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Assessing Environmental Pesticide Exposures in Chili Farming Communities of Ubon Ratchathani Province, Thailand

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University of Michigan
2006

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An abstract of
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ABSTRACT

Assessing Environmental Pesticide Exposures in Chili Farming Communities of Ubon Ratchathani Province, Thailand
By Priyanka Pathak

Pesticides are pervasively used in Thailand to ensure a productive crop yield and to keep the agricultural sector economically competitive in the global food market. With regular application, pesticides tend to enter the watersheds and soils where they persist for long periods of time resulting in community-wide, low-level, chronic pesticide exposure. Children are especially vulnerable to the health risks of chronic pesticide exposures even at low doses since these highly reactive chemicals are very likely to chemically interfere with their early stages of growth and development during in utero and postnatal periods. This study aims to evaluate possible pathways of insecticide exposure and to assess baseline exposures in the community in Ubon Ratchathani farms, most of which are involved in chili production. Our findings indicate that several pesticides are frequently found in the local water systems. Chlorpyrifos appears at higher levels in reservoir and drinking water than in ground water, although not statistically significantly higher. p,p'-DDE is found in all waters at approximately equivalent levels. Most other pesticides were found in 10-78% of the water samples tested. Similarly, urinary metabolites of chlorpyrifos and permethrin metabolites were found in virtually all of the farmer urine samples tested with mean concentrations ranging from 2-5 ng/mL. These data suggest that exposure to organophosphate and pyrethroid insecticides is pervasive in this farming region of Thailand and that water may be an important pathway of exposure for which mitigation strategies can be implemented.

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INTRODUCTION

Thai Agriculture

Thai farmers utilize pesticides to ensure a productive crop yield in order to remain economically competitive in the global food market. Although Thailand's agricultural sector contributes to 12.4% of the nation's GDP, 42.4 % of Thailand's workforce of 38.64 million people is employed in agriculture (Central Intelligence Agency, 2011). About half of the country's land is agricultural (Thapinta & Hudak, 2000). A number of key factors have led to increased use of agricultural pesticides – the declining price of rice that has led to the planting of other crops that require pesticides and the shortage of agricultural labor to tend to the crops as more of the agricultural population seeks opportunities in other occupations (Jungbluth, 1996). Additionally, development of insect resistance, industrialization of agriculture, and seasonal crop conversion independent of environmental conditions have also led to an increase in pesticide use (Panuwet et al., 2012). Several thousand metric tons of herbicides, fungicides, and insecticides are imported annually for use by Thai farmers; the quantity imported, in metric tons, doubled between 1987 to 1996 and increased by a factor of four within the last decade (Panuwet et al., 2012; Thapinta & Hudak, 2000). In 2010 the most imported insecticides, according to the country's Department of Agriculture were chlorpyrifos, fenobucarb, cartap hydrochloride, cypermethrin, and methomyl (Panuwet et al., 2012). Out of 15 Asian countries that were assessed, Thailand ranks third in pesticide use per hectare of land and third in percent of agricultural Gross National Product spent annually on pesticides (Walter-Echols & Yongfan, 2005). Despite the increasing reliance and heavy use of pesticides, health effects of environmental and occupational pesticide exposure in

Thailand has not been well-studied and is only recently gaining importance as a research subject.

Although agricultural use of organochlorine insecticides has been banned in the country since 1983, they were still used for malaria vector control in northern provinces until 1999 and continue to be used along coastal regions and neighboring countries (Cheevaporn, Duangkaew, & Tangkrock-Olan, 2005; Stuetz, Prapamontol, Erhardt, & Classen, 2001). On occasion, organochlorines are used illegally in agriculture (Stuetz et al., 2001). Farmers are also using banned organophosphate pesticides such as methyl parathion based on results from a recent biomarker study (Panuwet et al., 2008). Other organophosphates, carbamates and pyrethroids, which continue to be imported, are sold and used legally but with little regulatory oversight (Panuwet et al., 2012).

Few studies in Thailand have thoroughly investigated environmental pesticide exposure from agricultural sources. Monitoring agencies, specifically the Pollution Control Department (PCD) and the Department of Agriculture (DOA) can use evaluations that identify major pathways of exposure to develop effective exposure mitigation strategies to make recommendations to reduce exposures to insecticides and improve community health (Thapinta & Hudak, 2000).

Contaminated Bodies of Water

Pesticides sprayed in fields travel through soil and contaminate bodies of water such as streams, lakes, and rivers. Between 1985 and 1988 Thailand's National Environmental Board found 13 insecticides in water samples from major rivers: 9 organochlorines, 3 organophosphates, and 1 carbamate (Thapinta & Hudak, 2000). In 1997 the Pollution Control Department reported finding 10 organochlorines, 3 organophosphates, and 1

pyrethroid in bodies of water located in agricultural regions in northern and eastern Thailand (Thapinta & Hudak, 2000). Studies of coastal waters in 1981, 1991, 1994, and 2005 found declining levels of the organochlorine DDT in coastal regions, but the consistently measureable concentrations of lindane suggest continued use even after banning agricultural use of organochlorine pesticides (Cheevaporn et al., 2005). These studies, all of which evaluated green mussels and one that also included mullets and river sediments, did not evaluate groundwater, which is commonly used for drinking water as well as for watering crops on farms. However, one study evaluating vegetable farmers in southern Thailand found that farmers' artesian wells, which supplied water for consumption as well as pesticide mixing, were contaminated with organophosphate residues (Jaipieam et al., 2009).

Regardless, the Thai government has not set water quality standards for organophosphates, pyrethroids, and carbamates, which tend to contaminate the environment at higher levels than organochlorines (Thapinta & Hudak, 2000).

