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Residual Levels and Bioaccumulation of Chlorinated Persistent Organic Pollutants (POPs) in Vegetables from Suburb of Nanjing, People's Republic of China

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Vegetable is an essential constituent of the human diet. Growing demand for food as a result of increasing population has lead to a substantial increase in the application of agro-chemicals like pesticides and fertilizers, resulting in continued contamination of our environment and food (Agarwal 1976). Public concern over pesticide residues has risen over the past decades to the point, where it has become a significant food safety issue (Colume et al. 1999). The determination of pesticide residues in food has become an increasingly essential requirement for consumers, producers, and authorities responsible for food quality control (Aguilear et al. 2003).

Organochlorine pesticides (OCPs) were the most popular pesticides used in the world from the 1950s to 1970s. OCPs such as DDTs and its metabolites, hexachlorocyclohexane(HCH) and its isomers, hexachlorobenzene (HCB), aldrin, dieldrin, endrin and heptachlor are included in the group of chlorinated persistent organic pollutants (Wania and Mackay 1996). They have very low solubility in water, but relatively high solubility in lipids, and they are chemically stable and resistance to metabolism (Magdic et al. 1996). Despite the ban of OCPs application in agriculture for more than two decades, they are still ubiquitous and detectable in natural water (Matin et al. 1998), soil (Harner et al. 1999), air (Haenel and Siebers 1995) and food (Barkatina et al. 1999) throughout the world, which is posing a menace to human health and environment. OCPs have been linked to carcinogenicity and endocrine disruption in mammals. Therefore, concerns over toxicity are exacerbated by their hydrophobic properties mentioned above (Patlak 1996), which results in the bioaccumulation of OCPs in fatty tissues and biomagnification through the trophic web (Strandberg et al. 1998).

Since the 1970s, many developed countries such as USA, Canada, most European countries have banned or highly restricted organochlorine pesticides application in agriculture. Many OCPs have been replaced with less persistent organophosphate and carbamate pesticides (Edwards 1975). In many developing countries, however, the OCPs were widely used to increase food production and control plant disease and pests in cotton, cereals, fruits and vegetables for the growing population until the late 1980s (Adeyeye and Osibanjo 1999). In 1983, many OCPs such as DDT

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and HCH were banned for their use in agriculture in China. Although they were banned two decades ago, most of the OCPs currently still can be found in environment (Jiang et al. 2000) and food crops (Zheng et al. 1993) in China due to the persistent properties of them.

In view of their poisonous and persistent nature, there is a pressing need to control and monitor OCPs in the environment and food. The purpose of the present study was to reveal the residual levels and bioaccumulation of OCPs in representative vegetables from the suburb of Nanjing, China.

MATERIALS AND METHODS

All chemicals were of analytical grade or better. Standard organochlorine pesticides like *p,p'*-DDT , *p,p'*-DDE , *p,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, hexachlorocyclohexane (α-, β-, γ- and δ -HCH), dieldrin, endrin and hexachlorobenzene(HCB) were purchased from Dr. Ehrenstorfer Company (Agusburg, Germany). Other reagents and solvents (acetone, dichloromethane, petroleum ether, *n*-hexane, sulfuric acid and anhydrous sodium sulfate) with high quality, were obtained from Nanjing chemical reagent company. Stock standard solution of OCPs were prepared in *n*-hexane at concentration of 1 mg ml^{-1}, and then stored in volumetric flask in the dark below zero degree Celsius.

Vegetable samples were collected from one of the vegetable planting areas in the suburb of Nanjing ($32^o09'$-$32^o15'$N; $108^o43'$-$108^o50'$E), about three kilometers away from the north bank of the Yangtse River. The sampling was conducted twice in a year, in June 25th and October 20th, 2003. The edible part of 2.5 kg raw sample was reduced by quartering to about 500 g, then pulped in a high-speed blender, kept in a glass bottle and stored in refrigerator for analysis. Soil samples were also collected at the same time and in the same place as vegetables. At each site, soils of 7 sampling locations were mixed for representative sample, and nine selected sites were collected. The soil samples were air-dried, then passed through 2 mm mesh sieve, and stored at ambient temperature prior to the start of the experiments.

