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Short communication

GC-FPD measurement of urinary dialkylphosphate metabolites of organophosphorous pesticides as pentafluorobenzyl derivatives in occupationally exposed workers and in a general population in Shanghai (China)☆

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ABSTRACT

Measurement of organophosphorus (OP) pesticide metabolites in human biological fluids is an important biomarker of pesticides exposure. We measured the urinary excretion of OP pesticide metabolites to evaluate occupational and non-occupational exposure to OP pesticides in the Chinese population in Shanghai (Eastern China). We collected urine samples from 30 exposed workers in a dimethoate emulsion packing division and from 60 healthy adults without any report of occupational exposure. DMP, DMTP, DMDTP, DEP, DEDP and DEDTP were measured by GC-FPD after derivatization with pentafluorobenzyl bromide. The LOQ values (1 mL urine) were 2.0 μg/L for DMP and DETP, 4.0 μg/L for DEP and DEDTP, 8.0 μg/L for DMDTP, and 10.0 μg/L for DMTP. Dimethyl phosphates were detected in the majority of the urine samples, i.e., 90–100% in the exposed group and 80–87% in the control group. The concentration of the urinary diethyl phosphates DEP and DETP was above the LOQ values in 40 and 20% of samples for the exposed group, and in 50 and 30% of the samples for the control group, respectively. DEDTP was not detectable in the urine samples except for a post-shift exposed worker (detection frequency, 6.7%). Median creatinine-adjusted values (μg/g cr.) for DAP in Chinese with pre-shift, post-shift and without occupational exposure to OP were 316, 584 and 170 for DMP, below LOQ, 115 and 114 for DEP, 840, 1730 and 693 for DMTP, and 255, 756 and 135 for DMDTP, respectively. In all subjects, the highest excretion levels were found for DMTP. Several OP pesticide metabolites were frequently detected in urine samples of both populations studied.

1. Introduction

In China, organophosphorus (OP) compounds are widely used as insecticides in agriculture and residential settings. Measurement of OP pesticides metabolites in the urine samples of humans represents a useful approach to obtain reliable information about current exposure to occupationally exposed workers and to the general population. These biomarkers reflect cumulative exposure by all routes [1]. OP pesticides are mainly metabolized into any of the following compounds and excreted in urine (80–90% of the total dose within 48 h) [2]: dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP) and diethyl dithiophosphate (DEDTP) [3]. Measurement of these dialkyl phosphates (DAP) in human urine was proposed to be a sensitive indicator for non-occupational background exposure levels [4–6], and many biological monitoring studies on DAP were reported [7–9].

In the present paper, we report on the determination of DAP metabolites of OP pesticides in human urine by capillary gas chromatography with P-specific flame photometric detection (GC-FPD) after derivatization with pentafluorobenzyl bromide (PFBBr). Pentafluorobenzyl (PFB) derivatives were identified by GC–MS in the electron ionization (EI) mode. The GC-FPD method was applied to measure DAP concentrations in 120 urine samples from 90 subjects collected from a Chinese population in order to evaluate both non-occupational and occupational OP exposure levels. To our knowledge, this is the first report on the biological monitoring of these compounds among Chinese subjects in the Shanghai area.

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2. Experimental

2.1. Chemicals and materials

DMP (98% purity) was from Acros Organics. DEP (98.8% purity) was purchased from Accu Standard. The sodium salts of DMTP (99% purity) and DMDTP (85% purity) were from Applichem. DETP potassium salt (98% purity) and DEDTP ammonium salt (95% purity) were from Aldrich. Dibutylphosphate (DBP, 97% purity) was obtained from Fluka and used as an internal standard (IS). Stock solutions were prepared at a concentration of 4000 mg/L in acetonitrile and diluted with acetonitrile to each working standard solution at concentrations 4.0 mg/L. All the standard solutions were stored in the dark at 4 °C.

2.2. Study subjects

A total of 90 subjects were recruited in the study. Urine samples were collected with written informed consent from 60 healthy non-occupationally exposed volunteers (37 males, 23 females; mean age 23 years) and 30 occupationally exposed apparently healthy workers (20 males, 10 females; mean age 40 years). These workers packed dimethoate emulsion in the packing division of a pesticide factory. The package was semi-automated and the workers put the emulsion into bottles by hand. They worked for 8 h daytime, usually from 8:00 a.m. to 4:00 p.m. Preceding environmental monitoring data in this division showed that the airborne dimethoate concentrations varied from 0.25 to 11.0 mg/m^3 (median, 1.07 mg/m^3). The workers had dermal exposure to dimethoate sometimes because of liquid overflow, but no information on dermal contamination was available. They had no exposure to further organophosphates.

