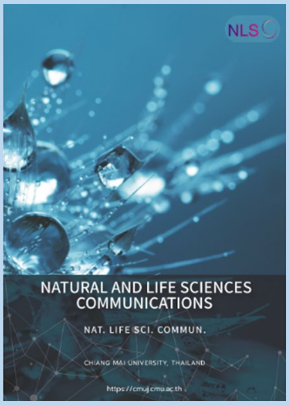
Research article



Association between Urinary Metabolites of Organophosphate Insecticides and Malondialdehyde Oxidative Stress Biomarker Among Adults in a Rural Area of Chiang Mai, Thailand: A Follow-Up Study in Two Post-Harvest Seasons

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Editor:
Nisit Kittipongpatana,
Chiang Mai University, Thailand

Article history:
Received: June 20, 2024;
Revised: January 23, 2025;
Accepted: January 24, 2025;
Online First: February 6, 2025
https://doi.org/10.12982/NLSC.2025.030

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ABSTRACT

The upper northern Thailand is well-known as abundant of variety of crop production, including several vegetables, longans, lychees, and mangoes, as well as paddy rice. In Thailand, village health volunteers (VHVs) are an essential component of the primary healthcare system. This study aims to determine the concentration of urinary dialkylphosphates (DAPs), the common organophosphate (OP) insecticide metabolites, and the relationship between DAPs and malondialdehyde (MDA), an oxidative stress biomarker among VHVs in two post-harvest seasons. The urinary DAPs and MDA were determined in two post-harvest seasons in 2021 and 2022. The results revealed the total DAPs and MDA concentrations were significantly higher in 2021 than in 2022, may cause by locked down during the COVID-19 pandemic in 2022, this made VHVs less faming activity. In 2021, the post-harvest season showed a significant association between total DMPs, with total DAPs and MDA (β = 1.53 and 1.59, respectively). While in the 2022 post-harvest season, there was a significant relationship between the total DEPs and MDA, with β = 1.44 and border line reached in total DAPs with β = 1.43. This study suggested that OP exposure in post-harvest season depends on insecticides used in each crop production. In addition, OP exposure can cause oxidative stress in the human body via urinary DAPs and MDA expressions among farmers. Hence the protection of insecticides exposure using personal protective equipment (PPE) is crucial.

Keywords: Organophosphate (OP), Dialkylphosphates (DAPs), Malondialdehyde (MDA), Oxidative stress, Post-harvest season

Funding: The authors gratefully thank Chiang Mai University, Chiang Mai, Thailand, for the research funding provided.

Citation: Thongjan, N., Prapamontol, T., Promkam, N., Somsunun, K., Naksen, W., Norbäck, D. 2025. Association between urinary metabolites of organophosphate insecticides and malondialdehyde oxidative stress biomarker among adults in a rural area of Chiang Mai, Thailand: A follow-up study in two post-harvest seasons. Natural and Life Sciences Communications. 24(2): e2025030.

INTRODUCTION

Organophosphate (OP) insecticides are a group of chemicals frequently employed for the purpose of managing pests such as insects, mites, and nematodes. Malathion, chlorpyrifos, diazinon, and parathion are all well-known examples of organophosphate insecticides. Although organophosphates are efficient in pest control, they can also present hazards to human health and the environment if not utilized correctly. They have linked to acute toxicity in both humans and wildlife, as well as causing many health complicated consequences with long term exposure (Ranjan and Jindal, 2022). Exposure to OPs raises considerable risks due to their neurotoxic properties in humans. High levels of organophosphate exposure irreversibly inhibit acetylcholinesterase, the enzyme responsible for the degradation of acetylcholine. This accumulation of acetylcholine leads to hypercholinergic symptoms (Costa, 2006; Jokanović, 2018). However, a predictive study reported that gender and behaviors were the predictive factors for AChE activity among rice farmers (Thiphom and Prapamontol, 2021). OP insecticides undergo metabolic processes in the body, resulting in the formation of different compounds. Dialkylphosphates (DAPs) are metabolites of organophosphate pesticides that are frequently used as biomarkers for evaluating human exposure to these pesticides. The six urinary DAPs metabolites of OP insecticides that are typically quantified include: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP), diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP). Metabolites of organophosphate pesticides can be identified in biological samples like urine, blood, and saliva, which can indicate recent exposure to these pesticides (Lu et al., 2005; Prapamontol et al., 2014; Sudakin and Stone, 2011).

