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Judul : Factors of Organophosphate Pesticide Exposure on School Children in An Agricultural Area, Indonesia

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Title and Abstract

Title Factors of Organophosphate Pesticide Exposure on School Children in An Agricultural Area, Indonesia

Abstract

Organophosphate is widely used in agriculture in Indonesia and contributes to a public health problem. However, the risk factors of organophosphate exposure, particularly in children living in the agricultural area, have not been described. The research aimed to assess the risk factors associated with organophosphate pesticide exposure on school children living in the agricultural area. This work was a cross-sectional study in 2017 with 166 school children were selected by simple random sampling. Structured questionnaires identified risk factors. Organophosphate metabolites detected by using LC-MS/MS. While chi-square and binary logistic tests as statistical analysis ($\alpha=0.05$; 95%CI). In 28.9% of subjects, organophosphate metabolites were detected. Cut the onion leaves ($p=0.002$, OR=3.33, 95% CI:1.55–7.15), the onion, pesticide equipment, or pesticide in their neighbors ($p=0.007$; OR=2.67; 95%CI:1.31–5.46) was associated with organophosphate pesticide exposure. Involvement in agriculture activities and the onion, pesticide equipment, or pesticide in the neighbor.

Indexing

Keywords	organophosphate, metabolites, risk factors, school children, agriculture
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experts and observers in the field of health education, which was established on September 1, 2014. The founder of this organization is the university of Teacher Training Education Institution (LPTK) which organizes public health education, namely Universitas Negeri Semarang, Universitas Negeri Malang and Universitas Negeri Gorontalo. Mutu Agreement No: 75/UN.37.1.6/KS/2018

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2. Revisi 1

Risk factors of organophosphate pesticide exposure on school children in an agricultural area, Indonesia

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Risk factors of organophosphate pesticide exposure on school children in an agricultural area, Indonesia

Abstrak

Organophosphate is widely used in agriculture in Indonesia and contributes to a public health problem. However, the risk factors of organophosphate exposure particularly in children living in the agriculture area have not been described. The research aimed to assess the risk factors associated with organophosphate pesticide exposure on school children living in the agriculture area. This work was across sectional study with 166 school children. Risk factors were identified by structured questionnaires. Organophosphate metabolites detected by using LC-MS/MS. Chi-square and binary logistic tests were employed for statistical analysis ($p < 0.05$; 95%CI). Organophosphate metabolites were detected in 28.9% of subjects. Cut the onion leaves ($p=0.002$, OR=3.33, 95%CI:1.55–7.15) and the presence of the onion, pesticide equipment, or pesticide in their neighbors ($p=0.007$; OR=2.67; 95%CI:1.31–5.46) was associated to organophosphate pesticide exposure. Involvement in agriculture activities and the presence of onion, pesticide equipment, or pesticide in the neighbor are risk factors of organophosphate pesticide exposure on school children.

Keywords: organophosphate, metabolites, risk factors, school children, agriculture

Introduction

The registered pesticides in Indonesia during 2011 to October 2016 tend to increase. The insecticide is the most pesticides which registered compare to other types of pesticides (Ministry of Agriculture Republic of Indonesia, 2016). Organophosphate insecticide used to protect the onion crops from pest (Badrudin and Jazilah, 2013) and may be applied up to three times a week along the growing season (Budiyono et al., 2015).

Besides farmers and farmworkers, 81.3% of children are involved in onion plantation activities (Budiyono et al., 2015). Unfortunately, assistance in the informal

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occupational sector may also increase the risk of pesticide exposure in these children(Gamlin et al., 2007).

The children may be exposed to pesticides in several ways, including their diet, behaviors, and other factors(Quirós-alcalá et al., 2011).Children who live in agricultural communities may be exposed to pesticides from nearby agriculture fields and parents bring the contaminants to home(Bradman et al., 2011; Curl et al., 2002).The mechanism of take-home exposure contributes to children's pesticide contamination(Curl et al., 2002; Simcox et al., 1995).

Children are more vulnerable than adults to pesticide exposure(Landrigan et al., 1999; Perkins et al., 2016). A previous study revealed that the children who live in agricultural communities have higher organophosphate metabolite levels in the urine(Arcury et al., 2007; Lu et al., 2000; Roberts and Karr, 2012).

Dialkyl phosphate (DAP) metabolites in urine are widely used to analyze the organophosphate pesticide exposure in humans(Bradman et al., 2013; De Alwis et al., 2008). The previous study revealed that DAP metabolites could be detected in 31.25% (Budiyono et al., 2015). However, the risk factors for pesticide exposure in school children remain unclear. Thus, this work seeks to determine which risk factors are related to organophosphate pesticide exposure in school children living in the agriculture area.

Methods

Study design and population

The research involved a cross sectional design and required interviews and laboratory analyses. The population of school children in elementary school is 1017 school children which were distributed at 4 elementary schools in Dukuhlo and Luwungragi

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villages the district of Brebes, Indonesia. A total of 166 subjects in 4th–6th-grade children of elementary schools were selected by simple random sampling.

Interviews and observations were conducted by a well-trained surveyor. The variables of interest during the interviews were demographic characteristics, parent's occupation, play around the onion farm, buy pesticides, formulate pesticides, spray pesticides, wash off the application equipment or parent's clothes after pesticide application, carry of the onion harvest to other places (e.g., at home, drying process, and processing unit), seek of the remains of onion harvest in the processing unit, cut off the onion leaves, use of the pesticide container as toys, use of anti-mosquito repellents at home, presence of pesticide or pesticide application equipment at home, presence of onion harvest at the house, presence of the onion harvest or pesticides or application equipment at the neighbors' house, and distance of the home from the onion farm (500 m).

Urine collection

Subjects were asked to collect first morning void or a spot urine samples (Hoppin et al., 2006; Kissel et al., 2005; Oates et al., 2014). The method is simple compared to the 24-hour urine samples. As well, vein-puncture tends to reduce participation rates (Wessels et al., 2003) and pesticide levels are usually higher in urine than in blood (Barr et al., 2004). Spot urine samples is a reliable sample method, and according to (Bradman et al., 2013) that DAP metabolites in single or multiple spot samples are strongly correlated with levels in same-day 24-hr samples. As much as 50–100 ml of urine was collected from each subject, after which the voids were sealed and put into a plastic bag. Plastic bags containing a void of urine were brought to school by the children and checked for

volume. The urine sample was placed in a urine specimen void, sealed and put back into the plastic bag. They were stored in an icebox (4°C, no preservative agent) (Attfield et al., 2014; Barr et al., 2004; Hoppin et al., 2006) and transported (<24 hours) to the laboratory.

