**Short Communication**

Relationship between Urinary Pesticide Metabolites and Pest Control Operation among Occupational Pesticide Sprayers

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Since the prohibition of organochlorines such as DDT in the 1960s, insecticides currently available on the market for agricultural and non-agricultural pest control purposes can be categorized into two major classes according to their chemical structures and properties, i.e., organophosphorus insecticides (OPs) and pyrethroids, both of which are widely used1). While both OPs and pyrethroids owe their insecticidal potency to their neurotoxicity, their actions are attributable to distinct mechanisms in that OPs work by inhibiting acetylcholinesterase (AChE)2) and pyrethroids by interacting with the voltage-gated sodium channels of the axon3). The widespread application of these pesticides is ascribable in part to the lower sensitivity of mammals to these compounds due to mechanisms including faster metabolic detoxification, though concern remains over their neurotoxicity2, 3), induction of oxidative stress4), immunotoxicity and reproductive toxicity in humans5, 6).

Statistics indicate that use of pesticides has been sustained around that level ever since7), implying that the practice of pesticide spraying has not declined for the past two or more decades. Despite the effort to reduce direct exposure to pesticides among occupational pesticide control operators (PCOs) by utilization of protective equipment, complete elimination of exposure is considered unattainable. Thus, exposure to pesticides among PCOs is probably greater than among general populations.

After uptake in the human body, both OPs and pyrethroids are rapidly metabolized and excreted mainly into urine. With respect to the metabolism of OPs in the human body, two distinct pathways have been identified depending on whether or not oxidation to produce oxon-form intermediate products is involved in the course of the breakdown of OPs8). Oxon-form products are further metabolized and excreted in urine as either dimethyl phosphate (DMP) or diethyl phosphate (DEP), and non-oxidized products as either dimethyl thiophosphate (DMTP) or diethyl thiophosphate (DETP)9). Moreover, major pyrethroids in occupational use have an alcohol moiety which can be metabolized and renally eliminated as 3-Phenoxybenzoic acid (3-PBA)10).

Quantification of these end metabolites in urine of PCOs could serve as a monitoring tool for estimating an individual’s level of exposure to pesticide. Some human volunteer studies revealed a correlation between external exposure to OP or pyrethroids and biological monitoring values11–13). However, only a few studies have estimated the individual exposure amount in occupational settings14–16), where factors influencing the exposure level usually vary; highly elaborate techniques were employed in these dose evaluation studies. Because only limited data are available in Japan on the relationship between the exposure to pesticide and its metabolite concentrations in urine among PCOs17–19), we conducted a preliminary field study to examine whether the levels of urinary metabolites reflect the length of pest control operations which we consider to be a crude substitute for pesticide exposure levels.

**Subjects and Methods**

A total of 65 subjects (55 men and 10 women) engaged in the pest control occupation attended a mandatory health checkup program conducted in August 2006 and gave written informed consent to both answering a questionnaire covering the length of pest control working hours during the one week prior to the health checkup and providing blood and urine samples. Venous blood was drawn into heparinized tubes and stored at 4°C until assayed for erythrocyte AChE activity which was measured according to the modified acetylcholine-DTNB (5,5’-dithiobis-2-nitrobenzoic acid) procedure20) within 12 h after collection. The wake-up spot urine samples in the morning following the most recent pesticide spraying during the pre-checkup week were collected, while the morning urine samples on the checkup day were collected from those who had not sprayed pesticide during the past week. These urine samples were kept at −30°C until
Using gas chromatography-mass spectrometry (GC-MS) apparatus (Agilent 5975 MSD System), urinary OPs metabolites and 3-PBA were determined according to the previously reported procedure. GC-MS has been the technique of choice due to its high sensitivity which enables it to detect trace amounts of urinary metabolites. The determined concentrations of these urinary metabolites were corrected for the urinary creatinine concentration according to the alkaline picrate method.

The subjects were classified into two groups: non-sprayers who sprayed no pesticides during the pre-checkup week and sprayers who were engaged in spraying either OPs or pyrethroid pesticides during the same period. The sprayers were further dichotomized into either low-frequency PCOs (length of pest control operation <8 h/wk) or high-frequency PCOs (≥8 h/wk). Class-specific (OPs or pyrethroids) length of pest control operation was also summed and likewise dichotomized.

