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RESEARCH ARTICLE

Chlorpyrifos and other pesticide exposure and suspected developmental delay in children aged under 5 years: a case-control study in Phitsanulok, Thailand [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract
Background: Developmental delay among children under 5 years of age is a serious global public health problem and much research has been carried out to find potential causes. Pesticides - especially organophosphates - are suspected to be one of the main causes of the problem. This study aimed to investigate the association between pesticide use by the mother during pregnancy and preschool children development using a case-control study.
Methods: Data on prenatal and postnatal pesticide exposure of 442 children with suspected developmental delay, and 413 controls with normal development were included for analysis. The children were matched for gender, age, and residency. Data on pesticide exposure were collected via interview with the mother, and data on pregnancy outcomes abstracted from hospital records.
Results: Chlorpyrifos exposure significantly increased the risk of developmental delay with an odds ratio (OR) of 3.71 (95% CI 1.03-13.36) for ever use of the pesticide, and an OR of 5.92 (95% CI 1.01-34.68) for postnatal exposure (p <0.05). Some other pesticides also had a positive association with developmental delay but none were

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statistically significant ($p < 0.05$). Those pesticides were insecticide, fungicide, herbicide, and molluscicide. Individual pesticides with a positive association were glyphosate, paraquat, butachlor, methyl parathion (pholidon), savin, methomyl, endosulfan, carbosulfan, methamidophos, monochrotofos, mancozeb, and bordeaumixture.
Conclusions: This case-control study found that chlorpyrifos and some other pesticide exposure during pregnancy was positively associated with developmental delay in children aged under 5 years. Further research should be conducted to better understand this potential effect of pesticides on child neurodevelopment, and the public - especially those who plan to have families - should be informed.

Keywords
Developmental disorder, child developmental delay, neurodevelopmental toxicity, pesticides neurotoxicity, chlorpyrifos

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Abbreviations

ADHD, attention deficit and hyperactivity disorder; ASD, autism spectrum disorders; CPF, chlorpyrifos; DAP, dialkylphosphate metabolites; DD, developmental delay; IQ, intelligence quotient; OP, organophosphate; Ever, either prenatal exposure or postnatal exposure; PostN, postnatal exposure; PreN, prenatal exposure; SDD, suspected developmental delay.

Introduction

Developmental delay in young children is a global public health concern. A study of 35 low- and middle-income countries reported that one in every three children below five years of age fails to reach their developmental potential[1]. In Thailand, a national survey by the Ministry of Public Health reported that approximately 15% of children aged under 5 years are suspected to have a developmental delay (SDD)[2]. In addition to stunting, inadequate cognitive stimulation, iodine and iron deficiency, malaria, intrauterine growth restrictions, maternal depression, exposure to violence[3], exposure to environmental toxicants including phthalates, bisphenol A, flame retardants, polycyclic aromatic hydrocarbon (PAHs), gas cooking[4], and heavy metals[3].

Pesticides of the acetylcholinesterase inhibitor group are another set of compounds suspected to affect neurodevelopment. In laboratory studies, this type of pesticide has been found to affect neuron cell and synaptic functions[5]. Young children are also at a higher risk of pesticide effects because their bodies are not yet fully developed, and they also have a higher chance of exposure to environmental pesticides from engagement in high-risk behaviors, *e.g.* crawling on the floor, object-to-mouth behaviors, and playing with items found in the environment[6]. A recent literature review indicated that 45 out of a total of 50 articles found a positive association between delayed neurodevelopment in young children and OP exposure[7]. The neurological and behavioral developmental outcomes induced by pesticides include slower neonatal reflexes, delayed psychomotor and mental development[8], attention deficit[9], lower IQ[10], and autism spectrum disorder (ASD)[11,12].

Regarding individual pesticides, chlorpyrifos (CPF) is the only OP pesticide that has been extensively studied, with most studies finding a positive association between exposure to CPF and child developmental delay. In a study among inter-city minority communities in New York City, researchers reported a positive association between levels of CPF in umbilical cord plasma and neurodevelopmental delay[13]. Studies have also linked CPF exposure to poorer outcomes in working memory, visual motor coordination, color discrimination[14], and verbal comprehension[15], in addition to vision and hearing loss[16], and lower IQ[17]. There is limited evidence that CPF exposure might also relate to ASD[18]. On the other hand, a study of 2-year-old Mexican-American children found no association between cognitive function and CPF exposure[19], meaning this link is not yet conclusive.

Currently, in Thailand, the effects of CPF on child development have not been adequately studied. The objective of this case-control study was to analyze the association between suspected developmental delay (SDD) in children aged under 5 years living in Phitsanulok province, Thailand, and the use of CPF and other compounds during pregnancy. The results may be useful for SDD prevention, and for comparison to other similar studies in this field.

Methods

Study area

Phitsanulok province is in lower northern Thailand, located 370 km from Bangkok. It is a midsize province of 4,176 square miles, with nine districts, and a population of 866,891 people (density = 200 people per square mile). The capital city of the province is Muang district.

Study participants

This study used a case-control design. Children diagnosed with suspected developmental delay (SDD) (cases) were compared with normal children (controls) with respect to pesticides exposure of the mother during pregnancy. The cases were children aged under 5 years who had participated in the National Child Developmental Screening Program (DSPM), and had been assigned as SDD. In Thailand, under the DSPM, every child is screened for development progress at the ages of 9, 18, 30, and 42 months using a modified Denver Development Screening Test II (DDST-II). The screening is carried out by a trained nurse or health personnel at a health promoting hospital. In accordance with the DSPM manual, the children are evaluated in five skills, namely 1) gross motor skills), 2) fine motor skills, 3) receptive language skills, 4) expressive language skills, and 5) personal and social skills. If a child fails one or more of these skills, they are classified as having SDD. Children classified as having SDD were the target population in this study and were randomly selected to take part. The controls were children who attended the same hospital for the screening program but passed all five skills and were therefore classified as having normal development. The case and control groups were matched for gender, age, and area residence at assessment. Children with congenital anomalies or head trauma were excluded from the study.

Sampling and sample size

Participants were children who participated in the screening program in selected local hospitals in the Bang Rakam and Muang districts of Phitsanulok province, Thailand. These two of the nine districts in the province were purposively selected to represent a rural area (Bang Rakam district) and an urban area (Muang district) of the province. A total of 15 out of 21 local hospitals in the Bang Rakam district, and 10 out of 30 hospitals in the Muang district were randomly chosen using a simple lottery method. The mothers of every child with SDD who met the inclusion criteria from the selected hospitals were invited to take part in the study at their appointment. For each case, a child with normal development was randomly selected from the hospital database matching for gender, age, and area residence.