Oxidative stress can occur when there is an imbalance between free radicals and antioxidants in the body (Ayala et al., 2014). Oxidative stress can be a contributing mechanism in cases of acute OP insecticide poisoning (Rambabu et al., 2020). Lipid peroxidation is a metabolic process that causes oxidative deterioration of lipids by reactive oxygen species (ROS) that produced as the result of the metabolism of organophosphates by cytochrome P450s (Chambers et al., 2010). Lipid peroxidation forms several oxidation products, including lipid hydroperoxides (LOOH) and aldehydes such as malondialdehyde (MDA). MDA is commonly used as a biomarker of oxidative degradation of omega-3 and omega-6 fatty acids (Pizzimenti et al., 2010).

There are a few epidemiological studies that investigate the relationship between exposure to OP insecticides, which are measured by the presence of OP insecticide metabolites in urine, and the prevalence of MDA (malondialdehyde) in urine. An occupational study found that pesticide application workers exposed to OP pesticides had higher MDA levels than farmers in general (Surajudeen et al., 2014). Another study found a significant association between DAPs and various indicators of oxidative stress, such as malondialdehyde (MDA), isoprostane, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Lee et al., 2017). This indicates that exposure to DAPs possibly has a role in causing oxidative stress, which could potentially result in health problems such as damage to lipids and DNA. A recent study discovered a correlation between OP metabolites found in the urine of individuals from agricultural households and MDA (Abbasi-Jorjandi et al., 2020).

The region of upper northern Thailand is well-known for its agricultural activities, which include the cultivation of rice, the growth of fruit farms (such as lychee, longan, and mango), and the production of vegetables. Originally, these agricultural systems employed OP insecticides to control pests like rice stemborers, fruit flies, and aphids. The prevalence and severity of pest infestations in specific crops during different seasons could potentially impact the use of pesticides, including organophosphates (Trébuil et al., 2006).

Chiang Dao is a district situated in the northern region of Chiang Mai Province, Thailand. Agriculture is an important part of Chiang Dao's local economy and population sustenance. Local farmers cultivate a diverse range of crops well adapted to the specific climate and soil conditions of Chiang Dao. Rice cultivation is a particular industry in the district, involving the cultivation of both upland and lowland rice varieties in various areas. Additional agricultural produce comprises various fruits, such as lychee, longan, mango, and citrus fruits, along with vegetables, herbs, and flowers (Mekanupak and Sreshthaputra, 2016). Production in agriculture is contingent upon the crop type and season. The post-harvest season in Chiang Mai spans from January to May. During this time, farmers work on the cultivation and harvesting of various vegetables and fruits, including rice, maize, soybean, mango, longan, and tangerine (Chiang Mai Agricultural Extension Office, Thailand). The agricultural products exhibit seasonal variations from year to year (Office of Agricultural Regulation, Thailand), resulting in different pesticide usage.

Village Health Volunteers (VHVs) serve an important part in Thailand's primary healthcare system. These individuals are community members who voluntarily devote their efforts and time to advance public health and provide fundamental healthcare services at the local level. In Thailand, VHVs play a crucial role in connecting formal healthcare services with local communities. They empower individuals and communities by providing education, prevention strategies, and community-based interventions, enabling them to take responsibility for their own health and well-being. Their unwavering devotion and steadfastness make a substantial contribution to the success of Thailand's primary healthcare system.

However, the study examines the impact of OP insecticide exposure on oxidative stress in northern Thailand, a region where the primary agricultural sector is limited, particularly among adults. There is only one study that investigated DAPs for OP exposure effects on oxidative stress by measuring glutathione (GSH) and MDA in urine among children. They found the levels of total DAPs among children in the agricultural community were significantly higher than in the urban community. Besides, GSH levels among children in the agricultural community were significantly lower than those in the urban community but MDA levels did not differ. However, this evidence provides supporting OP exposure can cause oxidative stress in children (Sapbamrer et al., 2020).

This study measured the concentration of urinary DAPs and investigated the relationship between DAPs and MDA among rural villagers in two post-harvest seasons (2021 and 2022).