After exposed from the previous day, pre-shift urine samples were collected from the first morning urine of exposed workers in the next day. Post-shift urine samples were collected 4 h later after shift. First morning urine samples were collected from healthy adults without any report of occupational exposure as control samples. All samples were transferred into 10 mL polyethylene test tubes and sent immediately to the laboratory under normal temperature conditions where they were stored at −80 °C until analysis. Urinary creatinine analysis was performed according to the Jaffé reaction.

2.3. GC-FPD and GC–MS conditions

A GC-FPD (Shimadzu GC-14A) was used. The GC operating conditions were as follows. GC column, BP-10, 25 m × 0.33 mm i.d., 0.25 μm film thickness (SGE, Australia). Column temperatures, 110 °C (1 min) – 8 °C/min – 210 °C (1 min) – 20 °C/min – 280 °C (10 min). Injection port temperature, 280 °C; detector temperature, 300 °C. Nitrogen gas (99.99% purity) was used as carrier gas at a head pressure of 150 kPa. The detector gases used were air at 60 kPa and hydrogen at 80 kPa. The injection volume was 1.0 μL in the splitless mode (splitless time, 1 min). A GC–MS (HP5890-5973) was used for structural elucidation of PFB derivatives of DAP. Injector conditions and chromatographic conditions used were the same as for the GC-FPD. Operating conditions were as follows: carrier gas, helium gas (high purity grade) at a flow rate of 1.0 mL/min; ion-source temperature, 250 °C; electron ionization, 70 eV; interface temperature, 280 °C. Chromatographic peaks were identified by target and qualify ions for each PFB-DAP (Table 1).

2.4. Standard preparation and analytical procedure of DAP metabolites

DAP were measured in urine samples based on the modified method of Aprea et al. [10]. Pooled blank urine sample was obtained from several healthy donors who were not treated with any drugs and were not exposed to known chemicals before collection. The pooled blank urine was found to be free of DAP or below the respective LOQ values of the present method. One milliliter of urine was pipetted into a 10 mL screw-top glass test tube, 250 μL of a DBP (IS) solution (4.0 mg/L) were added. Subsequently, 4 mL acetonitrile were added and the sample was mixed. After vigorous mechanical shaking for 5 min, the test tube was centrifuged (1200 × *g*, 5 min, 25 °C). The supernatant fluid containing DAP and DBP was transferred into a clean screw-top glass test tube. Sample volume was then reduced at 70 °C to a volume of 0.5 mL with a gentle nitrogen stream. Residues were re-extracted with 3 mL of acetonitrile that contained 1 g of Na_2SO_4, were shaken for 10 min and then centrifuged. The resulting extract was repeatedly evaporated at 70 °C to 0.1–0.2 mL under a gentle stream of nitrogen. To the final extracts, 20 mg of K_2CO_3, and 25 μL of pentafluorobenzyl bromide (PFBBr) were added and heated at 50 °C for 16 h to covert the phosphate acids to their pentafluorobenzyl (PFB) esters. The PFB-DAP derivatives were dissolved in 100 μL of toluene for injection into the GC–FPD.

Table 1
Some characteristic ions in the electron ionization (EI) mass spectra of the pentafluorobenzyl esters of synthetic of dialkyl phosphates (DAP) and the internal standard dibutylphosphate (DBP).

DAP	Main conformation ion (*m*/*z*)
DMP	306 $[M]^{+\bullet}$, 307, 194
DEP	334 $[M]^{+\bullet}$, 278, 258
DMTP	322 $[M]^{+\bullet}$, 110
DMDTP	338 $[M]^{+\bullet}$, 157, 125
DETP	350 $[M]^{+\bullet}$, 169
DEDTP	366 $[M]^{+\bullet}$, 185, 157
DBP	335 $[M-55]^{+\bullet}$, 279

2.5. Assay validation

Method validation was performed using the pooled urine blank sample. Calibration curves were prepared with a spiked concentration of 4.0 mg/L of DAP solution (ranging from 10 to 30 μL) added to 1 mL of pooled blank urine prior to extraction. The final concentrations of urinary DAP were designed to range from 40 to 1200 μg/L (seven points). Standard curves were obtained by analyzing standard solutions in triplicate at different concentrations in the same range. Calibration and standards curves were presented as plots of the peak area ratio of analyte to IS versus the analytes concentration. Extraction recovery was evaluated by analyzing pooled urine spiked with DAP standards in triplicate at each concentration level of 40, 600, and 1000 μg/L. The within-series precision for our proposed method was examined by assaying the pooled urine spiked with the same DAP concentrations. The between-day precision was examined by a duplicate assay of the pooled urine spiked with DAP (40, 600, and 1000 μg/L). LOQ was defined as the lowest concentration that the analytical process can reliably differentiate from background levels. LOQ values were estimated when the signal was three times the background noise from the chromatograms at the lowest analyte concentration assayed.