The sample size was calculated to be 816 (408 cases and 408 controls) using OpenEpi online using the following assumptions: confident interval = 95%, power of detection = 80%, ratio of case to control = 1:1, proportion of control with exposure = 40, odds ratio = 1.5[20].

Questionnaire and data collection

Data on pesticide use and exposure during pregnancy was collected from the child's mother using a constructed questionnaire (provided as Extended data in English)[21]. Besides demographic data, the children's mothers were asked "yes" or "no" questions concerning prenatal and postnatal use of pesticides. The exposure period was classified as "ever" for any prenatal and/or postnatal exposure to pesticides, "prenatal" for prenatal exposure to pesticides, and "postnatal" for postnatal exposure to pesticides. Pesticides were categorized into insecticides, herbicides, fungicides, rodenticides, and molluscicides. Exposure data for 14 individual compounds that are commonly used in Thailand and around the world were also collected. There were also questions for potential confounding factors such as occupation, monthly income, education, cigarette smoking, and alcohol use of the mother. Data on health status and pregnancy outcomes including delivery method, gestation, birth order, birth weight, and breast-feeding history, were retrieved from the hospitals' medical records. Data collection took place in the participants' homes, and was conducted between January and May, 2019. Data were collected by 60 village health volunteers who were trained to use the questionnaire and conduct the interviews.

The questionnaire was constructed by literature reviewed. The content validity of the questionnaire was tested by three experts in pediatric, obstetrics and gynecology and family medicine, and occupational health nursing. The index of Item Objective Congruence (IOC) was between 0.67–1.00. The questions were also tested for sequencing and understanding with a group of 30 women with similar characteristics of the intended participants.

Data analysis

Demographic characteristics were analyzed with descriptive statistics and the results presented as frequency, percentage, mean, and standard deviation. Differences between groups were compared via t-test for continuous variables, and chi-square test for categorical data. The association between developmental delay and pesticide exposure was analyzed using multivariable logistic regression with odds ratios (OR) and a 95 percent confidence interval (CI) adjusted for mother age when pregnant (continuous), education (no school, primary school, secondary school, college degree), occupation (farmer, own business, civil servant, employee [formal], employee [general work], housewife, retired, unemployed), income (<5000 baht, 5000–9999, 10000–14999, 15000 or more), chronic disease (yes, no), alcohol consumption (yes, no), gestation (<37 weeks, 37 or more weeks), birth order (1, 2, 3 or more), delivery method (vaginal delivery, caesarean section, assisted delivery), baby weight (<2500 grams, 2500 grams or more), and breast-feeding (yes, no). Data was analyzed using IBM SPSS (version 26) software. Statistical significance was set at $p < 0.05$ (2-tailed test).

Ethical considerations

Ethical approval for this study was obtained from Naresuan University Institutional Review Board (Approval number 448/2019). Written informed consent to participate in the study and for attaining of their clinical details was obtained from the parents of the patients before data collection.

Results

Demographic data

From the dataset of 858 individuals, 855 records (413 cases, 442 controls) were used in the data analysis. Three records were not included for analysis because important information such as gender and age, was missing. Demographic data of the participants is shown in Table 1 and in the Underlying data[22]. Most of the mothers were in the youngest age group with an average age of about 25 years. Most of them finished secondary school and had a monthly income of about 10,000 Thai Baht (300 USD) which is the minimum wage for Thailand. Only about 10% of them were farmers, and therefore reported using pesticides. There was a significantly higher proportion of mothers working as private employees, housewives, and civil servants. Most of the participants were healthy and had never drunk alcohol. One participant reported smoking cigarettes, and thus, was excluded from the data analysis. Data from the child's medical records revealed that most of them, with the same proportion of case to control were born with spontaneous vaginal delivery (n=303, 69.5% and n=274, 67.5%, respectively). Compared to the control group, there were a higher proportion of cases born preterm and being the second or third child of the family. There was also a significant difference in birth weight and breastfeeding between groups. Although a higher percentage of cases had a birth weight of below 2,500 grams (n=64, 14.5% vs n=31, 7.6%, respectively), a lower percentage of them were not breast fed (n=25, 7.7% vs n=43, 12.8%, respectively).

Environmental pesticide exposure

Roughly half of the participants had lived in the community for more than 10 years. With an equal proportion in the case and control groups, about 35% of participants had a family member working on a farm, 20% often entered farmland, and 14% stored pesticides in the house (Table 1), yet around 70% lived within 1.0 km of farm land.

Association of pesticide use and developmental delay

There were only 47 (10.4%) case mothers and 46 (11.4%) control mothers who reported ever using any pesticides during pregnancy. Table 2 presented odds ratio of SDD by types of pesticides the children were exposed to during pregnancy. Types of pesticides and exposure periods that were positively associated with SDD were insecticides (PostN), fungicides (PreN), fungicides (PostN), herbicides (PostN), and molluscicides (PostN). However, none of these ORs were statistically significant ($p < 0.05$).

Of 14 individual pesticides, exposure to CPF during pregnancy was significantly associated with child developmental delay. The associated odds ratio was significant for CPF (Ever) (OR = 3.71, 95% CI 1.03-13.36), and CPF (PostN) (OR = 5.92, 95% CI 1.01-34.68) (Table 3). Risk of SDD were also increased with exposure to some other pesticides, including glyphosate(PostN), paraquat(PostN), butachlor (PostN), Methyl parathion/Pholidon(PostN), savin(PreN), savin (PostN),

Table 1. Demographic characteristics and some exposure factors of the study participants.