Pesticide metabolite analysis

Examination of the types and levels of organophosphate pesticide metabolites in the urine (ppm) was conducted by high-pressure liquid chromatography-tandem mass spectrophotometry (HPLC–MS/MS (Cartier et al., 2016) AB SCIEX API 4500™) to detect six DAP metabolites (Barr et al., 2004). The LC-MS method offers high sensitivity for all classes of pesticides (Alder et al., 2006; Margariti et al., 2007). The DAP metabolites which were detected in the urine specimens were diethyl dithiophosphate (DEDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), dimethyl phosphate (DMP), and dimethyl thiophosphate (DMTP). (Barr et al., 2004; Bravo et al., 2002; Cartier et al., 2016). The main procedure of analysis organophosphate pesticide metabolites described by Ueyama (Ueyama et al., 2014). Qualitative measurements were conducted by monitoring the ion ratio of two multiple reaction monitoring pairs for each compound. Quantitative determinations were calculated by single-point matrix-based calibration at the reporting limit (RL). The RL is the practical limit of quantification (LOQ); it is measured whenever batch analysis was performed. The precision of the batch analysis was checked, and findings fulfilled the declared laboratory quality control criteria. If a value below the RL, it means the value is out of range the calibration. The RL for DEDTP was 0.050 ppm, that for DEP was 0.100 ppm, that for DETP was 0.100 ppm, that for DMDTP was 0.050 ppm, that for DMP was 0.500 ppm, and that for DMTP was 0.500 ppm. The LOQ is the

lowest level at which the criteria for accuracy and precision are met; it is measured once during validation. The result of the analysis is presented in units of ppm.

Ethics

The study design was approved by the Commission on Health Research Ethics of the Faculty of Public Health at Diponegoro University, Indonesia. Participation in the research was voluntary and written informed consent was obtained.

Statistical analysis

The bivariate analysis used chi-square test was used to identify the selected risk factors (of a p-value ≤ 0.25). Then, the selected risk factors were included in the multivariate analysis used binary logistic test (Li et al., 2014; Sperandei, 2014). The selected variables were involvement in onion plantation activities, carry of the onion harvest to other places (e.g., at home, drying process, and processing unit), seek of the remain onion harvest in the field or processing unit, cut onion leaves, presence of the onion harvest at home, and neighbors storing the onion harvest, pesticides, or pesticide application equipment at their home.

The status of exposure to organophosphate pesticides was based on the presence or absence of six DAP metabolites in the urine of the subjects. If at least one of the six DAP metabolites was observed, the status of organophosphate pesticide exposure was considered positive (present); otherwise negative (absent). The dependent variable (organophosphate pesticide exposure) used in the study is a dummy variable that takes the value of one (1), indicating the presence of organophosphate pesticide metabolites in urine, and null (0), indicating the absence of organophosphate pesticide metabolites in the urine of the subjects. Multivariate analysis was performed to analyze the important risk factors of organophosphate pesticide exposure using the binary logistic test (backward Wald statistic method) at $\alpha = 0.05$. The risk factors were considered Exp. B

or odds ratio (OR), the 95% confidence interval (CI), and $p < 0.05$. All statistical analyses were performed using SPSS statistical software version 20.0 (IBM Corporation, 2011).

Results and Discussion

Demographic characteristics

The district of Brebes is the largest onion-producing district that produces onion in Indonesia. This district also is the biggest pesticides-using district in Indonesia. In table 1, all of the subjects lived in an agricultural area. The proportion of males samples was higher than the female samples. The average age of subjects was 9.9 years (ranged 8–12 years). In terms of the educational level of the parents of the subjects, the majority (82%) of their parents had lower education (no education, no completed elementary school, and completed elementary school). The majority of parents were 60.8% as a small trader, civil servant, and construction worker.

Table 1. Socio-demographic characteristic (n=166)

Variables	n (%)
Sex	
Male	53.6
Female	46.4
Place of living	
Agricultural area	100
Non-agricultural area	0
Job of the parents	
Farmer/farm worker	39.1
Others	60.8
Level of education of the parents	
No education	0.6
No completed elementary school	13.9
Completed elementary school	67.7
Completed junior high school	10.8
Completed senior high school	5.4
Higher education	1.8

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The DAP levels in the urine of the school children

Laboratory tests showed that organophosphate pesticide metabolites in the urine were found in 48 (28.9%) school children. Table 2, the levels of DAP metabolites in 166 subjects ranged from 0 parts per millions (ppm) to 0.223 ppm. The type of DAP metabolites detected in the urine were DEP, DETP, and DMTP. While, the DEDTP, DMDTP, and DMP were not detected in the urine samples.

Table 2. Levels of the six DAP metabolites in the urine of school children

DAP metabolites	Mean \pm SD (ppm)	Min. (ppm)	Max.(ppm)	n = 166 (%)
DEDTP	ND/0 \pm ND/0	ND/0	ND/0	0
DEP	0.00314 \pm 0.0075	ND/0	0.036	19.3
DETP	0.00125 \pm 0.0027	ND/0	0.018	27.1
DMDTP	ND/0 \pm ND/0	ND/0	ND/0	0
DMP	ND/0 \pm ND/0	ND/0	ND/0	0
DMTP	0.00417 \pm 0.0192	ND/0	0.223	26.5

ND=Not detected, SD=standard deviation, ppm=parts per millions

The average levels of DEP, DETP, and DMTP metabolites were higher in females (0.00349 ppm, 0.00126 ppm, and 0.00553 ppm, respectively) than in males (0.00283 ppm, 0.00125 ppm, and 0.00300 ppm, respectively), although no statistically significant different ($p = 0.427$, $p = 0.558$, and $p = 0.328$, respectively) (Table 3).

Table 3. Mean levels of organophosphate metabolites according to sex

Sex	Mean \pm SD (ppm)	p^a
DEP:		
Male	0.00283 \pm 0.00727	0.427
Female	0.00349 \pm 0.00789	
DETP:		
Male	0.00125 \pm 0.00286	0.558
Female	0.00126 \pm 0.00251	
DMTP:		
Male	0.00300 \pm 0.01044	0.328
Female	0.00553 \pm 0.02587	

^a Mann-Whitney

In terms of parental occupation, DEP and DETP levels were higher in school children with parents working as farmers or farm workers (0.00442 ppm and 0.00138 ppm) than in those whose parents had other jobs (0.00232 ppm and 0.00117 ppm). The DMTP levels were lower (0.00335 ppm) in school children with parents as farmers or farm workers than in children whose parents had other jobs (0.00470 ppm). However, no statistically significant difference in average levels of DEP, DETP, and DMTP based on job classification of parents ($p = 0.138$, $p = 0.548$, and $p = 0.652$, respectively) (Table 4).

Table 4. Mean levels of organophosphate metabolites according to job of the parents

job of the parents	Mean±SD (ppm)	p^a
DEP:		
Farmer/farm workers	0.00442±0.00922	0.138
Others	0.00232±0.00615	
DETP:		
Farmer/farm workers	0.00138±0.00264	0.548
Others	0.00117±0.00274	
DMTP:		
Farmer/farm workers	0.00335±0.01089	0.652
Others	0.00470±0.02303	

^a Mann-Whitney

The risk factors of the organophosphate pesticide exposure on school children

Table 5 showed the potential risk factors of the organophosphate pesticide exposure to children. Five variables to be involved in the binary logistic model (p -value < 0.25). The variables were carried onion harvest to other places; seek of the remain onion harvest in the field of the processing unit; cut the onion leaves; the presence of onion harvest at their house; the presence of onion, pesticide, or spray equipment at neighbor's house.

