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Pesticide poisoning and neurobehavioral function among farm workers in Jiangsu, People's Republic of China

Xujun Zhang [a,b,*], *Ming Wu* [c], *Hongyan Yao* [d], *Yaming Yang* [e], *Mengjing Cui* [a], *Zhibin Tu* [f], *Lorann Stallones* [g] *and Huiyun Xiang* [h]

[a] *Injury Prevention Research Institute, School of Public Health, Southeast University, Nanjing, Jiangsu Province, China*
[b] *Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu Province, China*
[c] *Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, Jiangsu Province, China*
[d] *Office of Epidemiology, Chinese Center for Disease Control and Prevention, Beijing, China*
[e] *Yixing Center for Disease Control and Prevention, Yixing, Jiangsu Province, China*
[f] *Nanchang Center for Disease Control and Prevention, Nanchang, Jiangsu Province, China*
[g] *Colorado Injury Control Research Center, Colorado State University, Fort Collins, CO, USA*
[h] *Center for Injury Research and Policy and Center for Pediatric Trauma Research, The Research Institute at Nationwide Children's Hospital, The Ohio State University College of Medicine, Columbus, OH, USA*

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ABSTRACT

Pesticides remain an integral part of agricultural activities worldwide. Although there have been a number of studies over the last two decades concerning the adverse effects of pesticide poisoning and chronic long term exposures on neurobehavioral function, the impact of recent pesticide poisoning and long term pesticide exposure on neurobehavioral function in Chinese farm workers has not been reported. China is the largest user of pesticides worldwide and figures suggest 53,300–123,000 Chinese people are poisoned every year. A case control study was conducted to examine the impact of recent pesticide poisoning on neurobehavioral function and the relationship between years worked in agriculture and lower performance on neurobehavioral tests. A total of 121 farm workers who self-reported recent pesticide poisonings within the previous 12 months (case group) and 80 farm workers who reported no pesticide poisoning in the previous 12 months (control group) were recruited from three areas of Jiangsu Province, China. The World Health Organization (WHO) recommended neurobehavioral core test battery (NCTB) was used to assess neurobehavioral functioning among cases and controls. Student's *t* tests and two-way covariance analysis (ANCOVA) were used to test for significant differences in the neurobehavioral test results between the groups. Scores on the Profile of Mood States (POMS) in the recently poisoned group were significantly higher for anger-hostility,

* *Corresponding author*. Injury Prevention Research Institute, School of Public Health, Southeast University Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, 87 Dingjiaqiao, Nanjing, Jiangsu 210009, China.
E-mail address: xjzhang@seu.edu.cn (X. Zhang).
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depression-dejection, tension-anxiety and lower for vigor-activity compared to controls ($p < .05$). Digit span, digit symbol, Benton visual retention and pursuit aiming scores were all significantly lower among the recently poisoned group compared to the controls ($p < .05$). Two-way ANCOVA indicated significantly lower performance in correct pursuit aiming and higher error pursuit aiming amongst the recently poisoned group and those who had worked for more than 30 years in agriculture ($p < .05$). These findings provide important preliminary epidemiological evidence regarding the association between occupational pesticide exposure and neurobehavioral functioning in Chinese farm workers.

1. Introduction

Pesticides are widely used in agriculture and play a crucial role in the control of insect pests, especially in developing countries. In China, more than 45,000 chemical products including about 770 approved pesticides are available (Hao, Li, Wang, Wu, & Song, 2013; Wu & Sun, 2004). Worldwide 5,211 million pounds (approximately 2.4 million tons) of pesticides are used (United States Environmental Protection Agency, 2011), with approximately half of those, or 1.2 million tons used in China (Yang, 2007). With the burden of feeding 20% of the world's population on only 7% of the world's arable land, Chinese farmers rely on pesticides to maintain high crop yields and China is currently the largest user, producer and exporter of pesticides (Yang, 2007). The World Health Organization (WHO) reported that about one million serious unintentional pesticide poisoning cases occur each year (Jeyaratnam, 1990); mostly in developing countries, particularly in Southeast Asia (London & Bailie, 2001; Xiang et al., 2000; Zhang et al., 2011). Chinese government figures show that between 53,300 and 123,000 people are poisoned by pesticides each year (Yang, 2007). Many Chinese farmers do not receive proper pesticide safety training, are not willing or able to invest in personal protective clothing and equipment and are therefore at greatly increased risk of pesticide poisonings (Yang, 2007; Zhang et al., 2011). A survey showed that many Chinese farmers suffered nervous system, gastrointestinal tract system and cardiovascular system problems due to pesticide poisonings, as well as eye problems, and skin and respiratory irritation (Zhang et al., 2011). Pesticide poisoning has been shown to be an important public health problem among Chinese farm workers (Xiang et al., 2000; Zhang et al., 2011).

