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Organophosphate Exposure: A Preliminary Assessment on the use of Pesticide Intensity Score to Evaluate Exposure among Fruit Growers

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Abstract

This study examines the influence of work hours, personal protective equipment use, and pesticide ingestion on the amount of urinary metabolites among fruit growers applying organophosphate pesticide. Thirty nine urine samples were collected from seven applicators before and after organophosphate applications. All dimethyl metabolites were present in day 1 morning urine samples for all workers. The arithmetic means for day 1 ranged from 21.5-94.17 µg/L DMP, 6.25-81.25 µg/L DMTP, and <LOQ-153.17 µg/L DMDTP. Day 2 urine samples had the highest amount of metabolites. The arithmetic means ranged from 25.8-558 µg/L DMP, 15.75-398 µg/L DMTP, 21.5-568.57 µg/L DMDTP, and <LOQ-17.67 µg/L DEP. The arithmetic means for day 4 ranges from 19.2-182 µg/L DMP, 13.33-138 µg/L DMTP, 22.75-157.83 µg/L DMDTP, and <LOQ-26 µg/L DEP. From the questionnaire, the exposure algorithm based on duration of hours worked, PPE use and pesticide ingestion showed poor relationship with urine concentration ($r=0.1847$). The linear relationship is not established due to variability within and between applicators.

Keywords: organophosphate; pesticide intensity score; urinary metabolites; fruit growers.

1. Introduction

Exposure to organophosphates has been linked with to both acute and chronic adverse health effects such as neurotoxicity and certain types of cancer (McDuffie 1994; Nurminen *et al.,* 1995). Exposure to organophosphates may be measured quantitatively by determining the concentration of biomarkers excreted in the urine. Degradation of most organophosphates produces three dimethyl phosphate biomarkers: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP) and three diethyl biomarkers: diethylphosphate (DEP), diethylthiophosphate (DETP) and diethyldithiophosphate (DEDTP). These metabolites are expected to be fully excreted within 48-72 hours after termination of exposure (WHO 1987; CDC 2003). Studies on OP exposure indicated that the dialkyl phosphate metabolites have been frequently detected in the urine of the general population (adults and children) (Aprea *et al.,* 1996; Aprea *et al.,* 2000; Fenske *et al.,* 2000; Garcia *et al.,* 2000; O'Rourke *et al.,* 2000; Heudorf and Angerer 2001; Curl *et al.,* 2003; Barr *et al.,* 2004; Saieva *et al.,* 2004; Bradman *et al.,* 2005; Bouvier *et al.,* 2006) and exposed workers (Hayes *et al.,* 1980; Coye *et al.,* 1986; He 1993; Nutley and Cocker 1993; Buchanan *et al.,* 2001; McCauley *et al.,* 2001; Cocker *et al.,* 2002; Coronado *et al.,*. 2004; Frenich *et al.,* 2004; Blanco *et al.,* 2005.).

The objective of this study was to determine the amount of urinary metabolites among fruit farmers exposed to three organophosphate pesticides: Guthion® (Azinphosmethyl), Lorsban® (Chlorpyrifos), and Imidan® (Phosmet). The influence of organophosphate spraying on the metabolites was characterized using the principal component analysis (PCA) and hierarchical cluster analysis (HCA). PCA is a data reduction technique that transforms the original variables to a set of new variables called principal components (PC). HCA is used to demonstrate clustering and grouping of data. PCA and HCA have been successfully used in exposure assessment especially in the area of occupational hygiene (Pio *et al.,* 1998; Burstyn *et al.,* 2002; Frenich *et al.,* 2002; Burstyn 2004; Preller *et al.,* 2004; Meijster *et al.,* 2004). Vidal *et al.* (2002) and Frenich *et al.* (2004) demonstrated, with PCA, unequal distribution of pesticide contamination as well as establishing the body parts that received the highest exposure in greenhouse applicators.

2. Materials and Methods

2.1. Sample study

Pesticide-using owner/operator (and their workers) ranging in age from 25 to 55 years of age were recruited. Selection of potential orchards was based on anticipated use of OP insecticides azinphos-methyl (Guthion®), chlorpyrifos (Lorsban®, Dursban®), and phosmet (Imidan®) during 1997 growing season. Control subjects were recruited among same sex, similar age, non-farming friends and relatives of the selected farmers and lacking significant potential for occupational pesticide exposure.

2.2. Urine sample collection

Pre-season urine samples were collected from the farmers in early March before spraying activities begin. They were asked to collect 60-hour urine specimens after each OP application/exposure event. Farmers collected all urine samples starting with the first-morning void (day 1) before application begins. Starting from the evening (8 pm) of day 1 through midnight of day 3, they were asked to collect the urine into a second bottle. This composite sample consisted of 48 hour urine sample. A day 4 urine sample was collected into a third bottle.

