ORIGINAL RESEARCH

Predictors of Detected Organophosphorus Pesticides Among Orang Asli Children Living in Malaysia



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Abstract

BACKGROUND Increasing use of pesticides in agriculture to control pest may result in permanent damage to the environment and consequently cause harmful health problems especially among infant and children. Due to pesticide's natural toxicity and its widespread use, it causes a serious threat to public health especially to this vulnerable group.

OBJECTIVE The purpose of this study was to determine the organophosphorus pesticide urinary metabolite levels and its predictors among Orang Asli children of the Mah Meri tribe living in an agricultural island in Kuala Langat, Selangor.

METHODS Data collection was carried out at an island in Kuala Langat, Selangor, where a total of 180 Orang Asli children of the Mah Meri tribe voluntarily participated in the study. Data were collected via a validated, modified questionnaire. Urinary organophosphate metabolites, namely dimethylphosphate, diethylphosphate, dimethylthiophosphate, dimethyldithiophosphate, diethylthiophosphate, and diethyldithiophosphate were measured to assess organophosphate pesticide exposure in children.

FINDINGS Eighty-four (46.7%) of the respondents were positive for urine dialkyl phosphate metabolites. In multivariable analysis, children who frequently consumed apples had 4 times higher risk of pesticide detection than those who consumed apple less frequently. In addition, those who frequently ate cucumbers had 4 times higher risk for pesticide detection than those who ate cucumbers less frequently. Children with a father whose occupation involved high exposure to pesticides (agriculture) had 3 times higher risk of pesticide detection than those with a father in a low-risk occupation (nonagriculture).

CONCLUSIONS Almost half of the children (46.7%) in the study area tested positive for urinary dialkyl phosphate metabolite levels. Most of the metabolite levels were equal to or higher than that reported in other previous studies. Major factors associated with pesticide detection in children in this study were frequent intake of apple and cucumber and fathers who are working in an agricultural area.

KEY WORDS organophosphorus (OP) pesticides, OP dialkyl phosphate (DAP) metabolites, Orang Asli, Malaysia, Mah Meri, children, predictors

The authors declare no conflicts of interest.

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INTRODUCTION

Pesticides have been widely used in agriculture, as they are often considered a quick, easy, and inexpensive solution to increase crop production and eliminate pests. Pesticides are classified on the basis of the targeted pests, such as herbicides, insecticides, fungicides, rodenticides, and nematicides. Although these chemicals have been banned or, in certain instances, their usage has been limited, strong evidence suggests that the widespread and excessive use of pesticides and their residues in agriculture has a negative impact on the environment and human health.

Increased use of pesticides in farming to control pests results in permanent damage to the environment and causes health problems, especially among vulnerable groups like children. Studies suggest that pesticide exposure may be one of the causes of several diseases, including cancer, as well as neurologic, mental, and reproductive effects, because pesticides are persistent and can accumulate within the food chain.¹ According to the US National Academy of Sciences, concerns about children's exposure to pesticides are valid because the level of exposure to neurotoxic compounds that is believed to be safe for adults could result in permanent loss of brain function if children are exposed during the prenatal and early childhood period of brain development.²

In addition to agricultural workers, populations such as children and women can also be exposed to pesticides via food, water, and air, especially among those living near pesticide exposure, such as in an agricultural area. Pesticide exposures are considered an important environmental threat to children's health, especially in rural and agricultural communities. In rural or agricultural areas, a large variety of chemicals and mixtures are used as pesticides, and children may be exposed to these pesticides from multiple sources because many pesticides are used at the same time. Children may be exposed in different settings by a variety of routes; for example, pesticides may be stored within the reach of children, may be present in food or drinking water, and may remain as residues from the treatment of crops or as a result of contamination.³ Therefore, children may receive high levels of exposure, leading to poisoning, or chronic and low-level exposure, which is linked to more elusive developmental and various other effects.