Table 5. Potential risk factors of organophosphate (OP) pesticide exposure in the school children

Potential risk factors	OP Pesticide Exposure		p-value	OR	95%CI	
	n=48, %Yes	n=118, %No			Lower	Upper
Parent's job						
Farmer/farm worker	20 (30.8)	45 (69.2)	0.805	1.11	0.68	1.79
Others	28 (27.7)	73 (72.3)				
Played at the farm or processing unit						
Yes	40 (30.1)	93 (69.9)	0.655	1.24	0.64	2.39
No	8 (24.2)	25 (75.8)				
Bought pesticides						
Yes	6 (26.1)	17 (73.9)	0.941	0.89	0.43	1.85
No	42 (29.4)	101 (70.6)				
Formulated pesticides						
Yes	0 (0.0)	4 (100.0)	0.464	-	-	-
No	48 (29.6)	114 (70.4)				
Sprayed pesticides						
Yes	2 (28.6)	5 (71.4)	1.000	0.99	0.29	3.26
No	46 (28.9)	113 (71.1)				
Washed the clothes or spraying equipment						
Yes	5 (35.7)	9 (64.3)	0.781	1.26	0.59	2.66
No	43 (28.3)	109 (71.7)				
Carried onion harvest to other places						
Yes	21 (43.8)	27 (56.2)	0.012*	1.91	1.21	3.03
No	27 (22.9)	91 (77.1)				
Seek of the remain onion harvest in the field or processing unit						
Yes	27 (39.1)	42 (60.9)	0.023*	1.81	1.12	2.92
No	21 (21.6)	76 (78.4)				
Cut the onion leaves						
Yes	36 (39.1)	56 (60.9)	0.002*	2.41	1.35	4.29
No	12 (16.2)	62 (83.8)				
Used pesticide containers as toys						
Yes	11 (32.4)	23 (67.6)	0.777	1.15	0.66	2.01
No	37 (28.0)	95 (72.0)				
Used anti-mosquitos						
Yes	39 (27.5)	103 (72.5)	0.448	0.73	0.41	1.31
No	9 (37.5)	15 (62.5)				
Presence pesticides, spray equipment at home						
Yes	18 (35.3)	33 (64.7)	0.307	1.35	0.83	1.29
No	30 (26.1)	85 (73.9)				
Presence of onion harvest at home						
Yes	19 (38.8)	30 (61.2)	0.104	1.56	0.97	2.51

No	29 (24.8)	88 (75.2)				
Presence of onion harvest, pesticide, or spray equipment at neighbor's home						
Yes	29 (40.3)	43 (59.7)	0.008*	1.99	1.22	3.25
No	19 (20.2)	75 (79.8)				
Distance from home to onion farm						
500 m	26 (28.3)	66 (71.7)	0.972	0.95	0.59	1.53
> 500 m	22 (29.7)	52 (70.3)				

The final results of the binary logistic analysis are shown in Table 6. Two risk factors of organophosphate pesticide exposure to the school children included cut the onion leaves ($p = 0.002$, OR = 3.33, 95% CI 1.55–7.15) and the presence of onion harvest, pesticides, or pesticide application equipment at the neighbors ($p = 0.007$, OR = 2.67, 95% CI 1.31–5.46).

Table 6. Risk factors of the organophosphate pesticide exposure in school children

Risk factors	B	p-value	Exp. (B)	95%CI for Exp. (B)	
				Lower	Upper
Cut the onion leaves	1.205	0.002	3.33	1.55	7.15
The presence of the onion harvest, pesticides, or pesticide application equipment at the neighbours	0.984	0.007	2.67	1.31	5.46
Constant	-2.121	0.000	0.120		

Organophosphate pesticide metabolites in school children

We detected DAP metabolites in the urine of school children. The DAP metabolites in the urine result from the metabolism of organophosphate pesticides (Bravo et al., 2002). The pesticides (Solomon et al., 2014) were absorbed through the lung (70%), oral (37%), skin (4%), distributed (K o-w) in fat (435: 1), brain (33: 1), liver (22: 1), kidneys (10: 1) (Eaton et al., 2008) and undergoing biotransformation (Tang et al., 2001). The DAP metabolites are widely used to assess organophosphate pesticides exposure to humans (De Alwis et al., 2008), usually via chromatography which presents high accuracy and precision (Bravo et al., 2002) and the LC-MS / MS method is a satisfactory

approach to detect of a low level of DAP metabolites in urine(De Alwis et al., 2008; Margariti et al., 2007). The DEP, DETP, and DMTP were detected in the urine of subjects, similar to the previous study(Budiyono et al., 2015). This chemical compound has been widely detected in farms, and continuous exposure of the school children to organophosphate pesticides is possible (Eskenazi et al., 1999) because the metabolites are biomarkers of chronic organophosphate pesticide exposure(Bradman et al., 2013; Kapka-skrzypczak et al., 2011). The metabolites also provide useful information on the effect of cumulative exposure to organophosphate pesticides(Barr et al., 2004).

Children can expose to the organophosphate pesticides at farmland and home or neighbors. Our study revealed that the chlorpyrifos and dimethoate pesticides were identified in the onion farm and at home. These organophosphate pesticides also were confirmed in small pesticide retails in the villages. The farmers or farmworker after sprayed onion plantation usually they left the container in the field, irrigation channel, or the roadside. They also brought the remains pesticides to their home and stored in the kitchen or surround the home.

The DEP and DETP are metabolites of chlorpyrifos pesticide, DMTP is a metabolite of dimethoate or chlorpyrifos-methyl(Bravo et al., 2002; Wessels et al., 2003). DETP and DMTP were the most commonly detected metabolites in the subjects. DETP was detected in 27.1% of the subjects, similar metabolites to a previous finding(Kissel et al., 2005). Organophosphate pesticide exposure to schoolchildren might have come from agriculture pesticide use, a residue of pesticide in house dust (Lu et al., 2000), or dust exposure from agriculture products or pesticides from neighbors(Goldmann, 2004; Suarez-Lopez et al., 2012).

School children may contact pesticides by their activities in agriculture, i.e., cut onion leaves, carry the onion harvest, and seek remains onion harvest in the field that

may contain pesticides. The pesticides then absorbed via skin, inhalation, and digestion(Quirós-alcalá et al., 2011). After absorption into the body, organophosphate pesticides are metabolized(Kumar et al., 2013). The time required for an elimination of the organophosphate pesticides in humans ranges from 2 hours to 41 hours(Egeghy et al., 2011). However, the peak rate of excretion of these metabolites in urine depends on the route of exposure(Griffin et al., 1999; Harnly et al., 2009; Meuling et al., 2005). Another study revealed that peak excretion has been observed to 6–24 hours later or more following dermal exposure in comparison with the oral route(Krieger et al., 2000). Chlorpyrifos takes a longer time (over 120 hours) for excretion(Meuling et al., 2005). The CDC predicted that the half-lives of organophosphate pesticides do not exceed a week in the human body (Centers for Disease Control and Prevention, 2009).

The quality of urine samples was ensured by practicing appropriate sampling and handling techniques. It is because the pesticides are volatile or semi-volatile organic materials and evaporate easily(Reeve, 2002). Urine was placed in sealed labeled voids and brought to the school at 07:00 AM. It was then placed in an icebox of 4 °C (Attfield et al., 2014; Barr et al., 2004; Hoppin et al., 2006). The longer analysis (e.g., 5 days) may be possible by storing the urine sample at 2 °C–8 °C(Delanghe and Speeckaert, 2014). The urine samples were transported to the laboratory within 24 hours to ensure the stability of the sample.