Characteristic	Control	Case	P-value
Child gender (n = 855)	n = 413	n = 442	0.916
Boy	222 (53.8)	236 (53.4)	
Girl	191 (46.2)	206 (46.6)	
Age of child at assessment, months (n = 855)	n = 413	n = 442	0.982
9	89 (21.5)	99 (22.4)	
18	112 (27.1)	121 (27.4)	
30	122 (29.5)	130 (29.4)	
42	90 (21.8)	92 (20.8)	
Mother characteristic			
Mother age when pregnant, years (n = 820)	n = 402	n = 418	0.228
≤25	225 (56.0)	210 (50.2)	
26–30	82 (20.4)	101 (24.2)	
31–35	62 (15.4)	61 (14.6)	
≥36	33 (8.2)	46 (11.0)	
Mean ± SD	25.36 ± 6.51	26.17 ± 6.72	0.080
Range	13–42	13–46	
Education of mother (n = 847)	n = 407	n = 440	0.883
no school	10 (2.5)	14 (3.2)	
primary school	59 (14.5)	66 (15.0)	
secondary school	292 (71.7)	315 (71.6)	
college degree	46 (11.3)	45 (10.2)	
Occupation of mother (n = 845)	n = 407	n = 438	0.014*
farmer	39 (9.6)	47 (10.7)	
own business	79 (19.4)	60 (13.7)	
civil servant	23 (5.7)	12 (2.7)	
Employee (formal)	59 (14.5)	77 (17.6)	
Employee (general work)	160 (39.3)	168 (38.4)	
housewife/ retired / unemployed	47 (11.5)	74 (16.9)	
Income of mother, baht (n = 813)	n = 394	n = 419	0.490
<5,000	84 (21.3)	93 (22.2)	
5,000–9,999	155 (39.3)	163 (38.9)	
10,000–14,999	74 (18.8)	64 (15.3)	
15,000 or more	81 (20.6)	99 (23.6)	
Mean ± SD	9854 ± 7352	10405 ± 8504	0.323

Characteristic	Control	Case	P-value
Cigarette smoking of mother (n = 856)	n = 414	n = 442	1.000
Never smoke	413 (99.8)	441 (99.8)	
Current smoker	1 (0.2)	1(0.2)	
Alcohol consumption of mother (n = 855)	n = 413	n = 442	0.039*
Never drink	400 (96.9)	435 (98.4)	
Used to drink	6 (1.5)	0 (0)	
Currently drink	7 (1.7)	7 (1.6)	
Mother having chronic disease (n = 844)	n = 409	n = 435	0.197
No	383 (93.6)	397 (91.3)	
Yes	26 (6.4)	38 (8.7)	
Child pregnancy/birth outcome			
Child gestation period, week (n = 825)	n = 398	n = 427	0.599
37 or more	351 (88.2)	371 (86.9)	
<37	47 (11.8)	56 (13.1)	
Birth order (n = 847)	n = 410	n = 437	0.023*
1	237 (57.8)	212 (48.5)	
2	125 (30.5)	158 (36.2)	
3 or more	48 (11.7)	67 (15.3)	
Delivery methods (n = 842)			0.288
vaginal delivery	274 (67.5)	303 (69.5)	
Caesarean section	126 (31.0)	131 (30.0)	
Assisted delivery	6 (1.5)	2 (0.5)	
Birth weight, gram (n = 850)	n = 410	n = 440	0.001*
2,500 or more	379 (92.4)	376 (85.5)	
<2,500	31 (7.6)	64 (14.5)	
Ever breast-feeding (n = 662)	n = 336	n = 326	0.040*
Yes	293 (87.2)	301 (92.3)	
No	43 (12.8)	25 (7.7)	
Pesticide environmental exposure			
Years of residence in the area (n = 850)	n = 412	n = 438	0.124
<5	77 (18.7)	97 (22.1)	
5–10	78 (18.9)	95 (21.7)	
11–20	84 (20.4)	63 (14.4)	
21–30	96 (23.3)	110 (25.1)	
31 or more	77 (18.7)	73 (16.7)	
Having family member working as a farmer (n = 830)	n = 399	n = 431	0.312
Yes	137 (34.3)	163 (37.8)	
No	262 (65.7)	268 (62.2)	

Characteristic	Control	Case	P-value
Distance from farm to home, km (n = 848)	n = 410	n = 438	0.280
<0.1	91 (22.2)	101 (23.1)	
0.1–0.5	111 (27.1)	97 (22.1)	
0.5–1.0	73 (17.8)	78 (17.8)	
2.0–5.0	76 (18.5)	104 (23.7)	
>5.0	59 (14.4)	58 (13.2)	
Frequency of farm enter (n = 855)	n = 413	n = 442	0.277
never	182 (44.1)	210 (47.5)	
<1 time per month	159 (38.5)	147 (33.3)	
>1 time per month	72 (17.4)	85 (19.2)	
Store pesticides in a house (n = 690)	n = 341	n = 349	1.00
Yes	50 (14.7)	51 (14.6)	
No	291 (85.3)	298 (85.4)	

Value expressed as number (percent) or mean ± standard error unless noted otherwise.

* Statistically significant difference with p value <0.05.

Table 2. Types of pesticides exposure of case and control and risk of suspected developmental delay.

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
Pesticide (Ever)				
No	366 (88.6)	395 (89.6)	1.0	1.0
Yes	47 (11.4)	46 (10.4)	1.03 (0.61-1.74)	0.97 (0.51-1.85)
Insecticide (Ever)				
No	373 (90.3)	403 (91.2)	1.0	1.0
Yes	40 (9.7)	39 (8.8)	1.03 (0.58-1.82)	0.97 (0.48-2.00)
Insecticide (PreN)				
No	375 (90.8)	410 (92.8)	1.0	1.0
Yes	38 (9.2)	32 (7.2)	0.89 (0.48-1.64)	0.84 (0.40-1.75)
Insecticide (PostN)				
No	397 (96.1)	421 (95.2)	1.0	1.0
Yes	16 (3.9)	21 (4.8)	1.42 (0.64-3.15)	1.61 (0.66-3.90)
Fungicide (Ever)				
No	389 (94.2)	412 (93.2)		
Yes	24 (5.8)	30 (6.8)	1.55 (0.79-3.05)	1.59 (0.71-3.56)
Fungicide (PreN)				
No	390 (94.4)	416 (94.3)	1.0	1.0
Yes	23 (5.6)	25 (5.7)	1.27 (0.61-2.62)	1.25 (0.54-2.91)