2.3. Sample analyses

Urine samples were frozen and sent to Pacific Toxicology Laboratory, California. USA. They were analyzed for the following six metabolites: DMP, DMTP, DMDTP, DEP, DETP, and DEDTP. The limit of quantification (LOQ) for DMP, DMTP, DEP, and DEPT was 5 µg/L urine; for DMDTP and DEDTP was 10 µg/L.

2.4. Pesticide exposure intensity score

Detailed analysis of activity of each worker was carried to determine what accounts for variation in the OP exposure. The questionnaire responses were used to develop pesticide exposure scenarios and exposure algorithm. The algorithm used in this study was based on Dosemeci *et al.* (2002), Dosemeci (2002), and Coble *et al.* (2005). The exposure algorithm developed by these researchers provided quantitative estimates of exposure intensity based on categorical responses to questions on mixing and application methods, repair activities, the use of personal protective equipments (PPE), and work hygiene. The algorithm and weights for the variables used by them contain four basic factors: (1) mixing status [MIX], (2) application method [APPLY], (3) equipment repair status [REPAIR] and (4) personal protective equipment [PPE]:

Intensity = (MIX + APPLY + REPAIR) x PPE

For this study, we used this general algorithm with some changes. Since everyone in this study used the same method of mixing, loading and application, we removed the MIX and APPLY variables. The exposure weight for [PPE] was adopted with some changes, separating PPE use during mixing and loading activities from the spraying activities. For detailed PPE exposure weights, refer to Dosemeci *et al.* (2002) and Coble *et al.* (2005). We asked the farmers whether they eat, drink, or smoke during work and include it as possible route of exposure through ingestion. However, we did not have information on [REPAIR]. We started with eight exposure variables, namely, the numbers of hours worked per application [HR], use of PPE during mixing and loading activities [PPE-MIX], using Dosemeciûs exposure weights, use of PPE during spraying [PPE-SPRAY], using Dosemeci’s exposure weights), [EAT] during work (1 = yes, 0 = no), [DRINK] during work (1 = yes, 0 = no), smoke during work ([SMK], 1 = yes, 0 = no), wash hands before eat ([WEAT], 0 = yes, 1= no), and wash hands before drink ([WDRINK], 0 = yes, 1= no). The numbers of hours worked was based on the start and finish times reported on the event questionnaire. [HR] was stratified into three categories: low (< 4 hours), medium (4- 10 hr), and high (>10 hr). After the PCA, the variable [MOUTH] (formerly [EAT], [DRINK], [SMK] variables) and variable [WASH] (formerly [WEAT] and [WDRINK] variables) were regrouped, as [INGEST]. Finally, four variables were retained, namely, [HR], [PPE-MIX], [PPE-SPRAY], and [INGEST].

Keeping these four variables, we rescored [HR], [PPE-MIX], [PPE-SPRAY], and [INGEST]:

HR
- 3= >10 hr (long)
- 2= 4-10 (medium)
- 1= <4 (short)

PPE-mix
- 3= None
- 2=Incomplete (Dosemici’s score 0.5)
- 1=Full (Dosemici’s score < 0.5)
- 0=Did not mix

PPE-spray
- 3= None

2=Incomplete (Dosemici's score 0.5)
1=Full (Dosemici's score < 0.5)
0=Did not spray

INGEST
1= Yes
0= No

The general algorithm for this study is then finalized as:

Pesticide Intensity Score = HR + PPE-MIX + PPE-SPRAY + INGEST

[PPE-MIX] represents the use of PPE when handling during mixing and loading chemicals into the spraying equipment. The [PPE-SPRAY] represents use of PPE during spraying activities. [INGEST] refers to smoking, eating or drinking and washing hands during work.

2.5. Statistical Analysis

All data were stored in an Excel spreadsheet. The non quantifiable metabolites (below the limit of quantification, LOQ) were calculated as LOQ/2. The data were found to be not normally distributed, thus logarithmic transformation was required. Multiple comparison tests were carried out using the Tukey's test. PCA and HCA were performed using SPlus 2000 Professional (MathSoft Inc., Seattle, Washington).

3. Results and Discussion

This study presented selected organophosphates (chlorpyrifos, phosmet, and azinphosmethyl) exposure assessment on seven fruit growers. Thirty nine urine samples were collected from seven applicators.

3.1. Work pattern and chemical use

Table 1 shows the detailed information of chemical use, work pattern and use of protection during work. Spraying season commenced in mid-April and ended in September, with peak sprayings in June. The number of spraying events varied from five to six, each was seven to 10 days apart. One "spray event" is considered as one spraying in a single sitting. "One cover" referred to a complete OP application to cover the whole orchard. For large orchards, it took several days to complete the whole orchard; hence, one cover required multiple spray events. In this study, one spray event constitutes one or more days of spraying to cover the orchard. Since the orchard was more than 500 acres, work hours were long. The total work hours also varied, depending on the wind conditions. Work hours ranged from 1-15.5 hours. All spraying activities would be stopped if wind speed exceeded 5 miles per hour. For this orchard, the most heavily used organophosphate was phosmet, formulated as Imidan 70WP (powder packed in a 22-lb bag or water soluble 4-lb packet) and chlorpyrifors formulated as Lorsban 50W (water soluble 1-lb packet). The most common method of handling was by pouring the powder from the bag or soluble packets directly into the spraying tank. Generally, mixing and loading tasks lasted for 10 minutes while spraying lasted for two hours. All of them used spray blast mounted on a tractor.

