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Urinary dialkyl phosphate metabolites of organophosphorus in pesticide sprayers of Mango Plantation in Malihabad Lucknow

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Abstract

Organophosphorus pesticides are the most commonly used pesticides in Mal and Malihabad areas of Lucknow. The agricultural workers, sprayers, formulators and applicators are exposed to occupational hazards of pesticides directly or indirectly. The present study was aimed to determine the knowledge about using OPs, safety practice and urinary dialkylphosphate metabolites on OP (Organophosphorus pesticides) sprayers in the Malihabad areas of Lucknow. Assessment of urinary biomarkers dialkyl phosphates is a recent development in the field of pesticide toxicology and is considered non-invasive and sensitive tool for early detection of body burden of organophosphates in the exposed workers. Exposure was assessed by measuring six urinary OP metabolites by Gas chromatography using a single flame photometric detector. The sample consisted of 31 men and 2 women aged 20 to 65 years old.

75% of OP pesticide sprayers had at least one urinary DAP metabolite above the limit of detection. The geometric mean (GM) and the geometric standard deviation (GSD) of DMP and DEP were 5.73 µg/g cr. (GSD 2.51), and 6.08 µg/g cr. (GSD 3.63), respectively. The percentage of sprayers with detectable DMP, DMDTP, and DMTP in urine was 73.72%, 3.03%, and 15.15%, respectively, while the corresponding values for DEP, DETP, and DEDTP were 49.44%, 36.36% and 15.15%, respectively. There was no significant association between the use of protection practices and the absence of urine OPs metabolites suggesting inadequate protection practices. The pesticide sprayers in Malihabad, Lucknow have significant exposure to OP pesticides, probably due to inappropriate protective practices.

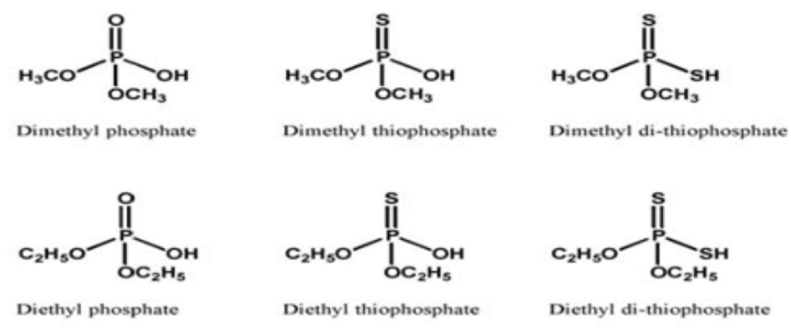
INTRODUCTION

Pesticides are extensively used throughout the world and in recent years, their use has increased considerably. Large amounts of these chemicals are released into the environment and many of them affect non-target organisms thereby poses potential hazard to human health. Organophosphorus pesticides are commonly used worldwide in agriculture and in pest control. The toxicity of these pesticides in humans is well documented. Pesticide exposure can occur through a number of sources such as contaminated soil, dusty work clothing, water, contaminated food and drift of a pesticide off target deposition [1, 2]. A high risk of occupational human exposure to OPs may occur in pesticide sprayers if they do not practice adequate protective measures [3]. The measurement of blood cholinesterase is used as a biological marker of OPs contamination. This is based on the fact that organophosphate pesticides inhibit the activity of both the cholinesterase (ChE) enzymes in the red blood cells (RBC ChE) and serum ChE (AchE) [4]. A 50% reduction in serum ChE activity from the baseline is an indicator of acute organophosphate toxicity. The RBC ChE activity, which is less rapidly depressed than the serum ChE activity (AChE), is a measure of more chronic exposure to organophosphates [4]. Although cholinesterase monitoring has the advantage of providing a measure of physiological response, it has disadvantages as well [5]. The absence of baseline values for an individual subject makes it difficult to know if an observed level of AChE or RBC ChE activity represents a depression by exposure to an OP or if the value is normal for the subject [6].