Contaminated Soil

Pesticide residue concentrations in soil tend to be higher than those found in water due to a couple of factors. First, the surface soils generally have direct contact with sprayed pesticides and secondly, soils can absorb pesticides (Thapinta & Hudak, 2000). In 1996 the Pollution Control Department detected 4 organochlorines – DDT, dieldrin, dicofol, and endosulfan; 3 organophosphates – parathion, dophos, and mevinphos; carbofuran, a carbamate; and permethrin, pyrethroid in agricultural soil samples from northern Thailand (Thapinta & Hudak, 2000). The following year the Department reported finding 10 varieties of organochlorienes (Thapinta & Hudak, 2000).

Ubon Ratchathani, Thailand

Ubon Ratchathani (Figure 1) is a northeastern province in Thailand and is the easternmost province bordering both Laos and Cambodia. It is also one of Thailand's largest chili growing regions (Siriwong et al., 2011). According to Thailand's Agricultural Extension Department, about 77% of the families in Ubon Ratchathani participate in agriculture (Taneepanichskul, 2009).

Figure 1

Map of Thailand with Ubon Ratchathani shaded in red
Source: *Wikipedia*

Thai farmers are likely exposed to agricultural pesticides occupationally and through their diet. Also, since farmers' residences are completely integrated within the fields, it is likely that the farming communities of Ubon Ratchathani receive appreciable exposure to pesticides from their environment. Chili growing farmers in this province predominantly use organophosphates insecticides such as profenofos and chlorpyrifos (Siriwong et al., 2011).

Organochlorines insecticide exposure is also of concern since biomarker studies have demonstrated that organochlorines and their residues can still be found in the environment (Asawasinsopon et al., 2006; Barr, Weihe, Davis, Needham, & Grandjean, 2006; Brock, Melnyk, Caudill, Needham, & Bond, 1998; Walker et al., 2003). Organochlorines have been detected in breast milk and blood samples of farming women from Chiang Mai province in northern Thailand (Asawasinsopon et al., 2006; Sapbamrer et al., 2008; Stuetz et al., 2001).

Similarly, pyrethroid and carbamate insecticide have been detected in environmental samples throughout Thailand in the previously mentioned studies.

Biomarker data also provide evidence of pesticide exposure in Thais. Orange farmers in the Northern Province of Chiang Mai had significantly higher levels of acetylcholinesterase inhibition, indicative of organophosphate exposure, and levels of p,p'-DDE and p,p'-DDT exposure compared to non-farming consumer reference group (Prapamontol et al., 2006). Urinary metabolite analysis of small scale male farmers from the same province showed evidence of exposure to pyrethroids and organophosphate insecticides and also demonstrated that the type of pesticides they were exposed to depended on the crops they were cultivating (Panuwet et al., 2008).

Pesticides have been identified as key threats to children's health in Southeast Asia, where exposure to industrial chemical toxicants, an emerging health threat, is adding to the existing health burden caused by infectious diseases among children (Suk et al., 2003). Children are exposed to pesticides via multiple pathways: in utero, hand-to-mouth behavior, postnatal dietary intake through breast milk and food, as well as para-occupational or take-home exposures from farming parents (Panuwet, Prapamontol, Chantara, & Barr, 2009; Rosas & Eskenazi, 2008). Although dietary exposure is the main source of pesticide exposure among most children, non-dietary exposure makes a contribution to the overall exposure (Fenske, Lu, Barr, & Needham, 2002; Koch, Lu, Fisker-Andersen, Jolley, & Fenske, 2002). Several studies have concluded that children living near farms using pesticide or whose parents are agricultural workers have higher exposure levels to pesticides compared to children whose environment or family members are non-agricultural (Fenske et al., 2002; Koch et al., 2002; Lu, Fenske, Simcox, & Kalman, 2000; Panuwet et al., 2009; Rosas & Eskenazi, 2008). Indeed, recent data generated in the Barr-Ryan lab at Emory University

demonstrates that Thai children have higher exposures to pesticides than children in the US (as assessed using urinary dialkylphosphate (DAP) metabolite concentrations used for detecting organophosphate exposure) during both growing and non-growing seasons compared to children living in non-agricultural areas (Figure 2).

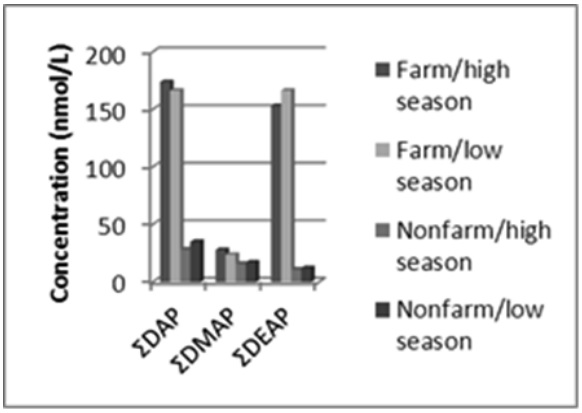


Figure 2

Dialkylphosphate concentrations in Thai farm and nonfarm children

To better understand exposures and exposure pathways in Ubon Ratchathani, a multi-pathway exposure study was designed with the following specific aims:

1) To identify predominant sources of pesticides in environmental media: surface soil, drinking water, groundwater, and reservoir water.
2) To quantify pesticides residues in surface soil, drinking water, groundwater, and reservoir water.
3) To compare insecticide levels between the tested environmental exposure pathways.
4) To assess farmer exposures by measurement of urinary metabolites of pesticides.

Given that agricultural surface soils have direct contact with sprayed pesticides and have the capacity to absorb organic compounds, we hypothesized that:

1) Three classes of insecticides (organophosphates, organochlorines, and pyrethroids) would be detected at higher concentrations in soil samples compared to water samples.
2) Water-soluble insecticides would be measurable in surface water, drinking water, and groundwater since soil can contaminate groundwater and surface water; groundwater was the main source of drinking water in the study community.
3) Over 50% of farmers would have detectable insecticide metabolites in their urine.