For quality assurance, an analytical run consisted of several unknown samples, calibration standards, pooled blank samples and three different levels quality control (QC) materials. Because no QC material was commercially available, we used spiked concentration of DAP standard solution in pooled blank urine. QC materials at three concentrations spanning the calibration range were analyzed concurrently with unknown samples to ensure the accuracy and precision of analytic data. A calibration plot for quantification was constructed for every analytical run, using 5–8 different calibration concentrations (40–1200 μg/L).

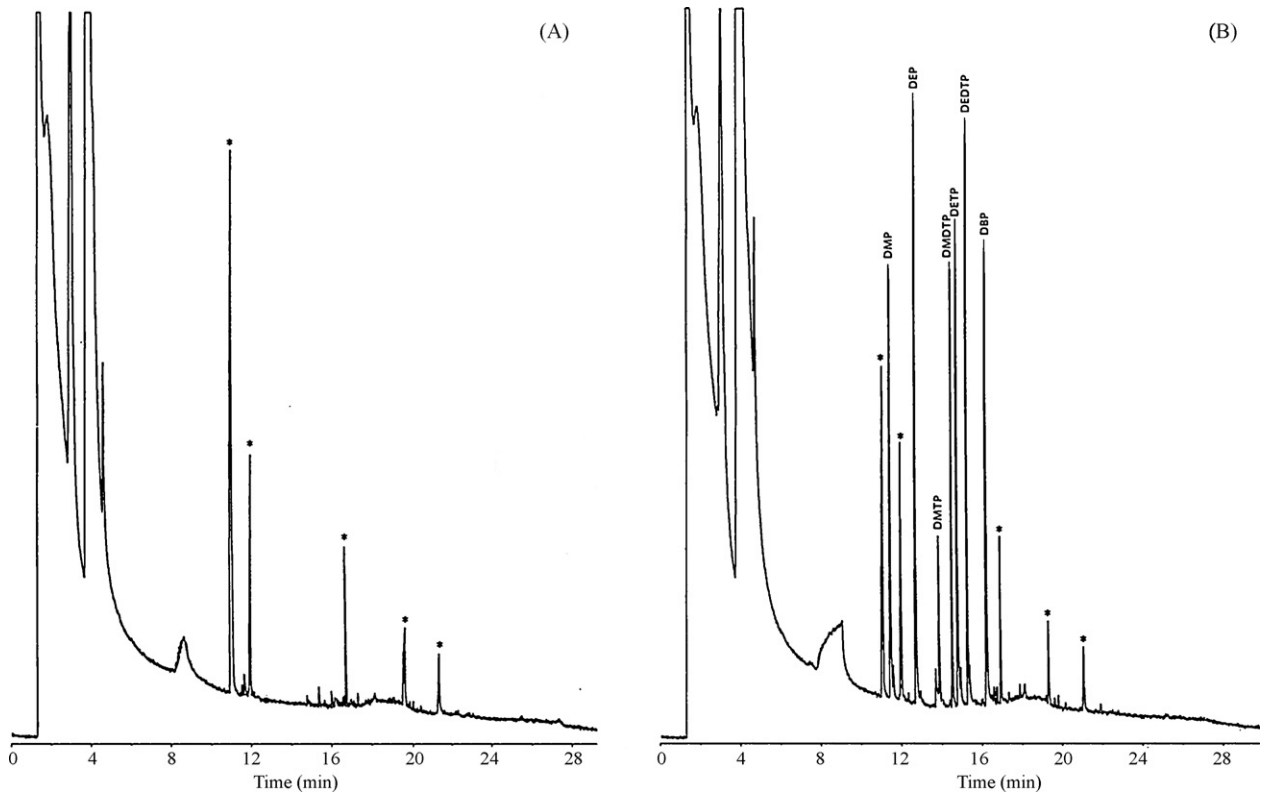

Fig. 1. GC-FPD chromatograms from the analysis as pentafluorobenzyl (PFB) derivatives of free-DAP in unspiked pooled urine (A) and from the same urine spiked with 1000 μg/L DAP (B). The retention times (min) were: 11.4 for DMP, 12.8 for DEP, 13.9 for DMTP, 14.5 for DMDTP, 14.8 for DETP, 15.3 for DEDTP, and 16.2 for DBP. Asterisks indicate unknown impurities.

