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# Agricultural Worker Exposure to and Absorption of Permethrin Applied to Cabbage

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Permethrin is an agrochemical belonging to the synthetic pyrethroid family, and is widely used as an insecticide in Japan on such produce as cabbage, Chinese cabbage, cucumbers, eggplants, pears, peaches, and mandarin oranges. Several studies have examined occupational exposure to permethrin (Kolmodin-Hedman et al. 1982; Adams et al. 1985; Cloud et al. 1987; Byers et al. 1992). However, there are no reports concerning exposure of agricultural workers to permethrin in Japan.

In Japan, agricultural workers who use agrochemicals have many problems with their health (Shiwaku et al. 1982; Manda et al. 1987). The purpose of the present study was to obtain basic data on health protection measures for the agricultural workers. We decided to investigate the state of exposure to agrochemicals in an agricultural worker (spreader) who spreads (applies) the permethrin on cabbage grown in an open field and a worker (assistant) who assists him.

Biological monitoring of metabolites in the urine is thought to be a valid indication of exposure. Accordingly, we focused on 3-phenoxybenzoic acid (3PBA), which is a metabolite of permethrin found in the urine (WHO 1990). According to the methods by Woolen et al. (1992), we investigated a method of analysis, and developed a simple and rapid gas chromatography-mass spectrometric analysis (SPE-GCMS analysis) employing solid phase extraction (SPE) in pre-treatment.

In this study, along with measurement of 3PBA in the urine of the spreader (applicator) and the assistant using SPE-GCMS analysis, we also recorded the amount of permethrin adhesion and amount of permeation on different parts of their bodies, as well as the concentration of airborne permethrin. As a result, we were able to ascertain the existing state of exposure of agricultural workers to agrochemicals and obtain information for further promotion of health protection measures for these workers.

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## MATERIALS AND METHODS

Woolen et al. (1992) extracted 3PBA from urine by adding concentrated sulfuric acid and degrading it by heat and then performing liquid-liquid extraction with diethyl ether. After centrifugation, the ether layer was placed in a centrifuge tube which contained anhydrous sodium sulfate, and mixed. After centrifugation, the ether layer was removed, and the aqueous phase was partitioned with ether again. After centrifugation, the ether layer was combined. The operations required by this method, such as centrifuging 3 times after the liquid-liquid extraction, are complicated. According, we applied the solid phase extraction method (Asakawa et al. 1989) using a cartridge column (Sep-Pak $C_{18}$, Waters) for the extraction of 3PBA from urine. We added 5 mL of urine to 1 mL of concentrated sulfuric acid, and performed the thermal decomposition. After cooling, we added 10 mL of distilled water, washed it beforehand with 5 mL of chloroform and 5 mL of methanol, and poured the remaining liquid into the Sep-Pak, which was prepared by eluting it twice with 5 mL of distilled water. After rinsing this Sep-Pak 4 times with 5 mL of distilled water, we eluted 3PBA in 5 mL of chloroform. Next, in order to esterify and measure 3PBA, we dried the solution using a stream of N,, added 200 µL of pentafluoropropionic anhydride and 1.50 µL of 2, 2, 3, 3, 3-pentafluoropropanol, sealed it hermetically, and heated it for 0.5 h at 90°C. After cooling, we added 100 µL of trifluoroacetic acid, sealed it hermetically and heated it for 0.5 h at 90 °C. After cooling, we dried it using a stream of $N_2$, dissolved it in 150 µL of ethyl acetate, and used that as the sample for GCMS analysis. The standard solution of 3PBA was also esterified and measured in the same way. We used 3-phenoxybenzoic acid, pentafluoropropionic anhydride, 2, 2, 3, 3, 3-pentafluoropropanol and trifluoroacetic acid (Wako Pure Chemical) for organic solvents and other reagents, or other comparable products on the market that are used for analysis of residual pesticides. The standard solution was in acetone. For the GCMS, we used Shimadzu GCMS-QP2000GF. Our measurement standards were: column; DB-SMS (J&W) 30 m X 0.32 mm I.D., 0.25 µm, carrier; He 10 mL/min., oven; 140 °C (2 min) to 200 °C at 8 °C /min, injection; splitless, 200 °C, m/z; 346, 197.