METHODS

Study Design

This study was a descriptive multi-matrix environmental exposure assessment that evaluated insecticide levels in four potential exposure pathways in agricultural communities in Ubon Ratchathani Province – groundwater, drinking water, surface water in the local reservoir, and surface soil from agricultural fields. In addition, general farmer exposure was evaluated in the community by the measurement of pesticide metabolites in urine samples. The study protocol was reviewed and approved by Emory's Institutional Review Board. All farmer participants provided informed consent prior to participation.

Sample Collection

Sample collection took place in chili growing farming communities in Huarua Sub-District, Mueang District, Ubon Rachathani Province, Thailand. Soil samples were collected from 51 locations within agricultural fields growing chili and various local vegetables. Field samples were collected according to established sampling protocols (Riederer et al., 2010). Each soil sample was a composite of 5 sub-samples of surface soil that were located within a 4 square meter area at randomly chosen locations within the boundaries of an agricultural field. Prior to desiccation, all soil samples were stored in air-tight plastic bags away from direct sunlight to prevent degradation of insecticides.

Additionally, 31 1-liter water samples were collected from groundwater sources, in-home drinking water storage, and surface water from the local reservoir. All water samples were covered with aluminum foil and stored in coolers with ice to prevent degradation of insecticides.

Similarly, urine samples were collected in regular urine collection cups and were transferred to storage vials for shipment. Farmers participated in this study on a voluntary basis. In order to be eligible to participate in the study, farmers had to be above age 18, grow chili or rice in the Huarua sub-district, and be directly responsible for pesticide application.

All samples, except soil, were shipped from Bangkok to Atlanta, GA on ice using World Courier Services. Soil samples could not be shipped from Thailand to the United States because of regulatory rules preventing their shipment.

Sample Preparation and Analysis

Water samples were loaded onto Strata-X polymeric solid phase extraction (SPE) cartridges in the laboratory at Chulalongkorn University. Each water sample, amounting to approximately 1-liter, was loaded into the cartridge over a time period ranging from 1 to several days using positive air pressure from squeezable hand pumps when necessary. Some samples, contaminated with sediment, were filtered using standard filter paper. After loading the water samples, cartridges were air-dried in a dark environment. They were then shipped to the Barr-Ryan Lab at Rollins School of Public Health, where the cartridges were washed with 4 ml of 5% methanol and water then eluted with 4 mL of 50:50 methanol:acetonitrile then 4 mL ethyl acetate elution. The eluates were combined, dried in a Turbovap evaporator at 40 °C using 25 psi of air and reconstituted in acetonitrile for a final drying step after which the extracts were reconstituted in 3:1 acetonitrile/toluene. All extracts were analyzed using gas chromatography-tandem mass spectrometry (GC-MS/MS) in the Barr-Ryan Lab using the method described in Appendix A and B. Quantitative values were obtained for two pesticides (chlorpyrifos and p,p'-DDT). Qualitative measurements (i.e., present/not present)

were obtained for chlorpyrifos-methyl, o,p-DDE, α-endosulfan, β-endosulfan, permethrin, cyfluthrin, cypermethrin, fenvalerate, and deltamethrin.

Urine samples were quantified for 3,5,6-tricholopyridinol, *cis*- and *trans*-dichlorovinylcyclopropane carboxylic acid (DCCA), malathion dicarboxylic acid and 3-phenoxybenzoic acid that are metabolites of chlorpyrifos/chlorpyrifos methyl, permethrin, malathion and pyrethroids, respectively, using a modification of a previously published method (Olsson et al., 2004). Briefly, urine sample aliquots were subjected to an enzyme digestion to liberate the target chemicals from their bound state. The urine was extracted using an OASIS solid phase extraction column (Phenomenex, Inc., Torrance, CA) and eluted with methanol. The extract was concentrated to dryness and analyzed using high performance liquid chromatography-tandem mass spectrometry according to the procedure shown in Appendix B. The target analytes, parent pesticides, limits of detection (LOD) and other pertinent information is given in Tables 1 and 2.

Table 1

Parent pesticides and target analytes			
	Insecticide Class	Parent compound	Analyte
In Water	Organochlorines	DDT	o,p-DDE
			α-Endosulfan
			β-Endosulfan
In Urine	Organophosphates	Chlorpyrifos Methyl	3,5,6-Trichloropyridiol
		Malathion	Malathion Dicarboxylic Acid
	Pyrethroids	Permethrin	3PBA, cis-DCCA and trans-DCCA
		Cypermethrin	3PBA, cis-DCCA and trans-DCCA
		Cyfluthrin	cis-DCCA and trans-DCCA
		Fenvalerate	3-PBA
		Deltamethrin	3-PBA
		Allethrin	3-PBA
		Resmethrin	3-PBA
		Deltamethrin	3-PBA

Table 2

Limit of Detection (LOD) values for insecticides in water	
Insecticide	ng
Chlorpyrifos Methyl	0.170
o,p-DDE	0.025
α-Endosulfan	0.022
β-Endosulfan	0.014
Permethrin	0.042
Cyfluthrin	0.027
Cypermethrin	0.052
Fenvalerate	0.096
Deltamethrin	0.667
Chlorpyrefos	0.150
p,p'-DDE	0.001

Statistical Analysis

Statistical analysis was done using the Wilcoxon-Mann-Whitney test to compare mean chlorpyrifos concentrations between groundwater and drinking water. A two-sample t-test was used to compare mean p,p'-DDE concentrations between groundwater and drinking water. Fisher's exact test was used to compare the distribution of eight other insecticides detected in groundwater and drinking water pathways for which there were only qualitative results. The limited number of samples would not produce statically powerful results using a Chi-square test. Prior to running statistical tests, concentrations that were below the LOD were imputed using the formula LOD/square-root(2). For chlorpyrifos the LOD was 0.15 ng. LOD values for other compounds are listed in Table 2, but no other values required imputation. All data were analyzed using SAS statistical software (SAS Institute, Cary, NC).

RESULTS

Water Samples

Detectable levels of pesticides were found in all categories of water samples: drinking water, ground water, and reservoir water. Results are shown in Table 3 and boxplots in Figures 3 and 4.