Among children, diet is another source of exposure to pesticides. Food consumption patterns place children in a special risk category for dietary pesticides, wherein trace quantities of pesticide residues are present on or in both plant and animal products. For children, the principle route of exposure is through ingestion from diet and breast milk.⁴ This finding is concurrent with a study in Washington that demonstrated that the dietary intake of organophosphorus (OP) pesticides represents the major source of exposure in young children.⁵

As children grow, they drink more water and eat more food per body weight than adults. Water and food containing pesticide residues may therefore be a source of chronic and low-level or high-level pesticide exposure. Growing food on or near contaminated soils and using contaminated water on crops or for washing puts children at risk.⁶

OP pesticides are the most commonly used insecticides in the world due to their effectiveness and their lack of persistence in the environment. OP pesticides include malathion, diazinon, chlorpyrifos, parathion, and many others. Once OP pesticides enter the body, they are rapidly metabolized and excreted in urine as dialkyl phosphate (DAP) metabolites. There are 6 DAP metabolites, and they are used to indicate OP pesticide exposure. Because these DAP metabolites do not hold any of the structures unique to the pesticides from which they were derived, it is impossible to identify individual pesticides. However, they can provide information about cumulative exposure to the OP class.⁷

In this study, urinary metabolite levels of OP pesticides provide useful information about cumulative exposure to OP pesticides as a class; however, there is no specific information about the specific pesticides to which a person had been exposed.^{8,9} The purpose of this study was to determine OP pesticide exposure and its predictors among Orang Asli children living in an agricultural island using urinary metabolite levels of OP pesticides as biomarkers.

METHODS

Study Design. This cross-sectional study was approved by the Research and Ethics Committee of the University Kebangsaan Malaysia Medical Centre (UKM 1.5.3.5/244/FF-2014-182/Dr.ZalehaMdIsa) and involved 180 Orang Asli Mah Meri children aged 7 to 12 years living in an island in Kuala Langat, Selangor. All participants in the study were volunteers, met the inclusion criteria, and agreed to provide a urine sample. Written informed consent was obtained from the parents or guardians.

Study Area. The island total area is 16,000 hectares, and more than three-quarters (11,700 hectares) of the area is planted with palm trees. It is also the settlement of the Mah Meri tribe, one of the aborigine tribes in Malaysia.¹⁰ The land was previously a forest consisting mainly of mangroves and later was converted into a plantation. Since the early 1920s, the island has been transformed into a complete agricultural land. Other than a palm oil plantation, there is also an 18-hole golf course located within the plantation that was built in 1968. Study Instruments. Data were collected using a validated, modified questionnaire, including a 24hour dietary recall, an adapted food frequency questionnaire consisting of 131 food items, and 6 OP DAP metabolites, namely dimethylphosphate (DMP), diethylphosphate (DEP), dimethylthiophosphate (DMTP), diethylthiophosphate, dimethyldithiophosphate (DMDTP), and diethyldithiophosphate. Urinary OP levels were detected using gas chromatography-mass spectrometry (Model GC-CP-3800 chromatograph, Model MS-1200L Quadrupole MS/MS [Varian Medical Systems]).

An interview with the parent or guardian was done concurrently with collection of urine samples. Interviews were conducted in the Malay language and were assisted by Tok Batin (head of the village), in which general information, such as the child's sociodemographic characteristics, parents' income and education level, occupation of the parents, length of stay at the current home, health and lifestyle, pesticide use at home, and dietary consumption of fruits and vegetables, was obtained. Daily dietary consumption was measured using 24-hour dietary recall. Parents or guardians were also asked about their children's usual consumption and serving size of food items listed in the food frequency questionnaire.

The food frequency questionnaire consists of 131 food items, in which the frequency of food consumption was classified using a 5-point scale ranging from 5 = daily, 4 = 2-3 times a week, 3 = once a week, 2 = once a month, 1 = never.¹¹ This scale was later divided into 2 categories: frequent intake and less-frequent intake. Scale points of 4 and 5 were categorized under frequent intake, while scale points of 1, 2, and 3 were categorized as less-frequent intake.

Spot urine samples were collected in 60-mL urine specimen containers during the time of interview and were maintained at 4°C (maximum duration of 24 hours) until they reached the Universiti

Kebangsaan Malaysia Medical Centre environmental health laboratory and were then frozen at -20°C. Within 1 month of collection, urine samples were transported to a private laboratory (Chromelab) where they were analysed. Urinary organophosphate metabolites were determined using a gas chromatography-mass spectrometry procedure,¹² which is sensitive enough for assessment of nonoccupational OP exposure. This method provides detection limits that meet lowlevel exposure assessment, such as for nonoccupational exposure to OP pesticides. The limit of detection for the 6 metabolites ranged from 0.1-0.15 ng/ mL, where DMP was 0.1 ng/mL, DEP 0.1 ng/mL, DMTP 0.15 ng/mL, DMDTP 0.1 ng/mL, diethvlthiophosphate 0.1 ng/mL, and diethyldithiophosphate 0.1 ng/mL.

