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# Current pesticide profiles in blood serum of adults in Jiangsu Province of China and a comparison with other countries

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ABSTRACT

Although various pesticides were used globally, the pesticides profiles in human blood serum remain largely unknown. We determined pesticide exposure profiles using solid-phase extraction and gas chromatography tandem with triple quadrupole mass spectrometry in 200 human blood serum samples from the adult population in Jiangsu Province, China. A systematic and comprehensive literature review was carried out to identify the articles investigating pesticide exposure and compare exposure data. Of the 88 pesticides, 76 were found in the blood serum of the population in Jiangsu Province. To the best of our knowledge, 58 pesticides were reported in human blood serum for the first time, and among these pesticides, parathion-methyl, pyrimethanil, fluacrypyrim, simazine, cloquintocet-mexyl and barban were debatable in more than half of the samples. By statistical comparison of the blood serum levels of pesticides between this study and other countries, we found the levels of several organochlorine pesticides were significantly higher in the female population of Jiangsu Province. Health risks related to the pesticide profiling were then revealed, which identified higher carcinogenic toxicity and teratogenic toxicity risk in the female adults of Jiangsu Province caused by organochlorine pesticide exposure. This study not only provides a high-throughput pesticide screening method for future studies of the exposome, but also presents the first human data on exposure to a number of pesticides. It may provide a knowledge database for the risk assessment and management of the pesticides.



## 1. Introduction

Because of the widespread use of pesticides, the global consumption of pesticides has reached 3 billion kg in recent years (Pimentel, 2009). Considering the resistance of pests, changes in the pesticides market, and some other reasons, the application of mixed pesticides may become more common than the use of single pesticides in practice (Saha et al., 2013). Consequently, contamination by multiple pesticides gains more and more global attention, especially in China, where a large volume of pesticides is used (Chowdhury et al., 2013; Knežević and Serdar, 2009; Li et al., 2014b). Jiangsu is one of the most densely populated and economically developed regions in China. The number of adults in Jiangsu accounted for the largest proportion. Therefore, studying the exposure to pesticides in adults in Jiangsu has important research and application significances for environmental health in China.

Pesticide exposure can increase the risk of a variety of diseases such as cancer (Koutros et al., 2015), nervous system diseases (Imanishi et al., 2013), reproductive disorders and endocrine abnormalities (Chevrier et al., 2011). Therefore, it is necessary to comprehensively evaluate the profiles of pesticide exposure for risk assessment based on pesticide exposure distribution characteristics, which can also provide a basis for risk management by controlling pesticide use in different regions around the world and among susceptible populations. However, methods for high-throughput determination of human internal pesticide exposure are still in their infancy. In the U.S., the Centers for Disease Control and Prevention (CDC) have started to monitor exposure to a variety of pesticides in the U.S. population (Centers for Disease Control and Prevention, 2009). However, the data are still lacking in China, and the

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potential contamination levels of a large number of pesticides in humans are still unknown.

In this study, by applying a new method to detect 88 major pesticides simultaneously using gas chromatography-triple quadrupole tandem mass spectrometry (GC–MS/MS), we obtained human internal pesticide exposure levels with 200 human blood serum samples from the population of Jiangsu Province of China. The 88 pesticides including organophosphorus, organochlorine, organic nitrogen, carbamates, pyrethroid and amide pesticides were selected based on the usage and exposure levels in the environment and human samples as well as potential health risk (Na et al., 2006; Centers for Disease Control and Prevention, 2009; Li et al., 2014a, 2014b; Liu et al., 2015) (Supplemental Table S1). Among the 88 pesticides, many of them have been used >0.1 tons in China in 2015 (Shu et al., 2016). The 88 pesticides also include the new pesticides recommended by the Ministry of Agriculture of China. A systematic and comprehensive literature review was then carried out to identify previous studies investigating pesticide exposure. By statistical comparison of the blood serum levels of pesticides between this study and other countries, we comprehensively described the distribution characteristics and health risks related to the pesticide exposure profiles in the Northern Hemisphere for the first time.

## 2. Materials and methods

### 2.1. Chemicals and reagents

All the standards and three kinds of isotope-labeled internal standards (Dimethoate D6, Alachlor D13, Chlorpyrifos D10) (Purity > 95%) were purchased from Dr. Ehrenstorfer Reference Materials (Augsburg, Germany). The chemical list is shown in Table S1, Figs. S1, S2 and S3. Dichloromethane, n-hexane, and toluene were purchased from Merck (Darmstadt, Germany). Deionized water was purified by an ELGA Purelab Ultra system (Vivendi Water Systems, Buckinghamshire, UK). Ammonium sulfate was purchased from Sigma-Aldrich (St. Louis, USA). According to previous studies (Yamamuro et al., 2014), the human blank serum was purchased from Sigma–Aldrich (St. Louis, USA).