Urine samples of our study were obtained in the rainy and dry seasons. It because the variation in DAP metabolite concentration is influenced by season (Attfield et al., 2014; Quirós-alcalá et al., 2011), even the previous study revealed that metabolites higher in the rainy season than in the sunny season (Lacasaña et al., 2010), while other study indicated higher DAP metabolites in the spring and summer months(Quirós-alcalá et al., 2011). The short half-life of organophosphate pesticides in

urine as biomarkers depend on seasonal measurements, repeated measurements can provide more opportunities to identify risk factors(Attfield et al., 2014; Wessels et al., 2003).

The levels of the pesticide metabolites detected may also be influenced by demographic variations, the DAPs levels of this work did not show significant differences between demographic subgroups. Our findings showed that gender was not significantly associated with a status (presence or absence) of organophosphate pesticide exposure. The subjects were living in the same geographical area there was no different pesticide exposure. Sex and race/ethnicity did not significantly affect DAP levels(Barr et al., 2004; Huen et al., 2012).

The subjects in the study ranged from 8–12 years old. By the age, children 6–11 years of age showed significantly higher DAP levels compared to adults(Barr et al., 2004; Curl et al., 2002; Perkins et al., 2016). It likely children absorb more pesticides from their environment than adults(Landrigan PJ et al 1999). Children are also less able to detoxify their body from organophosphate pesticides, which mean they are more vulnerable to expose pesticide(Garry, 2004).

The findings should be followed up by measuring the health outcome of organophosphate pesticide exposure to children in an agricultural area. Several studies have shown an association with pesticide exposure and health effects. Exposure to organophosphate pesticides may be responsible for endocrine disruption, poor reproductive health in children, developmental abnormalities (Diamanti-Kandarakis et al., 2009; Lacasaña et al., 2010), poorer mental development 51, and the deficit of IQ points among children(Bouchard et al., 2011).

Risk factors of organophosphate pesticides exposure to school children

The children could be exposed to organophosphate pesticides through their involvement in the onion farm activities (cut the onion leaves). The children that were involved in the cut of onion leave had 3.33 times to be exposed to organophosphate pesticides than children that were no involved. Neighbors also had a potential source of pesticide exposure. Play in the neighbor's home also a risk of exposed to pesticides, because there was the presence of pesticides, the pesticide application equipment, or the onion harvest in the neighbors. The pesticides can be transported from neighbors to the children via drift. The children that had the neighbors which stored onion harvests, the application equipment, and pesticides had 2.67 times to be exposed to organophosphate pesticides than children that had no neighbors which stored them.

The residues of pesticides in the onion were approximately several days to a week(Mahugija et al., 2017). The farmers or farm workers sprayed of pesticide on an onion plant more three times a week until several days before harvesting. They conducted the activity for protecting the crops and don't want to take risk of failing harvest. It is because the onion crops may be attacked by many diseases and pests at different growth stages (Mishra et al., 2014). Larvae and adults of the pest live in the leaf sheath and stalk, causing serious damage to the crop(da Silva et al., 2015). Farmers generally use organophosphate pesticides (e.g., chlorpyrifos) to eradicate the pest. Farmers or farm workers used more than 3 types of pesticides as they believe doing so can ensure the successful control of these pests. These behaviors can make insects more resistant to pesticides(Sarwar and Salman, 2015). The more pesticides used the more pesticide residues in the onion plant. The pesticide residues in the onion plant will expose to the children via skin and inhalation when they were in contact with them (i.e., cut the onion leaves with the bared hands and did no use a masker). The activities such as carry the onion harvest seek the remains of onion harvest in the field or the onion

processing unit can produce dust. The dust that may contain pesticides can inhale into the lungs and have potential risks to be exposed to pesticides. Some of the school children involved in agriculture along the onion plantation season and the dust exposure can happen continuously.

Neighbors' home is another source of exposure to pesticides in children. The pesticides came from farmland and onion harvest. The farmers sprayed pesticides 1–5 days before harvesting. It can contaminate the onion crop. Recent studies have shown that the soil in onion crops and onion bulbs contain pesticides (Akan et al., 2013; Aktar et al., 2009; Jamaluddin et al., 2015). The most common pesticides in these materials are dichlorvos, diazinon, chlorpyrifos, and fenitrothion. Farmland is a significant source of pesticide exposure to children (Goldmann, 2004), agricultural pesticides move from the workplace to residential environments through the activities of farmworkers; this mechanism is called take-home pesticide exposure. The take-home exposure pathway contributes to residential pesticide contamination in agricultural homes where young children are present (Curl et al., 2002). People who live in the agriculture community have the potential to expose pesticides. Organophosphate pesticide metabolites have been detected in inhabitants, both farm and non-farm workers, of an agricultural community (Harnly et al., 2009; Huen et al., 2012). Urinary pesticide metabolite levels in the children did not differ across parental occupational categories (Fenske et al., 2002). A total of 39.2% of the 166 of the school children included in this work were farmers or farm workers, and average levels of DEP metabolites were higher in school children with parents as farmers or farm workers than in those whose parents held other jobs. However, the average levels of DETP and DMTP metabolites were lower in school children who had parent farmers or farm workers than in those who did not. Another study revealed that children living with parents that work with agricultural

pesticides or who live in proximity to pesticide-treated farmlands more exposed to pesticides than other children living in the same community(Lu et al., 2000). Because farmworkers are more likely than other workers to be exposed to pesticides. Farmers who mix, load, and spray pesticides can be exposed to pesticides due to spills, splashes, and direct spray contact(Damalas and Koutroubas, 2016). Even the children who do not have parents as farmers or farm workers, the source of pesticide exposure likely from neighbors that stored onion harvest or pesticide spray equipment that may contain pesticides. A lack of knowledge may influence the behaviors (Reeves et al., 2003), and nearly 70% of the parents reported an educational level of elementary school (i.e., completed 6 years of formal education). Lower education may influence their behaviors for application of the pesticides(Jin et al., 2017). Thus, the level of knowledge of farmers or farm workers as regards pesticide handling and personal protective equipment must be elevated.

In general, the farmers store the onion harvest or pesticides or their application equipment in the house, especially in the terrace, in the kitchen, in the living room, and the yard. They stored onion seeds in the kitchen and mix it with pesticides to prevent degradation. The behaviors have the potential to expose pesticides. The onion harvest or onion seeds produce dust, which can be inhaled or absorbed through the skin. Drift from onion seeds or pesticides or their pesticide application equipment in the home presents a possible pathway of exposure. Some studies, for example, found pesticide in fruit, seeds, and leaves and 25% of the samples of onion have been detected chlorpyrifos (2.12 ± 0.10 mg/kg) (Mahugija et al., 2017).

The pesticides and the equipment used to apply them are usually stored in the kitchen, close to the stove and tableware. Previous work revealed that pesticides found in application equipment(Damalas and Eleftherohorinos, 2011). Even, the percentage of

parents that stored pesticides or application equipment in their home was relatively low (30.7%), these practices still puts children at the risk of pesticide exposure through drift. Pesticide storage in the home associated with pesticide levels in the dust(Damalas and Eleftherohorinos, 2011). Household dust containing pesticides is significantly associated with dimethyl DAP levels in child urine(Curwin et al., 2007; Liroy et al., 2000; Shalat et al., 2003).