3.2. Urinary metabolites

Urine were collected and analyzed for creatinine and six OP metabolites (DMP, DMTP, DMDTP, DEP, DETP, and DEDTP). The metabolites were expressed as volume concentration (μg/L) and creatinine adjusted concentration (μg/g creatinine). Table 2 shows the metabolite concentration for each applicator and the control group for each spray events. The baseline samples were urine taken before spraying activities began. Metabolites were not detected in three workers (W2, W4 and W6) but four workers (W1, W3, W5, and W7) had DMP, DMTP, and DMDTP in their urine. The DMP concentration for W7 was 89 μg/L. All dimethyl metabolites were present in day 1 morning urine samples for all workers. The arithmetic means ranged from 21.5-94.17 μg/L DMP, 6.25-81.25 μg/L DMTP, and <LOQ-53.17 μg/L DMDTP. W1 is the only person who had DEP in his urine. Other workers, except for W7, reported using chlorpyrifos but no DEP was detected. Based on four days of observation, day 2 urine samples (48 hr composite samples) had the highest amount of metabolites. The arithmetic means ranged from 25.8-558 μg/L DMP, 15.75-398 μg/L DMTP, 21.5-568.57 μg/L DMDTP, and <LOQ-17.67 μg/L DEP. Previously mentioned literatures suggested that the metabolites would be cleared from the body within five to seven days, and azinphosmethyl and phosmet generated DMP, DMTP, and DMDTP metabolites, while chlorpyrifos generated DEP and DETP (Coye *et al.,* 1986b; He 1993). From this study the excretion of metabolites in some sprayers was still evident until day 4 after spraying. The arithmetic means ranged from 19.2-182 μg/L DMP, 13.33-138 μg/L DMTP, 22.75-157.83 μg/L DMDTP, and <LOQ-26 μg/L DEP. For the control persons, some metabolites were detected in their urine. The arithmetic means ranged from <LOQ-36.15 μg/L DMP, <LOQ-55.05 μg/L DMTP, <LOQ-42.4 μg/L DMDTP, <LOQ-13.4 μg/L DE, and <LOQ-6.5 μg/L DETP.

Fig. 1 shows the pattern of urinary metabolites taken from day 1, day 2, and day 4. The observed variability in the excretion level is reflective of the type of chemicals used for each application event as well as the degree of protection. We, however, could not comment on the biological variability. In general, the level of urinary metabolites on day 2 reached the peak and started to decrease on day 4. We assume the same pattern for W6, although urine samples for day 1 were not available. In general, the level of urinary metabolites on day 2 reached the peak and started to decrease on day 4. The urinary metabolites excreted by the applicators in this study were comparable with the Florida citrus sprayers and harvesters (Barr *et al.,* 2004). Fenske *et al.,* (2005) reported high DMTP level among the apple thinners (50th percentile, 530 g/L) but the adult farm workers were 50 times lower. We believe that this study gave a clear picture of the profile of metabolite excretion by the applicators since the urine samples collected were not spot samples. For each person, the frequency of urine samples collected is at least five times. The observed variability in the excretion level is reflective of the type of chemicals used for each application event as well as the degree of PPE use. In general, the excretion of metabolites was at maximum 48 hours after spray and could still be detected after 96 hours.

The urinary metabolites excreted by the applicators in this study were comparable with the Florida citrus sprayers and harvesters (Barr *et al.,* 2004) and the existing OP database collected during the National Health and Nutritional Examination Survey (NHANES 1999-2000) (Center for Disease Control 2003). Fenske *et al.,* (2005) reported high DMTP level among the apple thinners (50th percentile, 530 μg/L). From this study, the metabolite concentration detected in some of the control group was high, at levels similar to the 95th percentile group of the United States general population. This study gave an accurate picture of the profile of metabolite excretion by the applicators since for each person multiple urine samples were collected, from two to eight samples.