The nervous system is the target of many pesticides (Casida & Durkin, 2013) and both acute and chronic neurotoxic effects in humans have been reported following exposure to organophosphates (OP), carbamates, pyrethroid and organochlorine compounds (Beseler & Stallones, 2013; Bradberry, Cage, Proudfoot, & Vale, 2005; Jokanovic & Kosanovic, 2010; London et al., 2012; Rohlman, Anger, & Lein, 2011; van Wendel de Joode et al., 2001; Wesseling et al., 2002). In a recent systematic review, depression and suicide were shown to be associated with previous poisonings in studies published within the last 15 years (Freire & Koifman, 2013) . While there is scientific evidence of an association between pesticide exposure and depression or suicide, the evidence is limited to selected populations using varying approaches (Freire & Koifman, 2013), therefore a need exists to provide further support for the hypothesis of an association between organophosphate pesticide exposures and mood disorders. Other classes of pesticides including herbicides, fungicides and fumigants have also been associated with chronic neurotoxicity effects in humans (Baldi et al., 2001; Beseler & Stallones, 2003; Bradberry, Proudfoot, & Vale, 2004; Greenwood, 1985; O'Malley et al., 2011) but there is much less scientific evidence supporting the role of these pesticide classes relative to organophosphate compounds.

In China, OP insecticide poisoning accounts for 74.03% of all pesticides poisoning reported by the National Institute of Occupational Health and Poison Control at the Chinese Center for Disease Control and Prevention (Chen, Wang, & Yin, 2005). Among the different types of pesticides, OP insecticides have been the most intensively studied. The acute neurotoxic effects of OPs are caused by the inhibition of acetylcholinesterase in the synaptic cleft and therefore OPs increase cholinergic activity. Cholinergic symptoms following acute poisoning to these chemicals include dizziness, cramps, nausea, vomiting, abdominal pain, numbness and/or tremors in the extremities, fatigue, headaches, excessive salivation, diarrhea, generalized weakness, respiratory problems, and blurred vision (Sapbamrer & Nata, 2014; Stallones & Beseler, 2002).

In addition to acute neurotoxic effects, OP pesticide poisoning may cause chronic, long-term effects including delayed onset peripheral neuropathies (primarily affecting the extremities), neuropsychological changes, and neurobehavioral changes (Beseler et al., 2008, 2006; Rosenstock, Keifer, Daniell, McConnell, & Claypole, 1991; Stallones & Beseler, 2002; Steenland et al., 1994; Wesseling et al., 2002). These may occur 2–3 weeks after a single exposure, when symptoms and signs of the cholinergic crisis have subsided (Costa, 2008). For example, the Intermediate Syndrome which results in weakness of the muscles involved in respiration and proximal limb muscles, typically starts 24–96 h after an acute poisoning event. It is relatively rare, affecting only a proportion of cases, but is reported more frequently in developing countries (Abdollahi & Karami-Mohajeri, 2012; Karalliedde, Baker, & Marrs, 2006; Senanayake & Karalliedde, 1987). The late onset of respiratory failure associated with the intermediate syndrome is a major contributor to the high morbidity, mortality, and cost of OP poisoning treatment (Abdollahi & Karami-Mohajeri, 2012). Neuropsychological impairment and increased psychiatric morbidity has frequently been identified in individuals with a history of acute OP poisoning. For example, Stallones et al. reported an association between OP poisoning and high depressive symptoms (Stallones & Beseler, 2002). Significant differences between individuals with previous acute OP poisoning and non-poisoned controls were reported on neuropsychological tests including intellectual functioning, academic skills, abstraction and flexibility of thinking, and motor skills (Savage et al., 1988). In another study, the OP poisoned group had significantly worse performance on five of six subtests of a WHO neuropsychological test battery and on 3 of 6 tests assessing verbal and visual attention, visual memory, visuomotor speed, sequencing and problem solving, and motor steadiness and dexterity (Rosenstock et al., 1991).

