Exposure of chlorpyrifos in toddlers living in an agricultural area in Sakon Nakhon province, North-East Thailand

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Satinee Siriwat
College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand
Juthasiri Rohitrattana
Thailand Institute of Occupational Safety and Health, Bangkok, Thailand
Thitirat Nganchamung
College of Medicine and Public Health, Ubon Ratchathani University College, Ubon Ratchathani, Thailand
Parichat Ong-artborirak
College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand
Mark Robson
School of Environmental and Biological Sciences, Rutgers The State University of New Jersey, New Brunswick, New Jersey, USA, and
Wattasit Siriwong
College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand

Abstract

Purpose – Children living in agricultural areas are exposed to pesticides in their living areas and through activities of daily living. These exposures may lead to adverse health effects. The purpose of this paper is to investigate household environmental and behavioural factors associated with chlorpyrifos exposure and resultant adverse health effects in children living in an agricultural community.

Design/methodology/approach – A cross-sectional study was conducted including 65 toddlers (age of 12–36 months) and their parents were face-to-face interviewed from January to February 2016. Toddler's hands and feet, toys and floors were wiped for chlorpyrifos residue analysis. The wipes were extracted and analysed by gas chromatography with a flame photometric detector, and blood cholinesterase activity was measured by the EQM Test-mate (model 400).

Findings – The average age (± standard deviation) of children was 19.9 (±5.9) months. Chlorpyrifos detections were 61.5 per cent (hands), 57.1 per cent (toys), 53.8 per cent (floors) and 30.8 per cent (feet). The highest chlorpyrifos residue concentration was detected on toy surface (3.022 $\mu g/m^2$). Chlorpyrifos residues on hands and feet were positively correlated with concentrations on floors and toys (Spearman's ρ, $p < 0.01$). Multiple linear regression analysis revealed that more frequent hand washing ($\beta = -0.236$, $p = 0.067$) and showering ($\beta = -0.240$, $p = 0.056$) was negatively associated with chlorpyrifos residue on children's body. House cleaning frequency was significantly associated with an increase in haemoglobin-adjusted erythrocyte cholinesterase ($\beta = 0.251$, $p < 0.05$).

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Originality/value – Chlorpyrifos exposures found in the children household area through their activities and behaviours can cause several adverse health effects. The circumstances associated with chlorpyrifos exposure should be mitigated and reduced to improve the household environment of children living agricultural areas.

Keywords Young children, Agricultural area, Blood cholinesterase, Chlorpyrifos

Paper type Research paper

Introduction

Agriculture is the primary occupation in Thailand, and 46.54 per cent of the land area is devoted to agriculture. Most of agriculture area (63.85 per cent) is located in the northeastern part of the country[1]. Pesticides are commonly used in agriculture to control weeds, insects and diseases[2]. In 2010–2015, Thailand pesticide imports exceeded 130,000 tons per year[3]. Organophosphate (OP) insecticides are a group of chemical compounds that are highly toxic and used for the control insects for crop in agriculture, and they are highly toxic to nervous system and known as cholinesterase inhibitor[4]. Chlorpyrifos is a popular organophosphate insecticide that is intensively used in agricultural areas in Thailand[5]. The report case of pesticide poisonings in 2014 showed that toddlers under four years of age had a high incidence rate at the age of three[6]. Children are much more susceptible during different life stages owing to their dynamic growth and developmental processes as well as physiological, metabolic, behavioural differences and hygiene which may result in significantly greater exposures to environmental contaminants than adults[7]. Children have a larger skin surface area per kilogram body weight; therefore, they may have a higher chance of dermal exposure than adults[8]. Children who live in agricultural community have much higher exposure to pesticide more than children in non-farm areas[9–11]. Recreational activity in farming areas where pesticides have been used increases children's exposure to pesticide. Hand-to-mouth and object-to-mouth (pica) behaviours in children aged one to three years old can also lead to increased pesticide exposure[12, 13]. Family members who work as farmers may bring home pesticide and contaminate the living environment with pesticide residues on clothes or shoes[8, 12, 14].

The measurements of OP exposure can be analysed by urinary metabolite concentration and blood cholinesterase level[15–18]. Similar to carbamate insecticide, OP is a potent inhibitor of cholinesterase, including erythrocyte cholinesterase (AChE) and plasma cholinesterase (PChE). The levels of AChE and PChE in blood samples are used to indicate cholinesterase activity which is related to acute or chronic health effects[14, 19]. The effect of chlorpyrifos exposure and residues on health symptoms may include contact dermatitis, nausea, sweating, diaphoresis, lacrimation, diarrhoea, salivation and headache[4].