3.3. Pesticide intensity score

To estimate exposure to organophosphate, we adopted and revised the Dosemeci (2002) and Coble *et al.,* (2005) general algorithm calculations. Initially, we started with eight exposure variables: hours work [HR], protection during mixing and loading (PPE-MIX), protection during spraying [PPE-SPRAY], smoke [SMK], [EAT], [DRINK] during work, wash hand before eat [WEAT], and wash hand before drink [WDRINK]. Since these variables are highly correlated, we attempted to examine these variables using PCA. From PCA model 1 (Fig. 2), we observed that variables [EAT], [DRINK], [SMK] are placed relatively close together and the [WEAT] and [WDRINK] as another group. From the first PCA model, we renamed [EAT], [DRINK], and [SMK] variables as [MOUTH] and [WEA]T and [WDRINK] as [WASH]. We then performed the second PCA. The PCA model 2 (Fig. 3) illustrates the position of each exposure variables. From model 2, we regrouped the [MOUTH] and [WASH] variables into a new variable called INGEST. Revision to the Dosemeci (2002) and Coble *et al.,* (2005) general algorithm calculation was necessary because we lacked certain information that is included in the original algorithm. For example, our questionnaire did not ask about the equipment repair status [REPAIR] therefore we were not able to include this variable in our algorithm. However, we observed that they repaired the equipment themselves. The mixing status [MIX] and the application method [APPLY] variables were also excluded because all of them mix, load, and apply in all spray events. We did not include the method of handling as one of our variables because all of them used similar methods.

For PPE use during mix and spray activities, an exposure score developed by Dosemeci *et al.,* (2002), and Dosemeci (2003) was used. The Dosemeciûs pesticide reduction factor ranged from 0.1-1. A score of 1 indicates 0% protection and 0.1 means complete body protection. We had separated information on PPE use during mixing- loading stage and spraying therefore our algorithm consist of [PPE-MIX] and [PPE-SPRAY] variables. We did not consider other exposure variables such as the type and the amount of pesticides used, the method of handling, and the size of orchard because all of the subjects who participated in this study were from the same farm and were using the same chemicals.

We applied PCA to reduce the number of highly correlated variables. We concluded that there are four variables that influence variability in exposure, namely, ingestion of pesticide (via eating, drinking, or smoking) while at work, the use of PPE during spray, duration of work hours per application event, and PPE use during mixing and loading activities. From this analysis, we observed that most of the variations come from PPE use during spray where these applicators tend to use complete PPE during mixing and loading activities but not during spraying.

According to the Pesticide Exposure Assessment Study, factors that were found to be predictors of urinary herbicide levels were pesticide formulations, the use of protective clothing, the type of application equipment, handling and personal hygiene (Arbuckle *et al.,* 2002). We finally retained four variables [HR],

Table 1. Chemical use, work pattern, and protective score by individual applicators

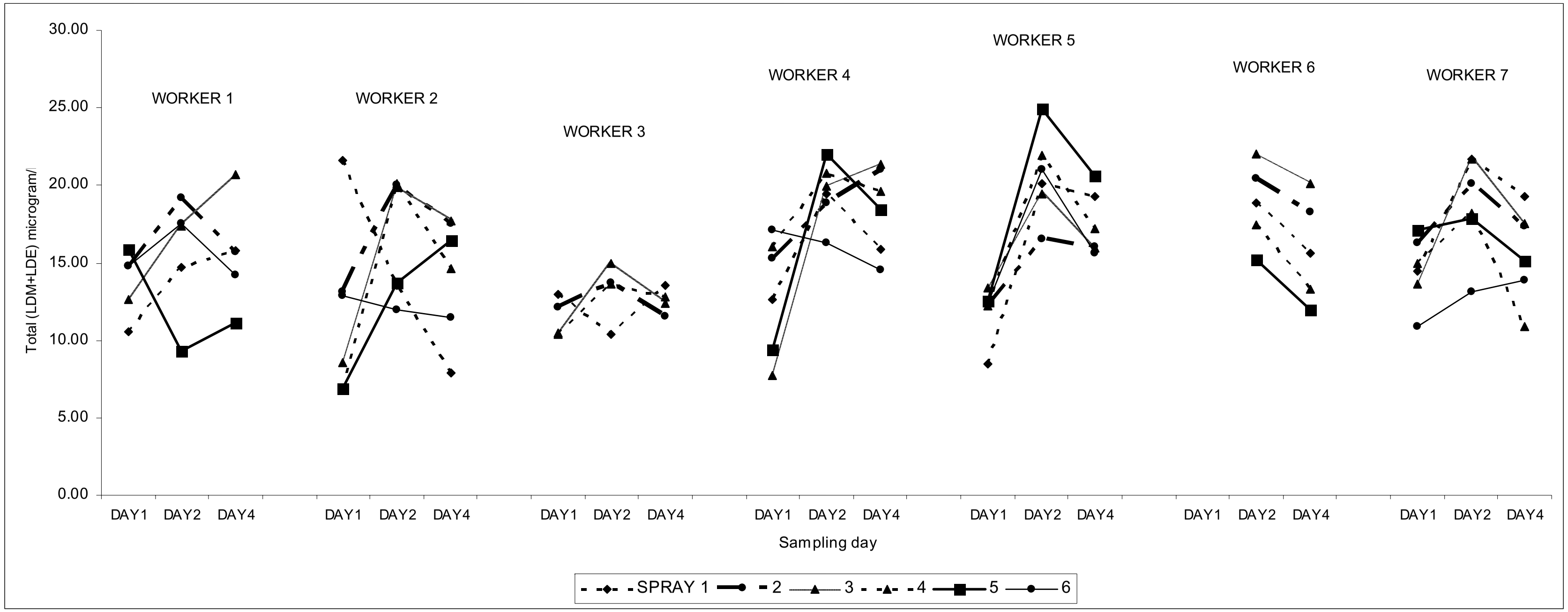
NAME	Spray event	Pesticide	Total hours work/spray event	Days/spray event	PPE mix-load score[1]	PPE Spra yscore[1]
W1	1	Phosmet	10	3	0.1	0.1
	2	Chlorpyrifos	15	4	0.1	0.5
	3	Phosmet	9	2	0.5	0.5
	4	Phosmet	2.5	1	0.5	0.5
	5	Phosmet	4.5	1	0.5	0.5
	6	Phosmet	7	3	0.1	0.5
W2	1	Phosmet	21	3	0.1	0.1
	2	Chlorpyrifos, Phosmet	15.5	3	0.1	0.1
	3	Phosmet	10	2	0.1	0.8
	4	Phosmet	6	3	0.1	0.5
	5	Phosmet	9.5	2	0.1	0.8
	6	Phosmet	4.25	1	0.1	0.5
W3	1	Phosmet	6	1	0.7	0.3
	2	Unknown	7.5	2	0.7	0.7
	3	Chlorpyrifos	10	2	0.7	0.7
	4	Phosmet	4.5	1	0.7	0.7
W4	1	Phosmet	13	3	0.5	0.5
	2	Chlorpyrifos, Phosmet	13	3	0.5	0.5
	3	Phosmet	13	3	0.5	0.5
	4	Phosmet	9	3	0.5	0.5
	5	Phosmet	10.5	2	0.5	0.5
	6	Phosmet	3.5	1	0.5	0.5
W5	1	Phosmet	1	1	0.7	0.3
	2	Chlorpyrifos,Phosmet	15.25	3	0.7	0.7
	3	Phosmet	6.5	2	1	1
	4	Phosmet	7.25	3	0.7	0.7
	5	Phosmet	6	2	0.7	0.7
	6	Phosmet	4	1	0.7	0.7
W6	1	Phosmet	15	3	0.1	0.5
	2	Chlorpyrifos	13	3	0.5	0.5
	3	Phosmet	6	2	0.5	0.5
	4	Phosmet	4.5	1	0.5	0.8
	5	Phosmet	4.75	2	0.5	0.8
	6	Phosmet	4.25	1	0.8	0.5
W7	1	Phosmet	16	3	0.1	0.8
	2	Phosmet	10	2	0.1	0.5
	3	Phosmet	9	3	0.1	0.5
	4	Phosmet	8	4	0.1	0.5
	5	Phosmet	10.5	2	0.1	0.5
	6	Phosmet	5	2	0.1	0.5

