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Short communication

# Environmental pyrethroid and organophosphorus insecticide exposures and sperm concentration☆

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**Abstract**

*Background:* There is growing concern that poisoning and other adverse health effects are increasing because organophosphorous (OP) insecticides are now being used in combination with pyrethroid (PYR) insecticides to enhance the toxic effects of PYR insecticides on target insects, especially those that have developed PYR resistance.
*Objectives:* We conducted a pilot biomonitoring study to determine whether men in our reproductive cohort study were being exposed to pesticides environmentally by virtue of frequenting an agricultural setting.
*Methods:* We screened 18 randomly selected urine samples collected from male participants of reproductive age for 24 parent compounds and metabolites of pesticides and examined the results in relation to sperm concentration.
*Results:* Results showed high prevalence of exposure to OP and PYR pesticides and our preliminary analyses provided some suggestion that the higher exposure group had lower sperm concentration.
*Conclusions:* The potential of OP/PYR mixtures to have enhanced human toxicity needs more research attention.
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*Keywords:* Organophosphorous; Pyrethroid; Insecticides; Pesticides; Reproduction; Male; Semen quality; Hormones; Chinese; Pesticide mixtures

## 1. Background

### 1.1. Scope and context of pyrethroid use

Pyrethroids (PYRs), a family of synthetic insecticides, are the most frequently used home and garden insecticides in the US, where the most recent data from the EPA estimate that 240 million applications of PYRs are made annually [1]. In addition to domestic use in pet shampoos, lice treatments, household insecticide sprays, and aerosol bombs, the consumption of food containing PYR residues, particularly vegetables and fruits, remains the primary route of exposure in the US population [2]. PYRs are an important class of pesticides because they rapidly paralyze insects, have low mammalian toxicity relative to other classes of pesticides, and are less persistent in the environment [2]. In countries where malaria is endemic, the use of PYR-treated bed nets and the burning of mosquito coils are important methods of vector control and expose large numbers of people to PYRs in their own homes. Despite widescale use, relatively little is known about the human health effects of environmental exposure to PYRs [2].

PYRs exert their effects on insects by prolonging the open phase of sodium channels when a nerve cell is excited. Low-level chronic exposure to PYRs usually does not cause neurological symptoms in mammals, largely due to rapid metabolism and excretion. Although not well studied, elimination appears to follow first-order kinetics, with elimination half-times in humans ranging from 6.4 to 16.5 h, depending upon the specific PYR and the specific exposure route. For most PYRs, elimination is nearly complete within 5 days of exposure, although certain isomers

*Abbreviations:* PYR, pyrethroid insecticides; OP, organophosphorous insecticides

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can persist in the body for longer periods [2]. Information on the specific enzymes involved in the metabolism of PYR compounds is limited. Metabolism appears to involve non-specific microsomal carboxylesterases and microsomal mixed function oxidases, which are located in nearly all tissue types [3–5]. Other important enzymes include alcohol and aldehyde dehydrogenases, which Choi et al. [6] demonstrated catalyzed the metabolism of intermediate breakdown products of permethrin. These breakdown products have been shown to be more potent endocrine disruptors than their parent compound in recombinant yeast with human estrogen and androgen receptors [7]. Since microsomal enzymes play an important role in the metabolism of PYRs, it is expected that many tissue types are potentially capable of rapidly metabolizing these compounds, with a particularly important role for the liver [2].

Organophosphorous pesticides (OPs) are being increasingly used in combination with PYRs because they can synergistically increase the effects of PYRs. This is particularly true in pest populations that, through natural selection for efficient PYR metabolism, have developed PYR-resistance. A number of studies have recommended that bed nets be treated with PYR/OP combinations to heighten their effectiveness in repelling mosquitoes in regions with PYR-resistant mosquitoes [8–10]. Similarly, as strains of PYR-resistant insects have increased in recent years in China, there has been increasing application of combined insecticides [11]. At present, more than 916 kinds of pesticide mixture preparations have been marketed in China, and 94.3% are produced domestically (Niu and He, 2001, cited in [11]). Nearly all the major kinds of insecticide mixtures contain OP insecticides and there are at least 197 OP/PYR mixtures on the market in China [11].