An alternative approach to biological monitoring for OPs is based on the analysis of six dialkyl phosphates metabolites in urine as DMP (Dimethylphosphate), DMTP (Dimethylthiophosphate), DMDTP (Dimethyldithiophosphate), DEP (Diethylphosphate), DETP (Diethylthiophosphate) and DEDTP (Diethyldithiophosphate) [5, 7, 8]. Urinary metabolites may be detected for several days after exposure and in association with lower levels of exposure than those required for cholinesterase inhibition [9]. The determination of these metabolites is used to monitor occupational exposure to OP pesticide [10]. OP metabolites are often the preferred method for pesticide measurements because their collection is non invasive, they are easily measured [11] and are more sensitive than ChE activity (can detected at lower levels of OP exposure) [7]. First morning void samples may accurately represent total daily exposure [12]. However, there are also disadvantages which include variation in urine and consequently the concentration of OPs. This may solved by creatinine correction in urine samples. Metabolites measured in urine are also not pesticide specific and they may enter the body from other exposure sources [5]. Despite these disadvantages, measurements of dialkyl phosphates metabolites are one of the commonly used markers of OPs exposure. The OPs are hydrolyzed rapidly to six dialkylphosphate metabolites detectable in the urine, which may be measured for several days after exposure [6].

Abundant research studies have been carried out to assess the human exposure to these pesticides and measurement of urinary dialkyl phosphate metabolites of the OP pesticides has been a

Abbreviations: Organophosphorus (OPs), Dimethylphosphate (DMP), Dimethylthiophosphate (DMTP), Dimethyldithiophosphate (DMDTP), Diethylphosphate (DEP), Diethylthiophosphate (DETP), Diethyldithiophosphate (DEDTP), Dialkyl phosphates (DAP), Indian Institute of Toxicological Research (IITR)



Chemical structures of dialkyl phosphate metabolites

Figure 1. Dialkyl phosphate metabolites.

popular method for such studies [13, 14]. Six DAP metabolites are the most commonly measured, namely, dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl di-thiophosphate (DMDTP), and diethyl di-thiophosphate (DEDTP) (Figure-1**)**. Determination of these metabolites provides information on cumulative exposure to OP pesticides as a class although the metabolites cannot be directly associated with a given pesticide. The majority of analytical methods for DAP determination involve the isolation of the metabolites from the sample matrix followed by derivatization and quantification using a variety of isolation techniques, derivatizing agents, and instrumentation [15].

Several occupational and environmental studies that have tested for all six DAP metabolites have reported that DMTP was the metabolite most often detected or was detected in the highest concentrations [16-20]. In most of these studies, specific OP exposure information was unavailable, but the DMTP finding indicated higher exposures to dimethyl OPs (e.g. dimethoate , parathion methyl and azinphos-methyl) as dimethyl OPs produce only dimethyl metabolites (DMP, DMTP, DMDTP) just as diethyl OPs produce only diethyl metabolites (DEP, DETP, DEDTP).

There is a need for evaluation of the adverse health effects due to its exposure on agricultural workers. No systematic study has been carried out regarding the health status of pesticide sprayers in mango plantation at Malihabad after IITR study in Lucknow during 1990. Due to the introduction of new pesticides viz. cypertherin, quinalphos, cypermethrin formulators and deltamethrin along with monocrotophos and endosulfan (used in previous study, 1990) in the mango plantation, a detailed investigation on the health profile in sprayers due to exposure of pesticides is very much warranted. Thus, the aim of the study was to assess the present health status of pesticide sprayers after the introduction of new pesticides and spraying techniques adopted in mango plantation in Malihabad, Lucknow.

MATERIALS AND METHODS

Study design

The sample size was drawn from the Malihabad (Mango belt of Lucknow, Uttar Pradesh). This is a cross-sectional descriptive study, based on interviews and collection of urine samples of 33 OP pesticides applicators (31 men and 2 women). The requirements to participate in the study were to have worked with pesticides and lived in Malihabad at least for two years before the study. Age of subjects ranged from 20 to 65 years.