Table 2. Summary statistics of urinary metabolites excreted by seven farmers during the application season

Sprayer	Sample day	Statistic summary	Metabolite concentration (µg/L)					
			DMP	DMTP	DMDTP	DEP	DETP	DEDTP
W1	Baseline		<LOQ	7	<LOQ	<LOQ	<LOQ	<LOQ
	First Morning void before spray (n=5)	Arithmetic mean	24.8	28.3	18.2	13.2	<LOQ	<LOQ
		Standard deviation	7.92	20.29	12.87	9.20		
		Range	14-34	<LOQ-54	5-36	6-29		
	Day 2 composite samples (n=6)	Arithmetic mean	25.83	71.08	63.33	17.67	<LOQ	<LOQ
		Standard deviation	6.68	37.72	36.57	12.78		
		Range	18-33	<LOQ-106	<LOQ-119	<LOQ-34		
	Morning void day 4 sample (n=)	Arithmetic mean	19.2	52.8	55.6	26	<LOQ	<LOQ
		Standard deviation	3.7	40.29	56.43	26.42		
		Range	15-24	19-112	LOQ-121	LOQ-61	LOQ-16	
	Control	Arithmetic mean (range)	28.5	55.05	42.4	<LOQ	<LOQ	<LOQ
W2	Baseline		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	First Morning void before spray (n=6)	Arithmetic mean	29.67	81.25	153.17	<LOQ	<LOQ	<LOQ
		Standard deviation	39.79	175.59	335.85			
		Range	<LOQ-108	<LOQ-439	<LOQ-838			
	Day 2 composite samples (n=6)	Arithmetic mean	361.67	106.5	81.17	<LOQ	<LOQ	<LOQ
		Standard deviation	311.01	100.14	110.06			
		Range	28-916	13-239	LOQ-282			
	Morning void day 4 sample (n=6)	Arithmetic mean	32.17	49.75	70.83	6.33	LOQ	LOQ
		Standard deviation	19.66	39.02	67.68	7.80		
		Range	7-50	<LOQ-98	<LOQ-193	<LOQ-22		
	Control	Arithmetic mean (range)	12.05	19.9	29.75	<LOQ	<LOQ	<LOQ