2.6. Statistical analysis

We used urinary creatinine levels to normalize the concentration of detectable metabolites based on the dilution of the urine. The concentrations were presented in units of microgram of DAP per gram creatinine (μg/g cr.). We used the median, geometric mean, geometric standard deviation, 25th, 75th and 95th percentiles to describe the results. The significance of the differences among individual characteristics for each metabolite levels was estimated by non-parametric tests, i.e., Mann–Whitney. Undetectable urinary DAP concentrations were recorded as half of the LOQ values [11]. The 0.05 level of probability was used as the criterion of significance. All the analyses were performed using SPSS10.0 software and Microsoft Excel 2000.

3. Results

3.1. Method validation

Typical GC-FPD chromatograms from analyses of unspiked blank urine and blank urine spiked with DAP metabolites are shown in Fig. 1. Owing to the difficulty in obtaining urine samples really free of DAP, calibration curves were obtained by subtracting the basal DAP concentrations measured in the unspiked pooled urine from those measured in the spiked urine. The calibration curves for DAP metabolites are shown in Fig. 2. The calibration curves were linear for all the DAP metabolites in the range of 40–1200 μg/L. DETP and DEDTP with higher correlation coefficients of more than 0.9925 for DMP, 0.9975 for DEP, 0.9980 for DMTP, 0.9985 for DMDTP, 0.9990 for DETP and 0.9995 for DEDTP, respectively. LOQ values were 2.0 μg/L for DMP, 4.0 μg/L for DEP, 10.0 μg/L for DMTP, 8.0 μg/L for DMDTP, 2.0 μg/L for DETP and 4.0 μg/L for DEDTP.

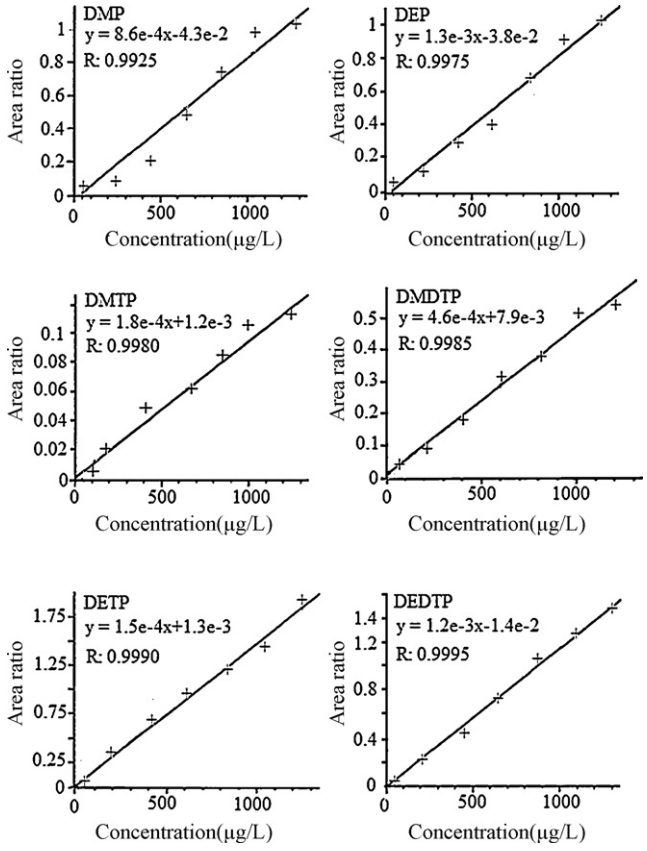

Fig. 2. GC-FPD calibration curves for the investigated DAP in a pooled urine sample.