Neurobehavioral changes are often the earliest indication of exposure to neurotoxicants and neuropsychological assessment has an important role to play in detecting and evaluating the effects of neurotoxic substances (Moser, 1990; Tilson, 1990). Although a number of studies have been published over the last two decades concerning the adverse effects of recent pesticide poisoning on neurobehavioral function, the impact of self-reported recent pesticide poisoning on neurobehavioral function in Chinese farm workers has not been reported, even though China is the largest user of pesticides worldwide. The objective of this study was to examine the association between self-reported recent pesticide poisoning and neurobehavioral deficits in a group of farm workers in Jiangsu Province, the People's Republic of China. We designed a study that had two key aims: (1) To evaluate the impact of recent pesticide poisoning (in the last 12 months) on neurobehavioral function, and (2) to explore the relationship between lifetime exposure to pesticides (i.e., working years) and neurobehavioral functioning. We hypothesized that farmers who self-reported recent poisoning (the case group) were significantly more likely to have neurobehavioural deficits than farmers who did not report a poisoning event in the last 12 months (control group). We also hypothesized that as years of agricultural work increased, the likelihood of experiencing neurobehavioural deficits in both the case and control groups would also increase. The interaction between these variables was also explored.

2. Material and methods

2.1. Participants

The study population was selected using stratified-cluster sampling of villages in three areas in Jiangsu Province, China. The study area was designed to include the Northern, Central and Southern regions of the province. One village was selected from each geographic region. Eligible participants included anyone aged 18 years and over who came into contact with pesticides while performing agricultural activities in the 12 months preceding the beginning of the study. A cross sectional survey was conducted among 1600 farm workers who were interviewed from July 2009 to August 2011. The survey yielded 1490 valid questionnaires, an overall response rate of 93.1%. Reasons for non-response included farmers who were absent from the village when the survey was conducted ($n = 97$) and farmers who provided incomplete data on the questionnaires ($n = 13$). Participants reporting an attempted suicide using pesticides, and pesticide poisonings which occurred during the production, transportation, or marketing of pesticides (any non-agricultural work-related situation) were excluded from our study. Pesticides were identified according to trade name and active ingredients, focusing on insecticides, herbicides, and fungicides. Insecticide sprayers in the study group applied mixed preparations containing organophosphates, pyrethroids, and carbamates.

Study participants for this study were recruited from the 1490 participants who completed the original questionnaire. The case and control groups were selected based on whether or not they had experienced recent pesticide poisoning symptoms in the 12 months prior to the survey. Of the 1490 respondents, there were 121 reported cases of pesticide poisoning (8.1%) in the previous 12 months. The farm workers for the control group ($n = 80$) were selected from the same village but did not report a pesticide poisoning in the previous 12 months. Cases and controls were matched by area, gender, age, education, working years, tobacco use, alcohol use, and general physical condition. Participants were excluded if they had reported amyotrophic lateral sclerosis, Parkinson's disease, retinopathy or macular degeneration, or were taking medicines which could affect nervous system functioning, as these conditions may influence the results of neurobehavioral testing.

Informed consent was obtained from participants in accordance with ethical guidelines. The institutional review board of Southeast University reviewed and approved the study procedures.

2.2. *Occupational pesticide poisoning*

Sixty-six potential symptoms of poisoning of organophosphates, pyrethroids and carbamates were included in the questionnaire. In addition to general symptoms, the list included symptoms specifically related to the skin, eyes, nervous system, respiratory system, gastrointestinal tract, urogenital system, and cardiovascular system. In our study, respondents who self-reported having had two or more of the listed symptoms within 24 h after applying pesticides were considered to have suffered recent pesticide poisoning during the past year. This is the standard case definition of pesticide poisoning used in China. Previous researchers used a similar approach to identifying a possible pesticide poisoning (Thundiyil, Stober, Besbelli, & Pronczuk, 2008).