Study area

The study was conducted in the neighboring villages of Lucknow in North India during the period of October to November 2010. The study included the total rural population of 33 OP pesticide applicators spraying a number of organophosphate pesticides in the mango plantation of Malihabad village of Lucknow. Pesticide sprayers are exposed to OPs during prolonged periods of time. The OP pesticides mainly sprayed where *Methamidophos,* Dichlorvos, *Disulfoton, Diazinon,* Methyl parathion, Phorate, *Pyrazophos, Profenophos,* Chlorpyrifos, *Coumaphos, Azinphos methyl*, *Fenitrothion,* Monocrotophos, *Triclorphon,* Ziazinon, Acephate, Chlorpyrifos, Malathion, Quinalphos, Profenofos.

Population recruitment

The sprayers participating in the study were identified and recruited by agronomic engineers working in Malihabad areas of Lucknow U.P India. From the universe of sprayers in Malihabad, 59 of them accepted to participate in the study. Out of these, only 33 satisfied the inclusion criteria as: i) to be working as pesticide sprayer for at least 2 years; ii) to have used pesticide the last week before questionnaire application; iii) to have used pesticide the day before the urine collection; iv) to agree to participate in the study. All participants in the study were instructed to carry out work activities according to their normal practice. The questionnaire was administered by trained interviewers to each pesticide applicator to obtain information on socio-demographic characteristics; agricultural work practice, and knowledge and practice of safety guidelines for pesticide use. Sprayers were asked to define how frequently they used OPs pesticides. Data related to the kind of pesticides used, kind of protective measures used during application and management of pesticides and clothes after pesticide application were also recorded.

Urine collection and storage

One day after OP pesticide application, each worker was provided with one polyethylene urine collection bottle and instructed to collect urine sample from the first morning void. All the collected urine samples were immediately placed inside a plastic container with ice and transported to the medical centre for freezing at -20°C. The time between urine collections to freezing processing was 1015 minutes. After collection was completed, all samples were shipped frozen to the IITR, Lucknow where they were stored in a -70°C freezer until extraction. Urine pH was not adjusted prior to freezing.

Freeze-dried urine samples were derivatized with a benzyltolytriazine reagent to produce benzyl derivatives of alkylphosphate metabolites. A saturated salt solution was added to the tubes and the benzyl derivatives were extracted with cyclohexane and analyzed by Gas chromatography with flame photometric detection. Likewise the quality control was made in-house by spiking normal urine sample. Two levels of in-house made urine controls were run. Six dialkyl phosphates metabolites were measured in the urine samples. The assay was run with a reagent water blank and urine blank. The recovery rate ranged from 82 to 125% of expected value.

The metabolites included in this study were DMP, DMTP, DMDTP, DEP, DETP and DEDTP. The limit of detection was 5µg/l for DMP, DEP, DETP and DMTP, and 10µg/l for DEDTP and DMDTP. Creatinine was also measured in the urine samples by a colorimetric method (Creatinine Procedure No 555; Sigma Diagnostics, St Louis, Mo). Its measurement was used to adjust results of OP metabolites (µg/gram creatinine) to avoid the variable dilution caused by the different hydration states of the sample donor.

Data analysis

Data recorded in the questionnaires were introduced in a database Excel. Statistical analysis was performed using the statistical package STATA (version 8.0) for personal computer. Descriptive data were presented as arithmetic means or geometric means and standard deviation (SD), as well as frequencies. The percentage of subjects with detected OPs metabolites in urine (percentage of samples above detection limit for each analyte) was also calculated. Subjects were also divided in a group with at least one kind of protection against OPs contamination and a group not using protection during pesticides application. In other case, subjects were grouped according the use of OPs pesticides: use frequently (group 1) or less frequently (group 2). Comparisons between groups were performed with Student's t test (parametric statistics) or Mann-Whitney test (non parametric statistics). P-value below 0.05 was considered as statistically significant.