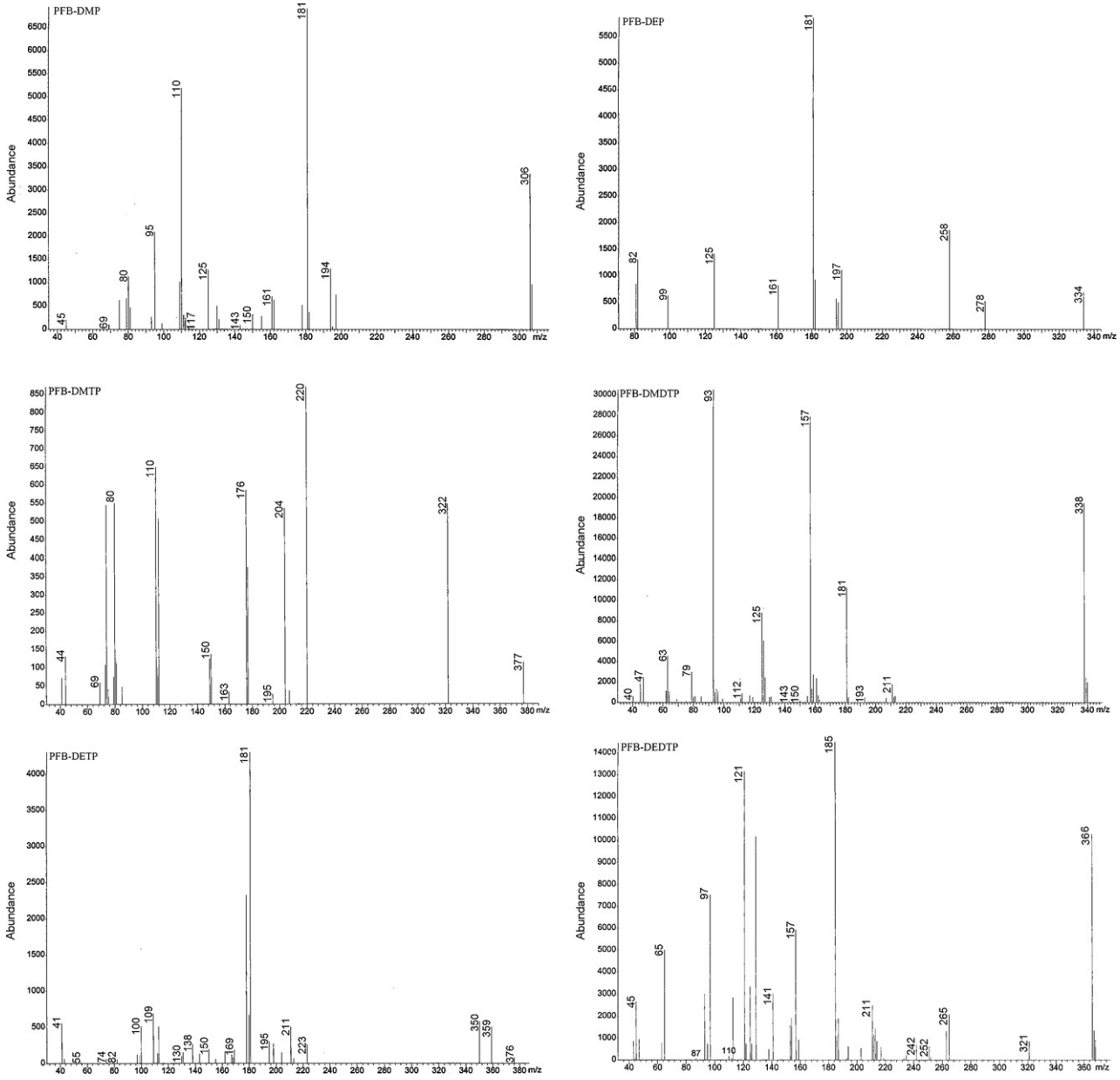

Fig. 3. Electron ionization (EI) mass spectra of the pentafluorobenzyl (PFB) ester derivatives of the synthetic DAP analyzed in the present study. Note: *m*/*z* 377 in DMTP spectrum, and *m*/*z* 359 and *m*/*z* 376 in DETP spectrum are due to unknown interferences.

Inter-assay precision (RSD, %) in spiked pooled urine samples ranged from 3.3 to 13.8%. Intra-assay precision was determined by triplicate analysis during 6 consecutive days and ranged between 3.0 and 12.6%. The mean relative recovery was 62.5–75.6% for DMP, 84.5–95.3% for DEP, 63.8–75.6% for DMTP, 68.1–78.5% for DMDTP, 82.7–97.6% for DETP, and 86.1–95.4% for DEDTP.

In our study, GC–MS was used for target analyte identification. The EI mass spectra of PFB-DAP derivatives are shown in Fig. 3.

3.2. Urinary DAP concentration in the study subjects

There are large variations in DAP concentrations in biological samples. We evaluated the normality of the data distribution and we found the creatinine-adjusted data were neither normally nor log-normally distributed. Therefore, non-parametric statistics (Mann–Whitney *U*-test) was used to determine the statistical significance of differences between general population group and

Table 2
Statistic categories of DAP (μg/g cr.) levels in samples according to gender.

Control group	Statistic categories	DAP metabolites					
		DMP	DEP	DMTP	DMDTP	DETP	DEDTP
Females	GM	218	114	676	195	63	<LOQ
(*n* = 23)	GSD	2.0	1.4	2.4	2.1	1.9	<LOQ
Males	GM	162	109	645	251	59	<LOQ
(*n* = 37)	GSD	2.4	2.2	1.9	2.1	1.9	<LOQ

Note: GM, geometric mean; GSD, geometric standard deviation.

Table 3a
Creatinine-adjusted levels (μg/g cr.) of DAP in spot urine from healthy unexposed volunteers ($n = 60$) and occupationally exposed workers ($n = 30$).