2.3. *Test of neurobehavioral function*

Neurobehavioral function was assessed in our study by using the WHO recommended neurobehavioral core test battery (NCTB) (Anger, 2003; World Health Organization, 1986). The NCTB is a widely used assessment established by WHO for early detection of neurotoxicity effects of compounds in the workplace. The NCTB provides a set of rapid, stable, and sensitive behavioral tests and is extensively used in neurotoxicology to assess changes of behavior in the presence of neurotoxic chemicals (Anger, 2003; World Health Organization, 1986). The NCTB contains domains of mood called the Profile of Mood States (POMS) questionnaire, simple reaction time, digit span, Santa Ana dexterity, digit symbol, Benton visual retention, and pursuit aiming. Seven sections of NCTB assessed neurobehavioral function in the areas of emotional state, learning, memory and hand-eye coordination.

2.4. *Statistical analysis*

Data were entered into EpiData 3.0 and statistical analysis was done using SAS 9.1 (SAS Institute, Cary, NC). First, differences in area, gender, age, education, working years, tobacco use and alcohol use between the case group and control group were evaluated by χ^2 analyses and Student's *t* test. Second, Student's *t* tests were performed to compare for significant differences in the neurobehavioral functioning between the case group and the control group. Third, two-way covariance analysis (ANCOVA) was used to test for significant differences in the neurobehavioral test results between the two working year groups, controlling for gender, age and school education level. A total of 18 ANCOVAs were carried out with each neurobehavioral test (dependent variable). The main variables of interest were pesticide poisoning in the previous 12 months and working years in agriculture ($\leq$30 *vs* > 30 working years). Interaction of recent pesticide poisoning and working years was assessed with ANCOVA. Effect sizes were used to evaluate difference of effects in the neurobehavioral functioning between case group and control group (Cohen, 1988).

Table 1 – Comparison of characteristics of case group ($n = 121$) and control group($n = 80$).

	Case group	Control group	Statistics
Area			$\chi^2 = .01$, $p = .995$
Northern Jiansu	67	44	
Central Jiangsu	19	13	
Southern Jiangsu	35	23	
Gender			$\chi^2 = .00$, $p = .952$
Men	63	42	
Women	58	38	
Age (mean ± SD)	53.12 ± 9.53	52.86 ± 10.19	$t = .18$, $p = .858$
Age (years)			$\chi^2 = .04$, $p = 1.00$
28–35	4	3	
35–44	21	14	
45–54	39	26	
55–64	48	31	
>64	9	6	
Education (years)			$\chi^2 = .01$, $p = .995$
<7	45	30	
7–9	68	45	
>9	8	5	
Working years (mean ± SD)	33.28 ± 9.18	33.11 ± 9.70	$t = .13$, $p = .901$
Working years			$\chi^2 = .70$, $p = .404$
≤30	40	22	
>30	81	58	
Tobacco use			$\chi^2 = .00$, $p = .972$
Current	33	22	
Never/former	88	58	
Alcohol use			$\chi^2 = .00$, $p = .981$
Yes	18	12	
No	103	68	

3. Results

As shown in Table 1, survey area, gender, age, education, working years, tobacco use and alcohol use did not differ significantly between the case and the control groups ($p > .05$). Patterns of medicine use, medical conditions, psychiatric conditions or physical condition, and sleep patterns at night before testing were similar between the two groups.

Table 2 presents results of neurobehavioral function testing. Compared to the control group, POMS scores in the recent pesticide poisoning group were significantly different for anger-hostility, depression-dejection, tension-anxiety and vigor-activity ($p < .05$). For the simple reaction time test, the fastest reaction time, slowest reaction time, and mean reaction time, lower performance was observed in the recent pesticide poisoning group compared to the control group, but the differences were not statistically significant ($p > .05$). Forward digit span, backward digit span, digit symbol, Benton visual retention, correct pursuit aiming and error pursuit aiming scores were all significantly lower in the recent pesticide poisoning group than in control group ($p < .05$). The Santa Ana dexterity scores were not statistically significantly different between the two groups ($p > .05$).