RESULTS

The mean age of participants was 34.0 ± 11.5 years (mean ± SD). 54.5% of pesticide sprayers were of age between 20 to 34 years. The period of time that subjects worked as pesticide sprayers was 8.55 ± 7.45 years (Table 1). 57.6% of sprayers had finished high school. In relation to protective measures used during pesticide application, 21 out of 33 applicators (62%) reported the use of some kind of protection at work. None of the pesticide sprayers in Malihabad used all the protective measures that normally are required. 43 percent of them reported the use of only a plastic cover for their back as a measure of protection (Table 1). Nobody used gloves. In addition, 21% of pesticides applicators ate their food within or near to the place of work and 91% used irrigation water for washing their hands before eating food (data not shown). Table 2 shows that most used OPs pesticides were Methamidophos (45%), Triclorphon (45%), Methyl Parathion (32%), Monocrotophos (25%), and Fenitrothion (15%). The less used OPs were Profenophos (7%), Dicrotophos (9%), Pyrazophos (9%), Diazinon (8%), Azinphos methyl (6%), Disulfoton (6%) and Malathion (1%). 14 sprayers used most frequently methamidophos, 10 used frequently Parathion methyl and 8 used Monocrotophos. These three pesticides are considered highly toxic [21].

Moreover, 20 sprayers (61%) wore work clothing at home and washed them after getting home, whereas 5 (15%) of the sprayers kept work clothing at home and then used them again. Eighteen (55%) kept pesticides in a separate room and 14 (38%) used them

Table 1. Characteristics of selected pesticide sprayers in Mango plantation of Malihabad, Lucknow

Characteristics	No (%)
Age (years)	
20–24	9 (26.3)
25–34	9 (26.3)
35–49	12 (36.3)
>50	3 (9.1)
No. of years working as applicator	
2 to 5	16 (48.5)
6 to 9	4 (12.1)
10 to 20	10 (30.3)
>20	3 (9.1)
Protective measure in use	
Boots	8 (24)
Apron	1(3)
Mask	2 (3)
Gloves	0 (0)
Glasses	2 (6)
Respirator	2 (6)
Plastic for the back	15 (43)
Waterproof garment	1 (3)

Table 2. Types of organophosphate pesticides most frequently reported to be used in Malihabad by the selected pesticide sprayers.

Characteristics	No (%)	Toxicity	Methylated	Ethylated
Pyrazophos	3 (9)	1		+
Coumaphos	3 (9)	1		+
Fenitrothion	4 (15)	1	+	
Diazinon	2 (8)	1		+
Dicrotophos	3 (9)	1	+	
Profenophos	3 (7)	1		+
Disulfoton	2 (6)	1		+
Azinphos methyl	2 (6)	2		
Malathion	1 (3)	2	+	
Triclorphon	14 (45)	2	+	
Monocrotophos	8 (25)	4	+	
Methyl Parathion	10 (32)	4	+	
Methamidophos	14(45)	4	+	

1: Low toxicity; 2: Moderate toxicity, 3: High Toxicity; 4: Very High toxicity. + Metabolite present in urine

Table 3. Activities of pesticide sprayers during the previous week of the study

Previous week	No (%)
Takes shower after a working day	
How many security components have you used during Application the previous week?	22 (67)
None	14(38)
1 to 2 components	22(65)
Reason for not using all protective measures	
Lack of economic resources	19 (58)
Ignorance	6 (18)
Uncomfortable use	8 (24)
Have you ever mixed pesticides when fumigating?	28 (85)
After pesticide application	
Were some parts of your body (arms, legs) moist with pesticide?	16 (48)
Were your whole body moist with pesticide?	15 (46)
Were not your body moist with pesticide?	2 (6)

Table 4. Concentration of Dialkylphosphates (μg/g Creatinine) in the urine of 33 sprayers of Malihabad Lucknow