W3	Baseline		48	9	<LOQ	<LOQ	<LOQ	<LOQ
	First Morning void before spray (n=4)	Arithmetic mean	48.75	6.25	21.5	<LOQ	<LOQ	<LOQ
		Standard deviation	27.32	4.63	11.09			
		Range	18-84	<LOQ-12	<LOQ-28			
	Day 2 composite samples (n=4)	Arithmetic mean	72.75	15.75	28.75	<LOQ	<LOQ	<LOQ
		Standard deviation	42.38	15.56	37.04			
		Range	35-117	6-39	<LOQ-83	LOQ-15		
	Morning void day 4 sample (n=4)	Arithmetic mean	53.75	13.88	22.75	<LOQ	<LOQ	<LOQ
		Standard deviation	23.92	7.77	17.33			
		Range	32-87	<LOQ-20	<LOQ-40			
	Control	Arithmetic mean (range)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
W4	Baseline		<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	First Morning void before spray (n=6)	Arithmetic mean	21.5	45.17	57.83	<LOQ	<LOQ	<LOQ
		Standard deviation	17.53	37.45	48.12			
		Range	<LOQ-47	<LOQ-93	<LOQ-126			
	Day 2 composite samples (n=6)	Arithmetic mean	127.83	260.5	305.17	<LOQ	<LOQ	<LOQ
		Standard deviation	77.12	145.89	184.51			
		Range	41-240	95-456	91-545	LOQ-11		
	Morning void day 4 sample (n=6)	Arithmetic mean	82.17	160.5	228.17			
		Standard deviation	67.46	98.83	157.83			
		Range	16-193	41-312	52-520	LOQ-16		
	Control	Arithmetic mean (range)	<LOQ-5.1	<LOQ	34.8	<LOQ-5	<LOQ	<LOQ
W5	Baseline		20	6	11	<LOQ	<LOQ	<LOQ
	First Morning void before spray (n=8)	Arithmetic mean	40.94	15.88	19.5	<LOQ	<LOQ	<LOQ
		Standard deviation	24.96	8.03	9.44			
		Range	<LOQ-86	<LOQ-26	<LOQ-30			
	Day 2 composite samples (n=7)	Arithmetic mean	558	398	304	<LOQ	<LOQ	<LOQ
		Standard deviation	534.15	419.16	298.61			
		Range	89-1710	76-1298	74-932			
	Morning void day 4 sample (n=5)	Arithmetic mean	178.86	138	122			
		Standard deviation	141.39	93.70	70.61			
		Range	48-413	53-316	43.212			
	Control	Arithmetic mean (range)	36.15	<LOQ-6.1	<LOQ-212	13.4	LOQ	LOQ
	Baseline		LOQ	LOQ	LOQ	<LOQ	<LOQ	<LOQ

	First Morning void before spray (n=2)	Arithmetic mean	35.75	15.75	<LOQ	<LOQ	<LOQ	<LOQ
		Standard deviation	47.02	18.74				
		Range	<LOQ-69	<LOQ-29	<LOQ-12			
	Day 2 composite samples (n=5)	Arithmetic mean	409.4	164.2	152	<LOQ	<LOQ	<LOQ
		Standard deviation	368.84	142.62	107.06			
		Range	70-916	41-380	44-325			
	Morning void day 4 sample (n=5)	Arithmetic mean	182	71.8	83	<LOQ	<LOQ	<LOQ
		Standard deviation	199.36	71.29	73.59			
		Range	21-490	14-188	11-189			
	Control	Arithmetic mean (range)	33.7	(<LOQ-5.2)	<LOQ	6.05	<LOA-6.5	<LOQ
	Baseline		89	9	19	LOQ	LOQ	LOQ
W7	First Morning void before spray (n=6)	Arithmetic mean	94.17	15.5	44.17	LOQ	LOQ	LOQ
		Standard deviation	14.65	12.97	32.47			
		Range	69-112	<LOQ-41	<LOQ-90			
	Day 2 composite samples (n=6)	Arithmetic mean	83.83	122.83	568.57	6.17		
		Standard deviation	41.31	68.87	469.08	3.10		
		Range	26-150	13-184	20-1250	<LOQ-10		
	Morning void day 4 sample (n=6)	Arithmetic mean	76.75	59.17	84.33			
		Standard deviation	50.67	40.09	77.78			
		Range	<LOQ-127	20-132	LOQ-199	LOQ-19		
	Control	Arithmetic mean (range)	(<LOQ-16.5)	(<LOQ-7.8)	(<LOQ-23.1)	<LOQ	<LOQ	<LOQ



(DMP: Dimethylphosphate, DMTP:Dimethylthiophosphate, DMDTP : Dimethyldithiophosphate, DEP: Diethylphosphate, DETP: Diethylthiophosphate, DEDTP : Diethyldithiophosphate. Limit of quantification (LOQ): DMP, DMTP, DEP,DETP = 5 µg/L; DMDTP, DEDTP= 10 µg/L)

Figure 1. The sum of log transformed values of dimethyl phosphates (DMP) and diethyl phosphates (DEP) from day 1- day 4 urine samples for each applicant for six spray events.