Previous studies in Thailand have shown that children (six to eight years old) living in rice production areas had significantly higher urinary metabolite concentrations of OP than aquaculture farm children[20, 21]. Pre-school and school-aged children in Thailand had significantly higher urinary metabolite concentrations of OP exposure than the American children[11, 22, 23]. Based on the earlier studies, we can infer that Thai children are at significant risk from pesticide exposure, especially children who live in and around agricultural communities. This study investigated the association of chlorpyrifos residues and blood cholinesterase activity with behavioural factors among young children living in an agricultural community.

Materials and methods

Study area and participants

This study was cross-sectional in design that collected information on study exposure and outcome during the dry season (January to February 2016). It was conducted in an agricultural area located in Khamin and Chaingkhruea sub-district, Muang Sakon Nakhon district, Sakon Nakhon province which is located in the northeastern part of Thailand. This region is agriculturally the most intense area that has been supported by irrigation to cultivate crops all year. Households in this area are surrounded by active agricultural activities that include the intensive use of chlorpyrifos. The major crops in this area are as follows: rice, watermelons, cantaloupes, chilies, cucumbers, canna flowers and vegetables[24]. Data from previous research were designed for calculating this study sample size[25] which was calculated by formula from Lemeshow *et al.*[26]. Approximately, 88 toddlers were randomized to 65 participants. Total of 65 toddlers aged between 12 and 36 months who born and live in agricultural area (distance from farming area to house < 50 m) were recruited by random sampling. If a family selected had more than 1 toddlers, the youngest one was selected because they were assumed to stay more in the house rather than going outdoor. Data were collected after toddlers and family caregivers received the study information and were enrolled as subjects. Informed consent was obtained by participant's parents or caregivers. This study was approved by the ethics review committee for research involving human research subjects, health science group, Chulalongkorn University, Thailand (COA No. 221/2015).

Questionnaire. A structured questionnaire was used to interview parent or caregiver by face-to-face technique. The questionnaire was developed from Rohitrattana[25] including demographic information, children activities, environmental factors and health effects. And the validity was examined by three experts.

Sample collection. Wipe samples were collected from children's hands, feet, floors/wooden beds and toys. Two 4×4 inch cotton gauzes wetted with 3 ml of 40 per cent isopropanol were prepared for wiping with modified technique from Clifton *et al.*[27] and Lu *et al.*[28]. The area for wipes sampling was based on answer from the questionnaire in children activities part. To collect the floors/wooden beds wipe samples, area 30×30 cm in the main living space was wiped by prepared gauze[29]. The toy wipe samples were wiped in 10×10 cm of surface areas[29]. Hands and feet wipe samples were collected by prepared gauzes, one gauze for each hand/foot side[28]. After that, the wipe sample was covered in aluminium foil and kept in a zip-lock bag. All samples were transported and analysed for chlorpyrifos at laboratory in the College of Public Health Sciences, Chulalongkorn University.

Wipe sample analysis. All surface wipe samples were extracted with modified technique from Anastassiades *et al.*[30]. The gauze pads wipe samples were added in Erlenmeyer flask with 25 ml acetonitrile (HPLC grade). Then, the sample was shaken for ten minutes, and gauze pads were separated from the solvent. Next, nitrogen gas 99.95 per cent was used for solvent vaporisation at 40 ± 2 °C to nearly dry off sample and 1 ml of 0.1 per cent acetic acid was added to acetonitrile. And then, the sample was removed to Eppendorf tube and added 25 mg of primary secondary amine (PSA) and 150 mg of $MgSO_4$. The tube was mixed by vortex tool at 1 and 2 min at 6,000 rpm centrifugation for clean-up. Finally, the sample was sucked (liquid) to 1.5 ml vial for analysis of chlorpyrifos, cypermethrin and permethrin by gas chromatograph (GC). Chlorpyrifos concentrations analysis was performed by using an Agilent 7890 gas chromatography with a flame photometric detector (GC-FPD). A chromatography column HP-5 (30 m length, 0.250 mm diameter, 0.25 μm film thickness) was used. The column temperature was raised from 80 °C at 12 min to 195 °C, at 2 °C/min to 210 °C (held for 3 min), at 15 °C/min to 225 °C (held for 2 min), and at 40 °C/min to 275 °C (held for 7 min), respectively.