Metabolite	% positive	n	Mean ± SD	GM (GSD)	25th Percentile	Median	75th Percentile	90th Percentile	Range
DMP	73.68	24	8.38 ± 7.76	5.73 (2.51)	2.65	6.83	10.02	19.90	1.18–36.67
DEP	49.42	16	14.16 ± 22.21	6.08 (3.63)	1.94	4.36	15.54	37.70	1.01–109.6
DETP	36.35	12	16.07 ± 28.47	5.81 (4.07)	1.65	3.33	16.2	20.17	1.01–147.8
DEDTP	3.03	5	8.09 ± 8.51	5.74 (2.14)	3.19	4.7	10.4	21.06	2.02–38.82
DMDTP	15.15	5	4.50 ± 4.20	3.15 (2.29)	1.65	2.38	6.73	11.79	1.01–15.63
DMTP	15.15	1	8.65 ± 14.74	5.25 (2.24)	3.08	4.39	6.58	47.46	2.02–82.93

as soon as they were bought, while3 (9%) kept them at home. Twenty-six (79%) of the sprayers prepared themselves the backpacks ("mochilas") containing the pesticides (data not shown). Sixty-five percent of the sprayers used at least one safety measure to avoid pesticide contamination. However, 38% did not use any safety clothing, and 58% of sprayers did not use adequate safety devices, mainly due to low economic resources (Table 3). Among the six urine dialkylphosphate metabolites measured, DMP was detected in 73.68% and DEP in 49.44% of applicators. DMDTP was the less frequent metabolite observed (one subject) with a value of 83 µg/g cr. The geometric mean (GM) and geometric mean standard deviation (GSD) of DMP and DEP was 5.73 µg/g cr. (GSD 2.51), and 6.08 µg/g cr. (GSD 3.63) respectively (Table 4). Grouping the sprayers according to the use of at least one measure of protection or not, the levels of dialkylphosphate metabolites in urine were not different between groups (P > 0.05) (Table 5). Applicators were also grouped as highly frequent users of OPs pesticides (55%) or less frequent user of OPs pesticides (45%), but this was not associated with metabolite level (P > 0.05) (Table 6). Data were assessed by ANOVA.

DISCUSSION

The use of pesticides was perceived to be associated with long term and unknown health effects. Lack of administrative regularity capacities and agriculture developmental programs partially cause the unsafe use of pesticides in the developing countries including India. We studied a population of pesticide sprayers in the rural region of the Lucknow (Uttar Pradesh) in the Malihabad. Methamidophos and Trichlorfon were the OPs most frequently used (45%) and both are methylated pesticides. These OPs are considered by the World Health Organization [22] as highly hazardous (Class I-b) and moderately hazardous (Class II), respectively. By comparison, for instance, in the Yakima Valley (Washington State) in the United States the most commonly used pesticide was the azinphos-methyl, classified as level I toxicity [23]. We measured six dialkylphosphate metabolites in urine of OP pesticide sprayer from the Malihabad Lucknow, and shown that 75% of them exhibited at least one OP metabolite in urine. In the Malihabad, the most common metabolite found was DMP (73.68%) followed by DEP (49.44%). Other studies with measurements of dialkyl phosphates showed that DMP was also the most common metabolite in urine [24]. Some studies in US farm workers showed that DMP was the most frequently detected metabolite (33%) followed from DMTP detected in 28% of the workers [25].

However, others authors in Washington, US showed that DMTP was more frequent than DMDTP and DMP [23]. In other studies, the frequencies of detection of OPs metabolites found in urine of farm workers were as follows: 96 and 94% (Barr, 2004); 83 and 99% CDC [25]; 51% and 68% [26] and 53 and 71% (NHANHES 1999-2000) for DMP and DEP, respectively. In Malihabad of Lucknow, the frequencies of detection of DMP and DEP were 73.68% and 49.44% respectively. The data of OPs metabolites in urine should be interpreted carefully since

Table 5. Dialkyl phosphate (DAP) metabolites in urine in pesticide sprayers in Malihabad Lucknow according to use of protective measures