Results of the two-way ANCOVA are presented in Table 3. There was a significant main effect of recent pesticide poisoning on depression-dejection, fatigue-inertia, tension-anxiety, forward digit span, and backward digit span such that participants in the case group showed relatively poorer performance than those in the control group ($p < .05$). In addition, the significant main effect of working years indicated that farmers with higher working years in agriculture also exhibited lower performance in terms of reaction time and digit symbol ($p < .05$). Interaction effects were also found for correct pursuit aiming and error pursuit ($p < .05$). For farmers who were in the case group, those who had less than 30 years potential exposure scored higher on the pursuit aiming test (number correct) than those who had more than 30 years exposure. In contrast, the control group showed the opposite pattern. For error scores, cases performed similarly regardless of years working in agriculture; whilst controls with less exposure scored higher than those with more than 30 years of exposure. Why this might be the case is unclear.

4. Discussion

Insecticides are not species-selective with regard to their toxicity; mammals including humans are highly sensitive to the toxic effects of these compounds (Costa, 2008). Behavioral toxicology is becoming increasingly important in risk assessment, because neurobehavioural problems are often one of the first signs of neurotoxic exposure (Yuan et al., 2006). Results from our study indicate that self-reported pesticide poisoning in the 12 months prior to the survey led to measurable deficits in farm workers' neurobehavioral functioning. In addition we found those individuals who spent

Table 2 – Neurobehavioral test results by case group (mean ± SD, $n = 121$) and control group(mean ± SD, $n = 80$).

Test item	Case group	Control group	Statistics	ES
Mood states				
Anger-hostility	17.16 ± 9.27	14.53 ± 8.32	$t = 2.05$, $p = .042$	.30
Confusion-bewilderment	8.93 ± 4.06	8.36 ± 3.98	$t = .98$, $p = .327$	.14
Depression-dejection	15.65 ± 6.41	12.34 ± 5.53	$t = 3.78$, $p < .001$	.55
Fatigue-inertia	9.71 ± 4.52	8.52 ± 4.18	$t = 1.88$, $p = .061$	.27
Tension-anxiety	9.85 ± 6.13	8.03 ± 6.06	$t = 2.07$, $p = .040$	.30
Vigor-activity	17.38 ± 7.02	19.71 ± 6.68	$t = 2.35$, $p = .020$	.34
Simple reaction time				
Fastest time	250.05 ± 49.22	246.81 ± 58.34	$t = .42$, $p = .672$	.06
Slowest time	454.76 ± 265.01	445.31 ± 177.50	$t = .28$, $p = .780$	.04
Mean time	263.40 ± 121.36	237.06 ± 97.55	$t = 1.63$, $p = .106$	.24
Digit span				
Digit span forward	10.21 ± 2.96	11.52 ± 2.67	$t = 3.19$, $p = .002$	.46
Digit span backward	6.06 ± 2.39	6.79 ± 2.55	$t = 2.06$, $p = .040$	.30
Santa Ana dexterity				
Preferred hand	15.58 ± 2.60	16.14 ± 2.58	$t = 1.49$, $p = .135$	.22
Non-preferred hand	14.45 ± 2.54	15.04 ± 2.32	$t = 1.67$, $p = .097$	.24
Digit symbol	22.93 ± 10.23	27.20 ± 12.65	$t = 2.63$, $p = .009$	.37
Benton visual retention	6.72 ± 1.85	7.38 ± 1.91	$t = 2.44$, $p = .015$	.35
Pursuit aiming				
Total pursuit aiming	101.32 ± 24.12	102.54 ± 22.72	$t = .36$, $p = .720$	.05
Correct pursuit aiming	79.72 ± 23.33	88.30 ± 21.75	$t = 2.62$, $p = .011$	.38
Error pursuit aiming	24.57 ± 11.31	20.62 ± 15.12	$t = 2.12$, $p = .036$	.30

ES: effect size.

more years engaged in agricultural work performed more poorly on tests of reaction time and psychomotor speed.