### 1.2. Effects of pyrethroids on male reproduction

A number of studies in both animals and humans have shown that exposure to PYRs affects semen parameters including sperm concentration (or count), motility and morphology. Kumar et al. [12] dosed adult male Swiss albino mice with the PYR cypermethrin, and found a significant elevation in the number of abnormally shaped sperm heads in higher dose groups as compared to controls. Mani et al. [13] exposed male rats to fenvalerate, a PYR, by inhalation for 4 h/day, 5 days/week for 3 months. They reported that exposure was significantly associated with a reduction in the weight of testes, epididymal sperm counts, and sperm motility. Elbetieha et al. [14] exposed adult male Sprague–Dawley rats to tap water containing graduated doses of cypermethrin for 12 weeks. Compared to rats that consumed lower doses, those with higher doses had significantly reduced fertility, epididymal and testicular sperm counts, as well as daily sperm production. The testes of treated animals were infiltrated with congested blood vessels with marked hemorrhage and a significant accumulation of connective tissue surrounding the seminiferous tubules, which contained a large number of immature spermatids.

Ratnasooriya et al. [15] studied the effects of one PYR, lambda-cyhalothrin (trade name ICON), on male rat reproductive behavior. Male rats were gavaged daily for seven consecutive days with different doses of ICON (63 and 100 mg/kg) or vehicle (distilled water). Their sexual behavior and fertility were evaluated at different time points during treatment and post-treatment using receptive females. Treatment had no effect on fertility, but sexual competence was seriously impaired: libido (assessed in terms of pre-coital sexual behavior, and numbers of mounting, intromission and ejaculation), sexual arousability/motivation (in terms of latencies for mounting, intromission and ejaculation), sexual vigor (judged by frequencies of mounting and intromission or copulatory efficiency). In addition, ICON suppressed intromission ratio, indicating erectile dysfunction. These effects on sexual function had a rapid onset and were reversible. ICON-induced sexual dysfunction was mediated by multiple mechanisms, mainly toxicity, stress, sedation and possibly via GABA neurotransmitters and dopaminergic systems.

In a case–control analysis, Kamijima et al. [16] found that compared to non-exposed controls, the percentages of slow progressive and non-progressive motile sperm among sprayers of OP and PYR pesticides were twice as high ($p < 0.05$), and that of rapid progressive sperm tended to be lower ($p = 0.06$). In another study of occupational exposures in China, Tan et al. [17] compared sperm parameters of 32 employees in a pesticide factory who directly handled pesticides, 46 employees in the same factory who worked in office roles and did not directly handle pesticides, and 22 workers in a non-pesticide manufacturing environment. Air monitoring over 3 days showed that ambient air concentrations of fenvalerate (but not toluene or xylene) were significantly higher in the workplaces of the exposed workers compared to the office workers in the same factory or workers outside the factory. Compared to non-exposed workers, exposed workers had significantly decreased sperm motility and count.

The developmental toxicity of PYRs in humans has not been determined due to a lack of studies focused on developmental outcomes. Standard developmental tests in animals have not demonstrated significant developmental toxicity. However, more targeted testing has suggested persistent neurotoxic effects in animals exposed in utero and or via lactation [2].

### 1.3. Pyrethroids and endocrine hormones

Prior studies have documented the potential hormonal effects of PYRs in several model systems including rats, recombinant yeast, and human cells *in vitro*. Mani et al. [13] found a decrease in marker testicular enzymes for testosterone biosynthesis viz. 17-beta-hydroxy steroid dehydrogenase (17-beta-HSD) and glucose-6-phosphate dehydrogenase (G6PDH), leading to net decrease in serum testosterone concentration in a group of rats exposed to one-fifth LC50 of fenvalerate (20% EC) by inhalation (4 h/day, 5 days a week) subchronically for 3 months. Hu et al. [18] exposed adult male Sprague–Dawley rats to different oral doses (0, 2, 4, 12 and 60 mg/kg) of fenvalerate for 15 days and 30 days. They found that at 15 days, serum follicle stimulating hormone levels markedly increased in rats exposed to fenvalerate of $\leq$12 mg/kg groups and serum levels of luteinizing hormone increased in 12 mg/kg group. In addition, testosterone levels in testis homogenates decreased after being treated with doses of $\geq$12 mg/kg groups compared with the con-