Stored vegetable samples were taken out from refrigerator and restored to ambient temperature. An amount of 2.0 g chopped sample was placed in mortar, 20.0 g anhydrous sodium sulfate and 1.0 g quartz sand were also added, then completely powdered and blended. After homogenization, the sample was transferred into a glass chromatographic column (30.0 cm × 1.5 cm). Then the glass column was blocked with glass wool at its bottom and top. 30.0 ml of mixed solvent (*n*-hexane-acetone 4:1, v/v) was slowly passed through the glass column and the eluate was collected in 100 ml separating funnel. In order to remove the colored matter of vegetables, 10.0 ml sulfuric acid was added, and the sublayer of sulfuric acid was discharged after shaking for five minutes. Latter, 10.0 ml 2% sodium sulfate solution was added to remove water-soluble impurities. The extracts were cleaned up by 6.0 ml solid-phase extraction (SPE) column after the organic phase was concentrated to 2.0 ml in a rotary evaporator. The SPE column contained 1.0

g anhydrous sodium sulfate layer at the bottom, 1.0 silica gel layer in the middle and another 1.0 g anhydrous sodium sulfate layer at the top. The SPE column was cleaned with 8.0 ml petroleum ether at first, and then 2.0 ml extract was added. Subsequently, the eluted analytes were collected in a glass pear bottle after 10.0 ml mixed solvent (dichloromethane-petroleum ether 1:9, v/v) was passed through the SPE column. The eluate was concentrated to about 1.0 ml in a rotary evaporator, then transferred into 1.0 ml volumeter and blown to scale by gentle N_2 stream. After the above procedures, the sample was diverted to 1.0 ml injection vial for analysis by GC-ECD with auto-sampler. In addition, 2.0 g air-dried soil samples underwent ultrasonic extraction over 2.0 h with 30.0 ml of *n*-hexane and acetone (v/v, 4:1). The soil extract was cleaned up by the same procedure mentioned above. The BAF (bioaccumulation factor) calculated as the ratio of the content of OCPs in vegetables over that in soil.

The determination of chlorinated POPs was carried out by using Agilent 6890 gas chromatography equipped with a ^{63}Ni Electron Capture Detector (ECD) and Agilent 7683 auto-sampler. Chromatographic separation was achieved by using an HP-5 fused capillary column (30 m length, 0.32 mm internal diameter, 0.25 µm film thickness). The column temperature was programmed from 60°C (1 min) to 140 °C (5 min) at 20 °C min^{-1} and then to 280 °C (5 min) at 12 °C min^{-1}. The temperature of injector and detector were 225°C and 280 °C respectively. The highly purified N_2 as carrier gas, and the flow velocity was at 1.5 ml min^{-1}. 1µl was injected and operated in the splitless mode.

For recoveries, OCPs were spiked to carrot and cabbage vegetable samples at 50 and 200 ng g^{-1} respectively. In order to ensure acceptable simulation of the real situation, the spiked samples were allowed to stand for 24 hours at ambient temperature in a closed system to avoid contamination. Each sample was spiked in triplicate and then analyzed using the proposed method.

The mean content of OCPs in vegetables given in this paper is expressed as nanograms per gram on a fresh weight basis of the particular matrix.

RESULTS AND DISCUSSION

Carrot and cabbage were chosen as reference matrices for the recovery assay. Spiking levels and mean recoveries from fortified crop samples in triplicate experiments for each matrix are in Table 1. From the twelve pesticides tested, DDT and its metabolites DDE and DDD gave better recoveries in two vegetables than HCH and HCB. Most of the OCPs gave recoveries between 80% and 120%, their relative standard deviation ranged from 3% to 15%, which suggested that the extraction and the clean-up procedure could be considered reliable enough for multi-residue screening. But the recoveries of dieldrin and endrin were very low, only between 20% and 50%. This may be due to the fact that, during the procedure of sulphuration, part of dieldrin and endrin was lost in the presence of sulfuric acid. This also implies that, the method is unsuitable for analysis of these two organochlorine pesticides.