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
Fungicide (PostN)				
No	404 (97.8)	424 (96.1)	1.0	1.0
Yes	9 (2.2)	17 (3.9)	2.29 (0.86-6.11)	2.42 (0.82-7.14)
Herbicide (Ever)				
No	372 (90.1)	404 (91.4)	1.0	1.0
Yes	41 (9.9)	38 (8.6)	0.99 (0.56-1.74)	0.94 (0.48-1.86)
Herbicide (PreN)				
No	375 (90.8)	408 (92.3)	1.0	1.0
Yes	38 (9.2)	34 (7.7)	0.89 (0.49-1.63)	0.87 (0.43-1.73)
Herbicide (PostN)				
No	399 (96.6)	424 (95.9)	1.0	1.0
Yes	14 (3.4)	18 (4.1)	1.24 (0.53-2.92)	1.36 (0.53-3.47)
Rodenticide (Ever)				
No	397 (96.1)	425 (96.4)		
Yes	16 (3.9)	16 (3.6)	1.03 (0.44-2.42)	0.92 (0.36-2.35)
Rodenticide (PreN)				
No	397 (96.1)	427 (96.8)	1.0	1.0
Yes	16 (3.9)	14 (3.2)	0.93 (0.39-2.23)	0.81 (0.31-2.14)
Rodenticide (PostN)				
No	407 (98.5)	433 (98.2)	1.0	1.0
Yes	6 (1.5)	8 (1.8)	1.03 (0.30-3.59)	1.28 (0.34-4.86)
Molluscicide (Ever)				
No	392 (94.9)	420 (95.2)	1.0	1.0
Yes	21 (5.1)	21 (4.8)	0.96 (0.45-2.02)	0.92 (0.39-2.16)
Molluscicide (PreN)				
No	392 (94.9)	423 (95.9)	1.0	1.0
Yes	21 (5.1)	18 (4.1)	0.82 (0.37-1.77)	0.78 (0.32-1.87)
Molluscicide (PostN)				
No	404 (97.8)	429 (97.3)	1.0	1.0
Yes	9 (2.2)	12 (2.7)	1.56 (0.55-4.44)	2.11 (0.66-6.75)

Ever, either prenatal exposure or postnatal exposure; PreN, prenatal exposure; PostN, postnatal exposure.

* Statistically significant with p value <0.05.

**Adjusted for mother age when pregnant (continuous), education (no school, primary school, secondary school, college degree), occupation (farmer, own business, civil servant, employee [formal], employee [general work], housewife/ retired / unemployed, income (<5000 baht, 5000–9999, 10000–14999, 15000 or more), chronic disease (yes, no), alcohol consumption (yes, no), gestation (<37 weeks, 37 or more), birth order (1, 2, 3 or more), delivery method (vaginal delivery, caesarean section, assisted delivery), baby weight (<2500 grams, 2500 grams or more), and breast-feeding (yes, no).

Table 3. Individual pesticide exposure of case and control and risk of suspected developmental delay.

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
Glyphosate				
Glyphosate (Ever)				
No	377 (91.7)	409 (92.5)	1.0	1.0
Yes	34 (8.3)	33 (7.5)	1.07 (0.58-1.97)	0.93 (0.46-1.90)
Glyphosate (PreN)				
No	379 (92.2)	413 (93.4)	1.0	1.0
Yes	32 (7.8)	29 (6.6)	1.02 (0.54-1.94)	0.92 (0.45-1.91)
Glyphosate (PostN)				
No	400 (97.3)	426 (96.4)	1.0	1.0
Yes	11 (2.7)	16 (3.6)	1.25 (0.51-3.07)	1.32 (0.49-3.55)
Paraquat (Ever)				
No	381 (92.7)	417 (94.3)	1.0	1.0
Yes	30 (7.3)	25 (5.7)	1.02 (0.52-2.00)	0.88 (0.40-1.91)
Paraquat (PreN)				
No	383 (93.2)	419 (94.8)	1.0	1.0
Yes	28 (6.8)	23 (5.2)	0.96 (0.47-1.93)	0.85 (0.38-1.88)
Paraquat (PostN)				
No	403 (98.1)	431 (97.7)	1.0	1.0
Yes	8 (1.9)	10 (2.3)	1.44 (0.45-4.60)	1.63 (0.46-5.73)
Butachlor (Ever)				
No	400 (97.3)	435 (98.4)	1.0	1.0
Yes	11 (2.7)	7 (1.6)	0.87 (0.29-2.62)	0.88 (0.27-2.92)
Butachlor (PreN)				
No	400 (97.3)	437 (98.9)	1.0	1.0
Yes	11 (2.7)	5 (1.1)	0.58 (0.17-1.99)	0.58 (0.15-2.18)
Butachlor (PostN)				
No	407 (99.0)	436 (98.6)	1.0	1.0
Yes	4 (1.0)	6 (1.4)	2.06 (0.51-8.31)	2.85 (0.61-13.24)
Methyl parathion/ Pholidon (Ever)				
No	393 (95.4)	426 (96.8)	1.0	1.0
Yes	19 (4.6)	14 (3.2)	0.92 (0.37-2.31)	0.89 (0.32-2.48)
Methyl parathion/ Pholidon (PreN)				
No	394 (95.6)	427 (97.0)	1.0	1.0
Yes	18 (4.4)	13 (3.0)	0.91 (0.35-2.40)	0.95 (0.33-2.76)

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
Methyl parathion/ Pholidon (PostN)				
No	406 (98.5)	431 (98.0)	1.0	1.0
Yes	6 (1.5)	9 (2.0)	1.82 (0.53-6.29)	2.19 (0.57-8.40)
Savin (Ever)				
No	399 (97.3)	429 (97.5)	1.0	1.0
Yes	11 (2.7)	11 (2.5)	1.56 (0.55-4.43)	1.58 (0.50-4.96)
Savin (PreN)				
No	399 (97.3)	429 (97.5)	1.0	1.0
Yes	11 (2.7)	11 (2.5)	1.56 (0.55-4.43)	1.59 (0.51-5.00)
Savin (PostN)				
No	408 (99.3)	433 (98.4)	1.0	1.0
Yes	3 (0.7)	7 (1.6)	2.07 (0.51-8.37)	2.86 (0.62-13.27)
Chlorpyrifos (Ever)				
No	400 (97.3)	428 (97.1)	1.0	1.0
Yes	11 (2.7)	13 (2.9)	2.88 (0.91-9.16)	3.71 (1.03-13.36)*
Chlorpyrifos (PreN)				
No	401 (97.6)	430 (97.5)	1.0	1.0
Yes	10 (2.4)	11 (2.5)	2.34 (0.71-7.69)	2.97 (0.80-11.07)
Chlorpyrifos (PostN)				
No	406 (99.0)	433 (98.2)	1.0	1.0
Yes	4 (1.0)	8 (1.8)	4.18 (0.88-19.84)	5.92 (1.01-34.68)*
Methomyl (Ever)				
No	397 (96.6)	432 (98.0)	1.0	1.0
Yes	14 (3.4)	9 (2.0)	0.64 (0.25-1.68)	0.63 (0.22-1.80)
Methomy l(PreN)				
No	397 (96.6)	433 (98.2)	1.0	1.0
Yes	14 (3.4)	8 (1.8)	0.55 (0.20-1.50)	0.54 (0.18-1.61)
Methomyl (PostN)				
No	408 (99.3)	435 (98.6)	1.0	1.0
Yes	3 (0.7)	6 (1.4)	1.72 (0.41-7.25}	2.52 (0.52-12.23)
Endosulfan (Ever)				
No	394 (95.9)	431 (97.7)	1.0	1.0
Yes	17 (4.1)	10 (2.3)	0.76 (0.31-1.83)	0.65 (0.25-1.73)
Endosulfan (PreN)				
No	394 (95.9)	432 (98.0)	1.0	1.0
Yes	17 (4.1)	9 (2.0)	0.67 (0.27-1.67)	0.58 (0.21-1.56)