MATERIAL AND METHODS

Study population and questionnaire data collection

The study population were village health volunteers (VHVs) who lived in Ping Khong sub-district, Chiang Dao district, Chiang Mai province, Thailand. The VHVs are a group of adults in the village who volunteer to work on public health communication between health stations and villagers in their own village. All 99 VHVs residing in the villages were extended invitations and informed about the study, and 95 VHVs participated. We followed up with the participants and collected data twice post-harvest. The first time, we collected in March 2021 (n = 95), and the second time was in April 2022, with only 65 VHVs participating. Before the questionnaire and urine sample collection, all participants signed a consent form following the ethical approval statement from the Research Institute for Health Sciences, Chiang Mai University (No 8/2021).

The questionnaire data was collected through individual interviews. In the context, demographic data, including age, gender, nationality, education, smoking status, occupation, and chronic health disease, including biomass burning, were collected from the question of burning in a house area or burning around a house area (yes or no). All participants were evaluated for height and weight for body mass index (BMI) calculations. Data on pesticide use was collected for farmers.

Organophosphate insecticides application

The application of OP insecticide was forecasted based on data regarding agricultural crops obtained from the Chiang Mai Agricultural Extension Office in Thailand (Chiang Mai Agricultural Extension Office, Thailand). A list of agricultural crops planted and harvested from January to May in 2021 and 2022 was compiled and specified for OP insecticide application. In addition, the classification of OP insecticides is divided into methyl OP and ethyl OP metabolites.

Urine sample collection

Each participant's spot urine was collected in a 100 mL cup. Each urine sample was aliquoted into a 10 mL tube, labeled, and then cooled in an ice box before being sent to the toxicology lab at Chiang Mai University's Environment and Health Research Unit (RIHES). Then, it kept at -20 °C until analysis.

Urinary biomarkers analysis

Dialkylphosphate

Six dialkylphospahtes (DAPs) were analyzed using the previous method (Prapamontol et al., 2014). Briefly, the urine sample (5 mL) was pipetted into 2 g of sodium chloride. After that, dibutyl phosphate (DBP) as an internal standard and 6 M hydrochloric acid were added. The extraction was processed twice: adding 5 mL of 1:1% v/v mixed acetone and ethyl acetate to the mixture, shaking for 3 min, and centrifuging (2,000 rpm) for 3 min. Then, we collected the supernatant into a bottle that contained 20 mg of potassium carbonate (K_2CO_3). The combined supernatant was evaporated by a rotary evaporator to almost dryness. The 2 mL of acetonitrile was added and then brought to dryness. The dried residue was re-dissolved with 3.0 mL of acetonitrile and transferred to a clean screw test tube containing 20 mg of K_2CO_3. Then, derivatize with pentafluorobenzyl bromide (PFBBr) and overnight incubate at 50 °C. After derivatization, water was added to the sample and extracted with 5 mL of hexane twice. The supernatant was dried under a gentle stream of nitrogen and concentrated with 200 µL of toluene before being analyzed by a gas chromatograph with a flame photometric detector (GC-FPD).

Malondialdehyde (MDA) analysis

Fifty microliters of the urine sample were pipetted into a glass test tube. Then, 300 µL of 0.5 mol/L phosphoric acid and 150 µL of 10 mmol/L 2-thiobarbituric acid (TBA) were added, and the sample was mixed. The mixture was then incubated at 95 $^{\circ}$C for 1 hour. After incubation, 500 µL of methanol was added and centrifuged (800 × g) for 5 min. After 5 min of centrifugation, the sample was filtered by a 0.2 µm syringe filter and analyzed by High Performance Liquid Chromatography with ultraviolet detection (HPLC-UV), as previously described in detail (Lee and Kang, 2008).

Statistical analysis

All statistical analyses were calculated separately for the two post-harvest seasons (2021 and 2022). The demographic and pesticide use of farmer participant data were analyzed using descriptive statistical analysis and a chi-square test, indicating number, prevalence, and p-value. The urinary DAPs and MDA concentrations were analyzed with concentrations that were above the limit of detection (LOD), and the lower LOD concentration was calculated with LOD/2 when the geometric standard deviation (GSD) was 3 or greater and LOD/√2 for GSD was lower than 3. The geometric mean (GM) and GSD were reported for the 50% above

LOD (Hornung and Reed, 1990; Panuwet et al., 2008). All urinary concentrations were log-10 transformed before comparison and association analysis. A pair sample t-test was used for comparing the urinary DAPs and MDA concentrations of the same person between 2021 and 2022 post-harvest seasons. The association between DAPs and MDA was analyzed by a linear regression model adjusted with eight co-variables, including gender, age, BMI, education, nationality, smoking status, medical condition, and occupation (farmer and non-farmer). The statistical analysis was conducted using SPSS 26 and STATA 14, with a significant level of 5%. Linear regression models were interpreted as beta (β) with 95% confidence intervals (CI).