Table 1. Recovery (%) and relative standard deviation (RSD) (n=3) of twelve organochlorine pesticides spiked to carrot and cabbage in two different concentrations.

Pesticides	Carrot				Cabbage			
	50 ng g^{-1}		200ng g^{-1}		50ng g^{-1}		200ng g^{-1}	
	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
o,p'-DDT	116.1	5.6	107.6	7.5	116.8	10.6	106.8	3.7
o,p'-DDE	105.5	5.5	114.2	23.3	109.7	10.3	125.8	2.3
p,p'-DDT	112.8	9.3	112.7	7.1	113.8	7.6	110.4	6.7
p,p'-DDE	105.9	11.3	114.4	14.8	110.3	9.3	117.2	8.9
p,p'-DDD	105.1	10.2	112.9	10.2	115.4	12.9	123.9	12.6
α-HCH	92.1	6.6	90.2	13.2	105.7	9.1	96.3	11.1
β-HCH	81.6	3.1	76.2	7.8	91.0	8.6	79.1	8.3
δ-HCH	96.4	5.9	95.5	13.7	110.3	8.8	96.5	7.4
γ-HCH	73.4	4.0	74.3	9.2	80.8	5.8	81.2	13.7
HCB	83.1	3.5	86.8	11.6	105.7	7.5	82.8	11.5
Dieldrin	51.6	9.5	42.4	5.4	35.8	3.9	32.1	7.8
Endrin	25.4	9.6	24.2	4.9	36.7	4.4	24.5	5.7

Residual levels of twelve OCPs in ten vegetables are in Table 2 and Table 3. From these tables, it can be clearly seen that, twelve OCPs were all detectable in all vegetables. The total OCPs residual level in carrot was over 100 ng g^{-1}, which was the highest residual level among all vegetables investigated. The residual levels of OCPs in garlic leaf (96.2 ng g^{-1}) and spinach (95.3 ng g^{-1}) were slightly less than that in carrot. However, the residual levels in the rest of the vegetables were relatively low and had no significant difference. It ranged narrowly from 56.8 to 64.0 ng g^{-1}, accounting for 60% to 70% of the total OCPs residual level in carrot, garlic leaf and spinash. In each vegetable, there was the same residual order of OCPs: ΣDDTs $>\Sigma$HCHs$>$dieldrin, endrin and HCB. It can be known from Table 3 that, DDTs and HCHs are the main OCPs residues in vegetables, accounting for 50% to 75% and 14% to 28% of all OCPs residues detected respectively.

Table 3 also brings out the comparison of the residual levels of OCPs in vegetables with the maximum residue limits (MRL) of China (National standard GB/T 5009.19-1996). Although the residual levels are generally below the MRL of China, OCPs in all vegetable samples are 100% detectable. Meaning that, the environment where these vegetables are grown might be contaminated by OCPs. In OCPs contaminated soil, some food crops may accumulate OCPs via different pathways, namely (1) adsorption to the root surface, (2) root uptake and transport to the shoot, (3) absorption of volatile OCPs from soil and some other pollution sources, (4) foliage and fruits contamination by soil particle, and deposition of airborne OCPs (Trapp and Mcfarlane 1995). Through these ways, OCPs may be bioaccumulated in different food crops and then biomagnified through different food chains, which poses a menace to food security and human health.

Table 2. Organochlorine pesticide residues in selected vegetables (ng g^{-1} fresh wt).