In the statistical analyses, the difference and trend of mean erythrocyte AChE between non-sprayers, low-frequency PCOs, and high-frequency PCOs were tested by analysis of covariance (ANCOVA) incorporating possible confounding variables, age (years), gender (male or female), drinking habit (yes or no), and the Brinkman index; the trend test was based on the linear contrast. Further ANCOVA analyses were performed to model associations of the length of pest control operation with four urinary biomarkers: 3-PBA, dialkylphosphate (DAP), a total of DMP and DEP (DMP+DEP, i.e., metabolites of oxon-form intermediate products), and a total of DMTP and DETP (DMTP+DETP, i.e., metabolites of non-oxidized intermediate products). We confirmed that the distribution of concentrations of urinary metabolites was normalized by logarithmic transformation. Besides, logistic regression analyses were conducted to estimate odds ratios of high levels of urinary metabolites among low-frequency and high-frequency sprayers compared to the referent non-sprayers; the confounding variables in the logistic model were the same as in the ANCOVA analysis. The cut-off limits which defined the high outcome values were: >550 nmol/g cre for DAP, >500 nmol/g cre for DMP+DEP, >50 nmol/g cre for DMTP+DETP, and >100 nmol/g cre for 3-PBA. All statistical analyses were performed using SPSS version 11.5J for Windows with the significance level set at \( p < 0.05 \).

### Results

Three PCOs reporting extremely long operating hours of ≥30 h/wk were excluded from the analysis, leaving data from 17 non-sprayers and 45 sprayers. Comparison of the basic characteristics showed no statistically significant differences between the non-sprayers and sprayers with regard to age and smoking/drinking habits (Table 1). A comparison of biomarkers showed...
significantly higher concentrations of 3-PBA, DAP, and DMP+DEP among sprayers, but no significant differences in the means of erythrocyte AChE and DMTP+DETP. The relationships between the class-specific (i.e., OP or pyrethroid) length of pest control operation with DAP and 3-PBA are illustrated in Fig. 1, with significant Spearman’s rank correlation coefficients of 0.60 and 0.61, respectively.

Multivariate adjustment resulted in significant differences in the means of 3-PBA, DAP, and DMP+DEP among the non-sprayers, low-frequency PCOs, and high-frequency PCOs with a significant linear trend (Table 2). Trichotomized hours of operating OPs were also significantly related to the adjusted means of 3-PBA.

Table 2. Relationship between length of pest control operation during pre-checkup week and measurements of exposure biomarkers by classes of sprayed pesticides

<table>
<thead>
<tr>
<th>Length of pest control operation (h/wk)</th>
<th>Non-sprayer</th>
<th>Sprayer</th>
<th>p for difference&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p for trend&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>&lt;8</td>
<td>≥8</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>25</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte AChE activity (µmol/ml/min)</td>
<td>3.2 (0.11)</td>
<td>3.4 (0.11)</td>
<td>3.4 (0.13)</td>
<td>0.31</td>
</tr>
<tr>
<td>3-PBA (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.7 (1.3)</td>
<td>9.6 (1.3)</td>
<td>43.2 (1.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DAP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>122.7 (1.3)</td>
<td>183.9 (1.3)</td>
<td>624.0 (1.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMP+DEP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.9 (1.4)</td>
<td>111.3 (1.4)</td>
<td>442.6 (1.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMTP+DETP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20.6 (1.4)</td>
<td>27.3 (1.4)</td>
<td>30.1 (1.5)</td>
<td>0.77</td>
</tr>
<tr>
<td>Organophosphorous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>26</td>
<td>21</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte AChE activity (µmol/ml/min)</td>
<td>3.3 (0.094)</td>
<td>3.4 (0.13)</td>
<td>3.4 (0.15)</td>
<td>0.54</td>
</tr>
<tr>
<td>3-PBA (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.0 (1.3)</td>
<td>9.6 (1.4)</td>
<td>29.0 (1.4)</td>
<td>0.015</td>
</tr>
<tr>
<td>DAP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>134.4 (1.3)</td>
<td>250.4 (1.3)</td>
<td>712.7 (1.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMP+DEP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>78.9 (1.3)</td>
<td>116.7 (1.5)</td>
<td>466.7 (1.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMTP+DETP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.0 (1.3)</td>
<td>43.8 (1.5)</td>
<td>34.7 (1.6)</td>
<td>0.15</td>
</tr>
<tr>
<td>Pyrethroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>35</td>
<td>21</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte AChE activity (µmol/ml/min)</td>
<td>3.3 (0.089)</td>
<td>3.4 (0.13)</td>
<td>3.3 (0.21)</td>
<td>0.84</td>
</tr>
<tr>
<td>3-PBA (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3 (1.2)</td>
<td>22.3 (1.3)</td>
<td>70.9 (1.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DAP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>158.6 (1.3)</td>
<td>297.9 (1.4)</td>
<td>454.9 (1.8)</td>
<td>0.081</td>
</tr>
<tr>
<td>DMP+DEP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>85.5 (1.3)</td>
<td>209.2 (1.5)</td>
<td>325.7 (2.0)</td>
<td>0.045</td>
</tr>
<tr>
<td>DMTP+DETP (nmol/g cre)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.5 (1.3)</td>
<td>28.2 (1.5)</td>
<td>41.6 (2.0)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Values are given as least-squares mean (S.E.M.) for erythrocyte AChE activity and least-squares geometric means (geometric S.E.M.). <sup>a</sup> p values by analysis of covariance to adjust for age, gender, drinking habit and Brinkman index. <sup>b</sup> Test for trend was preformed using linear contrast on the assumption of equal spacing from non-sprayer to high-frequency sprayer. <sup>c</sup> Log-transformed values were used for statistical analyses. AChE, acetylcholine esterase; 3-PBA, 3-phenoxybenzoic acid; DAP, dialkyl phosphate; DMP, dimethyl phosphate; DEP, diethyl phosphate; DMTP, dimethyl thiophosphate; DETP, diethyl thiophosphate.