RESULTS

Participant demographic

The demographic data were reported in Table 1 with age, gender, body mass index (BMI) range, nationality, education, smoking status, occupation (farmer and non-farmer), and medical condition (at least one chronic illness). In 2021 and 2022 post-harvest season, the results were the same. In 2021, the mean age was 47.3 years old, and in 2022, it was 47.5 years old. Most participants were female, both in 2021 (57.9%) and 2022 (60.0%), and the BMI range of participants was obese I (40.0% and 38.5% in 2021 and 2022, respectively), following the normal range in 2021 (21.1%) and overweight in 2022 (21.5%). In terms of nationality and education, most participants were Thai in both 2021 (52.6%) and 2022 (69.2%), with the secondary school showing the highest level of education: 55.8% in 2021 and 67.7% in 2022. Furthermore, most participants were non-smokers, with 87.4% in 2021 and 86.2% in 2022.

Table 1. Demographic data of participants separate post-harvest in 2021 and 2022.

	2021 post-harvest (N = 95)	**2022 post-harvest (N = 65)**
Age (Mean (Min-Max))	47.3 (19-74)	47.5 (20-75)
Gender		
Male	40 (42.1)	26 (40.0)
Female	55 (57.9)	39 (60.0)
BMI		
< 18.5 (Underweight)	3 (3.2)	3 (4.6)
18.5 - 22.9 (Normal range)	20 (21.1)	10 (15.4)
23.0 - 24.9 (Overweight)	17 (17.9)	14 (21.5)
25.0 - 29.9 (Obese I)	38 (40.0)	25 (38.5)
≥ 30 (Obese II)	17 (17.9)	13 (20.0)
Nationality		
Thai	50 (52.6)	45 (69.2)
Others (Hmong, Palaung, Lahu)	45 (47.4)	20 (30.8)
Education		
Primary school and lower	37 (38.9)	16 (24.6)
Secondary school	53 (55.8)	44 (67.7)
University	5 (5.3)	5 (7.7)
Smoking		
Non-smoking	83 (87.4)	56 (86.2)
Active smoking	12 (12.6)	9 (13.8)
Occupation		
Non-farmer	20 (21.1)	15 (23.1)
Farmer	75 (78.9)	50 (76.9)
Medical condition (at least one chronic illness)		
No	56 (58.9)	36 (55.4)
Yes	39 (41.1)	29 (44.6)

Descriptive of pesticides application

Table 2 shows the pesticide use profile of farmers compared between two years of post-harvest season. Most of the pesticide use profile variables showed no difference, except for pesticide spay, where 2021 was higher than 2022 (68.1% and 46.0%, respectively) (*P* = 0.015).

Table 3 presents the forecasts for OP insecticide application across two post-harvest seasons. The major agricultural crops cultivated and harvested from January to May in 2021 and 2022 comprised rice, maize, soybean, onion, mango, longan, lychee, and tangerine, with methyl OP insecticides being the most utilized.

Table 2. Prevalence of pesticides use factors of farmer separate post-harvest in 2021 and 2022.

	2021 post-harvest (n = 75)	2022 post-harvest (n = 50)	*P*-value*	n (%) N = 125
Pesticide use	68 (90.7)	48 (96.0)	0.258	116 (92.8)
Number of years for pesticide use				
≤ 5 years	15 (20.3)	9 (18.4)	0.952	24 (19.5)
6 - 10 years	15 (20.3)	11 (22.4)		26 (20.8)
11 - 20 years	16 (21.6)	9 (18.4)		25 (20.0)
> 20 years	28 (37.8)	20 (40.8)		48 (39.0)
Total number of days per week worked in the field; day(sd)	4.63 (2.40)	4.51 (2.40)	0.791	4.58 (2.39)
Total number of hours per day worked in the field; hour(sd)	6.19 (3.46)	6.24 (2.92)	0.927	6.21 (3.26)
Own Farm	70 (93.3)	45 (93.8)	0.927	115 (92.0)
House located in farm	20 (27.0)	18 (36.0)	0.288	38 (30.4)
Pesticide preparation	48 (64.9)	31 (62.0)	0.745	79 (63.7)
Pesticide spray	49 (68.1)	23 (46.0)	0.015	72 (57.6)
PPE use				
No	9 (12.3)	1 (2.0)	0.117	10 (8.2)
Sometimes	50 (68.5)	39 (79.6)		89 (73.0)
Always	14 (19.2)	9 (18.4)		23 (18.9)

Note: * *P*-vale test by chi-square

Table 3. OP insecticides information for agricultural crops in two post-harvest seasons.