The most commonly used class of pesticides in this study were insecticides (84.0%) followed by herbicides (9.6%), and fungicides (6.4%). It is noteworthy that about 60% of farm workers in China still use WHO categoryIorganophosphates, although these pesticides have been banned for more than 10 years (Wang et al., 2008). CategoryIinsecticides are more acutely toxic in nontarget species compared to other categories of pesticides, and some of them, most notably the organophosphates are involved in a large number of human poisonings and deaths each year (Costa, 2008). Although recent studies reported that the neurological outcomes related to pesticide exposure among farm workers have focused on OP insecticides exposure; many non-OP insecticides have some neurotoxic potency as well (Bradberry et al., 2004; Bradberry et al., 2005; Greenwood, 1985; O'Malley et al., 2011; Starks et al., 2012; van Wendel de Joode et al., 2001; Wesseling et al., 2002).

In our study, occupational pesticide poisoning reported in the past 12 months was associated with increased negative emotions and decreased positive emotions among farm workers. The negative emotions in the farm workers who self-reported recent pesticide poisoning, such as anger-hostility, depression-dejection and tension-anxiety were significantly more common than in the control group. Vigor-activity was significantly decreased in the recently poisoned group compared with controls. Occupational pesticide poisoning was also associated with decreased reaction speed, short-term memory deficits and loss of coordination. Farm workers who self-reported pesticide poisoning in the previous 12 months performed worse on tests of auditory memory, psychomotor speed and accuracy, visual perception/memory than the control group. These differences in the emotion and behavior of recently poisoned farm workers compared with controls suggests recent pesticide poisoning may have a crucial role to play in the aetiology of these symptoms. However, we also found an association between the number of years engaged in agricultural work and lower performance on neurobehavioural tests, but we are unable to determine whether this is because those with longer durations of exposure have a history of more acute poisoning events, or whether cumulative low level exposure in the absence of acute poisoning events, is also harmful. Obtaining reliable and valid estimates of exposure is notoriously difficult in retrospective studies making it difficult to determine the neurotoxicity of low level versus high level exposure. A limitation of our study is the possibility that some of the control group workers may have had an acute pesticide poisoning event in the years prior to the 12 months reference period, but failed to report this in our study. However, this would be more likely to bias our findings in relation to the toxicity of high level/acute poisoning to a null finding when comparing the two groups. As we were unable to tell whether or how often people might have been acutely poisoned during the years they have worked with pesticides, we were unable to determine the precise reason duration of exposure is associated with neurobehavioural deficits (i.e., the precise role of low level *vs* high level exposure). Future research needs to obtain more accurate estimates of exposure history, something which is difficult to achieve in retrospective studies, but prospective studies could be undertaken in which exposure is determined by the use of biomarkers (e.g., blood or urine levels of pesticide metabolites). This would allow more detailed analyses of dose–response relationships.

Table 3 – Two-way ANCOVA for the relationship between neurobehavioral test results and study group (case/control) by working years.