Chlorpyrifos was detected in 22 of 31 (70%) water samples; the median concentration was 0.22 ng/L. Median chlorpyrifos concentrations were similar in drinking water and reservoir water: 0.51 ng/L and 0.52 ng/L, respectively. Groundwater had the lowest median chlorpyrifos concentration at 0.21 ng/L. Chlorpyrifos concentrations varied most in drinking water samples (std. error = 0.190 ng/L) and least in reservoir water samples (std. error = 0.05). The Wilcoxon-Mann-Whitney test results suggest that there was no statistically significant difference between log-transformed concentration distributions of groundwater and drinking water (z= 0.033; p-value 0.742).

p,p'-DDT degradate**,** p,p'-DDE, was detected in all 31 water samples; the median concentration was 0.022 ng/L. Drinking water and reservoir water had similar median levels of p,p'-DDE: 0.027 ng/L and 0.022 ng/L, respectively). Ground water had the lowest median concentration at 0.018 ng/L. Concentrations varied most in groundwater samples (std. error = 0.072 ng/L) and least in drinking water samples (std. error = 0.027 ng/L). Two-sample t-test comparing log-transformed concentration distributions of DDE between groundwater and drinking water samples suggest that there was no statistically significant difference (t = -0.010; p-value = 0.990).

Table 3

Distribution of clorpyrifos and p,p-DDE concentrations in water pathways

			ng/L								
							Percentile				
Insecticide	Total	Missing	Mean	SE	Min	Max	25th	50th	75th	90th	95th
All Samples	**31**										
Chlorpyrifos	22	9 (29%)	0.320	0.165	0.156	0.578	0.192	0.217	0.515	0.548	0.562
p,p'-DDE	31	0 (0%)	0.042	0.056	0.003	0.302	0.011	0.022	0.066	0.087	0.102
Ground Water	**17**										
Chlorpyrifos	13	4 (24%)	0.227	0.096	0.156	0.531	0.175	0.210	0.218	0.255	0.531
p,p'-DDE	17	0 (0%)	0.048	0.072	0.006	0.302	0.010	0.018	0.069	0.068	0.302
Drinking Water	**10**										
Chlorpyrifos	5	5 (50%)	0.402	0.190	0.192	0.562	0.198	0.511	0.548	0.562	0.562
p,p'-DDE	10	0 (0%)	0.034	0.027	0.003	0.094	0.011	0.027	0.052	0.075	0.094
Reservoir Water	**4**										
Chlorpyrifos	4	0 (0%)	0.520	0.050	0.457	0.578	0.486	0.523	0.554	0.578	0.578
p,p'-DDE	4	0 (0%)	0.035	0.035	0.011	0.087	0.014	0.022	0.057	0.087	0.087

Abbreviations: SE, standard error; Min, minimum value; Max, maximum value

Figure 3

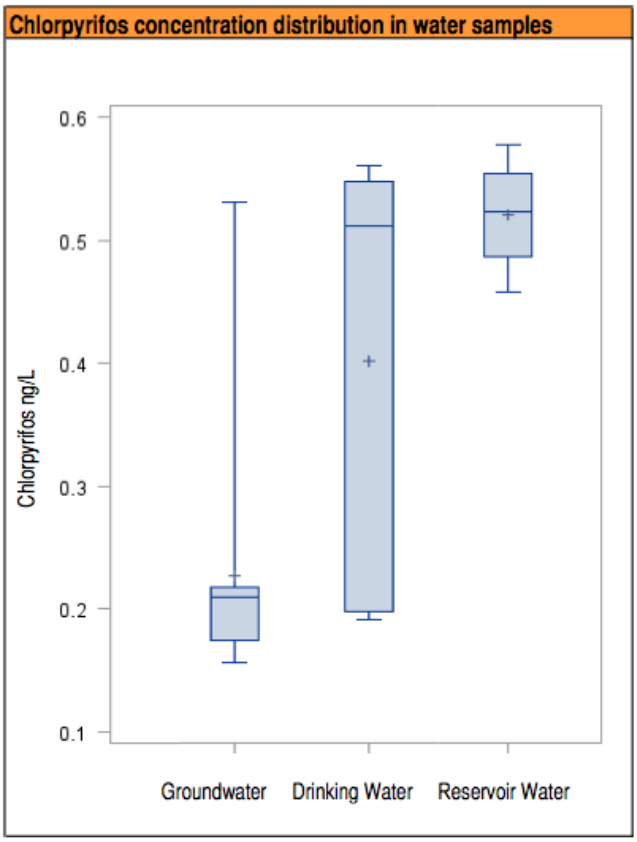
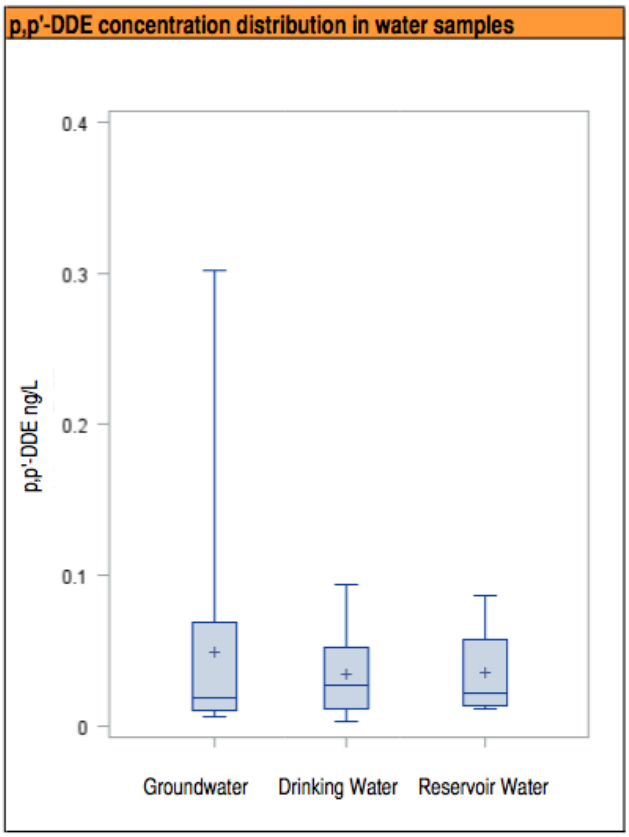