Agriculture crop[a]	OP insecticides	DMAP[b]	DEAP[c]
Rice	Chropyrifos		✓
	Fenitrothion	✓	
	Malathion	✓	
Maize	Chropyrifos		✓
	Diazinon		✓
Soybean	Pirimiphos-methyl	✓	
	Triazophos		✓
Onion	Methamidophos	✓	
	Momocrotophos	✓	
Strawberry	Chropyrifos		✓
	Prothiofos		✓
Mango	Malathion	✓	
Longan	Acephate	✓	
	Edifenphos		✓

Agriculture crop[a]	OP insecticides	DMAP[b]	DEAP[c]
	Malathion	✓	
	Momocrotophos	✓	
	Triazophos		✓
Lychee	Chropyrifos		✓
Tangerine	Dimethoate	✓	
	Ethion		✓
	Malathion	✓	
	Methamidophos	✓	

Notes: a = Agriculture crops that are planted and harvested between January and May in 2021 and 2022, as cited by the Chiang Mai Agricultural Extension Office in Thailand (Chiang Mai Agricultural Extension Office, Thailand)
b = DMAP: Dimethyl alkyl phosphate including DMP, DMTP and DMDTP
c = DEAP: Diethyl alkyl phosphate including DEP, DETP and DEDTP

Urinary DAPs and MDA

DAPs

The six DAPs metabolites (DMP, DMTP, DMDTP, DEP, DETP and DEDTP) concentration with creatinine adjusted, total DMPs, total DEPs and total DAPs were reported in Table 4. The results show the highest abundance found in DEP for 2021 (100 %) and in DETP for 2022 (93.8 %). Fow the lowest frequency found in DMDTP for both 2021 and 2022 (14.7 % and 4.6 % respectively). In each year, the found total DMPs was higher than total DEPs with 68.3 nmol/g Cr for 2021 and 38.6 nmol/g Cr in 2022. Moreover, the pair t-test of total DMPs, total DEPs and total DAPs between same person of participant between two years (n = 65), showed the concentration of total DMPs, total DEPs and total DAPs in 2021 were significantly higher than 2022 with p = <0.001, <0.001 and <0.001 respectively.

In addition, when we compared the total DMPs, total DEPs and total DAPs between farmer and non-farmer separate 2021 and 2022 (Table 5), there were no differences between farmer and non-farmer for total DMPs, total DEPs and total DAPs in both 2021 and 2022.

MDA

The MDA concentrations of two post-harvest seasons in 2021 and 2022 (n = 65) were compared by pair t-test, found that the MDA concentration in 2021 is significantly higher than 2022 (P < 0.001), We determined MDA concentration and calculated in unit µg/g Cr, found the GM of MDA concentrations in 2021 and 2022 were 86.89 µg/g Cr and 58.75 µg/g Cr respectively, and there were no differences between farmer and non-farmer (Table 5).

Table 4. Concentrations of urinary OP metabolites and MDA in two post-harvest seasons.