trol group. In 30 days, serum contents of follicle stimulating hormone were significantly elevated in the doses of ≥12 mg/kg groups and homogenate levels of testosterone were diminished in the low dose group. Elbetieha et al. [14] exposed adult male Sprague–Dawley rats to tap water containing graduated doses of cypermethrin for 12 weeks. Compared to rats which consumed lower doses, those with higher doses had significantly reduced serum levels of testosterone, follicle-stimulating hormone, and luteinizing hormone.

There is evidence that the metabolic products of at least one PYR pesticide, permethrin, have greater endocrine disrupting effects than the parent compound. Using recombinant yeast expressing human estrogen and human androgen receptors as a model system, Tyler et al. [7] found that permethrin had estrogenic activity and was a weak estrogen agonist. Three-phenoxybenzyl alcohol (a permethrin metabolite) had both estrogenic and antiandrogenic activity, with potencies more than 100-fold greater than that of the parent compound permethrin. Two of the three permethrin derivatives tested (3-phenoxybenzoic acid (3PBA) and permethrin cyclopropane) also had antiestrogenic activity, with potencies of approximately 100-fold and 1000-fold lower than that of 4-OH-tamoxifen, respectively, indicating permethrin metabolism products potentially have more potent endocrine disrupting properties than their parent compound.

Prior studies have indicated that several PYRs, including fenvalerate, permethrin, and fenothrin (sumithrin), are androgen antagonists. Eil and Nisula [19] reported that of several PYRs examined, only the pyrethrins (50% inhibition) and bioallethrin (43% inhibition) were able to displace [3H] testosterone from sex hormone binding globulin (SHBG) when tested at a concentration of $10^{-4}$ M in human genital skin fibroblasts. These data indicated that the PYRs, as a class of non-steroidal compounds, can interact competitively with human androgen receptors and SHBG, and suggest a mechanism by which chronic exposure to PYRs may result in disturbances in endocrine effects relating to androgen action.

### *1.4. Synergism of pyrethroid and organophosphorous pesticide exposures*

Pesticide mixtures can result in interactive effects rendering the mixture more toxic than the additive effects of each compound [20]. Carboxylesterases, enzymes that detoxify PYRs, are inhibited by OPs, therefore potentially increasing PYR toxicity [21]. Due to widespread and long term PYR use, insect strains resistant to PYR toxicity are increasing. Entomology studies have shown that this phenomenon is due to selection for increased esterase isoenzyme production, which allows rapid metabolism of PYR pesticides [22]. A number of studies have shown that in resistant strains, the use of OP pesticides in conjunction with PYRs cause synergy in the effects of PYRs on insect populations. This effect occurs because OPs bind to the active sites of the metabolic enzymes, thus limiting the rate of PYR detoxification. These enzyme inhibition studies are supported by bioassay data using non-toxic doses of OPs as synergists for PYRs. Authors have concluded that use of OP synergists in the field may have the potential to restore some PYR susceptibility in insects [22].

Mammalian resistance to PYRs is based on a capacity for rapid detoxification by ester hydrolysis, and a number of the carboxylesterases responsible for this are also inhibited, or competed for, by OPs. Some OPs, including the cotton defoliant s,s,s-tributyl phosphoro trithionate, can enhance PYR toxicity [23,21]. In rats given permethrin, the coadministration of a sublethal dose of the OP methylparathion significantly enhanced permethrin toxicity, decreasing the permethrin $LD_{50}$ by 57% [24]. Some OPs have a greater potential to synergize PYRs than others, and methylparathion is a strong carboxylesterase inhibitor. Hence, pesticide mixtures may potentiate toxicity, and produce unexpected acute poisoning, or unanticipated chronic health effects, particularly if a sufficiently high exposure to one component saturates the metabolic capacity to dispose of a second component [21].