Data Analysis. Data analysis was done using the Statistical Package for Social Sciences Version 20 (IBM Corp). Since the distribution for DMP and DMTP were skewed, nonparametric tests were used for these variables. A statistical probability of .05 and below was considered significant. All urine samples under the limit of detection were assumed to be 0. Statistical analysis was used to examine the association between sociodemographic factors, health, lifestyle and environmental factors, frequency of food intake, and urinary metabolites of OP as the outcome variable.

RESULTS

A total of 189 Orang Asli Mah Meri children participated in this study; however, only 180 children fulfilled the inclusion criteria, while 9 others had incomplete information. Table 1 presents the sociodemographic characteristics of 180 Orang Asli Mah Meri children. The majority (54.4%) of the respondents were boys, and 57.8% of the children were of younger age (7-9 years old). The majority of the mothers (87.2%) and fathers (71.1%) had primary school or lower education; most of the mothers were housewives (80.5%), and a total of 26.1% of fathers worked in the agricultural area. Parents working in the agricultural area were highly exposed to pesticides compared with those who worked in other sectors. The median household income was RM500 per month. Almost half of the parents were smokers (56.1%), and 51.7% had a history of pesticide usage at home. Seventy percent of the children had lived on the island for <10 years.

Table 2 shows the children's urinary DAP metabolite levels; out of 180 respondents, 84

Variable	N = 180	%	Mean	SD	Median	25th Percentile	75th Percentile
Sex							
Male	98	54.4					
Female	82	45.6					
Age							
7-9 y old	104	57.8					
10-12 y old	76	42.2					
Weight (kg)			25.90	9.57			
Height (cm)			124.55	12.3			
Mother's education							
Primary and below	157	87.2					
Secondary and above	23	12.8					
Mother's occupation							
Housewife	145	80.6					
Fisherman	23	12.8					
Office/administration	12	6.6					
Mother's occupation that is	exposed to pest	icides					
Low	180	100.0					
High	0	0					
Father's education							
Primary and below	128	71.1					
Secondary and above	52	28.9					
Father's occupation							
Agriculture	47	26.1					
Fisherman	45	25.0					
Office	33	18.3					
Traditional craft/others	55	30.6					
Father's occupation that is e	xposed to pesti	cides					
Low (nonagriculture)	133	73.9					
High (agriculture)	47	26.1					
Household income (RM)					500.00	300.00	900.00
Use of pesticides at home							
Yes	93	51.7					
No	87	48.3					
Parents smoking status							
Yes	101	56.1					
No	79	43.9					
Length of residence							
≥10 y	126	70.0					
<10 y	54	30.0					

(46.7%) were positive for urine DAP metabolite levels. Out of the 6 urinary DAP metabolites analyzed, only 3 were detected: DMP (46.7%), DEP (16.7%), and DMDTP (3.3%). In this study, the mean DMP concentration was 4.68 ± 7.66 ng/ mL, the mean for DEP was 12.33 ± 4.94 ng/mL, and the mean DMDTP was 0.37 ± 0.19 ng/mL. The maximum value for DMP and DEP was 39.77 ng/mL and 23.53 ng/mL, respectively. Out of 84 children who were positive for urinary DAP levels, a total of 48 children (26.7%) were detected with only DMP, 30 (16.7%) children were detected with DMP and DEP, and 6 (3.3%) children were detected with DMP and DMDTP.