	2021 post-harvest (n = 95)							2022 post-harvest (n = 65)							*P*-value*
	% >LOD	Max	GM	GSD	P50	P75	P95	% >LOD	Max	GM	GSD	P50	P75	P95	
OP metabolites (μg/g Cr)															
DMP	33.7	35.7	NC	NC	5.65	10.5	21.5	18.5	24.6	NC	NC	<LOD	5.63	10.4	
DMTP	21.1	7.14	NC	NC	1.09	1.98	3.61	66.2	3.61	0.72	2.10	<LOD	1.32	2.61	
DMDTP	14.7	3.57	NC	NC	0.56	0.97	2.10	4.6	7.93	NC	NC	<LOD	<LOD	0.93	
DEP	100	106	3.12	2.27	2.93	4.20	14.0	86.2	17.8	1.08	2.23	1.01	1.64	3.42	<0.001
DETP	83.2	25.0	1.09	2.35	1.00	1.86	5.12	93.8	9.89	0.41	2.20	0.38	0.57	1.75	<0.001
DEDTP	29.5	16.4	NC	NC	0.91	1.52	3.49	29.2	3.24	NC	NC	<LOD	0.73	1.35	
Total DMPs (nmol/g Cr)	50.0	398	68.3	2.10	65.0	114	224	69.2	229	38.6	2.01	34.6	64.3	113	<0.001
Total DEPs (nmol/g Cr)	100	758	34.6	2.14	31.4	47.8	137	96.9	124	12.4	2.14	10.4	18.8	53.7	<0.001
Total DAPs (nmol/g Cr)	100	1,151	109	2	104	178	302	96.9	237	54.0	1.92	48.8	87.8	172	<0.001
MDA (μg/g Cr)	100	381	90.6	1.70	84.2	129	222	100	199	58.8	1.54	56.7	73.2	139	<0.001

Notes: DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DEP, diethylphosphate; DETP, diethylthiophosphate; DEDTP; diethyldithiophosphate; MDA, malondialdehyde; μg/g Cr, microgram per gram creatinine; nmol/g Cr, nanomole per gram creatinine, Total DMPs = DMP + DMTP + DMDTP; Total DEPs = DEP + DETP + DEDTP; Total DAPs = Total DMPs + Total DEPs
Molecular weight; DMP, 110 g/mol; DMTP, 142 g/mol; DMDTP, 158.17 g/mol; DEP, 153.09 g/mol; DETP, 169.16 g/mol; DEDTP, 203.25 g/mol.
LOD, limit of detection; Max, maximum concentration; GM, geometric mean; GSD, geometric standard deviation; P50, 50th percentile; P75, 75^{th} percentile; P95, 95th percentile; NC, Not calculated
* *P*-value test by pair-sample t-test

Table 5. Urinary DAPs and MDA concentration (µg/g Cr) stratified by farmer and non-farmer separate two post-harvest seasons.

2021 post-harvest season	Farmer (n = 75)		Non-Farmer (n = 20)		*P*-value*
	GM	GSD	GM	GSD	
DAPs (nmol/g Cr)					
Total DMPs	70.1	2.07	62.0	2.25	0.516
Total DEPs	33.4	1.96	39.3	2.84	0.393
Total DAPs	108	1.92	112	2.34	0.818
MDA (µg/g Cr)	88.7	1.74	97.9	1.58	0.467
2022 post-harvest season	**Farmer (n = 50)**		**Non-Farmer (n = 15)**		***P*-value**
	GM	**GSD**	**GM**	**GSD**	
DAPs (nmol/g Cr)					
Total DMPs	38.5	2.04	38.8	1.96	0.968
Total DEPs	11.2	1.88	17.1	2.87	0.160
Total DAPs	51.8	1.89	61.7	2.05	0.371
MDA (µg/g Cr)	58.4	1.56	59.9	1.50	0.845

Note: * *p*-value test by independent-samples t-test

Associated between co-variates and oxidative stress.

We analyzed associations between the malondialdehyde (MDA) and the co-variates including gender, BMI, age, nationality, active smoking, chronic health conditions, education, and occupation (farmer and non-farmer), separate two post-harvest seasons (Table 6). In 2021, there were no association between MDA and 8 co-variates. However, we found significant relationship between MDA and medical condition and smoking in 2022 with β = 1.32 (*P* = 0.023) and β = 1.40 (*P* = 0.037) respectively, no significant association for others 6 co-variates (gender, BMI, age, nationality, education, and occupation; farmer and non-farmer).

Table 6. Association between co-variates and MDA.