DAP	Detection rate (%)	Median	GM	P25	P75	P95	Range
DMP							
Controls	85	170	166	100	254	398	<LOQ–1026
Pre-shift	100	316	371	236	590	933	139–1761
Post-shift	100	584	741	406	1106	1738	210–6663
DEP							
Controls	50	114	110	<LOQ	197	324	<LOQ–383
Pre-shift	40	<LOQ	102	<LOQ	242	251	<LOQ–273
Post-shift	53	115	105	<LOQ	134	178	<LOQ–205
DMTP							
Controls	80	693	661	404	1104	1778	<LOQ–3187
Pre-shift	100	840	891	486	1639	2951	192–5584
Post-shift	100	1730	1479	959	2450	3890	178–6740
DMDTP							
Controls	86.7	135	126	68	219	380	<LOQ–784
Pre-shift	90	255	302	189	541	912	<LOQ–1649
Post-shift	96.7	756	832	365	1920	3162	<LOQ–3581
DETP							
Controls	30	<LOQ	60	<LOQ	91	146	<LOQ–186
Pre-shift	20	<LOQ	78	<LOQ	<LOQ	186	<LOQ–191
Post-shift	26	<LOQ	74	<LOQ	121	126	<LOQ–147
DEDTP							
Controls	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pre-shift	0	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Post-shift	6.7	<LOQ	47	<LOQ	<LOQ	<LOQ	37–59

Note: GM, geometric mean; Cr, creatinine; P, percentile.

exposed group. Urinary DAP levels in the males were not significantly higher than in the females in the present study (Table 2). This observation is similar to that found by Barr et al. in an American population [12].

The distribution of the DAP metabolites in all the samples analyzed summarized in Table 3a for creatinine-adjusted and in Table 3b for unadjusted concentrations. For the exposed workers, higher positive percentages emerged for alkyl phosphates, particularly for the methyl metabolites (DMP, 100.0%; DMTP, 100.0%; DMDTP, above 90.0%). Urinary ethyl metabolite concentrations (DEP, DETP and DEDTP) were above the LOQ values in 40.0, 20.0%, but also below LOQ in the pre-shift groups. The proportion of positive samples was very similar in exposed group between pre-shift and post-shift. In the general population and occupationally exposed group, DMP was detected most frequently followed by DMTP and DMDTP. Among the analytes DMTP was detected at the highest concentrations. However, DEDTP was not detectable, except for post-shift exposed worker with the detection frequency of 6.7%. All groups had similar concentrations of DETP and DEP.

From the above data, we also found DMP, DMTP and DMDTP were detected with the highest frequency in nearly 100% of the exposed group, about 10% higher than in the general population group; however, DETP was detected much less frequently (30, 20 and 26.7%, respectively), whereas DEDTP was undetectable in most urine samples. For urinary DAP, the detection rates of methyl phosphates metabolites (DMP, DMDTP and DMTP) in the control group of our study subjects were similar to those reported

Table 3b
Unadjusted levels (μg/L) of DAP in spot urine from healthy unexposed volunteers ($n = 60$) and occupationally exposed workers ($n = 30$).

DAP	Mean ± SD	GM	Median	P25	P75	P95	Range
DMP							
Controls	90 ± 43	70	86	63	112	181	<LOQ–223
Pre-shift	161 ± 81	145	137	101	186	371	77–428
Post-shift	328 ± 183	288	277	201	418	757	139–805
DEP							
Controls	16 ± 22	5	<LOQ	<LOQ	38	61	<LOQ–74
Pre-shift	14 ± 18	6	<LOQ	<LOQ	29	50	<LOQ–55
Post-shift	36 ± 75	11	28	<LOQ	40	223	<LOQ–47
DMTP							
Controls	302 ± 193	220	270	150	439	758	<LOQ–841
Pre-shift	361 ± 166	326	325	240	453	751	120–833
Post-shift	663 ± 317	580	575	451	859	1347	132–1531
DMDTP							
Controls	59 ± 51	36	47	20	88	169	<LOQ–207
Pre-shift	114 ± 77	79	100	64	167	282	<LOQ–320
Post-shift	383 ± 240	281	381	176	553	934	<LOQ–980
DETP							
Controls	8 ± 13	2.4	<LOQ	<LOQ	15	41	<LOQ–57
Pre-shift	6 ± 11	1.8	<LOQ	<LOQ	<LOQ	38	<LOQ–47
Post-shift	10 ± 17	2.3	<LOQ	<LOQ	5	59	<LOQ–63
DEDTP							
Controls	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Pre-shift	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Post-shift	3 ± 4	2.3	<LOQ	<LOQ	<LOQ	19	<LOQ–23

Note: GM, geometric mean; P, percentile.

Table 4
Median values of DAP concentrations (μg/g cr.) in reported studies.