Anti-mosquito pesticides (e.g., coils, repellents, sprays) used in the home were another source of pesticide exposure in children(Landrigan et al., 1999; Roberts and Reigart, 2013). A large proportion (85.5%) of the subjects' parents reported using anti-mosquito pesticides at home; however, no significant association between this factor and pesticide metabolite levels was observed in the school children ($p = 0.448$). The pesticide that used for anti-mosquitos was a different type of active ingredient with the pesticide metabolites that were detected in the urine of children.

Children can be exposed to pesticides by drift. Pesticide drifts are any airborne movement of pesticides away from the intended target, including droplets, dust, volatilized vapor-phase pesticides, and pesticide-contaminated soil particles(Kegley et al., 2003). The distance of the schools to the onion farm is relatively close (approximately 650 m in average), and children who live or attend school near farmlands are particularly vulnerable to pesticide(Alarcon et al., 2005; Damalas and Koutroubas, 2016; Kegley et al., 2003). Lower organophosphates metabolite levels correlated with increasing distance from farmland(Coronado et al., 2011). In this work, the distance of the home to the agricultural area did not significantly influence pesticide exposure ($p 0.950$). It is likely because the sources of pesticide exposure may not only from onion field 7 but also from home (Damalas and Eleftherohorinos, 2011; Shalat et al., 2003)and their neighbor(Suarez-Lopez et al., 2012). It revealed that neighbors

stored the onion harvest, pesticides, or pesticide application equipment (p 0.007, OR = 2.67) were risk factors of organophosphate pesticide exposure in children.

Children may participate in agricultural work that involves the use of pesticides or come into contact with pesticide-treated foliage (Roberts and Karr, 2012). Children also work during the planting and harvesting seasons to help their parents and neighbors (Nugroho, 2013). Pesticides present in the soil and onion crops evaporate with increases in air temperature (Damalas and Eleftherohorinos, 2011), and increasing air temperature from 5 °C to 25 °C can increase dust production by 10%–30% (Løfstrøm et al., 2013). In this work, children who cut onion leaves were 3.35 times more likely to be exposed to organophosphate pesticides than those who did not (p = 0.002).

The farmers and farm workers (71% of 50 respondents) in the area of study usually spray onion crops with pesticides 1–5 days before harvest (Basuki, 2009). The children's activities could have exposed them to pesticides. Pesticides may exist as residues in the foliage of onions and cutting of the leaves could generate dust. The dust that evaporated and dissipated, which is eventually inhaled by the school children who involved in onion farms activities, especially those who do not wear masks while working. The vapored pesticide and particulate of soil are easy to inhale. Providing education to the parents and the children to prevent pesticide exposure is necessary. Moreover, determine the impact of pesticide exposure on children's health, research on endocrine disorders, and child growth and development in the agricultural communities is also necessary.

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2. Revisi 2

Risk factors of organophosphate pesticide exposure on school children in an agricultural area, Indonesia

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Risk factors of organophosphate pesticide exposure on school children in an agricultural area, Indonesia

Abstract

Organophosphate is widely used in agriculture in Indonesia and contributes to a public health problem. However, the risk factors of organophosphate exposure, particularly in children living in the agriculture area, have not been described. The research aimed to assess the risk factors associated with organophosphate pesticide exposure on school children living in the agriculture area. This work was a cross-sectional study in 2017 with 166 school children were selected by simple random sampling. Structured questionnaires identified risk factors. Organophosphate metabolites detected by using LC-MS/MS. Chi-square and binary logistic tests were employed for statistical analysis ($\alpha=0.05$; 95%CI). Organophosphate metabolites were detected in 28.9% of subjects. Cut the onion leaves ($p=0.002$, OR=3.33, 95% CI:1.55–7.15) and the presence of the onion, pesticide equipment, or pesticide in their neighbors ($p=0.007$; OR=2.67; 95%CI:1.31–5.46) was associated to organophosphate pesticide exposure. Involvement in agriculture activities and the presence of onion, pesticide equipment, or pesticide in the neighbor are risk factors of organophosphate pesticide exposure on school children.

Keywords: organophosphate, metabolites, risk factors, school children, agriculture

Introduction

The registered pesticides in Indonesia during 2011 to October 2016 tend to increase. The insecticide is the most pesticides which registered compare to other types of pesticides (Ministry of Agriculture Republic of Indonesia, 2016). Organophosphate insecticide used to protect the onion crops from pest (Badrudin and Jazilah, 2013) and may be applied up to three times a week along the growing season (Budiyono et al., 2015).

Besides farmers and farmworkers, 81.3% of children are involved in onion plantation activities(Budiyono et al., 2015).Unfortunately, assistance in the informal occupational sector may also increase the risk of pesticide exposure in these children(Gamlin et al., 2007).

The children may be exposed to pesticides in several ways, including their diet, behaviors, and other factors(Quirós-alcalá et al., 2011).Children who live in agricultural communities may be exposed to pesticides from nearby agriculture fields and parents bring the contaminants to home(Bradman et al., 2011; Curl et al., 2002).The mechanism of take-home exposure contributes to children's pesticide contamination(Curl et al., 2002).

Children are more vulnerable than adults to pesticide exposure(Perkins et al., 2016). A previous study revealed that the children who live in agricultural communities have higher organophosphate metabolite levels in the urine(Arcury et al., 2007; Lu et al., 2000; Roberts and Karr, 2012).

Dialkyl phosphate (DAP) metabolites in urine are widely used to analyze the organophosphate pesticide exposure inhumans(Bradman et al., 2013; De Alwis et al., 2008). The previous study revealed that DAP metabolites could be detected in 31.25% (Budiyono et al., 2015). However, the risk factors for pesticide exposure in school children remain unclear. Thus, this work seeks to determine which risk factors are related to organophosphate pesticide exposure in school children living in the agriculture area.

Methods

Study design and population

The research involved a cross sectional design and required interviews and laboratory analyses. The population of school children in elementary school is 1017 school children which were distributed at 4 elementary schools in Dukuhlo and Luwungragi villages the district of Brebes, Indonesia. A total of 166 subjects in 4th–6th-grade children of elementary schools were selected by simple random sampling.

Interviews and observations were conducted by a well-trained surveyor. The variables of interest during the interviews were demographic characteristics, parent's occupation, and potential risk factors of organophosphate (OP) pesticide exposure.

Urine collection

Subjects were asked to collect first morning void or a spot urine samples (Hoppin et al., 2006; Kissel et al., 2005; Oates et al., 2014). Spot urine samples is a reliable sample method, and according to (Bradman et al., 2013) that DAP metabolites in single or multiple spot samples are strongly correlated with levels in same-day 24-hr samples. As much as 50–100 ml of urine was collected from each subject. The urine sample was placed in a urine specimen void, sealed and put back into the plastic bag. They were stored in an icebox (4°C, no preservative agent) (Attfield et al., 2014; Barr et al., 2004; Hoppin et al., 2006) and transported (<24 hours) to the laboratory.

Pesticide metabolite analysis

Examination of the types and levels of organophosphate pesticide metabolites in the urine (ppm) was conducted by high-pressure liquid chromatography-tandem mass spectrophotometry (HPLC–MS/MS (Cartier et al., 2016) AB SCIEX API 4500™) to detect six DAP metabolites (Barr et al., 2004). The LC-MS method offers high sensitivity for all classes of pesticides (Alder et al., 2006; Margariti et al., 2007). The DAP metabolites which were detected in the urine specimens were diethyl dithiophosphate

(DEDTP), diethyl phosphate (DEP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), dimethyl phosphate (DMP), and dimethyl thiophosphate (DMTP). (Barr et al., 2004; Cartier et al., 2016). The result of the analysis is presented in units of ppm.