Bivariable analysis (Table 3) revealed a significant association between the father having an occupation involving exposure to pesticides and pesticide detection in children. Children with a father whose occupation involved high exposure to pesticides showed a higher percentage of pesticide detection (63.8%) compared with children with a

	Limit of									
	Detection				Standard			Martha		
	(ng/mL)	Ν	% of Detection	Mean	Deviation	Minimum	- Iviaximum	Median	25th Percentile	/Sth Percentile
DMP	0.10	84	46.7	4.68	7.66	0.11	39.77	2.15	0.75	4.32
DEP	0.10	30	16.7	12.33	4.92	1.24	23.53	11.57	8.98	15.77
DMDTP	0.15	6	3.3	0.37	0.19	0.17	0.60	0.40	0.18	0.53
DETP	0.10	-	0	-	-	-	-	-	-	-
DEDTP	0.10	-	0	-	-	-	-	-	-	-
DMTP	0.10	-	0	-	-	-	-	-	-	-
Total number		84	46.7							
of children										
with pesticides										
detected										
Total number		96	53.3							
of children with										
pesticides not										
detected										

father whose occupation involved less exposure to pesticides (40.6%) (P = .01).

Furthermore, there is a significant association between parents' use of pesticides at home and pesticide detection in children. Children whose parents used pesticides at home showed a higher percentage of pesticide detection (53.8%) compared with children whose parents did not use pesticides at home (39.1%). Therefore, those parents who used pesticides at home exposed their children to pesticides.

Variable	Pesticides Not Detected	Pesticides Detected	χ²	P Value	OR	95% CI
Sex						
Female	43 (52.4)	39 (47.6)	0.05	.83	0.94	0.52-1.69
Male	53 (54.1)	45(45.9)				
Age						
7-9 y old	54 (51.9)	50 (48.1)	0.2	.66	0.87	0.48-1.58
10-12 y old	42 (55.3)	34 (44.7)				
Mother's education						
Primary and below	86 (54.8)	71 (45.2)	1.03	.31	0.64	0.26-1.53
Secondary and above	10 (43.5)	13 (56.5)				
Father's education						
Primary and below	73 (57.0)	55 (43.0)	2.43	.12	0.60	0.31-1.14
Secondary and above	23 (44.2)	29 (55.8)				
Father's occupation expose	d to pesticides					
Low exposure	79 (59.4)	54 (40.6)	7.53	.01*	2.58	1.30-5.14
High exposure	17 (36.2)	30 (63.8)				
Use of pesticides at home						
No	53 (60.9)	34 (39.1)	3.89	.05*	1.81	1.00-3.28
Yes	43 (46.2)	50 (53.8)				
Parents smoking status						
No	41 (42.7)	38 (45.2)	0.12	.73	0.90	0.50-1.63
Yes	55 (57.3)	46 (54.8)				
Length of residence						
\geq 10 y	66 (52.4)	60 (47.6)	0.15	.70	0.88	0.46-1.67
<10 y	30 (55.6)	24 (44.4)				

	Pesticides Not Detected	Pesticides Detected	χ ²	P Value	OR	95% CI
Cucumber (Cucumis	sativus)					
Less frequent	90 (57.7)	66 (42.3)	8.93	<.001*	4.09	1.54-10.87
Frequent	6 (25.0)	18 (75.0)				
Long bean (Vigna un	iguiculata)					
Less frequent	87 (56.1)	68 (43.9)	3.51	.060	2.27	0.95-5.46
Frequent	9 (36.0)	16 (64.0)				
Pucuk paku (<i>Diplaziu</i>	m esculentum)					
Less frequent	70 (56.5)	54 (43.5)	1.56	.210	1.50	0.79-2.82
Frequent	26 (46.4)	30 (53.6)				
Potato sprouts (Mani	ihot esculenta)					
Less frequent	71 (58.7)	50 (41.3)	4.24	.040*	1.93	1.03-3.63
Frequent	25 (42.4)	34 (57.6)				
Carrot (Daucus caroto	a)					
Less frequent	93 (56.7)	71 (43.3)	6.98	.010*	5.68	1.56-20.6
Frequent	3 (18.8)	13 (81.3)				
Apple (Malus pumila))					
Less frequent	84 (60.9)	54 (39.1)	13.5	<.001*	3.89	1.83-8.25
Frequent	12 (28.6)	30 (71.4)				
Banana (<i>Musa</i> specie	s)					
Less frequent	93 (54.7)	77 (45.3)	1.43	.230	2.82	0.70-11.2
Frequent	3 (30.0)	7 (70.0)				

Table 4 shows an association between fruit and vegetable consumption and pesticide detection in Orang Asli children. There was a significant association between frequency of consumption of cucumbers, potato sprouts, carrots, and apples and pesticide detection in children. Children who frequently consumed cucumbers, potato sprouts, carrots, and apples showed a higher percentage of pesticide detection (75.0%, 57.6%, 81.3%, and 71.4%, respectively) compared with children who seldom consumed them (42.3%, 41.3%, 43.3%, and 39.1%, respectively).

