Accumulation levels and characteristics of some pesticides in human adipose tissue samples from Southeast China

Na Wang, Lili Shi, Deyang Kong, Daoji Cai, Yanzhong Cao, Yongming Liu, Guofang Pang, Rongbin Yu

1. Introduction

Pesticides have been used extensively to increase crop yield and produce high quality products for world-wide consumption (Widawsky et al., 1998). Stimulated by the success of organochlorine pesticides such as DDT and HCH, a steady stream of new insecticides, herbicides and fungicides have appeared on the market in China. Before banned in 1983, a great amount of DDT and HCH was used in the country. Afterwards, the total amount of other chemicals produced increased from 201 000 tons in 1985 to 13 84 600 tons in 2006 (Zhou and Jin, 2009) according to the China Statistic Yearbook (2007). As a consequence of the increasing use, pesticide residue-containing media may present commonly in our daily life.

A total of 58 pesticides including POPs pesticides, insecticide, acaricide, herbicide was studied in this research. These compounds were mostly produced and used extensively during last 50 years. POPs pesticides are a series of persistent, toxic and lipophilic compounds that are practically ubiquitous in the environment and may bio-accumulate and bio-magnify at all levels of the food chains, affecting top predators and humans (Juan et al., 2008). Because of the concerns, the agricultural use of DDT and HCH in China was banned since 1983. The other kinds of pesticides except chlor-dimeform and methamidophos were still in use for sustainable agriculture production. It was reported that Chinese farmers may apply more chemicals to their crops than their counterparts of almost any other country in the world (Huang et al., 2003), causing increasing concern about the status of the general public health.

One of the important routes for human exposure to pesticides is dietary intake of pesticides-containing products. Additional exposure can potentially occur from soil, drinking water, air, and through various sources in farmland and workplaces. Therefore, pesticide exposure through food consumption or physical contact during professional handling of pesticide may pose a potential risk to the general health of consumers in China.

Human samples such as breast milk, serum and adipose tissues have been used as biomarkers to assess the extent of previous exposure to the organochlorine pesticides (OCPs) in many recent reports (Erika et al., 2001; Chu et al., 2003; Jochen et al., 2008;
Pathak et al., 2008), due to their convenient and inexpensive availability. However, at present little data have been collected for the residue levels of pesticides other than (OCPs) in human bio-matrices, especially in human adipose tissue samples. These data would directly reflect the inner exposure levels and status of toxical substances, particularly substances with high lipophilicity.

The aim of this study was to determine concentrations of 58 pesticides in human adipose tissue samples from individuals of Southeast China and evaluate some rationale for their occurrence and potential health risks based on the results. Meanwhile, the potential influences of habitat environment, age, sex, and occupation on the tissue levels of the concerned pesticides were also investigated. This is the first comprehensive study of human adipose tissues in the southeast regions of China, consisting of as many as 633 subjects.

2. Experimental section

2.1. Sample collection and storage

Collection of human adipose tissue samples was conducted by the Ethics Committee of Nanjing Medical University in China between April 2008 and June 2009. A total of 633 human adipose tissue samples from patient abdominal operations were collected from the corresponding hospital of the three studied regions. All collected samples were placed into a glass vial in ice, labeled, and frozen to –70 °C until analysis. Donors of the adipose tissue samples had all lived in the area for at least 10 years at the time of sampling. Of the three studied regions, Nantong of Jiangsu Province is an agricultural region mainly producing cotton where the greatest amount of DDT was used 30 years ago. Hexian of Anhui Province is also an agricultural region representing a rural environment where kinds of pesticides were applied. The third sampling site of Dachang is in a chemical industrial area, representing an urban environment in Jiangsu Province. The map is given in Supplementary Information. The mean age of the studied subjects from Nantong was 46.3 years old, ranging from 17 to 91 years of age (with 15.1% of 15–25 years, 28.6% of 26–45 years and 56.3% of 46–91 years old). The mean age of the studied subjects from Hexian was 46.1 years old, ranging from 15 to 91 years (with 16.2% of 15–25 years, 33.8% of 26–45 years and 50.0% of 46–91 years old). The mean age of the studied subjects from Dachang was 36.5 years, ranging from 14 to 88 years (with 25.0% of 15–25 years, 25.8% of 26–45 years and 49.2% of 46–91 years old). After signing the informed consent, the subjects were interviewed in person by a trained researcher using a structured questionnaire for basic information the study protocol.

