Biological Monitoring of Pyrethroid Exposure of Pest Control Workers in Japan

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Abstract: Biological Monitoring of Pyrethroid Exposure of Pest Control Workers in Japan: Dong Wang, et al. Department of Radiological and Medical Laboratory Sciences, Nagoya University Graduate School of Medicine—Synthetic pyrethroids such as cypermethrin, deltamethrin and permethrin, which are usually used in pest control operations, are metabolized to 3-phenoxybenzoic acid (3-PBA) and excreted in urine. Though 3-PBA can be used to assess exposure to pyrethroids, there are few reports describing urinary 3-PBA levels in Japan. This study aimed to investigate the seasonal variation of the exposure levels of pyrethroids and the concentration of urinary 3-PBA among pest control operators (PCOs) in Japan. The study subjects were 78 and 66 PCOs who underwent a health examination in December 2004 and in August 2005, respectively. 3-PBA was determined using gas chromatography-mass spectrometry. The geometric mean concentration of urinary 3-PBA in winter (3.9 µg/g creatinine) was significantly lower than in summer (12.2 µg/g creatinine) (p<0.05). Geometric mean concentrations of urinary 3-PBA in the spraying workers and the not-spraying workers within 2 d before the survey were 5.4 µg/g creatinine and 0.9 µg/g creatinine for winter with a significant difference between the groups (p<0.05), and 12.3 µg/g creatinine and 8.7 µg/g creatinine for summer (p>0.05), respectively. A significant association of 3-PBA levels and pyrethroid spraying was thus observed only in winter. In conclusion, the results of the present study show that the exposure level of pyrethroids among PCOs in Japan assessed by monitoring urinary 3-PBA was higher than that reported in the UK but comparable to that in Germany. Further research should be accumulated to establish an occupational reference value in Japan. (J Occup Health 2007; 49: 509–514)

Key words: Biological monitoring, Pyrethroids, 3-Phenoxybenzoic acid, Pest control operators

Pyrethroids, a group of synthetic insecticides, started to be manufactured in the 1970s at the same time as the removal of organochlorine insecticides, such as DDT, from the consumer market. The synthetic pyrethroids not only inherit biologic activity (ability to kill insects) from their natural counterpart, pyrethrin, which is found in Chrysanthemums, but also show improvements in their environmental stability. Pyrethroids are widely used in agriculture, forestry, the textile industry, and public health programs worldwide. With the phaseout of organophosphorus pesticide (OP) use in residential environments in the United States, the consumer uses of pyrethroids have increased since the late 1990s. Although individual pyrethroid insecticides share some common physical and chemical properties as a group, their toxicological mechanisms vary in mammals. Pyrethroid insecticides are subject to review as potential developmental neurotoxicants in the light of their mode of action on voltage-sensitive sodium channels. In addition, permethrin, the most widely used pyrethroid insecticide, is suspected of being an endocrine-disrupting
chemicals, and along with fenvalerate, has been classified as a potential carcinogen at high exposure levels. Toxicological studies have also suggested that pyrethroids have a suppressive effect on the immune system and may cause lymph node and spleen damage.

Because of their widespread use, the exposure to pyrethroids among the general population is significant, and this has been shown in several biological monitoring studies. Pyrethroids such as cypermethrin, deltamethrin and permethrin are rapidly metabolized by a hydrolytic cleavage of the ester bond, followed by oxidation yielding a non-toxic acid metabolite, 3-phenoxybenzoic acid (3-PBA) (Fig. 1). The metabolite partly conjugated and mostly eliminated in urine was first identified as a valid human biomarker of pyrethroid exposure largely on the basis of data from animal studies. Subsequent human studies have produced data that support this premise and show the current exposure level. However, there are few reports describing urinary 3-PBA levels in Japan. The primary objective of this study was to investigate the seasonal variation of the concentration of urinary 3-PBA among Japanese pest control operators (PCOs) who are often exposed to pyrethroid insecticides during handling, mixing and spraying.