pesticides	carrot	radish	lettuce	cabbage	celery cabbage	garlic leaf	leek	spinach	tomato	pumpkin
o,p'-DDT	16.2±1.1	9.1±1.1	5.6±1.5	8.9±1.3	9.4±2.8	7.4±1.3	6.3±0.3	11.9±1.2	6.6±1.2	7.9±0.1
o,p'-DDE	14.3±2.0	5.8±1.0	3.1±0.9	5.7±0.9	4.2±2.3	7.8±2.2	2.0±0.2	8.1±3.0	2.3±0.3	3.0±0.07
p,p'-DDT	14.8±6.0	7.2±2.4	9.9±1.5	7.2±2.9	9.3±3.0	12.2±1.3	10.2±0.2	16.4±1.8	10.8±1.4	12.3±0.1
p,p'-DDE	17.2±3.9	8.8±1.1	7.0±1.4	8.4±0.8	7.0±1.7	10.0±3.3	4.1±0.6	12.1±3.1	5.4±1.2	6.5±0.2
p,p'-DDD	18.6±3.8	8.6±1.7	6.1±1.6	8.3±2.0	10.3±3.3	10.8±3.0	5.9±0.2	12.1±1.7	8.5±0.7	7.3±0.3
α-HCH	3.9±2.1	2.8±0.2	1.2±0.5	2.6±0.4	3.4±1.3	4.9±1.7	2.3±0.09	2.7±0.8	2.2±0.6	2.4±0.04
β-HCH	1.9±0.3	1.5±0.1	0.5±0.1	1.6±0.1	0.6±0.1	7.7±2.0	3.5±0.08	2.2±0.03	2.3±0.07	1.9±0.02
δ-HCH	6.5±2.0	4.4±1.3	2.2±1.0	4.0±0.5	2.6±1.0	9.3±1.9	4.2±0.05	5.0±0.5	4.0±0.3	4.3±0.07
γ-HCH	6.1±1.2	5.0±0.1	5.0±1.6	5.4±0.9	4.4±1.2	4.9±5.8	6.0±0.01	6.0±0.2	5.8±0.3	6.7±0.7
HCB	4.1±1.1	1.3±0.4	0.6±0.2	1.9±0.8	1.3±0.2	2.1±0.6	0.2±0.1	1.5±0.9	0.3±0.03	0.6±0.03
dieldrin	2.2±0.8	1.3±0.1	1.4±0.3	1.4±0.5	1.8±0.6	5.5±1.1	5.2±0.06	8.6±2.1	4.5±1.3	4.4±0.1
endrin	2.4±0.4	2.4±0.1	2.1±0.6	1.6±0.4	2.4±0.7	13.9±5.0	7.1±0.07	9.0±1.3	6.0±1.1	6.0±0.1
ΣOCPs	108.1	57.2	64.0	57.1	57.7	96.2	56.8	95.3	58.8	63.2

Table 3. Total DDT and HCH residues in selected vegetables compared with maximum residue limits of China (ng g^{-1} fresh wt).

pesticides	carrot	radish	lettuce	cabbage	celery cabbage	garlic leaf	leek	spinach	tomato	pumpkin	Maximun residue limit[a]
ΣDDT	81.1	39.5	31.7	38.5	40.2	48.2	28.5	60.6	33.6	37.0	100
ΣDDT/ΣOCPs, %	75.0	69.1	49.5	67.4	69.7	50.1	50.2	63.6	57.1	58.5	
ΣHCH	18.4	13.7	8.9	13.6	11.0	26.8	16.0	15.9	14.3	15.3	200
ΣHCH/ΣOCPs, %	17.0	24.0	13.9	23.8	19.1	27.9	28.2	16.7	24.3	24.2	

a: national standard (GB/T5009.19-1996 China); ΣOCPs: all OCPs listed in table 2; ΣHCHs: all HCH isomers; ΣDDTs: all DDT isomers

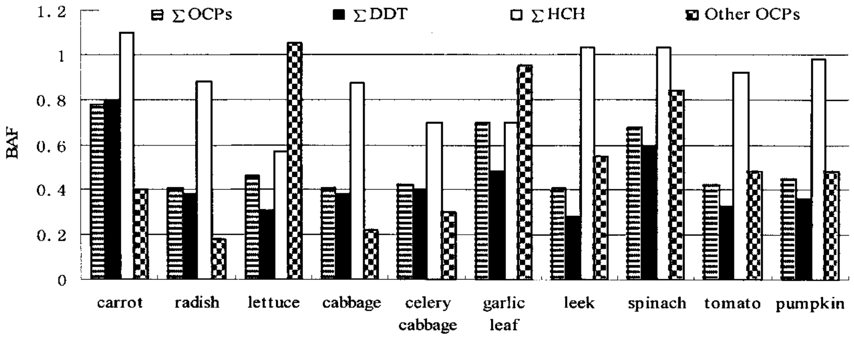

Figure 1. Bioaccumulation of chlorinated POPs in different vegetables from the suburb of Nanjing.