Ethics

The study design was approved by the Commission on Health Research Ethics of the Faculty of Public Health at Diponegoro University, Indonesia. Participation in the research was voluntary and written informed consent was obtained.

Statistical analysis

The bivariate analysis used chi-square test was used to identify the selected risk factors (of a p-value ≤ 0.25). Then, the selected risk factors were included in the multivariate analysis used binary logistic test (Li et al., 2014; Sperandei, 2014). The risk factors were considered Exp. B or odds ratio (OR), the 95% confidence interval (CI), and $p < 0.05$. All statistical analyses were performed using SPSS statistical software version 20.0 (IBM Corporation, 2011).

Results and Discussion

Demographic characteristics

The district of Brebes is the largest onion-producing district that produces onion in Indonesia. This district also is the biggest pesticides-using district in Indonesia. In table 1, all of the subjects lived in an agricultural area. The proportion of males samples was higher than the female samples. In terms of the educational level of the parents of the subjects, the majority (82%) of their parents had lower education (no education, no completed elementary school, and completed elementary school). The majority of parents were 60.8% as a small trader, civil servant, and construction worker.

Table 1. Socio-demographic characteristic (n=166)

Variables	n (%)
Sex	
Male	53.6
Female	46.4
Place of living	
Agricultural area	100
Non-agricultural area	0
Job of the parents	
Farmer/farm worker	39.1
Others	60.8
Level of education of the parents	
No education	0.6
No completed elementary school	13.9
Completed elementary school	67.7
Completed junior high school	10.8
Completed senior high school	5.4
Higher education	1.8

Source: Primary data, 2017

The DAP levels in the urine of the school children

Laboratory tests showed that organophosphate pesticide metabolites in the urine were found in 48 (28.9%) school children. Table 2, the levels of DAP metabolites in 166 subjects ranged from 0 parts per millions (ppm) to 0.223 ppm. The type of DAP metabolites detected in the urine were DEP, DETP, and DMTP. While, the DEDTP, DMDTP, and DMP were not detected in the urine samples.

Table 2. Levels of the six DAP metabolites in the urine of school children

DAP metabolites	Mean \pm SD (ppm)	Min. (ppm)	Max.(ppm)	n = 166 (%)
DEDTP	ND/0 \pm ND/0	ND/0	ND/0	0
DEP	0.00314 \pm 0.0075	ND/0	0.036	19.3
DETP	0.00125 \pm 0.0027	ND/0	0.018	27.1
DMDTP	ND/0 \pm ND/0	ND/0	ND/0	0
DMP	ND/0 \pm ND/0	ND/0	ND/0	0
DMTP	0.00417 \pm 0.0192	ND/0	0.223	26.5

Source: Primary data, 2017

ND=Not detected, SD=standard deviation, ppm=parts per millions

The average levels of DEP, DETP, and DMTP metabolites were higher in females than in males, although no statistically significant different (Table 3).

Table 3. Mean levels of organophosphate metabolites according to sex

Sex	Mean±SD (ppm)	<i>p</i> ^a
DEP:		
Male	0.00283±0.00727	0.427
Female	0.00349±0.00789	
DETP:		
Male	0.00125±0.00286	0.558
Female	0.00126±0.00251	
DMTP:		
Male	0.00300±0.01044	0.328
Female	0.00553±0.02587	

Source: Primary data, 2017

^a Mann-Whitney

In terms of parental occupation, DEP and DETP levels were higher in school children with parents working as farmers or farm workers than in those whose parents had other jobs. The DMTP levels were lower in school children with parents as farmers or farm workers than in children whose parents had other jobs. However, no statistically significant difference in average levels of DEP, DETP, and DMTP based on job classification of parents (Table 4).

Table 4. Mean levels of organophosphate metabolites according to job of the parents

job of the parents	Mean±SD (ppm)	<i>p</i> ^a
DEP:		
Farmer/farm workers	0.00442±0.00922	0.138
Others	0.00232±0.00615	
DETP:		
Farmer/farm workers	0.00138±0.00264	0.548
Others	0.00117±0.00274	
DMTP:		
Farmer/farm workers	0.00335±0.01089	0.652
Others	0.00470±0.02303	

Source: Primary data, 2017

^a Mann-Whitney

The risk factors of the organophosphate pesticide exposure on school children

Table 5 showed the potential risk factors of the organophosphate pesticide exposure to children. Five variables to be involved in the binary logistic model (p-value < 0.25). The variables were carried onion harvest to other places; seek of the remain onion

harvest in the field of the processing unit; cut the onion leaves; the presence of onion harvest at their house; the presence of onion, pesticide, or spray equipment at neighbor's house.

Table 5. Potential risk factors of organophosphate (OP) pesticide exposure in the school children

Potential risk factors	OP Pesticide Exposure		p-value	OR	95%CI	
	n=48, %Yes	n=118, %No			Lower	Upper
Parent's job						
Farmer/farm worker	20 (30.8)	45 (69.2)	0.805	1.11	0.68	1.79
Others	28 (27.7)	73 (72.3)				
Played at the farm or processing unit						
Yes	40 (30.1)	93 (69.9)	0.655	1.24	0.64	2.39
No	8 (24.2)	25 (75.8)				
Bought pesticides						
Yes	6 (26.1)	17 (73.9)	0.941	0.89	0.43	1.85
No	42 (29.4)	101 (70.6)				
Formulated pesticides						
Yes	0 (0.0)	4 (100.0)	0.464	-	-	-
No	48 (29.6)	114 (70.4)				
Sprayed pesticides						
Yes	2 (28.6)	5 (71.4)	1.000	0.99	0.29	3.26
No	46 (28.9)	113 (71.1)				
Washed the clothes or spraying equipment						
Yes	5 (35.7)	9 (64.3)	0.781	1.26	0.59	2.66
No	43 (28.3)	109 (71.7)				
Carried onion harvest to other places						
Yes	21 (43.8)	27 (56.2)	0.012*	1.91	1.21	3.03
No	27 (22.9)	91 (77.1)				
Seek of the remain onion harvest in the field or processing unit						
Yes	27 (39.1)	42 (60.9)	0.023*	1.81	1.12	2.92
No	21 (21.6)	76 (78.4)				
Cut the onion leaves						
Yes	36 (39.1)	56 (60.9)	0.002*	2.41	1.35	4.29
No	12 (16.2)	62 (83.8)				
Used pesticide containers as toys						
Yes	11 (32.4)	23 (67.6)	0.777	1.15	0.66	2.01
No	37 (28.0)	95 (72.0)				
Used anti-mosquitos						
Yes	39 (27.5)	103 (72.5)	0.448	0.73	0.41	1.31
No	9 (37.5)	15 (62.5)				

Presence pesticides, spray equipment at home						
Yes	18 (35.3)	33 (64.7)	0.307	1.35	0.83	1.29
No	30 (26.1)	85 (73.9)				
Presence of onion harvest at home						
Yes	19 (38.8)	30 (61.2)	0.104	1.56	0.97	2.51
No	29 (24.8)	88 (75.2)				
Presence of onion harvest, pesticide, or spray equipment at neighbor's home						
Yes	29 (40.3)	43 (59.7)	0.008*	1.99	1.22	3.25
No	19 (20.2)	75 (79.8)				
Distance from home to onion farm						
500 m	26 (28.3)	66 (71.7)	0.972	0.95	0.59	1.53
> 500 m	22 (29.7)	52 (70.3)				

Source: Primary data, 2017

The final results of the binary logistic analysis are shown in Table 6. Two risk factors of organophosphate pesticide exposure to the school children included cut the onion leaves ($p = 0.002$, OR = 3.33, 95% CI 1.55–7.15) and the presence of onion harvest, pesticides, or pesticide application equipment at the neighbors ($p = 0.007$, OR = 2.67, 95% CI 1.31–5.46).