Like PYRs, OP pesticides also have endocrine disrupting properties. Some OPs such as parathion and methylparathion are structurally similar to various hormones, including estrogens and may interact with hormone receptors and/or gene transcription. Prior epidemiologic work from our group showed that in Chinese pesticide factory workers, OP exposure was associated with decreased sperm concentration and motility [25], increased luteinizing hormone and decreased testosterone [26], and higher sex chromosome aneuploidy in sperm [27].

We are currently conducting a cohort study among couples of reproductive age in rural China. We first explored whether men in our reproductive cohort study were being exposed to pesticides environmentally by virtue of frequenting an agricultural setting. We then examined semen concentration in relation to pesticide exposure profiles.

## 2. Methods

The couples in our study are all young and newly married. Most of the men are not formally employed in agriculture and they do not personally apply pesticides. Instead, they seek out higher paying jobs (relative to farming) in service or skilled trades in surrounding cities, and make periodic visits home to their rural villages. Men working in surrounding cities were recruited into our study during visits home. Their home villages consist of agricultural households where older members of the family (parents, aunts, uncles) use pesticides to grow fruits and vegetables for household consumption and cotton and/or rice for market sale. Household pesticide use is also frequent to control mosquitoes indoors. To determine whether men were being exposed environmentally by virtue of frequenting this agricultural setting, we screened 18 baseline urine samples that were randomly selected from 202 men recruited at the beginning of our study in the winter of 2004 for 24 parent compounds and metabolites of pesticides. Urine samples were analyzed at the Centers for Disease Control, Pesticide Laboratory, and analysts were blind to study objectives and to the individual characteristics of the study samples. An herbicide/insecticide screen that employs a mass spectrometry-based method and quantification using the isotope dilution (ID) calibration [28] was used to screen urine for non-persistent pesticides including OPs, triazine herbicides, chloroacetanilide herbicides, phenoxyacetic acid herbicides and PYR insecticides. This is the same method and the same national laboratory that monitors non-persistent pesticides in the US general population, published in the Second National Report on Human Exposure to Environmental Chemicals [29].

Semen samples were also collected at baseline according to previously published guidelines [30]. We examined the sperm concentration characteristics for the 18 subjects in our pilot pesticide biomonitoring study. We dichotomized the

OP and PYR urinary metabolite levels into low and high exposure using median cut points and examined means and standard deviations for sperm concentration ($10^6$ per ml).

## 3. Results

The results (Table 1) show high prevalence of exposure to OP and PYR pesticides, but not triazines or phenoxyacetic acid herbicides.

These urinary concentrations are consistent with exposure to permethrin and cypermethrin because they share the same metabolites (3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (CDCCA); *trans*-dichlorodimethylvinyl-cyclo-propane carboxylic acid (TDCCA); 3-phenoxybenzoic acid (3-PBA)). Detecting PNP (*para*-nitrophenol), a parathion metabolite, in all of the samples tested is also consistent with 2004 pesticide sales data collected in our study area that showed parathion, which is used for killing cotton-related pests, to be one of the leading products sold.

Because these exposures are environmental rather than occupational, it is possible to compare them to US general population (NHANES 1999–2000) estimates of OP and PYR exposures (Table 2). For parathion (PNP) the prevalence of exposure and the concentration range was much higher than the US [31]. For 3PBA (permethrin metabolite) the prevalence of detection was much higher than US general population data and the geometric mean was lower than the US 95th percentile, but higher than the 75th percentile (0.67 (0.53–0.78)).

Table 3 details the descriptive results from examining sperm concentration by low and high pesticide metabolite levels, which provide some suggestion that the higher exposure group had lower sperm concentration. The crude difference in sperm concentration was statistically significant for DETP (absolute sperm concentration difference = −1.0 (95% confidence interval −1.8, −0.2).