Quality control. Average recovery of chlorpyrifos by analysing ten replicates at two spiked levels (0.1 and 1.0 μg) was in the acceptable range (108.1 per cent) with relative standard deviation (SD) of 4.0 per cent. Correlation coefficient (R^2) from the calibration curves of chlorpyrifos concentrations with analysis of three replicates was shown to be 0.999.

Quality control for pesticide residues analysis was acceptable following the Guidelines for Residues Monitoring in the European Union[31]. The limit of detection (LOD) and limit of quantitation (LOQ) were estimated from the chromatograms at signal to noise ratio (S/N), i.e. 0.01 (µg/sample) for LOD and 0.02 (µg/sample) for LOQ.

Blood cholinesterase test. Blood cholinesterase level was used as a measure of chlorpyrifos exposure. Finger-stick blood samples were collected from participants and analysed with AChE, haemoglobin-adjusted erythrocyte cholinesterase (HAChE), and PChE by the EQM Test-mate ChE Cholinesterase Test System (Model 400), following standard procedures[32].

Data and statistical analysis. Statistical analysis was performed using SPSS statistical software package (IBM SPSS Statistics 22.0). Descriptive statistics were analysed including frequency, mean and SD. Chlorpyrifos residue concentrations as below the LOD were replaced with one-half of the LOD [33]. χ^2 test was used for categorical variables. Spearman's correlations were considered for pesticide concentration on hands, feet, floors/wooden beds and toys. A linear regression was performed for factors associated with pesticide concentrations.

Results

The demographic data of participants in this study are presented in Table I. There were a total of 65 toddlers in this study, 39 (60 per cent) boys and 26 (40 per cent) girls. Participants had mean $\pm$ SD of age 19.92 ± 5.99 months, height 78.91 ± 6.60 cm, weight 10.70 ± 1.93 kg and BMI 17.20 ± 2.50. The average of playing duration was 7.26 ± 1.73 h/day. Toddler's behaviours included frequency of hand washing and shower, i.e. 3.86 ± 1.41 and 1.98 ± 0.54 times/day, respectively. Face touching and eating by bare hands were commonly found to be 55.4 and 67.7 per cent, respectively. Toddlers mostly spent time on house floor (52.3 per cent) and wooden bed (47.7 per cent) for their activities such as sitting, laying, sleeping, eating and playing. Residential environments showed everyday house cleaning (60 per cent), often window opening (63.1 per cent), house adjacent to farms (43.1 per cent) and exposed during pesticide spraying on farms (33.8 per cent).

The results of blood cholinesterase tests of participants are presented in Table II. The average of AChE was 2.38 ± 0.44 U/ml, HAChE 23.79 ± 4.76 U/g Hgb and PChE was 2.81 ± 0.81 U/ml.

For wipe samples, pesticide residues were collected from toddler's hands and feet, floors/wooden beds and toddler's toys. Concentrations residue of chlorpyrifos from surface wipe samples were typically detected on hands (61.5 per cent; 0.015 ± 0.026 µg/hands), followed by toys (57.14 per cent; 1.287 ± 0.757 µg/cm^2), floors/wooden beds (53.8 per cent; 0.030 ± 0.022 µg/m^2) and feet (30.8 per cent; 0.009 ± 0.006 µg/on feet), respectively (shown in Table III).

Since the chlorpyrifos residue concentrations were not normally distributed, Spearman's correlation was considered to determine the relationship between chlorpyrifos residue concentrations on surface wipe samples. The results presented significantly positive associations among chlorpyrifos residue concentrations on hands, feet, floors/wooden beds and toy, and Spearman's coefficient were in the range of 0.452–0.643 ($p < 0.001$) (Table IV).

Relationships between pesticide exposure and toddler's activities, behaviours and residential environments are shown in Table V. Significantly increased chlorpyrifos residue concentrations were found on toddler's feet who spent time on wooden beds ($\beta = -0.72$, $p = 0.003$). The concentrations on hands and feet were decreased after hygienic behaviours such as hand washing and showing. Hand washing significantly could reduce the pesticide residue concentrations on toddler's feet ($\beta = -0.302$, $p = 0.022$). Showering can reduce chlorpyrifos residue concentrations on hands ($\beta = -0.240$, $p = 0.056$) and feet ($\beta = -0.236$, $p = 0.068$).