DAP Metabolites	N	Protective Measures YES	NO	P
DMP	1	6.73 ± 0.66 (n = 15)	4.05 ± 1.06 (n = 2)	>0.05
DEP	5	20.69 ± 7.97 (n = 12)	6.87 ± 1.27 (n = 2)	>0.05
DETP	5	10.69 (n = 1)	6.28 ± 2.34 (n = 4)	>0.05
DEDTP	13	6.86 ± 1.07 (n = 3)	8.86 ± 3.98 (n = 3)	>0.05
DMTP	16	4.91 ± 1.35 (n = 3)	7.05 ± 0.84 (n = 9)	>0.05
DMDTP	23	21.61 ± 10.16 (n = 9)		

Data are mean ± SE N: number total of subjects for each DAP metabolite. n = number of subjects in each sub-group. P: Probability. NS: Not significant

Table 6. Dialkyl phosphate (DAP) metabolites in urine of pesticide sprayers in Malihabad Lucknow according to how frequently use OPs pesticides

DAP Metabolites	N	Organophosphorus pesticide use Yes	No	P
DMP	25	6.84 ± 0.67 (n = 13)	6.85 ± 0.81 (n = 11)	>0.05
DEP	17	8.01 ± 1.91 (n = 9)	24.38 ± 11.05 (n = 7)	>0.05
DETP	12	8.16 ± 2.28(n = 5)	27.55 ± 14.15(n = 7)	>0.05
DEDT	5	5.46 (n = 1)	8.55 ± 1.63 (n = 4)	>0.05
DMTP	5	2.94 (n = 1)	6.59 ± 1.89 (n = 4)	>0.05
DMDTP	1		12.96 (n = 1)	

exposure to these metabolites may also occur from dietary and or other environmental sources [27]. Geometric mean for DMP and DEP levels found in the pesticide sprayers of the Malihabad in Lucknow U.P was 5 times higher to those found in USA [20] in non-occupationally exposed men aged 20-59 years suggesting that values were related to direct pesticide exposure rather than exposure from another sources. This suggests that pesticide sprayers in Malihabad have a high risk of exposure and that high levels may be due to inappropriate practice of safety measurements of the guidelines for OPs handling. We surprisingly found that 38% of the applicators did not use any kind of protection. According to the interviewers, the main reason for not using protective clothing during pesticide application was economic. The same was found by other authors in agricultural farm workers in the Gaza Strip, Palestine [28]. The second reason for non-use was that they are not aware of Protection Guidelines. These Guidelines suggest the use of protective: work clothing, including protective gloves, footwear, outer garments, and eye and face protection, In fact, 46% of the applicators used a plastic cover to protect their backs as the only measure of protection against exposure to pesticides. These measures are usually used independently of the type of the pesticide. Our results showed no differences in OPs metabolites levels between applicators using or not using any kind of protective measures, suggesting that safety practices used by pesticide sprayers in Malihabad Lucknow are inadequate.

Sprayers also grouped as high frequently users or low frequently users of OPs pesticides showed no differences in the values of OPs metabolites in urine, suggesting that measurements were related to the last pesticide application, one day before the urine sample was requested. Our findings suggest the need for implementation of appropriate clothing and equipment for protection as well as a continuous training in the use of pesticides by the formulators, applicators, sprayers and farmers from this region. This concern should be extended to the farmers families since non-occupational exposure to agricultural pesticide can also be an important cause of contamination. For example, exposed farmers have been shown to track in residues and keep contaminates containers near the house [4]. Our study showed that pesticide sprayers get information but not training about handling OPs from the dealers. Dealers are not adequate persons to train farm-workers about the handling pesticides as it has been seen previously [21]. Another problem is the storage of OPs pesticides after they are acquired by sprayers In Malihabad, the 55% of interviewed pesticide sprayers reported that they use a separate room to keep the OPs. The rest of workers maintained the OPs at home. It is important to consider preventive options like elimination or substitution of certain compounds, reduction in use, integrated pest management, organic methods, among others.

CONCLUSION

The study concludes that pesticide sprayers in Malihabad, Lucknow have significant exposure to OP pesticides, probably due to inappropriate protective practices. Future work should evaluate the possible adverse health effects.

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