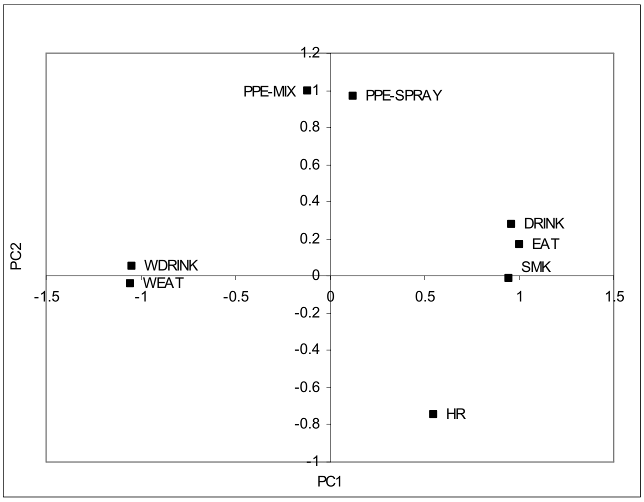


Figure 2. Principal Component Analysis. Distribution of eight exposure variables: HR, PPE-MIX, PPE-SPRAY, SMK, DRINK, EAT, WEAT and WDRINK), on the plane defined by the first and second component.

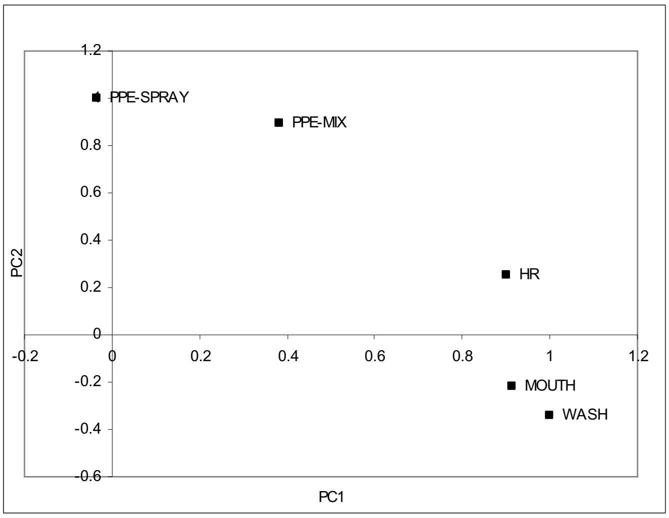


Figure 3. Principal Component Analysis. Distribution of reduced exposure variables: HR; PPE-MIX; PPE-SPRAY; MOUTH (from SMK, DRINK and EAT); WASH (from WEAT and WDRINK), on the plane defined by the first and second component.

[PPE-MIX], [PPE-SPRAY], and [INGEST] for pesticide intensity score. Using this algorithm, we calculated the pesticide intensity score, for each worker, as shown in Table 3. The scores for each person, over multiple observations (from several spray events), varies slightly, except for W2 and W5. The following are average scores for each worker: W1 5.67 (range 5-6), W2 6.60 (range 5-8), W3 7.00, W4 7.50 (range 7-8), W5 (6-8), W6 7.20 (range 7-8), and W7 6.40 (range 6-7). As expected, since the workers worked at the same orchard, they have similar work pattern therefore their exposure scores will not vary.

3.4. Relationship between the pesticide intensity score with urinary metabolites levels

From the pesticide intensity score, we predicted that the higher the score the higher is the urinary metabolite levels. To validate these scores, we used the metabolites taken from day 1, day 2 and day 4 samples against the pesticide intensity score. Fig. 4 illustrates the box plot of metabolite distribution of day 1 to day 4 (sum of log transformed dimethyl and diethyl phosphates) in urine grouped by pesticide score. Fig. 5 illustrates the relationship between metabolite concentrations with the pesticide score. There is no significant correlation between the two variables (*r = 0.1847, p=0.07*). The algorithm did not predict urine exposure. The linear relationship is not clear due to inter variability in metabolite levels among workers. In most literatures, researchers established very good and significant predictors that correlated well with urine metabolites (de Cock *et al.,* 1995; Hines and Deddens 2001; Arbuckle *et al.,* 2002; Harris *et al.,* 2002; Hardt and Angerer 2003). However, in the Hines *et al.,* (2008) study, the algorithm did not predict air, hand rinse and urine exposure. In future studies, we will include other factors into the algorithm when sprayers from other orchard s were included into the analysis.