The pesticide metabolites were highly correlated in two primary clusters: (a) DETP and DMTP and PNP and 3PBA; (b) TDCCA and PNP and 3PBA. We tested whether the log sperm concentration was different for the men who were in the high group for three or more pesticide metabolites ($n = 10$) versus the men who were in the high group for fewer than three metabolites ($n = 8$) (data not shown). Sperm concentration was lower in the group of men with more high exposures, however, the difference between the mean log sperm concentrations was not statistically significant (absolute difference (95% CI) = −0.3 (−1.3, 0.6)).

Table 1
Baseline urine concentrations (μg/l) of pesticides and metabolites from 18 randomly selected men, Anhui, China, 2004

| | LOD | *N* > LOD | Minimum | Median | Maximum |
|---|---|---|---|---|---|
| Organophosphates | | | | | |
| Dimethylphosphate | 0.50 | 5 | <LOD | <LOD | 27.9 |
| Dimethylthiophosphate | 0.50 | 16 | <LOD | 5.8 | 29.7 |
| Dimethyldithiophosphate | 0.25 | 3 | <LOD | <LOD | 20.2 |
| Diethylphosphate | 0.25 | 3 | <LOD | <LOD | 39.5 |
| Diethylthiophosphate | 0.25 | 18 | 0.53 | 4.1 | 42.6 |
| Diethyldithiophosphate | 0.25 | 2 | <LOD | <LOD | 2.9 |
| PNP (*para*-nitrophenol; metabolite of EPN, methyl parathion, parathion, other chemicals) | 0.14 | 18 | 1.1 | 5.3 | 48.0 |
| MDA (malathion dicarboxylic acid) | 0.29 | 1 | <LOD | <LOD | 0.3 |
| CMCH (metabolite of coumaphos) | 0.18 | 3 | <LOD | <LOD | 0.5 |
| TCPY (metabolite of chlorpyrifos) | 0.26 | 0 | | | |
| IMPY (metabolite of diazinon) | 0.69 | 1 | <LOD | <LOD | 1.4 |
| DEAMPY (metabolite of pirimiphos methyl) | 0.22 | 0 | | | |
| CIT (metabolite of isazaphos) | 1.50 | 0 | | | |
| Pyrethroids | | | | | |
| 3-Phenoxybenzoic acid | 0.10 | 18 | 0.5 | 1.1 | 25.3 |
| TDCCA (metabolite of permethrin, cypermethrin, cyfluthrin) | 0.35 | 10 | <LOD | 0.1 | 27.2 |
| 4F3PBA (metabolite of cyfluthrin) | 0.16 | 0 | | | |
| CDCCA (metabolite of permethrin, cypermethrin, cyfluthrin) | 0.23 | 2 | <LOD | <LOD | 12.1 |
| DBCA (metabolite of deltamethrin) | 0.12 | 0 | | | |
| Chloroacetanilide herbicides | | | | | |
| Metolachlor mercapturate (metabolite of metolachlor) | 0.16 | 0 | | | |
| Phenoxyacetic acid herbicides | | | | | |
| 2,4-Dichlorophenoxyacetic acid | 0.16 | 0 | | | |
| 2,4,5-Trichlorophenoxyacetic acid | 0.13 | 1 | <LOD | <LOD | 0.3 |
| Triazine herbicide | | | | | |
| Atrazine mercapturate (metabolite of atrazine) | 0.32 | 0 | | | |

LOD = limit of detection; CMHC = 3-chloro-4-methyl-7-hydroxycoumarin; TCPY = 3,5,6-trichloropyridinol; IMPY = 2-isopropyl-4-methyl-6-hydroxypyrimidinol; DEAMPY = 2-diethylamino-6-methyl pyrimidin-4-ol; CIT = 5-chloro-1,2-dihydro-1-isopropyl-[3H]-1,2,4-triazol-3-one; TDCCA = *trans*-dichlorodimethylvinylcyclopropane carboxylic acid; 4F3PBA = 4-fluoro-3-phenoxybenzoic acid; CDCCA = *cis*-dichlorodimethylvinylcyclopropane carboxylic acid; DBCA = *cis*-dibromodimethylvinylcyclopropane carboxylic acid.