Methods

Study design and subjects

The study population consisted of PCOs who worked for 14 companies located in the Chubu area of Japan. They were occupationally exposed to pyrethroid insecticides (phenothrin, etofenprox, cyphenothin and mainly permethrin). The health check-ups including routine clinical laboratory tests were conducted in December 2004 (winter), the off-season, and in August 2005 (summer), a relatively busy season for pesticide spraying. Seventy-eight and 66 workers took part in the winter and summer check-ups, respectively. Table 1 shows the profiles of the workers. All subjects were interviewed with a self-administered questionnaire asking about subjective symptoms and names of pesticides and their spraying frequencies. Urinary 3-PBA data were first analyzed according to the survey seasons, and then to the spraying frequency if the workers had sprayed insecticides within 2 days before the survey or not. This study protocol was approved by the Ethics Committee of the Nagoya University Graduate School of Medicine, Nagoya, Japan. All the subjects of the study gave their written informed consent.

Sample collection

Urine samples of each PCO were collected from the first voided morning specimen for the survey. After collection, the samples were transferred into 10 ml polyethylene test tubes and stored at −80°C without any pre-treatment until analysis. The creatinine level of each urine sample was determined according to the Jaffé reaction.

Urinary 3-PBA analysis

Urinary 3-PBA was determined using gas chromatography-mass spectrometry (GC/MS). The analytical method used was a modification of that...
described by the Schettgen et al. (20). The urine samples were subject to an acid-induced hydrolytic cleavage of the conjugates, followed by liquid extraction and methylation of free acid metabolites. Briefly, 5 ml of urine sample were spiked with 1 ml of HCl (6 mol/l) and 2-phenoxybenzoic acid (2-PBA, internal standard) and incubated in a water bath at 90°C for 60 min. Afterward, 8 ml of n-hexane were added, and the mixture was shaken vigorously for 10 min and centrifuged for 10 min. The resulting extract was evaporated to dryness with a gentle nitrogen stream. The residues were dissolved in 2 ml of H₂SO₄-Methanol (1:9, v/v), and incubated in a water bath at 60°C for 60 min. Then, 2.25 ml of NaOH, 1.5 ml of water and 6 ml of n-hexane were added. After vigorous mechanical shaking for 10 min and centrifuging for 10 min, the upper layer was passed through the SPE column. The extracts were then eluted with 10 ml of ether-n-hexane (15:85, v/v), and the eluate was evaporated at 60°C to dryness with a gentle nitrogen stream. The residues were dissolved in 0.2 ml of toluene and analyzed using GC/MS equipped with an auto sampler (PerkinElmer TurboMass Systems, Wellesley, MA, USA). The limit of detection (LOD) was 0.04 µg/l for 3-PBA.

Statistical analysis

Wilcoxon’s matched-pairs signed-rank test was applied for intra-individual differences of urinary 3-PBA levels of PCOs between the winter and summer surveys. The Mann-Whitney U-test was used for individual differences of urinary 3-PBA levels between those who sprayed within 2 d before the survey or not in winter and summer. Concentrations less than LOD (0.04 µg/l) were considered as half the LOD value. A two-tailed p value less than 0.05 was considered to indicate a statistically significant difference. These statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2003 (Microsoft Corporation, Seattle, WA, USA).

Results

According to the questionnaire, 14.1% (11 of 78 persons) and 36.4% (24 of 66 persons) of the PCOs sprayed pesticides within 2 d before the survey in winter and summer, respectively. Moreover, 37.1% (29 of 78 persons) and 95.4% (63 of 66 persons) of them had sprayed pesticides within a month before the survey in winter and summer, respectively. Hours of spraying pesticides during the above periods in summer tended to be longer than those in winter, but no significant difference was observed (Table 2).

Figure 2 shows the level of urinary 3-PBA among the same PCOs (n=44) who sprayed pyrethroid insecticides both in summer and winter. The levels of 43 workers in winter and 42 in summer were more than the LOD (0.04 µg/l). Geometric means of urinary 3-PBA in winter and summer were 3.9 µg/g creatinine and 12.2 µg/g creatinine, respectively, evidencing a statistically significant difference between the seasons.

The urinary 3-PBA levels of the PCOs who sprayed pyrethroids or not within 2 d before the survey are shown in Fig. 3. Of the total 78 PCOs, the levels of 10 and 55 operators who sprayed and did not spray the insecticides, respectively, were over the LOD in winter, and their geometric mean levels were 5.4 µg/g creatinine and 0.9 µg/g creatinine, respectively, with a statistically significant difference between the groups. In summer, of the 66 PCOs the levels of 22 spraying and 30 not-spraying operators were measurable, and their geometric mean levels were 12.3 µg/g creatinine and 8.7 µg/g creatinine, showing no statistically significant difference between groups. Regardless of whether there was a statistical significant difference or not between the groups, there was no significant difference in any exposure-related subjective symptoms or laboratory test result. Also, physical examination revealed no signs suggestive of neurotoxicity in any study subject.