	≤30 working years			>30 working years			Main effect A (Pesticide poisoning)	Main effect B (working years)	Interaction (A × B)
	Case group ($n = 40$)	Control group ($n = 22$)	ES	Case group ($n = 81$)	Control group ($n = 58$)	ES	Statistics	Statistics	Statistics
Mood states									
Anger-hostility	15.53 ± 6.49	13.81 ± 8.25	.23	19.28 ± 9.91	15.30 ± 8.12	.44	$F = 3.42, p = .066$	$F = .15, p = .703$	$F = 1.64, p = .202$
Confusion-bewilderment	9.01 ± 3.34	7.81 ± 3.27	.36	9.20 ± 4.01	8.49 ± 3.95	.18	$F = 2.37, p = .125$	$F = .53, p = .467$	$F = .15, p = .699$
Depression-dejection	15.14 ± 4.95	11.48 ± 4.95	.74	15.48 ± 6.97	13.23 ± 5.55	.36	**$F = 10.38, p = .001$**	$F = .25, p = .616$	$F = .37, p = .542$
Fatigue-inertia	9.96 ± 3.90	8.12 ± 5.49	.39	10.09 ± 4.64	7.61 ± 3.77	.59	**$F = 9.90, p = .002$**	$F = 2.18, p = .141$	$F = .611, p = .435$
Tension-anxiety	9.26 ± 6.08	8.47 ± 6.91	.12	10.42 ± 6.93	7.25 ± 6.58	.47	**$F = 4.02, p = .046$**	$F = .59, p = .443$	$F = .83, p = .364$
Vigor-activity	21.87 ± 6.99	22.30 ± 7.31	.06	17.20 ± 7.10	19.69 ± 6.26	.37	$F = 1.82, p = .179$	$F = 1.99, p = .160$	$F = .75, p = .388$
Simple reaction time									
Fastest time	228.54 ± 34.79	229.35 ± 52.40	.02	250.87 ± 42.82	245.47 ± 57.19	.11	$F = .21, p = .648$	**$F = 5.62, p = .019$**	$F = .38, p = .537$
Slowest time	415.66 ± 116.09	347.48 ± 155.65	.50	431.04 ± 166.51	407.02 ± 159.07	.15	$F = 3.90, p = .050$	$F = 1.84, p = .177$	$F = .68, p = .411$
Mean time	228.05 ± 50.30	254.21 ± 71.82	.42	255.78 ± 74.84	252.29 ± 87.90	.04	$F = .51, p = .475$	$F = 1.65, p = .201$	$F = .96, p = .329$
Digit span									
Digit span forward	10.02 ± 2.30	11.35 ± 2.57	.55	10.28 ± 2.76	10.57 ± 2.57	.11	**$F = 10.16, p = .002$**	$F = .44, p = .510$	$F = .19, p = .663$
Digit span backward	7.45 ± 2.68	7.80 ± 2.69	.13	6.22 ± 2.46	7.52 ± 2.58	.52	**$F = 4.29, p = .040$**	$F = 1.10, p = .295$	$F = 1.67, p = .198$
Santa Ana dexterity									
Preferred hand	16.51 ± 2.56	15.81 ± 2.15	.30	15.90 ± 2.60	16.40 ± 2.62	.19	$F = .03, p = .863$	$F = .41, p = .524$	$F = 2.52, p = .114$
Non-preferred hand	14.88 ± 2.29	15.51 ± 2.25	.28	14.75 ± 2.49	15.43 ± 2.35	.28	$F = 3.84, p = .052$	$F = .00, p = .954$	$F = .04, p = .835$
Digit symbol	29.35 ± 7.82	30.78 ± 9.30	.17	23.99 ± 8.79	27.63 ± 10.57	.37	$F = 1.25, p = .265$	**$F = 4.26, p = .040$**	$F = 1.67, p = .197$
Benton visual retention	7.64 ± 1.45	7.37 ± 1.64	.17	6.86 ± 1.82	7.47 ± 1.87	.33	$F = .39, p = .533$	$F = 1.36, p = .245$	$F = 2.00, p = .159$
Pursuit aiming									
Total pursuit aiming	110.87 ± 28.95	114.68 ± 23.99	.14	105.13 ± 22.90	105.48 ± 22.24	.02	$F = .19, p = .666$	$F = .11, p = .738$	$F = .28, p = .601$
Correct pursuit aiming	92.65 ± 23.59	85.87 ± 27.89	.26	80.45 ± 21.65	91.24 ± 20.66	.51	$F = .14, p = .710$	$F = .09, p = .766$	**$F = 7.44, p = .007$**
Error pursuit aiming	23.39 ± 21.01	33.33 ± 21.72	.47	24.24 ± 10.79	18.58 ± 13.85	.46	$F = .84, p = .362$	$F = 2.52, p = .114$	**$F = 11.34, p = .001$**

Covariates: gender, age, education; ES: effect size; A: with pesticide poisoning in the previous 12 months (case *vs* control); B: working years.