Co-variable	MDA			
	β	95% CI		*P* value
2021 post-harvest				
Gender	0.85	0.66	1.10	0.213
Body Mass Index	0.98	0.96	1.01	0.239
Age	1.01	1.00	1.02	0.110
Nationality	1.01	0.78	1.30	0.946
Education				
Primary	1.00	-	-	-
Secondary	0.99	0.76	1.28	0.938
University	1.09	0.66	1.81	0.739
Medical condition	1.01	0.80	1.28	0.908
Smoking	1.38	0.98	1.94	0.068
Farmer	0.97	0.73	1.30	0.840
2022 post-harvest				
Gender	0.88	0.70	1.10	0.245
Body Mass Index	1.00	0.97	1.02	0.663
Age	1.01	1.00	1.02	0.279
Nationality	0.92	0.71	1.19	0.528
Education				
Primary	1.00	-	-	-
Secondary	0.99	0.75	1.29	0.920
University	0.91	0.59	1.41	0.676
Medical condition	1.35	1.04	1.75	0.023
Smoking	1.40	1.02	1.93	0.037
Farmer	1.01	0.80	1.29	0.902

Note: Regression coefficient (β) with 95% confidence interval (CI) calculated by linear regression

Associated between DAPs and oxidative stress.

We analyzed associations between total DMPs, total DEPs and total DAPs and MDA in two post-harvest seasons (2021 and 2022) by linear regression adjusting for gender, BMI, age, nationality, active smoking, education, and occupation (farmer and non-farmer) (Table 7). In 2021, we found the associated of total DMPs and total DAPs with MDA, β = 1.53 and 1.59 respectively. In addition, total DEPs was associated with MDA in 2022, β = 1.44 and border line reached for total DAPs (β = 1.43, *P* = 0.051).

Table 7. Association between DAPs with MDA.

DAPs	MDA			
	β	95% CI		*P* value
2021 post-harvest				
Total DMPs	1.53	1.08	2.17	0.018
Total DEPs	1.37	0.98	1.90	0.063
Total DAPs	1.59	1.10	2.30	0.014
2022 post-harvest				
Total DMPs	1.30	0.93	1.83	0.120
Total DEPs	1.44	1.05	1.98	0.026
Total DAPs	1.43	1.00	2.06	0.051

Note: Regression coefficient (β) with 95% confidence interval (CI) calculated by linear regression gender, BMI, age, nationality, active smoking, chronic health conditions, education, and occupation (farmer and non-farmer).

DISCUSSION

We measured the dialkylphosphates (DAPs), which are a group of common organophosphate (OP) insecticides, divided into two years of sample collection time. When we compared DAPs concentrations between the same person on two post-harvest seasons (2021 and 2022), we found the concentrations of total DMPs, total DEPs, and total DAPs post-harvest in 2021 were significantly higher than post-harvest in 2022, indicating that OPs exposure was higher in 2022 among village health volunteers (VHVs). The reported result may be attributed to the differing applications of OPs between 2021 and 2022. Agricultural Regulation in Thailand indicated that pesticide imports in 2021 exceeded those in 2022, particularly for insecticides, which were imported in higher amounts in 2021 by approximately 10,000 tons. This indicates higher application of OPs in 2021 relative to 2022 (Office of Agricultural Regulation, Thailand). In addition, it may be the changing of lifestyle or agriculture habitat between the two years, especially from the COVID-19 pandemic, as the terrible period from the middle of 2021 to peak in the middle of 2022 caused a change in agricultural activity, with more people staying at home and fewer going out to work on farms (World Health Organization, WHO COVID-19 dashboard).

In addition, our investigation revealed DEP to be the most prevalent OP metabolites, in line with previous studies in Thailand that found DEP to have the highest detection percentage of OP metabolites (Sapbamrer et al., 2020; Suwannarin et al., 2021; Suwannarin et al., 2020). Particularly, studies have reported that chlorpyrifos, one of the ethyl OPs, is the most found OP residue (Prapamontol et al., 2020; Wongta et al., 2022). Comparing the concentration of OPs between the two groups (methyl and ethyl OPs), we found that the total DMP concentration is higher than the total DEPs concentration in both 2021 and 2022. This finding is consistent with previous research, which found the most methyl OP residues were used among farmers in northern Thailand (Sapbamrer and Hongsibsong, 2014), particularly in the rice growers, the main crop of this study area, that found high concentrations of DMP (Wongta et al., 2018). In addition, the levels of total DMPs, total DEPs, and total

DAPs were compared to those found in other studies, i.e., farm workers from the other districts in Chiang Mai province, and non-farmworkers in China and Japan (Table 8). The levels of total DMPs of present study is lower than those of Fang district in SAWASDEE birth cohort study, China and Japan studies, and total DEPs and total DAPs in the present study is lower than those all three studies.