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
Endosulfan (PostN)				
No	408 (99.3)	434 (98.4)	1.0	1.0
Yes	3 (0.7)	7 (1.6)	3.64 (0.75-17.68)	5.16 (0.86-31.21)
Carbosulfan (Ever)				
No	401 (97.6)	434 (98.4)	1.0	1.0
Yes	10 (2.4)	7 (1.6)	0.76 (0.26-2.22)	0.78 (0.24-2.52)
Carbosulfan (PreN)				
No	401 (97.6)	436 (98.9)	1.0	1.0
Yes	10 (2.4)	5 (1.1)	0.50 (0.15-1.69)	0.51 (0.14-1.90)
Carbosulfan (PostN)				
No	409 (99.5)	435 (98.6)	1.0	1.0
Yes	2 (0.5)	6 (1.4)	2.58 (0.50-13.42)	4.47 (0.71-28.71)
Methamidophos (Tamaron) (Ever)				
No	107 (99.0)	437 (99.1)	1.0	1.0
Yes	4 (1.0)	4 (0.9)	1.37 (0.30-6.17)	1.98 (0.39-10.37)
Methamidophos (PreN)	407 (99.0)	437 (99.1)	1.0	1.0
No	4 (1.0)	4 (0.9)	1.37 (0.30-6.17)	1.99 (0.38-10.37)
Yes				
Methamidophos (PostN)	409 (99.5)	438 (99.3)	1.0	1.0
No	2 (0.5)	3 (0.7)	1.54 (0.26-9.27)	3.12 (0.42-23.38)
Yes				
Monochrotofos (Ever)				
No	406 (98.8)	438 (99.3)	1.0	1.0
Yes	5 (1.2)	3 (0.7)	1.02 (0.20-5.11)	1.63 (0.29-9.28)
Monochrotofos (PreN)				
No	406 (98.8)	438 (99.3)	1.0	1.0
Yes	5 (1.2)	3 (0.7)	1.02 (0.20-5.11)	1.63 (0.29-9.28)
Monochrotofos (PostN)				
No	408 (99.3)	438 (99.3)	1.0	1.0
Yes	3 (0.7)	3 (0.7)	1.54 (0.26-9.27)	3.12 (0.42-23.38)
DDT (Ever)				
No	397 (96.6)	434 (98.4)	1.0	1.0
Yes	14 (3.4)	7 (1.6)	0.55 (0.20-1.50)	0.44 (0.14-1.33)

Pesticide use	Control	Case	OR (crude)	OR (adjusted)**
DDT (PreN)				
No	398 (96.8)	434 (98.4)	1.0	1.0
Yes	13 (3.2)	7 (1.6)	0.60 (0.22-1.69)	0.53 (0.17-1.64)
DDT (PostN)				
No	408 (99.3)	438 (99.3)	1.0	1.0
Yes	3 (0.7)	3 (0.7)	1.02 (0.20-5.11)	1.42 (0.24-8.44)
Mancozeb (Ever)				
No	404 (98.3)	430 (97.5)	1.0	1.0
Yes	7 (1.7)	11 (2.5)	1.73 (0.62-4.82)	1.86 (0.58-5.89)
Mancozeb (PreN)				
No	404 (98.3)	430 (97.5)	1.0	1.0
Yes	7 (1.7)	11 (2.5)	1.73 (0.62-4.82)	1.86 (0.58-5.89)
Mancozeb (PostN)				
No	409 (99.5)	436 (98.9)	1.0	1.0
Yes	2 (0.5)	5 (1.1)	2.58 (0.50-13.42)	3.94 (0.59-26.19)
Bordeaumixture (Ever)				
No	409 (99.5)	436 (98.9)	1.0	1.0
Yes	2 (0.5)	5 (1.1)	2.58 (0.50-13.42)	4.00 (0.61-26.33)
Bordeaumixture (PreN)				
No	409 (99.5)	436 (98.9)	1.0	1.0
Yes	2 (0.5)	5 (1.1)	2.58 (0.50-13.42)	4.00 (0.61-26.33)
Bordeaumixture (PostN)				
No	409 (99.5)	438 (99.3)	1.0	1.0
Yes	2 (0.5)	3 (0.7)	1.54 (0.26-9.27)	3.12 (0.42-23.38)

Ever, either prenatal exposure or postnatal exposure; PreN, prenatal exposure; PostN, postnatal exposure.

* Statistically significant with p value <0.05.

**Adjusted for mother age when pregnant (continuous), education (no school, primary school, secondary school, college degree), occupation (farmer, own business, civil servant, employee [formal], employee [general work], housewife/ retired / unemployed, income (<5000 baht, 5000–9999, 10000–14999, 15000 or more), chronic disease (yes, no), alcohol consumption (yes, no), gestation (<37 weeks, 37 or more), birth order (1, 2, 3 or more), delivery method (vaginal delivery, caesarean section, assisted delivery), baby weight (<2500 grams, 2500 grams or more), and breast-feeding (yes, no).

methomyl(PostN), endosulfan(PostN), carbosulfan(PostN), methamidophos(PreN), methamidophos(PostN), monochrotofos (PostN), mancozeb(PreN), mancozeb(PostN), bordeaumixture (PreN), and bordeaumixture(PostN); however, none were statistically significant.