The subject of our investigation was the application of pesticides to cabbage grown in an open field and was conducted in July, 1993. The work was performed by 2 people; a spreader and an assistant. A 1/2000 dilution of Adion (containing 20% permethrin) was applied successively to 3 fields (totaling 2000 m$^2$) of cabbage using a stationary power-driven sprayer. The spreader held a pole 360 cm in length which had 10 spray spouts (nozzles) that were attached from 240 cm to the end of the pole, a distance of 120 cm. Application was performed by advancing forward and moving the pole back and forth 180°. The assistant helped the application go smoothly by holding the hose, etc. During the working period (15:00-16:00) the temperature, humidity, globe temperature, WBGT and wind speed averaged 27.9 °C, 54.3% 34.2 °C, 24.7 °C and 1.6 m/sec, respectively. The spreader wore waterproof trousers and a usual (not waterproof) long-sleeved shirt. The assistant wore a usual trousers and long-sleeved shirt during the working period. They also wore a simple mask covering the nose and mouth, rubber gloves, and hat. A total of 110 L were applied and the entire time that it took to perform the application was around 0.5 h. The urine of the spreader and the assistant were sampled 7 times: before the application (2:00 PM), immediately after the application (5:00 PM) and before going to sleep (9:00 PM). On the next day, sampling was performed at 6:00 AM, at 4:00 PM and before going to sleep at 8:00 PM. Two days after the application, sampling was performed at 6:00 AM in the morning only. Moreover, to express the 3PBA concentration in per mg of creatinine, creatinine in the urine was measured by an automatic analyzer (Type 7050, Hitachi Co.), based on Jaffe's reaction (Jaffe 1886).

The concentration of permethrin in the air during application was sampled from around the mouth of spreader and 2 fixed points using a suction pump with an attached charcoal-silica gel tube (SIBATA SCI. TEC. LTD.). After elution with acetone, the samples were analyzed. To measure the amount of adherence and permeation of permethrin to different parts of the body, filter paper (Toyoroshi No63H) was applied to the surface of clothing and to the surface of the skin. This was collected after completion of the application and measured after elution with acetone. The standard permethrin solution we used was made by Nanogen and suspended with acetone. For GC, we used Shimadzu GC-7A. The measurement conditions were: co1umn; DB-5 (J &W) 30 m X 0.53 mm I.D., 1.5 pm, carrier; He 10 mL/min, oven; 260 °C, injection; 300 °C, detection; ECD.

## RESULTS AND DISCUSSION

Table 1 shows the recovery rate for each solvent when 150 µL of a 0.1 ppm standard solution of 3PBA was added to a Sep-Pak $C_{18}$. The 3PBA was almost completely eluted in 5 mL of chloroform.

Figure 1 shows the total ion chromatogram and mass spectrum of the esterified 3PBA. From the mass spectrum's peak retention time ($t_R$) of 7.90 min in the total ion chromatogram, we found a strong peak at ion mass number (m/z) 346 and 197. Then, we set the m/z to 346, 197 and measured the esterified 3PBA by Selected Ion Monitoring (SIM).