Factors	n (65) or mean ± SD	%
Characteristics		
Gender		
Boy	39	60
Girl	26	40
Age (month)	19.92 ± 5.99	
Height (cm)	78.91 ± 6.60	
Weight (kg)	10.70 ± 1.93	
BMI (kg/m^2)	17.20 ± 2.50	
Behaviours		
Frequency of hand washing (times)	3.86 ± 1.41	
Frequency of shower (times)	1.98 ± 0.54	
Face touching		
Always	5	7.7
Often	22	33.8
Sometime	36	55.4
None	2	3.1
Shoes		
Always	28	43.1
Sometime	37	56.9
Eating by		
Bare hand	44	67.7
Spoon or fork	21	32.3
Breast feeding		
Yes	13	20
No	52	80
Activities		
Playing duration (hour/day)	7.26 ± 1.73	
Spent time on		
Wooden bed	31	47.7
Floor	34	52.3
Activities		
Sleep	3	4.6
Outdoor playing	39	60
Indoor playing	23	35.4
Exposing during spraying		
Yes	22	33.8
No	43	66.2
Residential environments		
Window opening		
Every day open	41	63.1
Sometimes open	24	36.9
Housing adjacent to farms		
Yes	28	43.1
No	37	56.9
Frequency of floor cleaning		
Everyday	39	60
Sometime	26	40

Table I. Participants' characteristics, activities, behaviours and environmental conditions

Toddler's behaviours like non-food taking to mouth were likely to be related to decreased PChE level ($\beta = -0.169$, $p = 0.213$). More frequent house cleaning was associated with high HAChE level ($\beta = 0.251$, $p = 0.049$). Results for the relationship between pesticide exposure and health effects were presented in Table VI. General health effects (nausea, vomiting and anorexia) were associated with PChE ($p = 0.018$). Skin health effects (skin irritation and diaphoresis) were related to chlorpyrifos residue concentrations on hands and feet ($p = 0.052$), while eye health effects (irritation and lacrimation) were related to chlorpyrifos residue concentrations on hands ($p = 0.077$) and toys ($p = 0.086$).

Discussion

The AChE levels among toddlers (one to three years old) living in agricultural area in this study (2.38 ± 0.44 U/mg) were lower than those in previous studies. Rohitrattana *et al.*[34] found that AChE of children aged six to eight years living in rice farms in Thailand was 2.89 ± 0.34 U/ml for high pesticide use period. According to the study in Ecuador, Suarez-Lopez *et al.*[14] found AChE of 3.08 ± 0.51 U/ml among children (four to nine years old) living with flower plantation workers. Furthermore, AChE in our study was lower than the study among Indonesia children having age of seven to eight years (3.3 ± 0.5 U/ml) living in agricultural villages[35]. This may be explained by AChE level increasing linearly with the age of children[14]. Also, this is due to the fact that toddlers spent more time at home and presumably with more exposure to any insecticides than the older ones. Risk

Table II. Blood cholinesterase level

Blood cholinesterase	Mean ± SD	Range	Percentiles 25	50	75	95
AChE (U/ml)	2.38 ± 0.44	0.93–3.52	2.19	2.42	2.68	3.10
HAChE (U/g Hgb)	23.79 ± 4.76	9.90–40.20	21.30	23.90	26.00	32.16
PChE (U/ml)	2.81 ± 0.81	1.15–4.81	2.19	2.71	3.38	4.49

Notes: AChE, erythrocyte cholinesterase; HAChE, haemoglobin-adjusted erythrocyte cholinesterase; PChE, plasma cholinesterase

Table III. Chlorpyrifos residue concentrations

Surface wipe samples	Detected frequency ($n = 65$)	Mean ± SD	Min.	Max.	Percentiles 25	50	75	95
Hands (µg/hands)	40 (61.5%)	0.015 ± 0.026	< LOD	0.212	0.005	0.014	0.016	0.026
Toys[a] (µg/m^2)	20 (57.14%)	1.287 ± 0.757	< LOD	3.022	0.500	1.518	1.812	2.653
Floors/wooden beds (µg/m^2)	35 (53.8%)	0.030 ± 0.022	< LOD	0.096	0.010	0.033	0.041	0.081
Floors (µg/m^2)	15 (23.0%)	0.024 ± 0.019	< LOD	0.087	0.010	0.010	0.036	0.079
Wooden beds (µg/m^2)	20 (30.8%)	0.036 ± 0.024	< LOD	0.095	0.010	0.035	0.047	0.088
Feet (µg/feet)	20 (30.8%)	0.009 ± 0.006	< LOD	0.032	0.005	0.005	0.015	0.019