Discussion

Due to their low mammalian toxicity but high insecticidal activity, pyrethroids are being increasingly used for pest control. Consequently, PCOs are more often exposed to them during handling, mixing and spraying.

<table>
<thead>
<tr>
<th>Survey time</th>
<th>Within 2 d</th>
<th>Within a month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter (n=78)</td>
<td>14.1% (n=11)</td>
<td>37.1% (n=29)</td>
</tr>
<tr>
<td>Hours of Spraying (h)</td>
<td>3.4 ± 2.1</td>
<td>12.9 ± 11.9</td>
</tr>
<tr>
<td>(range)</td>
<td>(1–8)</td>
<td>(3–40)</td>
</tr>
<tr>
<td>Summer (n=66)</td>
<td>36.4% (n=24)</td>
<td>95.4% (n=63)</td>
</tr>
<tr>
<td>Hours of Spraying (h)</td>
<td>7.9 ± 4.3</td>
<td>20.1 ± 11.6</td>
</tr>
<tr>
<td>(range)</td>
<td>(1–14)</td>
<td>(4–52)</td>
</tr>
</tbody>
</table>
Therefore, a growing need for the assessment of pyrethroid pesticide exposure has been recognized. Under current exposure levels in the studied population, medical examinations including routine clinical blood tests showed no exposure-related hazardous effects on the health status of PCOs, although a previous study identified a slight deterioration in semen quality in work associated with spraying\(^{21}\).

All synthetic pyrethroids are rapidly metabolized by hydrolytic cleavage to form their corresponding metabolites, 1) acid moieties such as cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid or cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, and 2) alcohol moieties which are oxidized to 4-fluoro-3-phenoxybenzoic acid or 3-PBA. Both the acid moieties and the alcohol moieties in urine can be used as biological monitoring markers for pyrethroid pesticides\(^{22}\). Since most pyrethroids are metabolized to form 3-PBA, it is used as a general exposure marker of pyrethroids. The present study showed a relationship between the spraying of pyrethroid pesticides and the concentration of urinary 3-PBA among PCOs in Japan. The detected urinary concentrations were higher than those reported in an occupationally exposed population in the UK\(^{23}\), presumably attributable to shorter duration of spraying work in the UK, but were comparable to those reported in Germany\(^{17, 18, 24, 25}\). The intra-individual comparison of urinary 3-PBA levels reflected well the seasonal difference of occupational exposure to pyrethroids.

In our study, compared with winter, more PCOs sprayed pesticides within 2 d or within a month before the survey in summer. In particular, the number of workers who sprayed pesticides within a month before the survey in summer reached 95.4%, showing that summer was the busy season for workers. Elimination of pyrethroids appears to follow first-order kinetics, with elimination half-times in humans ranging from 6.4 to 16.5 h, depending upon the specific pyrethroid and the exposure route studied. For most pyrethroids, elimination is nearly complete within 5 d of exposure, although certain isomers can persist in the body for a longer period of time\(^{26}\). From the viewpoint of biological monitoring,
the level of urinary 3-PBA in summer was statistically significantly higher than in winter. This also corresponded with the result that the number of those spraying pyrethroids increased in summer. Thus, urinary 3-PBA is postulated to be a suitable biomarker for monitoring exposure to pyrethroid pesticides in Japan.

In the winter survey, there was a statistically significant difference in the level of urinary 3-PBA between the groups separated according to the spraying status, spraying or not spraying pyrethroids within 2 d before the survey. However, no statistical significant difference was found in the summer survey. We speculate that the residual effects of pesticide exposure 3–4 d before the survey might attenuate the difference between the groups in summer. Another possibility is that non-occupational background exposures or household use of pesticides might be involved.

The present study explored the urinary 3-PBA levels as a biological monitoring index for pyrethroid insecticides in PCOs in Japan, but could not establish a clear relationship in summer between the recent exposure and the metabolite levels. Further cumulative research must be done to establish an occupational reference value in Japan.

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References