Chlorpyrifos concentration distribution in water samples
Chlorpyrifos ng/L
0.6
0.5
0.4
0.3
0.2
0.1
Groundwater
Drinking Water
Reservoir Water

Figure 4



All samples were also tested for nine other compounds (see Table 4), which were assessed qualitatively. Among all 31 water samples, the following compounds were detected in a large proportion of the samples: o,p'-DDE and α-endosulfan were detected in 22 samples (71%), permethrin in 24 samples (77%), and cypermethrin in 20 samples (65%). A large proportion of the 17 ground water samples contained the following insecticides: α-endosulfan and permethrin were detected in 13 (76%) if the samples, o,p'-DDE in 10 samples (58%), and cypermethrin in 9 samples (52%). Among the 10 drinking water samples, o,p'-DDE was detected in 9 samples (90%), permethrin in 8 samples (80%), cypermethrin in 7 samples (70%), and α-endosulfan in 5 samples (50%). α-endosulfan and cypermethrin

were detected in all 4 reservoir samples; o,p'-DDE and permethrin were detected in 3 samples, and fenvalerate was found in 1 sample. None of the other compounds tested were detected in the reservoir samples.

Table 4

Insecticides detected in water samples								
	All Samples (31)		*Groundwater (17)*		*Drinking Water (10)*		*Reservoir Water (4)*	
Insecticide	**<LOD**	**DNQ**	**<LOD**	**DNQ**	**<LOD**	**DNQ**	**<LOD**	**DNQ**
Chlorpyrifos Methyl	31 (100%)	0 (0%)	17 (100%)	0 (0%)	10 (100%)	0 (0%)	4 (100%)	0 (0%)
o,p'-DDE	9 (29.03%)	22 (70.97%)	7 (41.18%)	10 (58.82%)	1 (10%)	9 (90%)	1 (25%)	3 (75%)
α-Endosulfan	9 (29.03%)	22 (70.97%)	4 (23.53%)	13 (76.47%)	5 (50%)	5 (50%)	0 (0%)	4 (100%)
β-Endosulfan	24 (77.42%)	7 (22.58%)	11 (64.71%)	6 (35.29%)	9 (90%)	1 (10%)	4 (100%)	0 (0%)
Permethrin	7 (22.58%)	24 (77.24%)	4 (23.53%)	13 (76.47%)	2 (20%)	8 (80%)	1 (25%)	3 (75%)
Cyfluthrin	30 (96.77%)	1 (3.23%)	16 (94.12%)	1 (5.88%)	10 (100%)	0 (0%)	4 (100%)	0 (0%)
Cypermethrin	11 (35.48%)	20 (64.52%)	8 (47.06%)	9 (52.94%)	3 (30%)	7 (70%)	0 (0%)	4 (100%)
Fenvalerate	27 (87.10%)	4 (12.90%)	15 (88.24%)	2 (11.76%)	9 (90%)	1 (10%)	3 (75%)	1 (25%)
Deltamethrin	28 (90.32%)	3 (9.68%)	15 (88.24%)	2 (11.76%)	9 (90%)	1 (10%)	4 (100%)	0 (0%)

Abbreviations: <LOD, number of samples in which insecticide was below "limit of detection"; DNQ, number of samples that had "detectible, but not quantifiable" amounts of insecticide;

Table 5 shows the results from conducting Fisher's Exact Test comparing groundwater to drinking water with respect to these insecticides. Since all p-values were well above the alpha level of 0.05, we can conclude that samples from the two sources do not differ significantly.

Table 5

Fisher's Exact Test results comparing distribution of insecticide concentrations in groundwater to drinking water samples	
	p-value
o,p'-DDE	0.190
α-Endosulfan	0.219
β-Endosulfan	0.204
Permethrin	1.000
Cyfluthrin	1.000
Cypermethrin	0.448
Fenvalerate	1.000
Deltamethrin	1.000

Urine Samples

We analyzed urine samples from 42 farmers in the region. 27 (64%) of the 42 farmers also completed a demographic survey that also assessed their practices with pesticides. Farmer demographics are shown in Table 6. Only 2 of the 27 had completed high school (7%), 10 and had a middle school education (37%), and 15 (56%) had only completed primary school. Out of all 27, 25 (92%) reported using pesticides. They reported using a wide range of personal protective equipment (PPE) such as head covering, fabric trousers, long-sleeved fabric shirt, rubber boots, and a face mask while working with pesticides. Table 6 also presents a detailed breakdown of types used during corresponding activities. When asked whether they used PPE while preparing individual pesticides, 20 of the farmers responded. Only 3 (15%) of the respondents reported using non-penetrable long-sleeved shirts and non-penetrable trousers. 19 farmers responded to the question about using PPE while mixing different prepared pesticides together prior to spraying. Only 2 (11%) reported using non-penetrable long-sleeved shirts, and 1 reported using a non-penetrable trouser

(5%). When asked about using PPE while applying pesticides, 18 farmers responded. Again, only 2 (11%) used non-penetrable long-sleeved shirts and 1 (6%) reported using non-penetrable trousers. The vast majority of the farmers reported using a variety of PPE made of fabric when working with pesticides.