Notes: [a]35 samples. LOD on hands = 0.01 µg/hands; LOD on feet = 0.01 µg/feet; LOD on floors or wooden beds = 0.02 µg/m^2; LOD on toys = 1 µg/m^2

Table IV. Bivariate associations among chlorpyrifos residue concentrations on wipe samples

Surface wipe samples	Spearman's ρ Correlation coefficient	p-value
Hands and feet	0.643	< 0.001**
Hands and floors/wooden beds	0.533	< 0.001**
Hand and toys	0.603	< 0.001**
Feet and floors/wooden beds	0.521	< 0.001**
Feet and toys	0.506	0.002**
Floors/wooden beds and toys	0.452	0.006**

Note: $^{**}p < 0.01$

Independent factors	Chlorpyrifos residue concentrations: Hands B	Hands Significance	Feet B	Feet Significance	Blood cholinesterase levels: HAChE B	HAChE Significance	PChE B	PChE Significance
Activities								
Playing duration (h/day)	0.009	0.944	−0.357	0.008*	−0.067	0.618	0.156	0.243
Sitting /laying on floor or wooden bed	−0.149	0.234	−0.372	0.003*	0.091	0.474	−0.130	0.305
Exposing during spraying	−0.080	0.541	0.044	0.743	−0.022	0.864	−0.018	0.889
Sleeping and playing duration	0.033	0.794	0.028	0.830	−0.060	0.638	−0.004	0.973
Behaviours								
Frequency of hand wash (times)	−0.236	0.067	−0.302	0.022*	0.201	0.126	0.086	0.518
Frequency of shower (times)	−0.240	0.056	−0.236	0.068	−0.029	0.826	0.033	0.803
Face touching	−0.159	0.246	−0.058	0.664	0.078	0.554	0.181	0.166
Taking non-food to mouth	−0.031	0.819	0.163	0.237	0.166	0.224	−0.169	0.213
Taking food to mouth	0.077	0.558	−0.045	0.738	0.066	0.626	0.032	0.811
Environments								
Frequency of house cleaning	−0.133	0.299	−0.042	0.753	0.251	0.049*	0.054	0.674
Window opening	0.057	0.649	−0.183	0.151	0.017	0.892	−0.041	0.747

Dependent factors

Notes: Significance was tested by linear regression. Adjust: surface area of hands and feet, age, gender for chlorpyrifos concentrations and age, gender for blood cholinesterase activity. *Significant at $p < 0.05$ level

Table V. Relationships between chlorpyrifos residue concentrations and activities, behaviours and environments of children living in agricultural area, North-East Thailand

Health effects	Chlorpyrifos concentrations: Hands	Feet	Hands and feet	Floors or wooden beds	Toys	Blood cholinesterase activity: HAChE	AChE	PChE
General	0.542	0.896	0.663	0.929	0.454[a]	0.663	0.188	0.018*
Skin	0.130	0.047*	0.052	0.478	0.700[a]	0.857	0.993	0.273
Eyes	0.077[a]	0.166[a]	0.105	0.427[a]	0.086[a]	0.321	0.618	0.224[a]
Respiration	0.753	0.583	0.919	0.919	0.968	0.156	0.839	0.683
Gastrointestinal	0.830	0.622	0.535	0.126	0.983	0.934	0.185	0.156

Pesticide exposure (p-value)

Notes: [a]Fisher's exact test. χ^2 test. *$p < 0.05$

Table VI. Relationships between health effects and pesticide exposures

behaviours and activities such as hand to mouth, object to mouth and playing on the floor may substantially increase the exposure to pesticide residue via multiple routes, oral and dermal[36, 37]. Differences in sociodemographic characteristics of these studies may be a potential factor on environmental exposure to pesticides[38].