4. Conclusion

There are four variables that influence organophosphate exposure, namely, ingestion of pesticide (via eating, drinking, or smoking) while at work, the use of

Table 3. Calculated values of pesticide intensity scores for each applicant during each spray events based on the exposure algorithm containing work hour, PPE use, and pesticide ingestion

Worker	Spray event	Pesticide intensity score	Average score
1	1	6	
1	2	6	
1	3	6	
1	4	5	
1	5	6	
1	6	5	5.67
2	1	8	
2	2	8	
2	3	6	
2	4	5	
2	5	7	
2	6	5	6.50
3	1	7	
3	2	7	
3	3	7	
3	4	7	7.00
4	1	8	
4	2	8	
4	3	8	
4	4	7	
4	5	8	
4	6	6	7.50
5	1	7	
5	2	8	
5	3	6	
5	4	7	
5	5	7	
5	6	6	
5	7	6	6.71
6	2	8	
6	3	7	
6	4	7	
6	5	7	
6	6	7	7.20
7	1	7	
7	2	6	
7	3	6	
7	4	6	
7	5	7	6.40

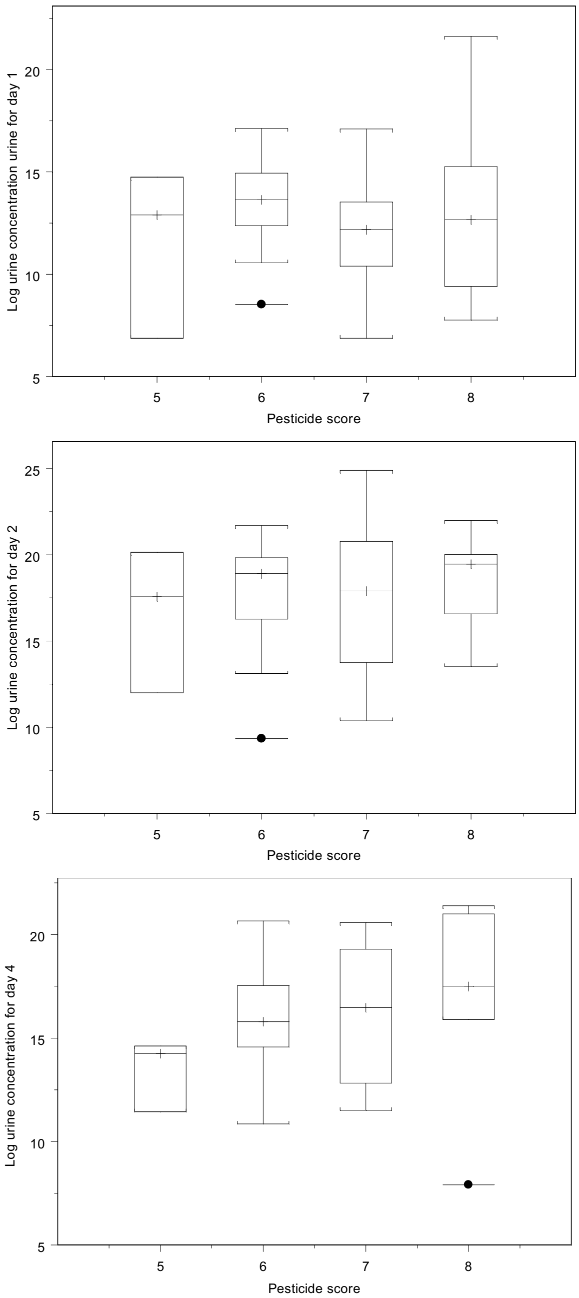


Figure 4. Box plots of all metabolites of day 1 to day 4 samples grouped by pesticide score

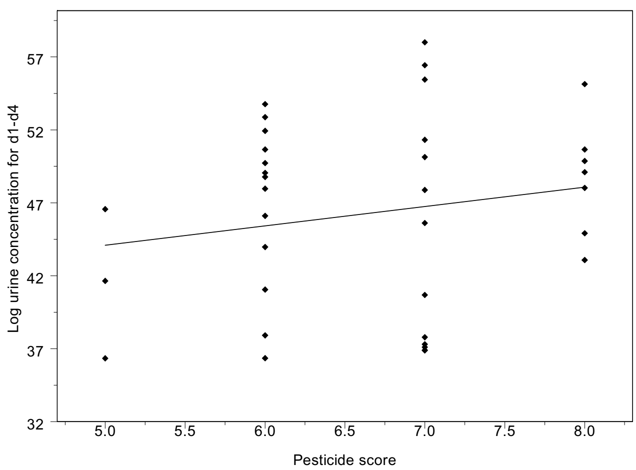


Figure 5. Pesticide intensity score verses urinary metabolites concentration (dimethyl and diethyl phosphates) for day 1, day 2 and day 4 samples (*r = 0.1847*)

PPE during spray, the duration of work hours per application event, and PPE-use during mixing and loading activities. From this analysis, we observed that most of the variations come from PPE use. From field observation, applicators tend to use complete PPE during mixing and loading activities but not during spraying. After employing PCA and intensity scores based on variables such as PPE use, duration of work hour, and personal hygiene, were used in the exposure algorithm. Based on the limited data, the algorithm scores did produce a clear linear relationship with the concentration of urinary metabolites. The lack of association may be due to variability in urine concentration among applicators despite having similar work pattern.

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