Table 6. Risk factors of the organophosphate pesticide exposure in school children

Risk factors	B	p-value	Exp. (B)	95%CI for Exp. (B)	
				Lower	Upper
Cut the onion leaves	1.205	0.002	3.33	1.55	7.15
The presence of the onion harvest, pesticides, or pesticide application equipment at the neighbours	0.984	0.007	2.67	1.31	5.46
Constant	-2.121	0.000	0.120		

Source: Primary data, 2017

Organophosphate pesticide metabolites in school children

We detected DAP metabolites in the urine of school children. The DAP metabolites in the urine result from the metabolism of organophosphate pesticides (Bravo et al., 2002). The pesticides (Solomon et al., 2014) were absorbed through the lung (70%), oral (37%), skin (4%), distributed (K o-w) in fat (435: 1), brain (33: 1), liver (22: 1),

kidneys (10: 1) (Eaton et al., 2008) and undergoing biotransformation (Tang et al., 2001). The DAP metabolites are widely used to assess organophosphate pesticides exposure to humans (De Alwis et al., 2008), usually via chromatography which presents high accuracy and precision (Bravo et al., 2002) and the LC-MS / MS method is a satisfactory approach to detect a low level of DAP metabolites in urine (De Alwis et al., 2008; Margariti et al., 2007). The DEP, DETP, and DMTP were detected in the urine of subjects, similar to the previous study (Budiyono et al., 2015). This chemical compound has been widely detected in farms, and continuous exposure of the school children to organophosphate pesticides is possible (Eskenazi et al., 1999) because the metabolites are biomarkers of chronic organophosphate pesticide exposure (Bradman et al., 2013; Kapka-skrzypczak et al., 2011). The metabolites also provide useful information on the effect of cumulative exposure to organophosphate pesticides (Barr et al., 2004).

Children can expose to the organophosphate pesticides at farmland and home or neighbors. Our study revealed that the chlorpyrifos and dimethoate pesticides were identified in the onion farm and at home. These organophosphate pesticides also were confirmed in small pesticide retailers in the villages. The farmers or farmworker after sprayed onion plantation usually they left the container in the field, irrigation channel, or the roadside. They also brought the remains pesticides to their home and stored in the kitchen or surround the home.

The DEP and DETP are metabolites of chlorpyrifos pesticide, DMTP is a metabolite of dimethoate or chlorpyrifos-methyl (Bravo et al., 2002; Wessels et al., 2003). DETP and DMTP were the most commonly detected metabolites in the subjects. DETP was detected in 27.1% of the subjects, similar metabolites to a previous finding (Kissel et al., 2005). Organophosphate pesticide exposure to school children might have come from agriculture pesticide use, a residue of pesticide in house dust (Lu

et al., 2000), or dust exposure from agriculture products or pesticides from neighbors(Goldmann, 2004; Suarez-Lopez et al., 2012).

School children may contact pesticides by their activities in agriculture, i.e., cut onion leaves, carry the onion harvest, and seek remains onion harvest in the field that may contain pesticides. The pesticides then absorbed via skin, inhalation, and digestion(Quirós-alcalá et al., 2011). After absorption into the body, organophosphate pesticides are metabolized(Kumar et al., 2013). The time required for an elimination of the organophosphate pesticides in humans ranges from 2 hours to 41 hours(Egeghy et al., 2011). However, the peak rate of excretion of these metabolites in urine depends on the route of exposure(Harnly et al., 2009; Meuling et al., 2005). Another study revealed that peak excretion has been observed to 6–24 hours later or more following dermal exposure in comparison with the oral route(Krieger et al., 2000). Chlorpyrifos takes a longer time (over 120 hours) for excretion(Meuling et al., 2005). The CDC predicted that the half-lives of organophosphate pesticides do not exceed a week in the human body (Centers for Disease Control and Prevention, 2009).

Urine samples of our study were obtained in the rainy and dry seasons. It because the variation in DAP metabolite concentration is influenced by season (Attfield et al., 2014; Quirós-alcalá et al., 2011), even the previous study revealed that metabolites higher in the rainy season than in the sunny season (Lacasaña et al., 2010), while other study indicated higher DAP metabolites in the spring and summer months(Quirós-alcalá et al., 2011). The short half-life of organophosphate pesticides in urine as biomarkers depend on seasonal measurements, repeated measurements can provide more opportunities to identify risk factors(Attfield et al., 2014; Wessels et al., 2003).

The levels of the pesticide metabolites detected may also be influenced by demographic variations, the DAPs levels of this work did not show significant differences between demographic subgroups. Our findings showed that gender was not significantly associated with a status (presence or absence) of organophosphate pesticide exposure. The subjects were living in the same geographical area there was no different pesticide exposure. Sex and race/ethnicity did not significantly affect DAP levels(Barr et al., 2004; Huen et al., 2012).

The subjects in the study ranged from 8–12 years old. By the age, children 6–11 years of age showed significantly higher DAP levels compared to adults(Barr et al., 2004; Curl et al., 2002; Perkins et al., 2016). It likely children absorb more pesticides from their environment than adults(Landrigan PJ et al 1999). Children are also less able to detoxify their body from organophosphate pesticides, which mean they are more vulnerable to expose pesticide(Garry, 2004).

Risk factors of organophosphate pesticides exposure to school children

The children could be exposed to organophosphate pesticides through their involvement in the onion farm activities (cut the onion leaves). Neighbors also had a potential source of pesticide exposure. Play in the neighbor's home also a risk of exposed to pesticides, because there was the presence of pesticides, the pesticide application equipment, or the onion harvest in the neighbors.

The residues of pesticides in the onion were approximately several days to a week(Mahugija et al., 2017). The farmers or farm workers sprayed of pesticide on an onion plant more three times a week until several days before harvesting. They conducted the activity for protecting the crops and don't want to take risk of failing harvest. It is because the onion crops may be attacked by many diseases and pests at different growth stages (Mishra et al., 2014). Larvae and adults of the pest live in the

leaf sheath and stalk, causing serious damage to the crop(da Silva et al., 2015). Farmers generally use organophosphate pesticides (e.g., chlorpyrifos) to eradicate the pest. Farmers or farm workers used more than 3 types of pesticides as they believe doing so can ensure the successful control of these pests. These behaviors can make insects more resistant to pesticides(Sarwar and Salman, 2015). The more pesticides used the more pesticide residues in the onion plant. The pesticide residues in the onion plant will expose to the children via skin and inhalation when they were in contact with them (i.e., cut the onion leaves with the bared hands and did no use a masker). The activities such as carry the onion harvest seek the remains of onion harvest in the field or the onion processing unit can produce dust. The dust that may contain pesticides can inhale into the lungs and have potential risks to be exposed to pesticides. Some of the school children involved in agriculture along the onion plantation season and the dust exposure can happen continuously.