Table 6

Demographics and PPE use of biomonitored farmers

	count	Total
Age	48.07 (8.28%)	27
Female	23 (85.19%)	27
Farm Owner	25 (96.15%)	26
Education		27
None	0 (0%)	
Primary Only	15 (55.56%)	
Middle School Only	10 (37.04%)	
High School Only	2 (7.41%)	
College	0 (0%)	
Use Pesticides	25 (92.60%)	27
Application Method		8
Spray	8 (100%)	
Fogger	0 (0%)	
Powder	0 (0%)	

	count	Total
Use PPE wile preparing pesticides		20
Non-penetrable long-sleeved shirt	3 (15%)	
Fabric long-sleeved shirt	18 (90%)	
Non-penetrable trousers	3 (15%)	
Fabric trousers	17 (85%)	
Rubber gloves	12 (60%)	
Rubber boots	16 (80%)	
Hat or head covering	17 (85%)	
Other (e.g. face masks)	13 (65%)	
Use PPE while mixing pesticides		19
Non-penetrable long-sleeved shirt	2 (10.53%)	
Fabric long-sleeved shirt	16 (84.21%)	
Non-penetrable trousers	1 (5.26%)	
Fabric trousers	16 (84.21%)	
Rubber gloves	12 (63.16%)	
Rubber boots	15 (78.95%)	
Hat or head covering	16 (84.21%)	
Other (e.g. face masks)	13 (68.42%)	
Use PPE while applying pesticides		18
Non-penetrable long-sleeved shirt	2 (11.11%)	
Fabric long-sleeved shirt	16 (88.89%)	
Non-penetrable trousers	1 (5.56%)	
Fabric trousers	16 (88.89%)	
Rubber gloves	11 (61.11%)	
Rubber boots	15 (83.33%)	
Hat or head covering	16 (88.89%)	
Other (e.g. face masks)	11 (61.11%)	

Abbreviations: PPE, personal protective equipment

Table 7 shows the distribution of urinary biomarkers examined in 42 farmers. Malathion dicarboxylic acid, a urinary biomarker for malathion was below the level of detection for all of the urine samples. 3,5,6-Trichloropyridiol, a biomarker for chlorpyrifos, was detected in 41 (98%) samples. The median concentration was 1.475 ng/mL. *cis*- and *trans*-DCCA, biomarkers for the pyrethroids permethrin, cypermethrin, and cyfluthrin, were detected in all samples; the median concentration was 4.357 ng/mL. 3PBA, biomarker for pyrethroids and pyrethroid breakdown products, was detected in 30 (71%) samples, with median concentration at 1.328 ng/mL.

Table 7

Urinary Biomarker Distribution

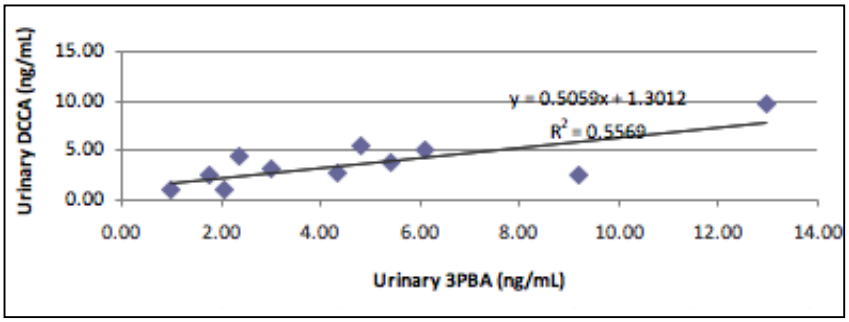
				(ng/mL)								
								Percentile				
Biomarker	No.	Missing	n <LOD	Mean	SE	Min	Max	25th	50th	75th	90th	95th
Malathion Dicarboxylic Acid	42	0 (0%)	42 (100%)									
3,5,6-Trichloropyridiol	41	1 (2%)	0 (0%)	3.091	3.999	0.290	20.180	0.784	1.475	2.944	7.121	11.109
DCCA	42	0 (0%)	31 (74%)	4.819	3.589	0.970	12.970	2.070	4.357	6.113	9.191	12.969
3-PBA	30	12 (71%)	0 (0%)	2.121	1.968	0.519	9.637	0.762	1.328	2.659	4.741	5.410

Abbreviations: DCCA, cis-DCCA and trans-DCCA; 3-PBA, 3-phenoxybenzoic acid; No., number; SE, standard error; Min, minimum value; Max, maximum value.

As shown in Figure 5, a high positive correlation ($R^2 = 0.560$) between 3PBA and DCCA existed among in 11 (26%) samples that contained both metabolites. Samples from 12 individuals (29%) had no detectable levels of DCCA and no detectible levels of 3PBA.

Figure 5

Correlation of Urinary DCCA and 3PBA Concentrations



DISCUSSION

Water

Chlorpyrifos concentration between the 17 groundwater and 10 drinking water samples were compared using the Wilcoxon-Mann-Whitney Test. A two-sample t-test could not be employed because chlorpyrifos concentrations were not distributed normally. Log transformed values of the concentrations also did not distribute normally. Concentrations had to be log-transformed in order to do a two-sample t-test comparing p,p-DDE concentrations between groundwater and drinking water. The original concentration values did not have a normal distribution, but once transformed, the assumptions for a t-test were met. The concentrations of chlorpyrifos were considerably less than OP pesticide concentrations found in Thai artesian well waters; however, we have no idea of the comparability of the methodology used for detection (Jaipieam et al., 2009). Jaipieam et al. reported average OP insecticide concentration in well water from agricultural communities (85 and 418 ng/L in dry and wet seasons, respectively) was higher than in non-agricultural communities (4 ng/L in both seasons). However, as reported, if the units measured were comparable, the values would have been comparable so we will discuss with these authors (our current collaborators) ways of cross validating our results. Measurable levels of DDT byproducts in the water samples are consistent with the findings of previous studies that demonstrate continued environmental contamination from previous use of DDT, the so-called legacy effect, or continued illicit use. Typically, the DDE/DDT ratio is evaluated to determine whether DDT was used recently; however, DDT was not measure in our samples, thus we cannot differentiate between current or past use.