Study	Study population	Sample size	Country	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
NHANES 1999–2000 [13]	Adults (20–59 years of age)	814	USA	0.76	1.90	<LOQ	0.86	0.25	0.08
NHANES 2001–2002 [14]	Adults (20–59 years of age)	1122	USA	<LOQ	<LOQ	<LOQ	<LOQ	0.490	<LOQ
NHANES 2003–2004 [14]	Adults (20–59 years of age)	937	USA	<LOQ	<1.67	<LOQ	<LOQ	<LOQ	<LOQ
Panuwet et al. 2008 [7]	Farmers (21–60 years of age) in Pong Yaeng	67	Thailand	<LOQ	0.71	0.12	2.60	0.80	0.14
Panuwet et al. 2008 [7]	Farmers (21–60 years of age) in Inthakhin	69	Thailand	<LOQ	0.49	<LOQ	<LOQ	0.94	<LOQ
Heudorf et al. 2001 [18]	Inhabitants (≥20 years of age)	484	Germany	15.5	13.5	<LOQ	2.10	<LOQ	<LOQ
Present study	Healthy students (21–23 years of age)	60	China	170	693	135	114	<LOQ	<LOQ

in some studies in USA populations [13], whereas ethyl phosphates (DEP, DETP and DEDTP) were detected much less frequently in our subjects. These differences could be due to the different exposure levels, LOQ values of the methods used and the sample timing. No significant differences existed for the detection rates of DAP between pre-shift and post-shift groups in our study.

Among the general population group, DMTP was the OP metabolite with the highest adjusted median level of 693 μg/g cr. The next biggest contributors were DMP (170 μg/g cr.) and DMDTP (135 μg/g cr.). The concentrations of DEP were higher than that of DETP. The adjusted median and geometric mean of DEDTP were below LOQ. Compared with the general population group, the concentrations of DMP, DMTP and DMDTP were significantly higher ($P < 0.05$) in pre-shift group, whereas there was no statistical difference with respect to DEP and DETP ($P > 0.05$). The concentrations of DEDTP were not detectable in both groups.

The concentrations of methyl phosphates metabolites in post-shift urine of exposed group also showed a significantly statistical difference ($P < 0.01$) suggesting a common source. DEP and DETP were detected more frequently in the post-shift group, but without a significant statistical difference ($P > 0.05$). Even after exposure, the median concentration of DEDTP was under LOQ with the detection frequency of 6.7%. It is possible that DEDTP is quickly metabolized to the corresponding monosulphated and oxidized metabolites for the lower detective percentage. Interestingly, DEDTP level was very low (2%) in a general population [13].

Urinary levels of most DAP increased after exposure. DMDTP level in post-shift urines was significantly higher than in pre-shift urines ($P < 0.01$) with a median level being nearly 3 times higher. Workers after exposure had statistically higher levels of DMP and DMTP than before exposure ($P < 0.05$). Urinary concentrations of DEP and DETP were not different in the pre- and post-exposure subjects ($P > 0.05$), whereas DEDTP levels became detectable first after exposure.

4. Discussion

Assessing exposure in occupations and elucidating predictors of those exposures are important. It is known that dimethoate is metabolized to DMP, DMTP and DMDTP in human body and that these metabolites are mainly excreted in the urine after exposure. In the present study we used a GC-FPD method to quantify DAP metabolites in urine after derivatization with PFBBr. Compared with the general population group, the concentrations of DMP, DMTP and DMDTP were significantly higher ($P < 0.05$) in the pre-shift group, whereas there were no statistical differences for DEP and DETP ($P > 0.05$). The concentrations of DMP, DMTP and DMDTP in the post-shift group also showed a significantly statistical difference ($P < 0.01$) with respect to the control group, indicating that packers are subjected to high exposure of OP. Our results suggest that they were derived from a common exposure source.

We found obvious higher creatinine-adjusted urinary DAP levels in the post-shift group than in the pre-shift group. DMDTP level in post-shift group was significantly higher than in pre-shift group ($P < 0.01$) with a median level being nearly 3 times higher. Packers after shift had statistically significantly higher levels of DMP and DMTP than before exposure. Urinary concentrations of DEP and DETP were not different between the pre- and post-exposure group, though being slightly increased. DEDTP levels became detectable first after exposure. These data show that hydrolysis products of dimethoate are rapidly excreted upon exposure. The first morning void samples were used to predict weighted-average daily metabolite concentration, and were often applied in cumulative risk assessment. DEP and DETP levels in both control and occupational group might partly be explained by non-occupational exposure.

Given the variability in urine dilution among the spot samples, we reported DAP levels in the Chinese population without and adjustment to urinary creatinine concentrations (Table 3). However, the levels of urinary DAP differed between our study and other studies. For example, NHANES (1999–2000) [14] reported

Table 5
Frequencies of detection (%) of DAP metabolites in general population-based studies.