In 2021 post-harvest season, we discovered a relationship between total DMPs and total DAPs and malondialdehyde (MDA), and a border line was reached for total DEPs. In 2022 post-harvest season, we discovered a relationship between total DEPs and total DAPs and MDA. Methyl and ethyl OPs may have been applied more frequently during different sample collection times, especially with maize crops, mango, and longans. According to the Agricultural Extension Office in Chiang Mai, mango production, particularly use of methyl OPs, was higher in 2021 than it was in 2022 at this study site, while longan and maize crops, for which ethyl OPs are common, output was higher in 2022 than in 2021 (Table 3). The primary use of methyl OPs and ethyl OPs for mango and maize, respectively, supported the association conclusion (Prapamontol et al., 2020; Suwannarin et al., 2020). However, our research confirmed the earlier study's findings that DAPs can impact oxidative stress; one occupational study reported that OP insecticide appliers had higher MDA than farmers in general (Surajudeen et al., 2014). Another study found associations between OP metabolites in urine and MDA in male farmers (Lee et al., 2017). Finally, one study found associations between OPs metabolites in the urine of members of farm families and MDA in the urine (Abbasi-Jorjandi et al., 2020). These farming studies, as well as our school study, indicate that OP insecticides could cause oxidative stress.

The study possesses several strengths and limitations. The study focuses on village health volunteers (VHVs), who have played an essential part in supporting the healthcare system and providing counseling to farmers in the local community. Therefore, this study's knowledge can significantly contribute to raising awareness about the use of pesticides by farmers. However, we did not gather data regarding the source of OP insecticide exposure in agricultural or residential environments. In addition, the identification of a single oxidative biomarker by a small number of participants might limit the ability to draw causal conclusions. Therefore, further research with a specific set of biomarkers is necessary to provide additional support for this issue.

Table 8. Comparison of total DMPs, total DEPs, and total DAPs concentrations (nmol/g Cr).

Study	Participants	City or country	Group of participants	Number of participants	DAPs concentration (nmol/g Cr)		
					Total DMPs	Total DEPs	Total DAPs
This study	Rural adults	Chiang Dao district, Chiang Mai province, Thailand	Year 2021	95	68.3	34.6	109
			Year 2022	65	38.6	12.4	54
(Baumert et al., 2022) (SAWASDEE birth cohort)	Pregnant women (farmworkers)	Fang and Chom Thong districts, Chiang Mai province, Thailand	All participants	330	58.8	74.7	147
			Fang site	108	76.7	164	272
			Chom Thong site	220	51.7	50.8	109
(Lin et al., 2021)	Non-farmworkers	China		114	208	71.0	317
(Hioki et al., 2019)	Pregnant women (non-farmworkers)	Japan		62	89.0	43.0	147

CONCLUSION

In the post-harvest years of 2021 and 2022, urinary dialkylphosphates (DAPs) were used to measure the exposure to organophosphate (OP) insecticides among rural village health volunteers (VHVs) in upper northern Thailand, of which 75% are farmers. The concentration of DAP differs slightly between the two periods due to the COVID-19 pandemic, which has implications for agricultural activity. The urinary total dialkylphosphates (DAPs) exposure was higher in 2021 compared to 2022, correlating with the critical period of the COVID-19 pandemic. Nevertheless, post-harvest exposure to OP insecticides may cause oxidative stress, particularly urinary malondialdehyde (MDA), to be more common among rural villagers in Chiang Mai, Thailand. This study suggested that OP exposure in post-harvest season depends on insecticides used in each crop production and can cause oxidative stress in the human body via urinary DAPs and MDA expressions among farmers. Hence the protection of insecticides exposure using personal protective equipment (PPE) is crucial.

ACKNOWLEDGEMENTS

The authors express their gratitude to all village health volunteers in Ping Khong subdistrict, Chiang Dao, Thailand, for their valuable contribution of data and samples to this study. We would like to thank the Research Institute for Health Sciences for their generous support of field and laboratory facilities.

AUTHOR CONTRIBUTIONS

Nathaporn Thongjan was responsible for data curation, laboratory analysis, data analysis, writing, and rewriting. Tippawan Prapamontol provided supervision, conceptualization, methodology, review, and rewriting, as well as funding acquisition. Dan Norback provided supervision and data analysis. Warangkana Naksen and Kawinwut Somsunun advised data curation and analysis. Nattawadee Promkam was responsible for data curation and laboratory analysis.

CONFLICT OF INTEREST

The authors declare that they hold no competing interests.

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