2.2. Sample preparation and purification

A total of 5 ± 0.01 g adipose tissue sample was extracted two times with 35 mL acetonitrile (plus 15 g anhydrous sodium sulfate). The concentrated supernatant, mixed with the internal standard (heptachlor-epoxide solution), was diluted to 10 mL with acetic ether-hexamethylene (1:1). After membrane filtration (0.45 μm), the sample solution was cleaned by gel-permeation chromatograph (GPC) to remove the lipid of the adipose sample. The eluate was concentrated to about 1 mL in 45 °C water bath with nitrogen before GC–MS/MS determination.

2.3. Instrumental analysis

A total of 58 pesticides were analyzed simultaneously by GC–MS/MS using an Agilent 7890 gas chromatograph coupled with Waters Quattro micro triple quadrupole MS/MS, operating in El mode. The final sample extract (1 μL) was injected in the splitless mode onto a DB-1701 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, USA) with helium as carrier gas at a constant flow rate of 1.2 mL min⁻¹. The injector temperature was 290 °C and the interface temperature was 250 °C. The oven temperature was held isothermally at 40 °C for 1 min, increased to 130 °C at 30 °C min⁻¹ then increased to 250 °C at 5 °C min⁻¹ with no hold time, and finally increased to 300 °C at 10 °C min⁻¹ for 5 min. Ionization energy was 70 eV. The mode of acquiring signal was Multiple Reaction Monitor (MRM), with which two parent-product ion transitions were monitored for quantification and qualification (See in Supplementary Information). The chromatogram of all the analytical pesticides was shown in Supplementary Information.

2.4. Quality control and assurance

The analytical method was subject to a validation process and showed no interference in the retention time (tR) region of the test substances. The levels of quantification (LOQ) of all pesticides in the lipid matrix were between 0.170 ng g⁻¹ and 204 ng g⁻¹ with recoveries at between 70.6% and 113%. The laboratory reagent, blank pig fat samples, and spiked pig fat samples, were treated and analyzed using the same method as the actual samples (1 reagent blank, 1 matrix blank, and 1 control sample for every 10 samples). The relative standard deviation (RSD) of all the 60 controls was between 8.93% and 12.6%, which showed that the sample processing was stable. The analytical work was conducted at the chemical laboratory at the Technological Center of Qin Huangdao Entry–Exit Inspection and Quarantine Bureau, an accredited testing laboratory in China.

3. Results and discussion

3.1. Pesticides levels in human adipose tissue

Table 1 shows the frequencies of detection, maximum and mean concentrations of detected pesticides in human adipose samples collected from three regions of Southeast China. It was found that POPs pesticides were frequently detected in samples, which included 2,4-DDD, 2,4-DDE, 2,4-DDT, 4,4-DDD, 4,4-DDE, 4,4-DDT, α-HCH, β-HCH, γ-HCH, δ-HCH, HCB and mirex, whereas the most detected species of other kinds of pesticides included dichloro (currently in use), methamidophos and chloridimeform (The last two have been banned).

The total DDT concentration ranged between 15.3 and 28 900 ng g⁻¹ fat with a mean value of 3710 ng g⁻¹ fat (n = 218) in Nantong, between <LOD – 17 400 ng g⁻¹ fat with a mean value of 2070 ng g⁻¹ fat (n = 198) in Hexian, and between 469 and 4850 ng g⁻¹ fat with a mean value of 969 ng g⁻¹ fat (n = 217) in Dachang. It was showed that the proportion of different DDT isomer existed in human adipose tissues was following the order of 4,4-DDE > 4,4-DDD > 2,4-DDD > 2,4-DDT > 2,4-DDT. The observed order was consistent with the bioaccumulation ability of DDT isomers, which is dependent on the human metabolism system. A main metabolite product of DDT was 4,4-DDE in the human adipose tissue due to its weak excretion ability among all the organochlorine pesticides. It was reported that the excreted daily dose of DDE was 1% of daily intake (Tu et al., 2001).