Study	LOD/LOQ	Participants	Country	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
Murphy et al. 1983 [15]	20 μg/L	NHANSII (1976–1980) 5976 Adults and children	USA	12	6	<1	7	6	<1
Aprea et al. 1996 [6]	~1 μg/L (<10 nmol/L)	124 adults	Italy	87	99	48	82	73	7
Aprea et al. 2000 [16]	2–3 μg/L	195 children	Italy	96	94	34	75	48	12
Hardt and Angerer 2000 [17]	1 μg/L (5 μg/LDMP)	54 adults	Germany	96	100	89	94	46	2
Heudorf et al. 2001 [18]	1 μg/L (5 μg/LDMP)	1146 adults, adolescents and children	Germany	79	87	32	78	45	2
CDC 2001[a] [19]	0.01–0.58 μg/L	703 adults, adolescents and children	USA	83	84	72	99	99	99
NHANES 1999–2000 [14]	0.01–0.58 μg/L	1949 adults, adolescents, and children	USA	53	64	53	71	53	56
Present study	2–10 μg/L	60 adults	China	85	80	86.7	50	30	<LOQ

[a] Non-weighed frequencies of detection.

the median value of DMP in general population as 0.76 μg/g cr., whereas our study revealed 170 μg/g cr., which is nearly 1000 times higher. All DAP levels in our study were significantly higher than those in USA and German general population and the Thailand farmers. This remarkable difference suggests that the Chinese general population in the Shanghai area experiences more exposure to OP than in many other countries.

Significantly higher concentrations of DMTP compared to other metabolites were found in NHANES (1999–2000, 2003–2004) and in our study. Also the lowest levels of DEDTP were more generally reported in some other populations and in our subjects. Despite different conditions for exposure based on dietary and physical behaviors, these findings seem to be common in different populations with respect to the higher DMTP concentrations and to the lower DEDTP levels. General population DAP data have been reported for populations in USA [12], Thailand [7] and Germany [17,18]. Tables 4 and 5 summarize these data including the results of the present study for comparison.

The sample size of German population ($n = 54$) [17] was similar to our population subset. Their frequencies of detection (LOD, 1–5 μg/L) ranged from 2 to 100%, with DMTP being the most frequently detected. The detection rate of DMDTP (86.7%) in our study was slightly lower than that reported for the German population (89%), whereas other frequencies of detection were much lower. The data from the Italian population ($n = 124$) [6] were double the size of our population, which were also derived from adults participants. Ethyl phosphates metabolites (DEP, DETP and DEDTP) were detected much less frequently in our study than in the Italian population. DMDTP was detected more frequently, whereas frequencies of detection of other methyl phosphates metabolites were lower.

Although direct comparison of these concentrations is problematic due to different pest control strategies and national conditions, the differences among our DAP data and other reported reference values may be caused by a variety of factors. Firstly, age group, sex, race/ethnicity, even geographic diversity affect the creatinine concentrations in the urine, creatinine adjustment in diverse populations would not be valid for comparisons of DAP concentrations among the demographic groups. Secondly, environmental factors such as air source and water container were not carefully studied in our analysis. There is no doubt that people in China have high exposure level to OP pesticides because of heavy use [20] and high residue in the common Chinese raw food [21]. Thirdly, our data were derived from a small size population which could not well represent the general Chinese population. Finally, due to the limitation of the apparatus and laboratory methods, the LOQ values of our method are relatively high compared to those in some other studies, which might lead to low detection rates. Nevertheless, the present method sounds reasonable and acceptable for the measurement of higher exposures to OP pesticides.

It is worth mentioning that the level of DAP metabolites excreted in urine was found to overestimate the OP pesticide exposure. It has been suggested that due to light energy or bacterial activity, degradation to the metabolites might occur in the foodstuff. Moreover, DAP are more persistent in plants and are produced at commonly measured levels than their parent OP insecticides [22]. Therefore, DAP in fruits and vegetables may confound biomonitoring in the area of OP insecticide exposure and risk assessment.

5. Conclusion

A GC-FPD method was developed, validated and used for the quantitative determination of OP pesticide metabolites in human urine after derivatization to their pentafluorobenzyl esters. Most urinary OP pesticide metabolites were detectable in the Chinese general population group in Shanghai area. DMTP was the OP pesticide metabolite with the highest adjusted median level of 693 μg/g cr. followed by DMP (170 μg/g cr.) and DMDTP (135 μg/g cr.). These observations indicate that the Chinese population has a high exposure to OP pesticides in the common life. The biological monitoring of DAP metabolites in occupationally and non-occupationally exposed subjects among Chinese population should be further explored.

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