Bioaccumulation referred to some toxic elements and persistent organic chemicals, which can be absorbed and accumulated from the environment by biological organism. Bioaccumulation factor (BAF) can be used as a parameter to predict the bioaccumulative ability of different organisms. The patterns of chlorinated POPs bioaccumulated in 10 vegetables can be seen in Fig 1. The BAF of total OCPs in different vegetables is between 0.41 and 0.78; moreover, the BAF in carrot, garlic leaf and spinach is higher than 0.7, which illuminates their relatively high accumulative ability. Although the absolute residual levels of HCHs in all vegetables investigated are obviously lower than those of DDTs, BAF of HCHs (0.70 to 1.10) is significantly higher than DDTs (0.28 to 0.80). This indicates that, the selected vegetables generally prefers the uptake of HCHs to that of DDTs. BAF of HCHs and DDTs in carrot maximal value reached 1.10 and 0.80 respectively, which implies that carrot can accumulate more HCHs and DDTs than other vegetables. This result is consistent with the conclusion of Lichtenstein's (Lichtenstein 1959), whose experiment testified that carrot can absorb and accumulate more chlorinated POPs from soil than other plants, because of the relatively high adipose substance such as fat and Vitamin A in its body. Compared with other vegetables, lettuce, garlic leaf and spinach can bioaccumulate more hexachlorobenzene (HCB), dieldrin and endrin because their BAF reached 0.80.

Studying the isomeric ratios and parent substance/metabolite ratios enables past and present pollutant emission sources to be distinguished (Harner et al. 1999).

The ratio of DDT / DDE and γ-/α-HCH can be used as a rough estimation of the new input of DDT and Lindane (almost pure γ-HCH) into the environment or not. If the ratio of γ-/α-HCH and DDT / DDE is higher than one, new inputs must be present in the environment (Mariana et al. 2003). It can be seen from Fig. 2 that the ratios of γ-/α-HCH and DDT / DDE in all vegetables are between 1.0 to 2.5 and 1.0 to 4.2 respectively. The ratios of γ-/α-HCH and DDT / DDE are higher than one in all vegetables, which reveals that those vegetables may accumulate more DDT and Lindane from the environment, and the environment in which those vegetables are grown might be polluted by DDT and Lindane recently.

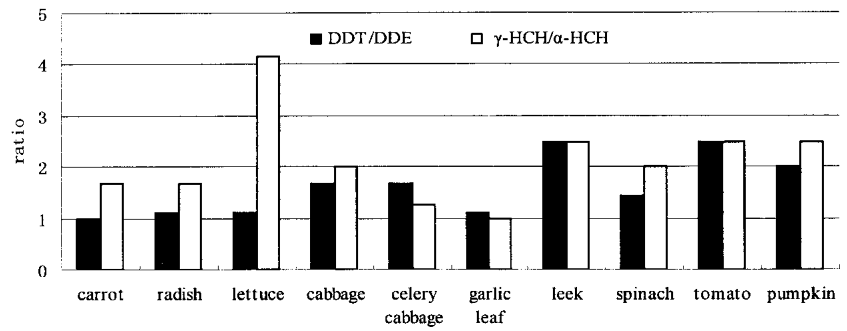

Figure 2. The ratio of DDT/DDE and γ-HCH/α-HCH in different vegetables

Although OCPs had been banned for agricultural use for two decades, Lidane and DDT are still used in China and other developing countries for non-agricultural purpose (Li et al. 1996). There is a possibility that, the HCHs and DDTs presented in the agricultural soil of this region came from atmospheric deposition or imported from OCPs emission areas through long distance transportation. On the other hand, DDT is also a main component of dicofol pesticides, which was widely used in China as a substitute of DDT a couple of years ago. This may be another way to increase DDT levels in the environment (Huang et al. 2000).

In order to reduce the threat of OCPs to food quality and human health, great measures should be taken to completely eradicate the production of OCPs and their use in agriculture. Furthermore, to decrease their accumulation in food chains, the scientific research on the degradation and immobilization of OCPs in soil should also be strengthened.

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