The total HCH concentration ranged between <LOD – 1950 ng g⁻¹ fat with a mean value of 191 ng g⁻¹ fat (n = 218) in Nantong, between <LOD – 3070 ng g⁻¹ fat with a mean value of 428 ng g⁻¹ fat (n = 198) in Hexian, and between <LOD – 3560 ng g⁻¹ fat with a mean value of 257 ng g⁻¹ fat (n = 217) in Dachang. It was showed that β-HCH was the main metabolite product of HCH isomers existed in the human
## Table 1

Results of 58 Pesticides in human adipose samples from three regions of Southeast China.

<table>
<thead>
<tr>
<th>Location</th>
<th>Frequency (%)</th>
<th>Max value (ng g⁻¹)</th>
<th>Mean value (ng g⁻¹)</th>
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<td>Nantong</td>
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<td>Dachang</td>
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<td>Total</td>
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*Note: LOD = Limit of Detection*
adipose tissue. Generally, the lipophilicity of HCH is less than other organochlorine pesticides, so the excreted efficiency of HCH is higher. However, β-HCH has intense resistance to the metabolic enzymes, resulting in the high bioaccumulation ability in organisms in the higher levels of biological food chain (Tu et al., 2001).

Table 2 shows the comparison of DDT and HCH levels between a previous result from Wuxi of Jiangsu Province, which is not far from Nantong in 1983 (Cai et al., 1983) and the present results. Wuxi was adjacent to Taihu River of China, and the pollution level of organochlorine pesticides in Wuxi was also high before 1983, which was similar to Nantong of our study (Cai et al., 1983). There is a significant decrease in DDT and HCH levels of the human adipose tissues in Southeast China over the past 30 years, due to the deregistration of these product uses. Meanwhile, the degraded rate of HCH was much quicker than that of DDT.

The concentration of HCB ranged between <LOD – 80.2 ng g⁻¹ fat with a mean value of 16.3 ng g⁻¹ fat (n = 218) in Nantong, between 6.65 and 336 ng g⁻¹ fat with a mean value of 39.4 ng g⁻¹ fat (n = 198) in Hexian, and between <LOD – 164 ng g⁻¹ fat with a mean value of 23.9 ng g⁻¹ fat (n = 217) in Dachang. The concentration of mirex ranged between <LOD – 16.3 ng g⁻¹ fat with a mean value of 1.49 ng g⁻¹ fat (n = 218) in Nantong, between <LOD – 15.3 ng g⁻¹ fat with a mean value of 1.63 ng g⁻¹ fat (n = 198) in Hexian, and between <LOD – 34.4 ng g⁻¹ fat with a mean value of 2.55 ng g⁻¹ fat (n = 217) in Dachang. Mirex is extensively used to prevent termite in Building area, so the detection frequency was high due to its properties as POPs. However, the limitation of exposure route to mirex leads to the low detected concentration in human adipose tissue.

The results are consistent with the chemical–physical properties of organochlorine pesticides. Their stability is responsible for their long persistence in humans and their high lipophilicity determines their accumulation and storage in the lipid fraction of tissues.

The concentrations of other pesticides were generally low comparing with that of POPs. It should be noted that the detection frequency of methamidophos was 22.0% and the max value of concentration was as high as 1030 ng g⁻¹ in Nantong. This maximum detection level has never been reported previously. Methamidophos was banned in China in 2008 due to the high oral toxicity of methamidophos (LD₅₀ 20–29.9 lgg⁻¹, rat oral) and neurotoxicity. This result was somewhat unexpected given the high water solubility of methamidophos, high potency to biodegradation, and low bioconcentration factor (Hu, 1997). However, the detection of methamidophos was confirmed in the laboratory through the analysis of the ratio of two parent-product ion transitions for quantification and qualification of the GC–MS/MS method.