The amounts of chlorpyrifos on hands were detected higher than those on feet. This might be due to more chance of exposure via hand contact with pesticide residue on toy, with found highest cholrpyrifos concentrations in this study, wooden bed/floor and other smooth surface such as furniture[37]. Floors/wooden beds wipe samples were detected with lower chlorpyrifos residue concentrations than toys wipe samples; this was because toddlers moved their toys everywhere (i.e. houses and farms) with them. There was a chance of toddler's toys to contact soil or get exposed to pesticides in the farms. Pesticide residues on toys were collected from toddler's toys; these are most favourite play items of children which may not be cleaned. Our result presented that chlorpyrifos residue concentrations on floors were detected slightly lower than on wooden beds. These findings agree with Qaundt *et al.*[39] that the floor may be source of the accumulation of pesticide residues in the household. The result can imply that the way Thai-house characters in rural area are commonly an opened-structure types with some add-on parts. Some houses do not have windows and doors; some houses have kitchen outside of the house, and some have terraces and spaces. Thai houses are designed for daily activities. There are open spaces and ventilation, which may increase pesticide exposures and dermal contract. Cleaning the environments in which the toddlers live such as floor or wooden bed could remove the pesticide residue; therefore, toddlers who stay on floor or wooden bed will be in less contact with the pesticide residue. In this study, both of wet mop and bloom were used for house cleaning. It is not enough to use a bloom to remove dust of pesticides on the floor/wooden bed. Pesticide accumulation in houses increases by spraying pesticides in farms[40]. Toddlers play with or crawl into soil and come in contact with contaminated pesticide at home surrounded by agricultural fields[41]. In addition, activity behaviours of toddlers such as frequency of face touching and playing in fields were exposed to pesticides on their hands and feet. Toddler's hand-to-mouth contact is more frequent and eating by bare hands may expose them to chlorpyrifos through ingestion. Chlorpyrifos residue concentrations in this study was less than other research works[28, 39, 42], based on activities and behaviours effects on pesticide exposures.

Linear association existed between pesticide exposure and daily activities of participants. This was explained by the frequency of showering and hand washing which decreased chlorpyrifos residue concentrations on hands and feet. The results shown suggest a significant negative association between chlorpyrifos residue concentrations on feet and playing duration as children may wear shoes and take a shower. Chlorpyrifos residue concentrations on feet were detected to be lower than chlorpyrifos residue concentrations on hands, floors/wooden beds and toys. However, chlorpyrifos residue concentrations on feet were positively related to chlorpyrifos residue concentrations on hands, floors/wooden beds and toys, which is similar to previous report of Qaundt *et al.*[39]. Hand washing and showering were a protective factor for pesticide exposure [43]. Linear regression models were adjusted for age and gender as factors associated to toddler's activities and behaviours. AChE activities can reflect organophosphates exposure, and age and gender are related to activities and behaviour of toddlers[14].

Health effects of pesticide exposure in this study mirror the general health effects including nausea, vomiting and anorexia that are related to PChE with acute exposure referring to an intense exposure over a short time period[8]. Skin health effects (skin irritation and diaphoresis) were associated with chlorpyrifos residue concentrations on hands and feet that were from exposure to pesticides by dermal contact. Chlorpyrifos residue concentrations on hands and toys were related to eye irritation and lacrimation. This may be due to toddlers making hand contact to their eyes and face. On further investigating, it was found that pesticides on toys also contribute to children rubbing their eyes. These observations were similar to other study findings on toddlers ($<$ 6 years old) who are exposed to pesticides at homes from toxicity category I and II pesticides[44]. Toddlers are susceptible and vulnerable to health effects from pesticide exposure because of their physiological characteristics and age-related behaviours[41].

Conclusions

Toddlers are exposed to pesticides by living in agricultural communities. Behavioural factors among toddlers affect the chlorpyrifos residue concentrations on children's hands and feet. Toddlers are exposed to pesticides from floors/wooden beds and toys in their living areas. Exposure to pesticides used in agriculture can affect blood cholinesterase levels in toddlers. Toddlers may be at a risk from residential exposure to pesticide. Moreover, toddlers have additional exposure pathways by ingestion and inhalation. This result suggests that toddlers should frequently wash their hands and take shower to reduce pesticide on hand, feet and body. In addition, properly organised environment and good personal hygiene practices, especially showering and hand washing, can prevent exposure to pesticide. The current study evaluated only pesticide exposure via dermal routes, mainly hands and feet. For further study, inhalation and ingestion routes should be investigated together with more specific biomarker, for example, urinary pesticide metabolite.

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Corresponding author
Wattasit Siriwong can be contacted at: wattasit.s@chula.ac.th