To our knowledge, this is the first study to examine the association between occupational pesticide poisoning and neurobehavioral function using the WHO recommended NCTB in Chinese farmers. Therefore, our results contribute to the scientific evidence about the long-term neurobehavioral effects of occupational pesticide poisoning in a population of workers previously unstudied. as called for in the recently published systematic review (Freire & Koifman, 2013). Whilst earlier work has been mostly consistent in demonstrating that acute poisoning with OP insecticides is associated with long-term neurobehavioral deficits, the effect on the nervous system of cumulative, low level exposure to OP insecticides, in the absence of a history of acute poisoning is still controversial (Baldi et al., 2011; Ismail, Bodner, & Rohlman, 2012; Mackenzie Ross, McManus, Harrison, & Mason, 2013; Meyer-Baron, Knapp, Schäper, & van Thriel, 2015; Rosenstock et al., 1991; Stallones & Beseler, 2002; Starks et al., 2012; Steenland et al., 1994; Wesseling et al., 2002). Although inconsistent findings have been published (Ames, Steenland, Jenkins, Chrislip, & Russo, 1995; Daniell et al., 1992; London, Nell, Thompson, & Myers, 1998; Steenland et al., 2000), an increasing number of studies of OP exposed farm workers have reported negative neurobehavioral consequences in terms of motor speed/coordination, reaction time, visual scanning and information processing speed, sustained attention, and memory (Baldi et al., 2001; Bazylewicz-Walczak, Majczakowa, & Szymczak, 1999; Fiedler, Kipen, Kelly-McNeil, & Fenske, 1997; Kamel et al., 2003; Mackenzie Ross et al., 2010; Pilkington et al., 2001; Rohlman et al., 2011; Roldan-Tapia, Parron, & Sanchez-Santed, 2005; Rothlein et al., 2006; Starks et al., 2012). In our study, associations observed between exposure to pesticides at a high enough concentration to cause individuals to report pesticide poisoning and measures of neurobehavioral function, are consistent with the results of these studies.

There are several limitations to be considered when interpreting the results of this study. First, the information on occupational pesticide poisoning in the past 12 months was collected through a cross-sectional survey. Recall bias might lead to an inaccurate estimation of recent pesticide poisoning. In addition, the effect of recent pesticide poisoning on neurobehavioral function might be underestimated because individuals in the control group could have experienced pesticide poisoning in the past. It may be difficult for them to remember and self-report all pesticide poisoning incidents over a 30–40 year period. However, if we assume that some of the control group workers also experienced pesticide poisoning but did not report it in our study, this recall bias would be more likely to obscure any association between recent pesticide poisoning and neurobehavioral function. Another limitation of this study is the fact that our sample of participants came from only one province in China and so the sample size was small and it is unclear how representative they were of all farm workers in China. Finally, it was not possible to determine which types of pesticides caused the neurobehavioral deficits described herein. Further investigation is necessary to differentiate the neurobehavioral effects of specific classes of pesticide.

A major strength of this study was the use of the widely used Chinese standard case classification matrix to identify recent pesticide poisoning cases. Based on this case definition matrix, laboratory confirmation is not required to define a case of recent pesticide poisoning. Use of this standard definition makes it possible to compare our results with other studies using self-reported symptoms in pesticide poisoning case determination.

5. Conclusions

In summary, our findings suggest that recent occupational pesticide poisoning is associated with reduced neurobehavioral function and increased psychiatric morbidity in Chinese farm workers. In addition, the number of years Chinese farm workers work in agriculture is also associated with reduced neurobehavioral function and increased psychiatric morbidity, but it is not possible to determine whether this is a result of cumulative, low level exposure or because they suffer more episodes of acute poisoning during their working life. This study provides important, preliminary epidemiological evidence regarding the association between occupational pesticide exposure and neurobehavioral changes in farm workers in China. However, further research is needed to explore the relationship further and to determine the relative contribution of low level versus high level exposure and ascertain whether particular pesticide classes are more harmful than others. It is also important to investigate whether health and safety training and the use of personal protective clothing would reduce the incidence of pesticide poisoning in Chinese farm workers.

Conflict of interest

None.

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