The detection frequency of dicofol was relatively high in all three regions but the mean value of concentration was low. Dicofol is an organochlorine pesticide which is not banned in China. Chlordimeform is an organic nitrogen insecticide banned in 1993 due to its carcinogenicity. Although its detection frequency was relatively high in all samples in the three regions, their mean values of concentration were low. The frequencies of the other detected pesticides, which are still used presently, were all very low, indicating that their ability of accumulation in adipose tissues was weaker than OCPs. However, the detection also reflected the use history and the amount of pesticides in the local region. For example, butachlor, chlorpyrifos, cypermethrin, metolachlor, pyridaben and trifluralin were the main pesticides used in Nantong of Jiangsu Province, and their use amounts were all large.

### 3.2. Region-dependent accumulation of pesticides

Concentration of POPs pesticides in human adipose tissues from different countries (Kashimoto et al., 1989; Greve and Zoonen,
1990; Tanabe et al., 1993; Nakamura et al., 1994; Gomez et al.,
1995; Waliszewski et al., 1996; Kang et al., 1997; ... South Vietnam, 1994; Korea, 1997; Japan,
1989; China: This study).

Table 2
Concentration of DDT and HCH in human adipose tissues between in 1983 and now.

<table>
<thead>
<tr>
<th></th>
<th>Number of sample</th>
<th>HCH (ng g⁻¹)</th>
<th>DDT (ng g⁻¹)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>α-HCH</td>
<td>β-HCH</td>
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<tr>
<td>Human adipose tissue (1983)</td>
<td>25</td>
<td>539</td>
<td>30 249</td>
</tr>
<tr>
<td>Human adipose tissue of Nantong (present)</td>
<td>218</td>
<td>18 81</td>
<td>189</td>
</tr>
<tr>
<td>Human adipose tissue of Hexian (present)</td>
<td>198</td>
<td>3 19</td>
<td>424</td>
</tr>
<tr>
<td>Human adipose tissue of Dachang (present)</td>
<td>217</td>
<td>1 79</td>
<td>253</td>
</tr>
</tbody>
</table>

3.3. Age- and gender-dependent accumulation of pesticides

In order to study the relation of the pesticide body burden with age, mean values in the different age categories were calculated. Three age groups have been chosen for the study, those less than 25 years old, the age group of 25–45 years old, and those older than 45 years old. Since POPs pesticides, such as DDT and HCH, were banned in China from 1983, the age group of less than 25 years old represents the population who should have less exposure to POPs pesticides, the age group of 25–45 years old represents the population who may have medium exposure to POPs pesticides, and the age group of more than 45 years old would represent the highest exposure population. Fig. 4 illustrates the mean total POPs pesticides concentrations in 3 age groups of the three studied regions. It is obvious that higher POPs pesticide levels were found in the older generation, indicating the increasing trend of POPs accumulation in human bodies as exposure time increases. This result is in agreement with other reports from other countries (Annika and Pekka, 2001; Monica et al., 2006). The age-dependent...
adipose samples and occupation of the donors. An independent targets to study the correlation between Pesticides levels in the farmers, the subjects in Hexian and Dachang were selected as the

3.4. Occupation-dependent accumulation of pesticides

In order to ensure the balance of numbers of farmers and non-farmers, the subjects in Hexian and Dachang were selected as the targets to study the correlation between Pesticides levels in the adipose samples and occupation of the donors. An independent sample t-test (confidence interval = 95%) was conducted using SPSS, and the result was listed in Table 3. The concentrations of DDT, HCH, HCB, Chlorodimeform in human adipose tissue of farmers are significantly higher than those of non-farmers (2-tailed significance at P < 0.05). It can be concluded that the potential exposure risks to pesticide applicators or farm workers are greater than to the general population exposed only to the trace levels of pesticides in food and/or water.