From this study, children who frequently consumed apples had a 4-times higher risk of pesticide detection compared with those who consumed apples less frequently. Those who frequently ate cucumber had a 4-times higher risk of pesticide detection than those who less frequently consumed cucumbers. Children whose father's occupation involved high exposure to pesticides (agriculture) had a 3-times higher risk for pesticide detection compared with children whose father's occupation did not (Table 5).

Variable	Crude OR (95% CI)*	Adjusted OR (95% CI) †	Wald Statistics $(df)^{\dagger}$	P Value
Apple				
Less frequent	1.00	1.00		
Frequent	3.89 (1.83-8.25)	3.86 (1.72-8.63)	10.80	<.010
Cucumber				
Less frequent	1.00	1.00		
Frequent	4.09 (1.54-10.87)	3.45 (1.20-9.85)	5.32	.020
Father's occupation that	at is exposed to pesticides			
Low exposure	1.00	1.00		
High exposure	2.58 (1.30-5.14)	2.85 (1.36-6.00)	7.66	.010

Pesticides	DMP	DMDTP	DEP
Azinphos methyl	Х	х	
Chlorethoxyphos			Х
Chlorpyrifos			Х
Dichlorvos (DDVP)	Х		
Diazinon			Х
Dimethoate	Х	Х	
Disulfoton			Х
Malathion	Х	Х	
Methidathion	Х	Х	
Parathion			Х
Phosmet	Х	Х	
Sulfotepp			х
Temephos	Х		

DISCUSSION

In this study, we used OP pesticide urinary metabolites as biomarkers for pesticide detection in children because these metabolites can provide an estimate of pesticide exposure via dermal, respiratory, and oral pathways and can be used to assess actual absorption. About 39 OP pesticides are used worldwide, and nearly all of them produce DAP metabolites. This method, therefore, provides a reliable exposure estimate for OP pesticides.

The OP pesticide urinary metabolites identified in the study were DMP, DEP, and DMDTP (Table 6). Of 84 (46.7%) children who had been detected as positive for urinary DAP levels, a total of 48 children (57.1%) were detected with only DMP exposure, 30 (35.7%) children were detected with DMP and DEP exposure, and 6 (7.1%) children were detected with DMP and DMDTP exposure.

In this study, the mean DMP concentration was 4.68 \pm 7.66 ng/mL, DEP was 12.33 \pm 4.94 ng/mL, and DMDTP was 0.37 \pm 0.19 ng/mL. In

a previous study in Chile,¹⁴ the mean DAP concentration in 190 children (aged 6-12 years old) was 5.2 ng/mL for DMP, 9.1 ng/mL for DEP, and 8.9 ng/mL for DMDTP. A study in eastern North Carolina¹⁵ reported that the mean DAP for 60 children aged 1-6 years was 5.14 ng/mL for DMP, 8.58 ng/mL for DEP, and 2.66 ng/mL for DMDTP.

Data from the National Health and Nutrition Examination Survey (NHANES 1999-2000) in the United States for children aged 6-11 years showed that the median level of urinary DAP metabolites was 1.58 ng/mL for DMP, 1.32 ng/mL for DEP, and 0.69 ng/mL for DMDTP.¹³ Therefore, the children in our study area had levels of DMP and DEP that were generally equal to or higher than those in the NHANES data or other studies (Table 7). Thus, it is important to ensure that there is an effort from higher authorities to reduce the exposure of these children to pesticides, especially among the Orang Asli community in the area, since there are no baseline data of DAP urinary metabolites among the Malaysian population to use for comparison.