Discussion

The results showed CPF exposure during pregnancy and childhood SDD, with an odds ratio of 3.71 (95% CI 1.03-13.36) for ever using the pesticide (either prenatal or postnatal exposure), 2.97 (95% CI 0.80-11.07) for prenatal exposure, and 5.92

(95% CI 1.01-34.68) for postnatal exposure (Table 3). Ever and postnatal exposure were found to be statistically significant. There was also a positive association, though not statistically significant, between SDD and other types of pesticides and individual compounds, including three herbicides [Glyphosate (PostN), Paraquat(PostN)), Butachlor(PostN)], two organophosphate insecticides [Pholidon (methyl parathion) (PostN), Tamaron (methamidophos)(PreN)], three carbamate insecticides [Savin(carbaryl), Methomyl(PostN), Carbosulfan(PostN)], and one organochlorine insecticide [Endosulfan(PostN)], and one fungicide [mancoceb(PreN)]. This is consistent with the literature: in an experimental study, CPF showed an ability to alter neuronal formation and structure in animal and human fetuses[23,24].

The results of epidemiological studies into SDD and pesticides have found a range of outcomes. In a study of Mexican American children aged 6–24 months, prenatal or child exposure to CPF was not associated with mental development, pervasive developmental disorder (a group of disorders characterized by delays in the development of socialization and communication skills), or behavioral problems[19]. However, several other studies have found a positive association between prenatal exposure to CPF and neurodevelopmental problems. A cohort study of three-year-old children from minority communities in New York City, USA, reported a high exposure group (CPF levels of >6.17 pg/g in the mother's plasma) to have a higher proportion of developmental delay, assessed by the psychomotor development index and the mental development index[13]. A more recent study in Costa Rica found 6–9-year-old children with higher CPF exposure to have several neurobehavioral problems, including poorer working memory, visual motor coordination, and color discrimination, as well as parent-reported cognitive problems/inattention, oppositional disorder, and attention deficit hyperactivity disorder[14]. A recent study of 9-month-old Thai infants reported an association between prenatal exposure to CPF and a reduction in grating visual acuity (OR = 0.64, 95% CI -1.22 to 0.06)[16].

One study has also linked prenatal exposure to CPF to lower IQ levels in children[17]. Similarly, a study among children aged 5.9–11.2 years linked prenatal exposure to CPF to brain anomalies[25]. This result has been replicated in a study of an adolescent group[26]. Neurotoxic deficits have also been associated with CPF exposure in high-exposure occupations[27].

For groups of pesticides, most literature has focused on OPs due to their neurological toxic effects. These studies, usually using a cross-sectional or a cohort study design, have found positive correlations between OP metabolite in the mother's urine and neurodevelopmental problems in the child[7,8]. Studies in the USA have reported prenatal exposure to OPs to increase the risk of abnormal reflexes in neonatal children (OR = 2.24, 95% CI 1.55-3.24)[28], and ADHD in male children at age five years (β = 1.3; 95% CI 0.4-2.1)[9]. Living in close proximity to agricultural areas using OPs and other pesticides during pregnancy has also been related to ASD and developmental delay[29]. A study in Taiwan using a case-control study design reported a dose-response relationship between OP metabolites in child urea and ADHD among children aged 4–15 years[11]. Studies have also linked OP, carbamate, and pyrethroid pesticide exposure to lower IQ[10,15,30]. A cohort study in Thailand also found lower motor and cognitive performance (using Bayley Scales of Infant and Toddler Development III [Bayley III]) among five-month old infants prenatally exposed to OP[31].

For other pesticides, data is limited. A study in Costa Rica reported a positive association of prenatal mancozeb exposure and lower social-emotional scores (β per 10-fold increase = -7.4 points [95% CI -15.2 to 0.4][measured by Bayley III]) in one-year-old infants[32]. This is consistent with the present study which also found an elevated risk of SDD among those exposed to mancozeb, with odds ratio of 1.87 (95% CI 0.59-5.93) for prenatal exposure, and OR of 3.97 (95% CI 0.60-26.38) for postnatal exposure (Table 3). A cohort study in Brittany, France, reported a negative association with neurocognitive development of 6-year-old children with prenatal exposure to pyrethroid[33]. A cohort study in 4-year-old children in Greece reported the association between prenatal exposure to the organochlorine compounds and neurodevelopmental effects[34]. A recent study in Indonesia reported a higher risk of small head circumference at birth to antenatal exposure to household non-OP pesticides (OR = -22.1 mm, 95% CI -36.5 to -7.6)[35].

Overall, the literature is limited and inconsistent regarding the critical duration of pesticide exposure developmental effects. In the current study, both prenatal and postnatal exposure was related to an increased risk of SDD (Table 3), yet only postnatal exposure was significant. However, most of the previous studies on the prenatal and postnatal effects of CPF on neurodevelopmental, reported a positive effect only with prenatal exposure[13,16,17,25]. Unfortunately, only a limited number of studies have examined the effects of child neurodevelopment from both prenatal and postnatal exposure. One study that did[36] report a negative association of children's developmental quotients (DQ) - a numerical indicator of a child's growth to maturity across a range of psychosocial competencies - with prenatal exposure to OP but not with postnatal exposure. On the other hand, a cohort study in China found both prenatal and postnatal OP exposure increased the risk of developmental delay especially in the adaptive development (self-care skills), among two-year old boys[37]. In a laboratory study, CPF caused neurobehavioral impairment to a zebrafish when the exposure occurred in either the fertilization stage or embryonic stage[38].

In the current study, there were some limitations that need to be mentioned. First, there was a smaller number of participants who had used pesticides during pregnancy than expected, which limits the power of association between the variables. In addition, it is difficult to study the effect of low-level pesticide exposure on growth and development because the outcomes can be affected by several factors including biological factors (*e.g.* stunting, infections, anemia, IUGR, preterm birth, birth weight, sex of the child, gestational age at delivery), psychosocial factors (*e.g.* inadequate cognitive stimulation, exposure to violence, maternal depression, household dysfunction), and maternal sociodemographic factors (*e.g.* poverty, low education, young age, smoking, drinking alcohol)[39]. There may also have been a problem with recall bias during the interviews where participants may not have recalled or did not know the name of the pesticides they'd used in the past. However, if this did occur, it would have happened equally between groups. It was also very likely that study participants were exposed to pesticides in the environment, and this information bias could lower the strength of the reported association.

Conclusion

This case-control study found a negative association between chlorpyrifos and some other pesticide exposure during pregnancy and preschool child development. This effect was found in both prenatal and postnatal exposure. More research, using a larger sample size, is still needed to identify more individual pesticides which may impact prenatal and postnatal growth and development of children. This potential effect of pesticides on child neurodevelopment should receive more attention by researchers, and the public, especially those who plan to have families, should be informed.