Chlorpyrifos methyl was not detected in any water samples, therefore Fisher's Exact

Test was not used to compare groundwater and drinking samples for this insecticide's concentration. Its absence indicates that the sampled farmers must not use 3,5,6-trichloropyridiol recently. Cyfluthrin, which was detected in only 1 groundwater sample, was also an unpopular insecticide in this community. The most common insecticide contaminants in the water samples were DDT byproduct o,p'-DDE, β-endosulfan, cyfluthrin, and deltamentrin. All four compounds were prevalent in all three examined pathways. Consult Table 4 for counts.

Urine

For the most part, urinary concentrations of pesticide metabolites were similar to those observed previously in the Northern region of Thailand (Panuwet et al., 2008). Concentrations of TCPY were similar in both sets of farmers, however, concentrations of pyrethroid insecticide metabolites were somewhat higher in the farmers from Ubon Ratchathani. The low frequency of detection of malathion dicarboxylic acid was also consistent with Panuwet et al. (2008).

Given that 100% of the farmers had concentrations of malathion dicarboxylic acid below the LOD, farmers were not likely exposed to appreciable levels of malathion in the day or two prior to sampling. The strong positive correlation between 3PBA and DCCA in urine samples (see Figure 5) suggests that these individuals were exposed to the parent compounds permethrin and/or cypermethrin from which these two metabolites are derived (Barr et al., 2010; Hardt & Angerer, 2003). 19 individuals (%) had detectable levels of 3PBA and no detectable levels of DCCA. These individuals were likely exposed to a variety of pyrethroids aside from permethrin and cypermethrin, 3PBA formed in the environment, and/or degraded pyrethroids (Centers for Disease Control and Prevention, 2010). None of

the individuals had only cis- and trans-DCCA without the detectible levels of 3PBA in their urine, meaning that these farmers were not exposed to Cyfluthrin. See Table 1 for a list of biomarkers with their parent compounds. The 12 individuals (29%) who had no detectable levels of DCCA and no detectible levels of 3PBA were most likely not recently exposed to pyrethroid insecticides.

Finally, although the farmer survey was useful in providing a general idea about the demographics of the participating farmers, it should be noted that the survey might not represent all of the study's farmers accurately since less than two-thirds of the farmers answered questions, and those were mostly the women.

Soil

It was not possible to transport the soil samples to Emory University in time for the completion of this study. SPE cartridges containing pesticide residues from all soil samples are expected to arrive at the Barr-Ryan Laboratory by July of 2012 and will be analyzed then.

CONCLUSIONS AND RECOMMENDATIONS

It is clear that current practices of agricultural insecticide use have led to the contamination of groundwater, drinking water, and reservoir water in this community with insecticides. Given the limited number of groundwater and drinking water samples, statistical analysis from this study could not demonstrate a significant difference between the two water pathways. DDE levels in the water and urine samples are consistent with previous, but not current use of DDT in the area. Results from the urinary biomarker analysis suggest that virtually all of these farmers were exposed to a variety of pyrethroids and their break-down products, as well as the organophosphate chlorpyrifos or chlorpyrifos methyl, but not malathion. They are also likely exposed to DDT-related chemicals via their water supply, in addition to several pyrethroids. This suggests that current PPE methods and pesticide exposure prevention efforts, neither in the home environment nor in the work environment, are sufficient in protecting farmers and their families from pesticide exposure.

Given the small ample sizes within each pathway of water in this study, further studies should be done comparing more water sources. Further studies should also investigate carbamate concentrations. Soil concentrations should also be taken into account when evaluating the level of environmental pesticide contamination. Thai regulatory agencies should consider prioritizing efforts to promote rural occupational health and rural environmental quality in these remote villages if further studies confirm the widespread contamination of soil and water. The Thai government could help farmers develop sustainable and economical alternatives, such as integrated pest management, to using pesticide and develop more effective PPE in the short-run.

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Appendix A

Insecticide Residue Analysis Method in Water Using GC-MS/MS

The method used for the analysis of 26 pesticides – organochlorine, organophosphorus, pyrethroid, and carbamate pesticides – was developed in-house in the Barr/Ryan Laboratory. The validation results indicate good precision, accuracy and limits of detection. Chromatographic separation was performed by Agilent 7890A Gas Chromatography (GC) with tandem mass spectrometry (Agilent Technologies, Waldbronn, Germany). The GC consisted of a temperature-stable column compartment. The GC and MS modules were programmed and controlled using Mass Hunter Software version B.03.01 (B2065) (Agilent Technologies, Waldbronn, Germany). Pesticides were analyzed by GC-QQQ-MS/MS (EI, 70eV). Analyses were carried out using a 7890A GC coupled to a 7000B MS (Agilent Technologies, Waldbronn, Germany). The system was fitted with a deactivated silica guard column (0.250 mm internal diameter (ID)) (Agilent Technologies, Santa Clara, CA USA) connected to a HP-5MS analytical column (30m x 0.250 ID x 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA USA). It was operated in pulsed splitless mode (25 PSI, 1.5 min) with an injector temperature of 250°C). The helium carrier gas flow rate was 1.2 mL/min and the oven temperature program was as follows: 100°C (2 min), 205°C (10°C/min) for 3 min, 280°C (10°C/min) for 4 min, and 310°C (25°C/min) for 12 min. The interface, source, and quadropole temperatures were set to 315°C, 300°C, and 150°C, respectively. Multiple reactions monitoring (MRM) was used during the mass spectrometric analysis of the target compounds. The selected MRM transitions, including their associated parameters, are summarized in Table A-1.

This protocol was developed by Dr. Ronald E. Hunter for the Barr-Ryan Laboratory.

