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# Urinary pyrethroid metabolites among pregnant women in an agricultural area of the Province of Jiangsu, China

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ABSTRACT

Pyrethroid pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases. Of particular concern is exposure of pregnant women and their fetuses because little is known about the potential developmental hazards of such exposure. Several studies have detected internal pyrethroid exposure in urine both in adults and children, but few published data are available on metabolites in pregnant women. The present paper provides data on pyrethroid pesticides exposure based on questionnaire items and measurement of maternal urinary metabolite levels among 1149 pregnant women living in agricultural area of Jiangsu Province, China in 2009–2010, none of which reported occupational exposure to pyrethroid insecticides during pregnancy. To assess exposure to pyrethroid pesticides, urine specimens were analyzed for three main metabolites of 3-phenoxybenzoic acid (3-PBA), *cis*-and-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (*cis*-DCCA and *trans*-DCCA) using a gas chromatography–mass spectrometry method. The limits of detection for three pyrethroid metabolites were 0.1 μg/L. All pyrethroid metabolites were found in more than 94.0% of the urine samples. Median unadjusted and creatinine-adjusted values for urinary pyrethroid metabolites in these females were 1.01 μg/L and 1.55 μg/g Cre for 3-PBA, 0.44 μg/L and 0.69 μg/g Cre for *cis*-DCCA, 1.17 μg/L and 1.86 μg/g Cre for *trans*-DCCA, respectively. About half (45.5%) of women reported that they or family members had applied commercially available indoor insecticides during pregnancy. Both the questionnaire and laboratory data revealed that exposure to pyrethroid pesticides was considerably widespread in our subjects. The median values of urinary metabolites in the present study were about 4–10 times higher as those of general population from the developed countries. Interestingly, we found there was a temporal season variation tendency in different months. Especially, the levels of urinary metabolites in summer were significant higher than those in winter. These data indicated the need to assess the potential adverse effects of pyrethroid pesticides exposure on fetuses and infants in order to take adequate measures to protect pregnant women from pesticide exposures during pregnancy.


## Introduction

Pyrethroids are a group of neurotoxic insecticides extensively used to control agricultural and domestic insect pests. They are ubiquitous components in the environment (Alavanja et al., 2004), and leading to increased concern about exposure of the general population and possible chronic effects on human health. In China, almost 3000 tonnes applications of pyrethroids are made annually according to the most recent statistic data from the State Statistics Bureau. Generally, pyrethroid pesticides are rapidly metabolized by esterases and renally eliminated with the excretion half time ($t_{1/2}$) for the metabolites being about 6 h (Leng et al., 1997) in human body. Therefore, biological monitoring of low-level pyrethroid metabolites in urine is an effective way to identify the pyrethroid exposure level in individuals. In recent years, owing to the improved analytical methodology for the pyrethroid metabolites, they can be used as the sensitive biomarkers to assess the level of occupational as well as environmental pyrethroid exposure (Leng and Gries, 2006; Perez et al., 2010; Ueyama et al., 2009; Williams et al., 2008). There are also some reports in which exposure to pyrethroid has been linked to health effects in epidemiological studies, such biological effects as immunotoxicological properties (Diel et al., 2003), sperm DNA damage (Meeker et al., 2008), or influence on male reproductive hormone levels (Han et al., 2008; Meeker et al., 2009).

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Biological monitoring studies indicate that pesticide exposures are widespread in the general population (Berman et al., 2011; Ye et al., 2008). Pyrethroid metabolites have been detected in urine to assess the internal exposure in both adults and children in the developed countries in numerous studies (Barr et al., 2010; Fortin et al., 2008; Heudorf and Angerer, 2001; Lu et al., 2006). Prenatal exposure may be a particular concern of pregnant women because experimental animal data and epidemiological studies have suggested that exposure to pesticides during pregnancy and early life may impair neurodevelopment in the offspring (Chanda and Pope, 1996; Julvez and Grandjean, 2009; Jurewicz and Hanke, 2008; Young et al., 2005). However, limited information is available for investigating levels of pyrethroid pesticides in the pregnant women. To our knowledge, there is no data available on the extent of pesticide exposure among agricultural region women during pregnancy in China. The aim of this study was to assess the exposure level of pyrethroid in pregnant women by collecting questionnaire data and urine samples from 1149 pregnant women between June 2009 and January 2010.

As a part of a research project, the present study was conducted to investigate the effects of exposure to environmental pollutants on fetal growth and neurodevelopment in a cohort of children delivered at Sheyang County Maternity Hospital in the Province of Jiangsu, China. In this paper we only present exposure data on pyrethroid pesticides as measured by questionnaire and maternal urinary metabolites among 1149 pregnant women.