3.5. Potential health risk

The potential health hazards of pesticide use have drawn increased attention globally (Blessing and Scott, 2003). Acute pesticide poisoning constitutes a serious problem in many countries. However, in recent years, public concern over the potential adverse health effects of pesticides has focused increasingly on a series of chronic end-points including cancer, developmental, reproductive, immunological, and a variety of neurobehavioral effects (Amr, 1999). These chronic health impacts may take a long time to appear and are difficult to trace back to specific reasons or polluting source (Blessing and Scott, 2003). Many animal studies have reported that some pesticides were potentially carcinogens or promoters of certain chronic disease, including DDT, the metabolites of DDT, HCH, HCB, heptachlor, and dichlorvos etc. (http://www.epa.gov/iris/). However, there have been no coherent human epidemiological studies to consistently show the definitive health impact of some pesticides. Much work has been started on pesticide residues, pollution and cumulative effects of pesticides in the developed countries. Kocan et al. (1994) have shown that the residues of HCH, DDE and DDT produced hazardous effects in human. Many researchers also tried to correlate various enzymes with the effects of pesticides, especially in the case of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) (Mistra et al., 1985; Kamal et al., 1990; Carvalho, 1991; Goel et al., 2000; Mani et al., 2001; Altuntas et al., 2002). There are increasing concerns that exposure of multiple pesticides at an unacceptable level and for a prolonged period may potentially affect the normal functioning of different organ systems and possibly produce characteristics of clinical effects such as hepatitis, dyspnea and burning sensation in urine (Azmi et al., 2006).

The number of volunteers in our study was too few to obtain a reasonable epidemiological conclusion to correlate the pesticides levels in human adipose tissues to certain health impact. However, through retrospective study, we investigated the death cases among residents in the three regions of Southeast China and analyzed the characteristics and causes of deaths. It is found that the mortality of malignant tumors during 2003–2005 in Nantong of Jiangsu Province was 230.77/100 000 and standardized mortality was 109.43/100 000, which was higher than the result of the Chinese third national retrospective investigation of causes of death (mortality of malignant tumors: 135.88/100 000 and standardized mortality: 91.24/100 000) (Huang et al., 2010). Nantong of Jiangsu province was a typical high cancer incidence area in China (Huang et al., 2010). Meanwhile, a study on the epidemic characteristics and trend for malignant tumor in Jiangsu province by Xue and Zhang (2006) proposed that the mortality of malignant tumors was higher in rural than urban area.

It is interesting to find that mortality of malignant tumors tends to associate with the pesticides levels in human adipose tissue. The sum of concentration values of all the detected pesticides in human
adipose tissue was 4.00 mg kg\(^{-1}\) in Nantong, 2.57 mg kg\(^{-1}\) in Hexian and 1.31 mg kg\(^{-1}\) in Dachang, respectively. Corresponding to the pesticides levels, the incidents of malignant tumors of the three regions also followed the order: Nantong > Hexian > Dachang. Moreover, the human epidemiological study was the future studying task.

4. Conclusion

Through a comprehensive study of pesticide levels and bioaccumulation characteristic in human adipose tissues among residents of Southeast China, we found that the levels of DDT and HCH were relatively high in the human adipose tissue, and a significant correlation was observed between age and POPs pesticide levels. Meanwhile, some pesticide residue levels were also found significantly correlated to occupation. It is interesting to find that mortality of malignant tumors tends to associate with the pesticides levels in human adipose tissue. The study has shown that many other pesticides were accumulated in human adipose tissues at the relative low concentration. As some pesticides are still used in some regions of China, these products may pose increasing concerns to the potential health hazards to human. The finding that methamidophos and chlordimeform can accumulate in human tissues was firstly reported in this study and deserves more investigation.

Acknowledgment

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2011.05.062.
**Reference**


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