Fig. 1. Relationship of class-specific length of pest control operation with DAP level (upper panel) and 3-PBA level (lower panel).
DAP, and DMP+DEP with a significant linear trend, whereas those of operating pyrethroids were significantly associated only with the adjusted means of 3-PBA. Erythrocyte AChE had no significant association with the length of either total or class-specific pesticide operating hours.

In the logistic regression analyses, high-frequency organophosphorous sprayers showed significantly high odds ratios of 30.7 and 16.0 in association with high urinary DAP and DMP+DEP concentrations, respectively, whereas low-frequency sprayers showed no significant associations (Table 3). Both low-frequency and high-frequency pyrethroid sprayers showed a significantly high level of 3-PBA with odds ratios of 10.9 and 34.2, respectively.

**Discussion**

A significant difference was observed among the means of the urinary pesticide metabolites according to the length of pest control operation. The means increased in the order of the three exposure categories and the linear trend was also significant. Since the length of pesticide operation was based on recall, misclassification of subjects by exposure status might be unavoidable. In this respect, we performed a sensitivity analysis to confirm that the statistical method used in this study was robust to variations caused by supposed misclassification of exposure levels (data not shown).

Further, we observed that urinary organophosphorous pesticides have a rather wide reference interval, and any baseline values should be determined using three blood samples collected at 1-wk intervals during the pre-exposure period. However, this procedure was difficult to perform among our subjects because they constantly underwent occupational exposure to some pesticides. An experimental human dosing approach is thus required to assess pre- vs. post-exposure intra-individual variations in the AChE activity.

Whereas significant associations of DAP and DMP+DEP with categorical OP exposure and 3-PBA with categorical pyrethroid exposure among PCOs were clearly demonstrated, urinary DMTP+DETP did not seem to be an effective biomarker for assessment of exposure to OPs in light of the observed lack of association with the pesticide operating hours. We speculated that, due to relatively rapid rates of metabolism in the oxidative and hydrolysis pathways ending in the excretion of DMTP or DETP compared to those ending in the excretion of DMP or DEP, the determined concentrations of these thioform metabolites might vary depending on the interval between exposure to OPs and urine sampling, which might attenuate the statistical power of detection. Although the significant relationship between OP exposure and 3-PBA cannot be biologically explained, it may possibly be attributable to the confounding effect of the intra-individual correlation between pyrethroid spraying and OP spraying hours.

Our results also demonstrate that the engagement in spraying either organophosphorous or pyrethroid pesticide over 8 h/wk is a strong independent risk factor associated with high levels of relevant urinary metabolites. Though the cut-off limits of biomarkers dichotomizing the subjects are somewhat arbitrary, the sharp rise in the odds ratio of >30-fold for high DAP or 3-PBA levels and 16-fold for high oxon-form metabolite levels compared to non-sprayers implies that the self-reported length of pest control operation serves as a rather important indicator of exposure.
sensitive indicator for estimating pesticide exposure risks. It should be noted that the level of urinary metabolites among the referent group was rather higher than among the general population, and possible underestimation of risks may have ensued.

This study has shown the usefulness of monitoring urinary metabolites as a biological marker of exposure levels among PCOs, which has often been demonstrated abroad but seldom in Japan.[17–19, 22]. However, key limitations regarding urine sampling and exposure assessment methods need to be addressed. First, the urinary concentrations of metabolites are subject to the exposure-sampling intervals because each pesticide has a different half-life. An orally administered organophosphate, diazinon, yielded peak urinary DAP levels at 2 h, and its urinary elimination half-life was reported to be 2 h in a human volunteer study,[29] whereas a chlorpyrifos volunteer study showed a much longer half-life of 15 h.[28] Moreover, the mean elimination half-life of an orally dosed pyrethroid, cypermethrin, was estimated to be 16.5 h.[12]. However, compound-specific kinetics of inhaled pesticides was not characterized in our study. Second, our study assumed that the length of pesticide spraying time reflects the individual exposure yet it lacked accurate dose evaluation, something which has been rarely investigated in occupational settings.[15]. Factors such as application method, use of personal protective gear, or work practices related to hygiene influence exposure levels and appropriate exposure assessments are challenging given these complexities.[27]. At this time, our results must be interpreted conservatively until some feasible tool is incorporated for individual-level exposure assessment.

References


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