Data availability

Underlying data

Figshare: child developmental delay and pesticide, Thailand. https://doi.org/10.6084/m9.figshare.13238501

This project contains the following underlying data:

- Child developmental delay and pesticide-database.csv (Collected demographic and child development data)
- Data dictionary-child development.docx (Word document containing dictionary for study dataset)

Extended data

Figshare: Questionnaire-child developmental delay and pesticide, https://doi.org/10.6084/m9.figshare.13238507.v2

This project contains the following extended data:

- Questionnaire-child development and pesticide.docx (Study questionnaire in English)
- Questionnaire pesticide and development-Thai.docx (Study questionnaire in Thai)

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✗

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This study aimed to evaluate the association between pesticide use by mothers during pregnancy and preschool children development using a case-control design in Thailand. They concluded that chlorpyrifos exposure during pregnancy was positively associated with developmental delay in children less than 5 years. I have some comments as listed below:

1. The cases of children included in the present study were "suspected developmental delay" rather than "confirmed developmental delay". Therefore, it would severely affect the results.

2. As the authors pointed out, there were only 47 (10.4%) of case mothers and 46 (11.4%) of control mothers reported ever using any pesticide during pregnancy. The case numbers were too small for comparison.

3. There were only 11 (2.7%) case mothers and 13 (2.9%) of control mothers had ever exposed to chlorpyrifos. Therefore, using these very small numbers of exposure to chlorpyrifos to evaluate the risk of developmental delay of children is not adequate at all. The results would severely mislead the readers.

4. The age ranges of mothers were between 13-42 and 13-46 in the control group and case group, respectively. Please re-analyze the mother's age by below 18 (or 20) vs. above 18 (or 20).

5. The only significant variable to developmental delay was the exposure to chlorpyrifos, and other variables were not statistically significantly associated with developmental delay. The authors calculated the crude odds ratios of chlorpyrifos ever exposure was 2.88, prenatal exposure was 2.34, and postnatal exposure was 4.18 as shown in Table 3. However, I found that it should be 1.10 in ever exposure, 1.02 in prenatal exposure, and 1.87 in postnatal exposure. Please recheck all variables' odds ratios carefully.

Is the work clearly and accurately presented and does it cite the current literature?
No

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Rehabilitation medicine; pediatric rehabilitation; developmental delay

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 31 Jan 2021

Chudchawal Juntarawijit, Naresuan University, Phitsanulok, Thailand

Reviewer II

This study aimed to evaluate the association between pesticide use by mothers during pregnancy and preschool children development using a case-control design in Thailand. They concluded that chlorpyrifos exposure during pregnancy was positively associated with developmental delay in children less than 5 years. I have some comments as listed below:

1. The cases of children included in the present study were “suspected developmental delay” rather than “confirmed developmental delay”. Therefore, it would severely affect the results.

We agree with the reviewer that using confirm developmental delay may be the best outcome variable for studying conclusion. However, a number of children with developmental delay in the study area was so small, we have to rely on Suspected Developmental Delay (SDD). SDD is an important outcome variable and it has been used widely either in public health surveys for early identification of the prevalence rate, or to study risk factors. For example, a large study in 8 counties of rural China by Yang *et al.* (2019)[1] published in BMC Pediatrics, use SDD variables to evaluate the

effects of care quality and development of children aged 1-59 months. Another study by Valla and team (2015)[2] also used SDD to study prevalence rates of developmental delay in Norwegian infants.

2. As the authors pointed out, there were only 47 (10.4%) of case mothers and 46 (11.4%) of control mothers reported ever using any pesticide during pregnancy. The case numbers were too small for comparison.

Yes, we admitted that sample size is the study's limitations. The number of mothers ever using pesticides is actually the exposure of interest. In this study, we collected data from about 800 participants, the minimum sample size required for this type of study, under the following assumption: 95% confident interval, power of detection = 80%, case to control = 1:1, odds ratio = 1.5, and control with exposure = 40% (as presented in the methods section). Unfortunately, only about ten percent of the mother had experience using pesticides. The problem was beyond our control, and with some other constraints, we have to report the result as such.

We did not completely agree that the sample size was 'too small'. The issue is rather subjective, and it should depend mainly on the purpose of the study, and the statistics used for the analysis. The problem of using a small sample size is the lack of power of detection and precision. As seen in the study results, OR and other statistical parameters will not be significant. A small sample size will not completely destroy the usefulness of the study if it was analyzed with proper statistics and clearly presented.

According to the following article (DOI: 10.22004/ag.econ.103771), sample size should not be a main concern for logistic regression. Thus, we believed the result is good enough to be presented to research community.

As said by P. Mean. "A small sample size does not mean that your results are "wrong". It means that the data is consistent with a wide range of possible hypotheses." (http://www.pmean.com/11/WideInterval.html)

3. There were only 11 (2.7%) case mothers and 13 (2.9%) of control mothers had ever exposed to chlorpyrifos. Therefore, using these very small numbers of exposure to chlorpyrifos to evaluate the risk of developmental delay of children is not adequate at all. The results would severely mislead the readers.

As mentioned before, the minimum sample size depends on the kind of statistic used for data analysis. If the sample size is too small, the OR obtained was not significant, as seen in many individual pesticides. This confirmed that the study results will not mislead the readers. We do the best to present study results. Data was analyzed with appropriate statistic, and the results were widely discussed. Therefore, readers can justify by themselves the reliability of the results.

It is not uncommon for studies to rely on small number of outcomes or exposure of interest, especially for rare diseases, e.g. developmental delay. For example, a study published in the Environmental Health Perspectives by Lui, *et al*. (2016), also included

only 310 mother-infant pairs when studying the effects of organophosphate exposure and developmentally delayed. In this study, it reported OR between 9.75 (95% CI: 1.28, 73.98, p = 0.028) and 12.00 (95% CI: 1.23, 117.37, p = 0.033), notice a wide confidence interval. This conclusion came from the data nearly all with <10 number of cases in each group of exposure of interest (please see the manuscript and its supplemental materials https://doi.org/10.1289/EHP196).