In this study, we also identified risk factors of OP pesticide exposure in children by determining the association between pesticide detection and sociodemographic factors as well as frequency of vegetable and fruit intake. From the analysis, parents' use of pesticides at home and a father's occupation in an agricultural area are significantly associated with detection of pesticide in children. This finding is consistent with another study in which children living in agricultural area may be exposed to pesticides through drifts during application and parental takehome exposures. Azaroff and Neas¹⁶ found that children who lived near an agricultural area experienced adverse health effects through environmental pesticide exposure even though they did not work in the field. Their parents may bring pesticide residues from the agricultural fields into their homes or pesticides may drift from fields into areas where the children play. In addition, children's behaviors,

OP Pesticide Urinary Metabolites Identified	Level Of OP Pesticide Urinary Metabolites Identified In This Study	Level of OP Pesticide Urinary Metabolites Identified in Chile	Level of OP Pesticide Urinary Metabolites Identified in Eastern North Carolina	Level of OP Pesticide Urinary Metabolites Identified in US (NHANES 1999-2000)
DMP	4.68±7.66 ng/ml	5.2 ng/ml	5.14 ng/ml	1.58 ng/ml
DEP	12.33±4.94 ng/ml	9.1 ng/ml	8.58 ng/ml	1.32 ng/ml
DMDTP	0.37±0.19 ng/ml	8.9 ng/ml	2.66 ng/ml	0.69 ng/ml

such as spending more time on the floor and engaging in hand-to-mouth or object-to-mouth habits, may cause ingestion of soil or house dust containing pesticide residues. A study done among preschool children in Seattle, Washington, found that children whose families reported garden pesticide use had significantly higher urinary metabolite levels.¹⁷

Studies have shown that living and working in agricultural areas increases the risk of delivering a child with a birth defect. For example, in California, mothers living in an area of high agricultural productivity or high pesticide use were found to be at greater risk of giving birth to children with limb defects compared with mothers living in areas with low pesticide use.¹⁸ Additionally, studies have implicated a link between pesticide exposure and impaired neurodevelopment as well as autism, hearing loss, intellectual impairment, and attention deficit hyperactivity disorder in children. A study done in 2010 among US children found that there was an association between children with a high urinary concentration of organophosphate metabolite and attention deficit hyperactivity disorder.¹⁹

Our findings also suggest that there is a significant association between intake of selected fruits and vegetables and children's pesticide detection, wherein those who frequently consumed cucumbers, potato sprouts, carrots, and apples had significantly higher levels of pesticide compared with those who consumed these foods less frequently. It is difficult to know the origin of fruits and vegetables consumed, but apples and carrots are usually bought at a nearby market, while cucumbers and potato sprouts are mainly found near the home. Therefore, it would be best to suggest proper washing of fruits and vegetables before they are consumed.

Diet is also a source of pesticide exposure in children. This is concurrent with a study finding in 2011 in which consumption of fruits containing residues of insecticides was the main factor contributing to pesticide exposure among schoolchildren in Chile.¹⁴ According to a report on pesticide exposure in children in 2012, the US Food and Drug Administration analyzed a total of 7234 food samples and found that 49% of fruits, 29% of vegetables, 26% of grain products, and 24% of fish/shellfish tested had detectable but legally allowable pesticide residues.²⁰

However, previous studies report that eating foods containing pesticide residues rarely causes significant toxicologic effects.²¹ Thus, concerns regarding pesticide residues in food and occurrence of cancer must be balanced with the health benefits of eating fruits, such as anticancer benefits. The results of the analysis conducted by Reiss et al²² showed that if half of the population of the United States were to increase consumption of fruits and vegetables in one meal a day, an estimated 20,000 cancers could be avoided every year. Meanwhile, the remaining residue of pesticides in fruits and vegetables also allow the addition of 10 cases of cancer per year. Therefore, the consumption of fruits and vegetables itself benefits health more than the adverse effects associated with pesticide residues on fruits and vegetables damage health.

CONCLUSIONS

In conclusion, almost half of the children (46.7%) in our study area were positive for urinary DAP metabolite levels, and most of them showed levels equal to or higher than in previous studies. The major risk factors associated with children's pesticide detection in this study were frequent intake of selected fruits and vegetables, namely apple and cucumber, and having a father who works in the agricultural area. Therefore, it is important to create awareness among parents, especially those of the Orang Asli community, regarding the importance of avoiding contamination of pesticides from the agricultural area and avoiding consuming fruits and vegetables that are not properly washed. It is hoped that this study can provide baseline information on exposure to OP pesticides among children in Malaysia, since exposure to pesticides is consistently associated with adverse health effects among children. Therefore, children's exposure to pesticides should be limited as much as possible.

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