Table 2
Baseline urine concentrations (μg/l) of pesticides and metabolites from 18 randomly selected men, Anhui, China, Winter 2004

| | Percentage of detections | Geometric mean | Maximum | S.D. | NHANES 95th percentile[a] |
|---|---|---|---|---|---|
| Organophosphates | | | | | |
| Dimethylthiophosphate (DMTP) | 89 | 5.7 | 29.7 | 10.1 | 38.0 (38.0–48.0) |
| Diethylthiophosphate (DETP) | 100 | 4.2 | 42.6 | 12.2 | 2.0 (1.5–2.8) |
| PNP (*para*-nitrophenol) | 100 | 6.9 | 48.0 | 12.7 | 4.5 (2.5–9.2) |
| Pyrethroids | | | | | |
| 3PBA = 3-phenoxybenzoic acid | 100 | 1.2 | 25.3 | 5.7 | 3.25 (2.5–6.7) |
| TDCCA | 55.5 | 0.8 | 27.2 | 6.4 | 2.56 (1.6–4.7) |

[a] Males and females 20–59 years of age; *source*: [31,29]; TDCCA = *trans*-dichlorodimethylvinylcyclopropane carboxylic acid.

Table 3
Crude geometric means of baseline sperm concentrations among men with lower or higher baseline urine concentrations of five commonly detected organophosphate and pyrethroid pesticides, Anhui, China 2004

| | Higher exposure | | Lower exposure | | Log (sperm concentration) difference (95% CI), high vs. low |
|---|---|---|---|---|---|
| | *n* | Geometric mean | *n* | Geometric mean | |
| Sperm concentration ($10^6$/ml) | | | | | |
| Organophosphates | | | | | |
| Dimethylthiophosphate (DMTP) | 9 | 46.4 | 9 | 60.3 | −0.3 (−1.2, 0.6) |
| Diethylthiophosphate (DETP) | 9 | 33.3 | 9 | 90.5 | −1.0 (−1.8, −0.2)* |
| PNP (*para*-nitrophenol) | 9 | 44.9 | 9 | 64.8 | −0.4 (−1.3, 0.5) |
| Pyrethroids | | | | | |
| 3PBA = 3-phenoxybenzoic acid | 9 | 48.0 | 9 | 60.0 | −0.2 (−1.1, 0.7) |
| TDCCA | 10 | 41.8 | 8 | 76.2 | −0.6 (−1.5, 0.3) |

TDCCA = *trans*-dichlorodimethylvinylcyclopropane carboxylic acid.
* $p < 0.05$.

## 4. Discussion

These biomonitoring pilot results suggest a specific exposure profile, and the specific pesticide exposure mixture is of concern because of possible synergistic effects on humans. All of the 18 males in our sample had diethylthiophosphate (DETP) and parathion (PNP) OP metabolites and all had phenoxybenzoic acid (3PBA) PYR metabolites. Urinary concentrations in our pilot sample were well above US general population levels.

The preliminary findings of sperm concentration patterns are comparable to the pesticide exposure and sperm parameter findings reported by Kamijima et al. [16]. Compared to non-exposed controls, the percentages of slow progressive and non-progressive motile sperm among 18 sprayers of OP and PYR pesticides were twice as high ($p < 0.05$), and that of rapid progressive sperm tended to be lower ($p = 0.06$). However, their study measured pesticide exposures using only self-report and OP/PYR exposure mixtures were not quantified separately.

Our pilot study is unique both in providing biomarker information of this specific OP/PYR exposure profile, and in examining sperm concentration in relation to specific OP and PYR metabolites. While the small sample size of this study had limited power for significance testing, these exploratory data are useful for hypothesis generation. We are examining this relationship further in a larger study of 202 men currently underway.

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