The following are a list of some other studies that have a small sample size but yet provide useful information:

- **Geetha, B., Sukumar, C., Dhivyadeepa, E. *et al.* Autism in India: a case–control study to understand the association between socio-economic and environmental risk factors. *Acta Neurol Belg* 119, 393–401 (2019). https://doi.org/10.1007/s13760-018-01057-4**
- **Rocha SGMO, Correia LL, Da Cunha AJLA, et al. Zika Virus Infection and Microcephaly: A Case-Control Study in Brazil. *Ann Glob Health*. 2019;85(1):116. Published 2019 Aug 28. doi:10.5334/aogh.2394**
- **El-Baz F., Ismael, NA., and El-Din, SMN. (2011). Risk factors for autism: An Egyptian study. Egyptian Journal of Medical Human Genetics. 12(1). DOI: 10.1016/j.ejmhg.2011.02.011**

4. The age ranges of mothers were between 13-42 and 13-46 in the control group and case group, respectively. Please re-analyze the mother's age by below 18 (or 20) vs. above 18 (or 20).

Thank you for suggestions, it is a good point. At first, we considered the best age to have a healthy baby is between 25 and 35 years of age, and thus using 25 as a cut point. However, actually, there is no scientific data to support the idea. So, we agree and decide to recategorize the age group to be <20, 20-25, 26-30, 31-35, and ≥36 years, as suggested.

5. The only significant variable to developmental delay was the exposure to chlorpyrifos, and other variables were not statistically significantly associated with developmental delay. The authors calculated the crude odds ratios of chlorpyrifos ever exposure was 2.88, prenatal exposure was 2.34, and postnatal exposure was 4.18 as shown in Table 3. However, I found that it should be 1.10 in ever exposure, 1.02 in prenatal exposure, and 1.87 in postnatal exposure. Please recheck all variables' odds ratios carefully.

Thank you so much for your effort to identify the problem. All the data was checked and the error was found only with the crude OR. Data in Table 2 and Table 3 has been revised. Sorry for the mistake.

References:

1. Yang, C., Liu, X., Yang, Y. *et al.* Quality of care and suspected developmental delay among children aged 1–59 months: a cross-sectional study in 8 counties of rural China. *BMC Pediatr* **19,** 41 (2019). https://doi.org/10.1186/s12887-019-1406-x

2. Valla, L., Wentzel-Larsen, T., Hofoss, D. *et al.* Prevalence of suspected developmental delays in early infancy: results from a regional population-based longitudinal study. *BMC Pediatr* **15,** 215 (2015). https://doi.org/10.1186/s12887-015-0528-z

3. de Moura DR, Costa JC, Santos IS, Barros AJD, Matijasevich A, Halpern R, Dumith S, Karam S, Barros FC. Risk factors for suspected developmental delay at age 2 years in a Brazilian birth cohort. *Paediatric and Perinatal Epidemiology* 2010; **24**: 211–221.

Competing Interests: No competing interests were disclosed.

Reviewer Report 18 January 2021

https://doi.org/10.5256/f1000research.30824.r76367

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Zhijun Zhou
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This manuscript reported the association between pesticide exposure of pregnant women and the suspected developmental delay of their children. It is interesting, but the quality of exposure assessment (description) is in question, only the questionnaire was used and there was obvious recall bias. How we can ensure the reality of pesticide exposure history? The authors should add more information on this point.

Besides, several pesticide exposures were reported to have association with suspected developmental delay, what about the biological mechanism? Please add more literature on animal studies to support your results. Currently, only the similar studies were mentioned.

Furthermore, the following minor comments should also be considered:

- The description that *"Three records were not included for analysis because important information such as gender and age, was missing"* in the section of results (Page 4) can't be understood, since you have data of all cases firstly, then select the reference.

- According to the description in the section of Sampling and sample size, all cases, including cases and controls, were from the database of children in the screening program. It is not case-control study, but cross-sectional study.

- About pesticide exposure history - is there data (records) of use of pesticides (types, amounts, use way, etc.) in these areas during the period when the mother were in pregnancy? It is important to use such data to confirm the answer of these mothers, specifically the use stage (pre-N, or post-N).

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Children environmental health; toxicology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 31 Jan 2021

Chudchawal Juntarawijit, Naresuan University, Phitsanulok, Thailand

Reviewer I

1. This manuscript reported the association between pesticide exposure of pregnant women and the suspected developmental delay of their children. It is interesting, but the quality of exposure assessment (description) is in question, only the questionnaire was used and there was obvious recall bias. <u>How we can ensure the reality of pesticide exposure history? The authors should add more information on this point.</u>

The risk of health effects associated with long-term exposure to pesticides is difficult to assess in epidemiologic studies. Direct measurement of exposure is often not feasible in large studies. Also, measurement of a biomarker in blood or urine is costly and represent a short-term exposure. For long-term exposure, using a questionnaire collecting data on duration and intensity of pesticide use might be more appropriate. This practice was found in a large study like Agricultural Health Study in the United

State[1].

It may inappropriate to discuss issue in the paper.

Besides, several pesticide exposures were reported to have association with suspected developmental delay, what about the biological mechanism? Please add more literature on animal studies to support your results. Currently, only the similar studies were mentioned.

Thanks for reminding the point. More information on biological mechanism has been added.

2. The description that *"Three records were not included for analysis because important information such as gender and age, was missing"* in the section of results (Page 4) can't be understood, since you have data of all cases firstly, then select the reference.

The information was missing during data entry. The problems occur with only a few cases thus it should not significantly affect the result.

3. According to the description in the section of Sampling and sample size, all cases, including cases and controls, were from the database of children in the screening program. It is not case-control study, but cross-sectional study.

Yes, all cases were from the same database. However, the study designed is a case-control study because case and control groups were selected based on their disease status (developmental delay). Then, pesticide exposure data in the past of the two groups were collected. If it was a cross-sectional study, all children should have been randomly selected, regardless of their developmental status, and the data on either diseases or exposure should have been collected simultaneously.

4. About pesticide exposure history - is there data (records) of use of pesticides (types, amounts, use way, etc.) in these areas during the period when the mother were in pregnancy? It is important to use such data to confirm the answer of these mothers, specifically the use stage (pre-N, or post-N).

We agree that the data will be useful. Unfortunately, there was no such data in the area, especially data of individual pesticides. As we mentioned before, using a questionnaire may be the best and the only way to collect data on long-term historical exposure of pesticides.

References:

[1] Coble J, Thomas KW, Hines CJ, Hoppin JA, Dosemeci M, Curwin B, Lubin JH, Freeman LEB, Blair A, Sandler DP, Alavanja MCR. An Updated Algorithm for Estimation of Pesticide Exposure Intensity in the Agricultural Health Study. *International Journal of Environmental Research and Public Health*. 2011; 8(12):4608-4622. https://doi.org/10.3390/ijerph8124608

Competing Interests: No competing interests were disclosed.