APPENDICES

Table A-1

Selected MRM Transitions and Parameters

		Pesticide	RT (min)	MW	3 Best Transitions - Natives						Transitions - ISTDs			
					Quant			Qual 1						
					Q1	Q3	CE	Q1	Q3	CE		RT	Q1	Q3
1	10.2	fenobucarb (fen)	10.69	207.3	121.2	103.1	20	121.2	51.2	40				
2	11.70	hexachlorobenzene (hcb)	11.95	284.8	284.0	249.1	25	284.0	214.2	40				
		atrazine (atr)	12.09	215.7	200.3	104.1	20	200.3	122.2	10				
3	12.57	propoxur (pro)	12.68	209.2	110.2	66.1	20	110.2	51.2	40				
		fonofos (fon)	12.68	246.3	246.2	137.2	5	109.1	63.1	15				
		bendiocarb (ben)	12.85	223.2	151.2	84.1	15	151.2	68.2	25				
		diazinon (dia)	12.85	304.3	304.3	179.3	15	179.3	121.0	40				
4	13.70	chlorpyrifos-methyl (chlm)	14.02	322.6	286.2	93.2	26	288.2	93.0	20	IS_chlm	13.96	291.9	274.0
													291.9	99.0
5	15.00	chlorpyrifos (cpy)	15.53	350.6	314.2	258.0	25	314.2	286.1	5	IS_cpy	15.39	324.0	292.0
													324.0	260.0
		parathion (par)	15.57	291.3	291.3	81.0	40	291.3	90.9	35				
											IS_par	15.43	301.0	115.0
		dicofol, p,p- (dic)	15.61	370.5	139.1	111.1	15	139.1	75.1	30				
6	16.10	heptachlor epoxide (hep)	16.61	389.3	353.1	263.1	10	353.1	282.1	15				
7	17.10	DDE, o,p- (ddeop)	17.49	318.0	246.2	176.2	35	248.2	176.3	30				
		prallethrin (pral)	17.49	300.4	123.2	87.1	15	123.2	105.2	20				
		endosulfan-α (endoA)	17.69	406.9	241.1	206.1	20	239.1	204.1	15				
8	18.15	DDE, p,p- (ddepp)	18.37	318.0	246.2	176.2	35	248.2	176.2	30	IS_ddepp	18.36	258.0	188.0
9	18.90	endosulfan-β (endoB)	19.22	406.9	241.1	206.1	15	239.1	204.1	20				
		DDT, o,p- (ddtop)	19.45	354.5	235.2	199.1	15	235.2	165.1	25				
10	20.50	piperonyl butoxide (pbo)	20.87	338.4	176.2	103.1	30	176.2	91.1	40				
		resmethrin (res)	20.92	382.5	171.2	143.2	5	123.2	95.2	6				
11	22.00	azinphos-methyl (azm)	22.25	317.3	160.2	77.2	20	132.2	77.1	15				
12	23.30	permethrin (per)	23.47	391.3	183.2	77.0	40	183.2	153.2	15				
			23.63											
13	24.00	cyfluthrin (cyf)	24.24	434.3	163.1	127.2	5	206.2	151.1	25	IS_cyp	24.79	170.0	134.0
			24.38											
			24.53									24.95	170.0	98.0
		cypermethrin (cyp)	24.68	416.3	163.1	127.1	15	181.2	152.2	25				
			24.82											
			24.93											
14	25.60	fenvalerate (fev)	26.15	419.9	125.2	89.0	20	167.2	89.2	40				
			26.53											
15	26.85	deltamethrin (del)	27.17	505.2	253.1	93.2	20	181.2	152.2	30				
			27.53											
16	28.00													

Appendix B

Urinary Pesticide Metabolite Analysis Using LC-MS/MS

The method used for the analysis of MDA, TCPY, DCCA, and 3-PBA was modified from the method developed by Olsson et al., (2004). Chromatographic separation was performed by Agilent 1200 High Performance Liquid Chromatography (HPLC) (Agilent Technologies, Waldbronn, Germany). The HPLC consisted of a binary pump, a degasser, a temperature-controlled auto sampler, and a temperature-stable column compartment. All of the HPLC modules were programmed and controlled using Mass Hunter Software version B.03.01 (B2065) (Agilent Technologies, Waldbronn, Germany). The Betasil-C18 analytical column (2.1x100 mm, 3 μm particle size, 110Å pore size, Thermo Fisher Scientific, Waltham, MA, USA) was placed in the column compartment with the temperature set at 45°C during analysis. The multi-step gradient elution using 0.1% acetic acid in water and 0.1% acetic acid in methanol was used for optimum separation of target compounds from other urine components. The total run time including the column re-equilibration was 19 minutes. The flow rate was set as 0.5 mL/min during the chromatographic separation.

A triple quadrupole 6460 tandem mass spectrometer (Agilent Technologies, Waldbronn, Germany) with a negative ion mode (jet-spray) electrospray ionization interface was used to analyze the samples. The following were the MS/MS setting parameters optimized to achieve the best overall sensitivity and selectivity: 300 °C Gas Temperature; 5 L/min Gas Flow; 325 °C Sheath Gas Temperature; 10 L/min Sheath Gas Flow; 35 psi Nebulizer; 3500v Capillary; 800v Nozzle Voltage. The mass spectrometer was programmed and controlled using Mass Hunter Software version B.03.01 (B2065) (Agilent Technologies, Waldbronn, Germany).

Multiple reactions monitoring (MRM) was used during the mass spectrometric analysis of the target compounds. The selected MRM transitions, including their associated parameters, are summarized in Table B-1.

This protocol was developed by Dr. Parinya Panuwet for the Barr-Ryan Laboratory.

Table B-1
Selected MRM transitions and associated parameters

Compound Name	ISTD	Precursor Ion	Product Ion	Dwell time (msec)	Fragmentor	Collision Energy (V)
MDA_L	Yes	280	147	200	80	1
MDA_Q		273	141	200	80	5
MDA_C		273	157	200	80	12
TCPY_Q		196	196	200	96	0
TCPY_C		198	198	200	96	0
DCCA_L	Yes	210	210	200	90	0
DCCA_Q		207	207	200	90	0
DCCA_C		209	209	200	90	0
3PBA_L	Yes	219	99	200	98	20
3PBA_Q		213	93	200	122	16
3PBA_C		213	169	200	122	8