## Materials and methods

### *Subject recruitment*

A total of 1149 healthy pregnant women who gave birth at the local maternity hospital took part in this investigation during June 2009 to January 2010. The average age and gestational week (mean ± SD) of participants were 26.00 ± 5.36 years old (range 17–45 years) and 39.98 ± 1.04 weeks, respectively. The studied participants reside at Sheyang County in Jiangsu Province (Southeast China), which is constituted of agricultural area. The objectives of the study were briefly explained to participants and a detailed information sheet about the project was distributed. Women who volunteered to participate in the study signed an informed consent form and agreed to donate venous blood and urine before delivery, and cord blood samples. They also agreed to answer a socio-economic questionnaire by interviewer and allowed access to their post delivery records. This study was carried out with the permission of the local authority and the Ethics Committees of Fudan University.

Exclusion criteria were women who had serious chronic diseases such as diabetes, hypertension, or thyroid disease or who developed a serious pregnancy complication that could affect fetal growth and development. Individuals with a liver or kidney disease were excluded from the study. Women were enrolled into the study once the maternal urine samples and questionnaires had been completed.

### *Questionnaire data*

Questionnaire was administered to the mothers by trained interviewers after they delivered within a week. It included information on maternal health, maternal education, household income, occupational history, maternal smoking, alcohol consumption, vitamin intake during pregnancy, and history of residential pesticide use. Information on pesticide use included whether or not any pest control measures were used by woman herself or by other household members during pregnancy and what types of measures were used. We have gathered validated questionnaires data from 1284 delivering women with a fairly high completion rate at 98.5%. The individuals who unfinished or without provided questionnaire were excluded from the study.

### *Urine sample collection*

All participants were asked to provide urine collections prior to delivery. Individuals without adequate urine sample were excluded from the study. Finally, we gathered 1193 spot urine specimens from the subjects. Urines were collected in the high-density polypropylene centrifuge tubes (Corning Incorporated, USA). All samples were immediately stored at −20 °C and shipped in a frozen state to the laboratory and kept frozen at −80 °C until analysis. The urine samples collected were measured for pyrethroid metabolites and creatinine.

### *Urine sample analysis*

The urine samples collected were measured for pyrethroid metabolites and creatinine. Urinary pyrethroid metabolites were measured using a sensitive and selective capillary gas chromatography–mass spectrometric detection (GC–MS) based on the slightly modified method of Kühn et al. (1996). Briefly, 5 mL of urine was transferred into a 10 mL screw-top glass test tube and 2-phenoxybenzoic acid (2-PBA) served as an internal standard was added. Subsequently, acidification with 1 mL of concentrated hydrochloric acid at 90 °C for 1 h and then cooling, the urine sample was followed by extraction with 4 mLof *n*-hexane. After vigorous mechanical shaking for 2 min, the test tube was centrifuged for 1 min at 1200 × *g*. The hexane layer was separated and transferred into a clean screw-top glass test tube, and the following extraction was repeated once. The combined organic layers were concentrated to almost dryness at 37 °C under a gentle stream of nitrogen. The residue was dealt with 3 mL of freshly prepared 10% (v/v) $H_2SO_4$ in methanol and heated at 75 °C for 1 h to covert the phenoxybenzoic acid and cyclopropane carboxylic acids to their methylated esters. The methylated derivatives were re-extracted with 3 mL of *n*-hexane that contained 3 mL of saturated NaCl solution, were adequately shaken and then centrifuged. The resulting extract was transferred and repeatedly evaporated at room temperature to almost dryness with a gentle nitrogen stream. The derivatives were dissolved in 100 μL of toluene for injection into the GC–MS.

A GC–MS system (QP2010 Shmadzu, Japan) equipped with an auto-sampler and coupled to a mass selective detector (MSD) was performed for metabolites analysis. The main operating conditions were as follows. A DB-5MS column (30 m × 0.25 mm i.d., 0.25 μm film thickness) was used for separated. The carrier gas flow was adjusted to 1.5 ml/min of helium. The oven temperature program was ramped from 90 °C (6 min hold time) to 200 °C at 10 °C/min (2 min hold time) and finally ramped at 20 °C/min to 250 °C with a hold time of 5 min. The injector and transfer line were kept at 250 °C and 280 °C, respectively. The volume of extract injected in splitless mode was 1.0 μL. The MS was operated in selected ion monitoring (SIM) mode, using electron ionization (70 eV) at 200 °C ionization source temperature. The limits of detection (LODs) for all targeted metabolites were defined as a signal-to-noise ratio of three, LODs for three pyrethroid metabolites were 0.1 μg/L. The intra-assay precision was determined six times with three quality control standards (2.0, 5.0 and 20.0 μg/L) as mean recoveries and relative standard deviations, and inter-assay precision was also tested with same quality control standards on five different days. The mean recoveries for three metabolites ranged from 96.2% to 105.8% at three quality control levels, and the mean intra- and inter-assay relative standard deviation for urinary metabolites under study were between 6.2% and 3.7%. For quality control, fortified manual