).

2. Materials and methods

2.1. Sample collection and preparation

The present study has been approved by the Human Investigation Ethics Committee of Department of Biology, Hong Kong Baptist University. Detailed information about blood donors and blood collection has been reported previously (Wang et al., 2012). Briefly, blood plasma from 54 females and 63 males was collected by the Hong Kong Red Cross from February to July 2011. All participants were Southern Han Chinese and determined to be eligible as blood donors based on the screening by nurses before recruitment. The age of blood donors ranged from 17 to 63 and therefore were classified into five age groups as ≤20 (n=9), 21–30 (n=36), 31–40 (n=22), 41–50 (n=28), and ≥51 (n=22). Because blood plasma in the present study was from those persons that are eligible as blood donors, children and people older than 65 were excluded. Samples of blood were collected with heparinized tubes, maintained at 4 °C, and centrifuged at 1000×*g* for 15 min to separate the plasma and kept at −20 °C until extraction. Lipid content was determined gravimetrically and the content of each sample was used to express concentrations of OCP on a lipid-normalized basis (Sjodin et al., 2001).

2.2. Extraction and instrumental analysis

Procedures for extraction and cleanup were based on those applied in previous studies (Qin et al., 2011; Tsang et al., 2011; Wang et al., 2011a) with slight modifications. Briefly, blood plasma was spiked with internal standards (PCB-60 and PCB-137, 10 ng for each sample) and allowed to equilibrate over-night. An accurate volume of serum (typically 2 ml) was mixed with 2 ml formic acid and 2 ml water and equilibrated by ultrasonic treatment for 20 min. Then, solid phase extraction (SPE) was performed by the use of 1 g/6 ml Extract-Clean, High-Capacity C18 end capped cartridges (AlltechAssociates Inc. Carnforth, UK). Cartridges were washed with dichloromethane (DCM) and activated by the use of methanol and Milli-Q water before extraction. Before the column became dry, plasma was added and dried for 15 min by aspiration of ambient air. The target compounds were then eluted by the use of 15 ml of n-hexane: DCM (1:1, v/v). The extract was concentrated to 1 ml before clean up and fractionation on a florisil column as described previously (Wang et al., 2011b). The extracts were concentrated and resolved in n-hexane. Deuterated recovery standard, 100 ng g^{-1} tetrachloro-m-xylene (TCmX), was added to all extracts prior to instrumental analyses. The final volume for instrumental analysis of all samples was 100 μl. OCPs were quantitatively analyzed by the use of a Hewlett-Packard (HP) 6890 N gas chromatograph (GC) coupled with a HP-5973 mass selective detector (MSD) and a 30 m×0.25 mm×0.25 μm DB-5 capillary column (J & W Scientific Co. Ltd., USA). The mass spectrometry mode is selected ion monitoring (SIM). Sample injection volume was 1.0 μl. The oven temperature was programmed to go from 100 °C (initial time, 2 min) to 175 °C at a rate of 15 °C min^{-1}, then 3 °C min^{-1} to 250 °C and held for 9 min. The 20 targeted OCP compounds included DDT and transformation products (*o,p′*-DDD, *p,p′*-DDD, *o,p′*-DDE, *p,p′*-DDE, *o,p′*-DDT and *p,p′*-DDT), HCHs (*α*-HCH, *β*-HCH, *γ*-HCH, and *δ*-HCH), CHLs (heptachlor, *trans*-Chlordane, *cis*-Chlordane, *trans*-Nonachlor, and *cis*-Nonachlor), DRINs (aldrin, dieldrin, and endrin), HCB (hexachlorobenzene) and mirex. The peaks of *p,p′*-DDD and *o,p′*-DDT were combined as one because they were extremely close and difficult to distinguish. Details of quantification parameters of target compounds are described in Table S1.

2.3. QA/QC

At regular intervals solvent blanks (n-hexane) were subjected to the entire analytical procedure to determine background interference. The blank values were not subtracted from the sample measurements because they are equal to the solvent baseline in most cases. For each set of 15 samples, a procedural blank, a sample duplicate, solvent spiked with known amount of OCPs, and known amount OCP standard solution were processed. Several quality control criteria were used to ensure correct identification and quantization of the target compounds: first, retention times matched with those of the authentic reference compounds; second, the ratios of the two characteristic ions were within 15% of the theoretical values; and third, the signal-to-noise (S/N) ratio was greater than 3 for the selected ions. If any of these three criteria failed, the congener was excluded. The limit of detection (LOD), defined as concentrations of analytes that gave rise to a peak with a signal-to-noise ratio (S/N) of 3, was determined for each analyte by the use of a batch standard (Table S1). Internal standards (PCB-60 and PCB-137) were used to determine the recoveries, which ranged from 87.5 to 107%. Recoveries of the solvent spiked samples for OCPs ranged from 91.2 to 109% (Table 1).

2.4. Data analyses

Concentrations of OCP were reported in units of ng g^{-1}, lipid weight (lw). Since recoveries were consistent and sufficiently great, concentrations were not adjusted for recovery. Statistical analyses were performed by the use of SPSS 17.0 for Windows. If the concentration of a congener was lower than the LOD, a value equal to half the LOD of the analytical method was attributed for statistical analysis, while it was set to zero for sum, mean and median calculations. Normality was confirmed by the Kolmogorov–Smirnov test. Independent t-tests were performed to compare concentrations of OCP in blood plasma of males and females. One-way ANOVA tests followed by Duncan's multiple range tests were used to determine if there were any significant difference ($p<0.05$) in concentrations of OCP among age groups. Pearson correlation coefficients were used to determine associations between concentrations of OCPs in blood plasma with the age and lipid contents. Linear regression analysis was used to test the correlations between marker substances and their corresponding groups.

3. Results and discussion

3.1. Concentrations of OCPs

Concentrations of 19 insecticides were quantified in plasma samples of males and females from Hong Kong (Table 1). Mirex was lower than the limit of detection in all samples of blood. Therefore, concentrations were not reported. Concentrations of ∑OCPs ranged from 294 to 9732 ng g^{-1} lw (mean 1894, median 1451 ng g^{-1} lw). Concentrations of ∑DDTs ranged from 172 to 8842 ng g^{-1} lw