### 2.2. Preparation of standards and QC samples

The stock solutions for each of the 88 pesticides were prepared by dissolving measured amounts of the compounds. Working standard solutions of all 88 pesticides ranging from 1.08 to 54,000.00 ng/mL were generated by diluting the stock solutions in dichloromethane. The working calibration standard samples and quality control (QC) samples were generated by 3990 μL of human blank serum with the enrichment of 10 μL of corresponding working standard solutions. The isotope labeled internal standard solution was also prepared in dichloromethane. These solutions were stored at −80 °C. The concentrations of pesticides in working calibration ranged from 0.0027 to 135.00 ng/mL for all the 88 pesticides. The concentrations of 88 pesticides in QC samples were 0.027, 0.27, 2.70 and 27.00 ng/mL. The working calibration standard samples and QC samples were next prepared following the sample preparation procedure. The QC samples were analyzed in parallel with unknown blood serum samples.

### 2.3. Sample preparation

Unknown blood serum samples (200 μL) were gently mixed with the isotope labeled internal standard solution containing Dimethoate D6, Alachlor D13 and Chlorpyrifos D10 to obtain a final concentration of 10 ng/mL for each internal standard, and then mixed with 200 μL saturated ammonium sulfate and vortexed for 30 s. After centrifuged (36,670 *g*) for 20 min, the samples were subjected to solid-phase extraction (SPE) using the ProElut C18 (200 mg/3 mL; 50/pk, Dikma, China). The SPE eluate was dried and reconstituted in 10 μL toluene. The reconstitution in the vial insert was then injected into GC–MS/MS for analysis. Additional information on Sample preparation can be found in Supplemental methods and Fig. S4.

### 2.4. GC–MS/MS analysis

Thermo Trace GC Ultra (Thermo Fisher Scientific, Inc., USA) was applied for the chromatographic analyses. A 45-min oven temperature program was used. After separation by GC, 88 pesticides were detected with a triple quadrupole TSQ 8000 mass spectrometer (Thermo Fisher Scientific Inc. USA). Additional information on the GC–MS/MS analysis method can be found in Supplemental methods and Table S1.

### 2.5. Participants and blood serum collection

Blood serum was collected from volunteers in affiliated hospitals of Nanjing Medical University. They were participants of NMU birth cohort and NMU infertility study covering Nanjing, Suzhou, Changzhou, Wuxi in South of Jiangsu and Huaian region in Northern Jiangsu, and were all healthy controls, covering age from 18.8 to 52.0 years, body mass index from 16.5 to 34.6. The detail and characteristics of the population have been described elsewhere (Tang et al., 2015; Xia et al., 2013). The 200 blood serum samples were randomly selected from the population for pesticide analysis (100 male and 100 female). The sample size was selected based on statistical consideration (Margarete, 1995; Yungtai et al., 2001), and was sufficient and representative based on the previous studies on the exposure level of pesticide in certain area (Whyatt et al., 2003; Sharma et al., 2015). The study protocols and informed consent were approved by the Institutional Review Board (IRB) of Nanjing Medical University prior to the study. All studies involving human subjects were conducted under full compliance with government policies and the Helsinki Declaration. All blood serum samples were stored at −80 °C.

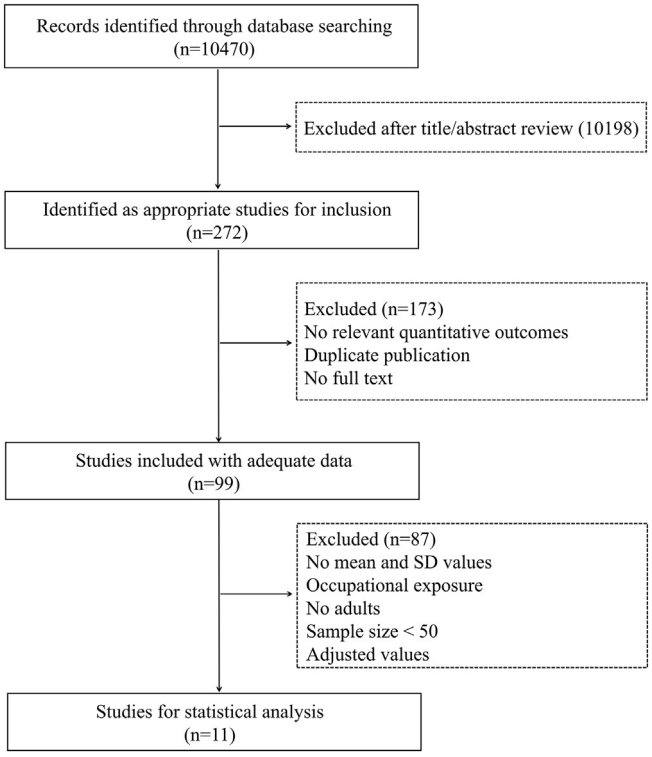


**Fig. 1.** Flow chart summarizing the inclusion of the studies. Because of a large amount of relevant literature on the p'p-DDD, o'p-DDT, p'p-DDT, HCH-α, HCH-β, and HCH-γ, we only selected the articles regarding these pesticides after 2010 for homogeneity.