Neighbors' home is another source of exposure to pesticides in children. The pesticides came from farmland and onion harvest. The farmers sprayed pesticides 1–5 days before harvesting. It can contaminate the onion crop. Recent studies have shown that the soil in onion crops and onion bulbs contain pesticides(Akan et al., 2013; Aktar et al., 2009; Jamaluddin et al., 2015). The most common pesticides in these materials are dichlorvos, diazinon, chlorpyrifos, and fenitrothion. Farmland is a significant source of pesticide exposure to children (Goldmann, 2004), agricultural pesticides move from the workplace to residential environments through the activities of farmworkers; this mechanism is called take-home pesticide exposure. The take-home exposure pathway contributes to residential pesticide contamination in agricultural homes where young children are present(Curl et al., 2002). People who live in the agriculture community have the potential to expose pesticides. Organophosphate pesticide metabolites have

been detected in inhabitants, both farm and non-farm workers, of an agricultural community(Harnly et al., 2009; Huen et al., 2012). Urinary pesticide metabolite levels in the children did not differ across parental occupational categories(Fenske et al., 2002). Average levels of DEP metabolites were higher in school children with parents as farmers or farm workers than in those whose parents held other jobs. Another study revealed that children living with parents that work with agricultural pesticides or who live in proximity to pesticide-treated farmlands more exposed to pesticides than other children living in the same community(Lu et al., 2000). Because farmworkers are more likely than other workers to be exposed to pesticides. Farmers who mix, load, and spray pesticides can be exposed to pesticides due to spills, splashes, and direct spray contact(Damalas and Koutroubas, 2016). Even the children who do not have parents as farmers or farm workers, the source of pesticide exposure likely from neighbors that stored onion harvest or pesticide spray equipment that may contain pesticides. A lack of knowledge may influence the behaviors (Reeves et al., 2003), and nearly 70% of the parents reported an educational level of elementary school (i.e., completed 6 years of formal education). Lower education may influence their behaviors for application of the pesticides(Jin et al., 2017).

In general, the farmers store the onion harvest or pesticides or their application equipment in the house, especially in the terrace, in the kitchen, in the living room, and the yard. They stored onion seeds in the kitchen and mix it with pesticides to prevent degradation. The behaviors have the potential to expose pesticides. The onion harvest or onion seeds produce dust, which can be inhaled or absorbed through the skin. Drift from onion seeds or pesticides or their pesticide application equipment in the home presents a possible pathway of exposure. Some studies, for example, found pesticide in fruit,

seeds, and leaves and 25% of the samples of onion have been detected chlorpyrifos (2.12 ± 0.10 mg/kg) (Mahugija et al., 2017).

The pesticides and the equipment used to apply them are usually stored in the kitchen, close to the stove and tableware. Previous work revealed that pesticides found in application equipment (Damalas and Eleftherohorinos, 2011). Even, the percentage of parents that stored pesticides or application equipment in their home was relatively low (30.7%), these practices still puts children at the risk of pesticide exposure through drift. Pesticide storage in the home associated with pesticide levels in the dust (Damalas and Eleftherohorinos, 2011). Household dust containing pesticides is significantly associated with dimethyl DAP levels in child urine (Curwin et al., 2007).

Anti-mosquito pesticides (e.g., coils, repellents, sprays) used in the home were another source of pesticide exposure in children (Roberts and Reigart, 2013). A large proportion (85.5%) of the subjects' parents reported using anti-mosquito pesticides at home; however, no significant association between this factor and pesticide metabolite levels was observed in the school children ($p = 0.448$). The pesticide that used for anti-mosquitos was a different type of active ingredient with the pesticide metabolites that were detected in the urine of children.

Children can be exposed to pesticides by drift. Pesticide drifts are any airborne movement of pesticides away from the intended target, including droplets, dust, volatilized vapor-phase pesticides, and pesticide-contaminated soil particles (Kegley et al., 2003). The distance of the schools to the onion farm is relatively close (approximately 650 m in average), and children who live or attend school near farmlands are particularly vulnerable to pesticide (Damalas and Koutroubas, 2016). Lower organophosphates metabolite levels correlated with increasing distance from farmland (Coronado et al., 2011). In this work, the distance of the home to the

agricultural area did not significantly influence pesticide exposure (p 0.950). It is likely because the sources of pesticide exposure may not only from onion field but also from home (Damalas and Eleftherohorinos, 2011; Shalat et al., 2003) and their neighbor (Suarez-Lopez et al., 2012). It revealed that neighbors stored the onion harvest, pesticides, or pesticide application equipment (p 0.007, OR = 2.67) were risk factors of organophosphate pesticide exposure in children.

Children may participate in agricultural work that involves the use of pesticides or come into contact with pesticide-treated foliage (Roberts and Karr, 2012). Children also work during the planting and harvesting seasons to help their parents and neighbors (Nugroho, 2013). Pesticides present in the soil and onion crops evaporate with increases in air temperature (Damalas and Eleftherohorinos, 2011), and increasing air temperature from 5 °C to 25 °C can increase dust production by 10%–30% (Løfstrøm et al., 2013). In this work, children who cut onion leaves were 3.35 times more likely to be exposed to organophosphate pesticides than those who did not (p = 0.002).

The farmers and farm workers (71% of 50 respondents) in the area of study usually spray onion crops with pesticides 1–5 days before harvest (Basuki, 2009). The children's activities could have exposed them to pesticides. Pesticides may exist as residues in the foliage of onions and cutting of the leaves could generate dust. The dust that evaporated and dissipated, which is eventually inhaled by the school children who involved in onion farms activities, especially those who do not wear masks while working. The vapored pesticide and particulate of soil are easy to inhale.

Conclusion

Involvement in agriculture activities and the presence of onion, pesticide equipment, or pesticide in the neighbor are risk factors of organophosphate pesticide exposure on school children.

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Factors of Organophosphate Pesticide Exposure on School Children in An Agricultural Area, Indonesia

Budiyono Budiyono, Suhartono Suhartono, Apoina Kartini



Abstract

Organophosphate is widely used in agriculture in Indonesia and contributes to a public health problem. However, the risk factors of organophosphate exposure, particularly in children living in the agricultural area, have not been described. The research aimed to assess the risk factors associated with organophosphate pesticide exposure on school children living in the agricultural area. This work was a cross-sectional study in 2017 with 166 school children were selected by simple random sampling. Structured questionnaires identified risk factors. Organophosphate metabolites detected by using LC-MS/MS. While chi-square and binary logistic tests as statistical analysis ($\alpha=0.05$; 95%CI). In 28.9% of subjects, organophosphate metabolites were detected. Cut the onion leaves ($p=0.002$, OR=3.33, 95% CI:1.55–7.15), the onion, pesticide equipment, or pesticide in their neighbors ($p=0.007$; OR=2.67; 95%CI:1.31–5.46) was associated with organophosphate pesticide exposure. Involvement in agriculture activities and the onion, pesticide equipment, or pesticide in the neighbor.

Keywords

organophosphate, metabolites, risk factors, school children, agriculture



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