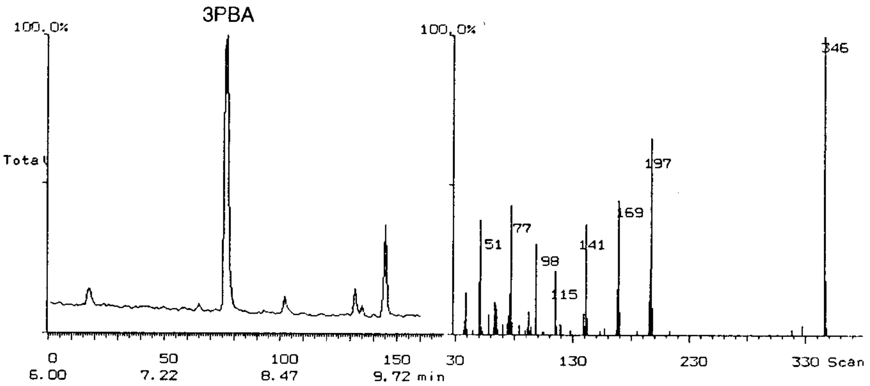

Figure 1. Total ion chromatogram and mass spectrum of esterified 3PBA

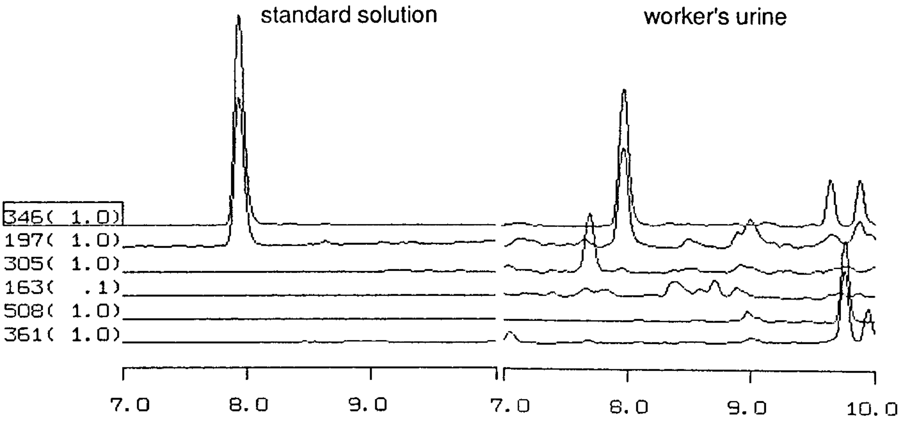

Figure 2. SIM chromatograms of esterified 3PBA

In worker's urine sample, a peak was obtained at the same $t_R$ (7.9 min) at m/z 346, 197, the same as for the standard 3PBA solution.

Table 1. Sep-Pak $C_{18}$ recoveries of 3PBA for each solvents

| Solvents | | Recoveries |
|---|---|---|
| Methanol | 5mL | -a |
| n-Hexane | 5mL | trace |
| Diethyl ether | 5 mL | 49.2% |
| Chloroform | 5mL | 91.4% |

a; unable to measure because extract was precipitated

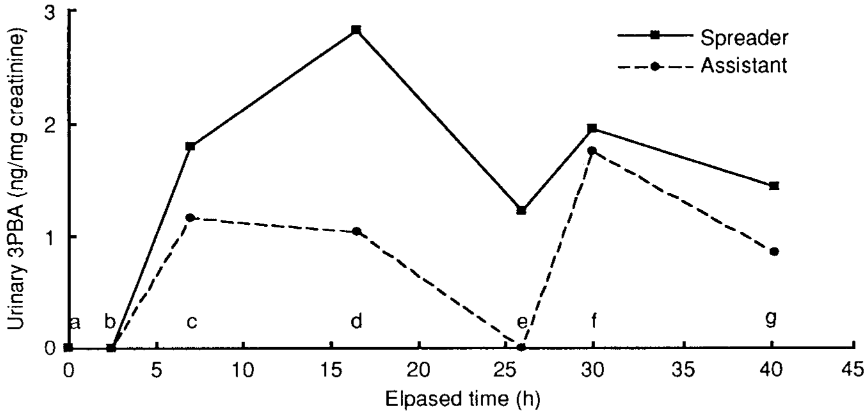

Figure 3. Urinary 3PBA concentration of agricultural workers applied permethrin
a; before application, b; after application, c; before sleeping. d; morning,
e; one day after application, f; before sleeping, g; morning

The chromatogram is shown in Figure 2. In the urine sample of the agricultural worker spreading Adion, which contains permethrin, a peak was obtained at the same $t_R$ of m/z 346, 197, the same as for the standard 3PBA solution. Also, the recovery rate of 3PBA in urine was over 90%, and the detection lower limit (N.D.) was 0.7 ppm at a urine concentration when the amount of the GCMS injection was 2 µL . This suggested that it is possible to measure 3PBA in the urine by SPE-GCMS analysis using SPE in the pre-treatment that we devised for this experiment. In this regard, m/z 305, 163 and 508, 361 are considered to be the m/z of metabolites in the urine other than permethrin (WHO 1990; Woollen et. al. 1992). The peak at m/z 508, 361 under the GCMS measurement conditions of our experiment suggested the existence of derivatives of 4-hydroxy-3-phenoxybenzoic acid in the urine.

Next, we measured the 3PBA in the urine of the agricultural worker who had spread Adion containing permethrin using this SPE-GCMS analysis. The results are shown in Figure 3. The spreader was N.D. before and directly after applying the permethrin, but before going to sleep on the same day, 2.9 ng/mL (1.8 ng/mg creatinine) was detected and on early next morning, 5.1 ng/mL (2.8 ng/mg creatinine) was detected. On the early morning of the third day, it was 1.4 ng/mL (1.4 ng/mg creatinine). 3PBA was also found in the urine of the assistant, although the levels were lower than that in the spreader. The pattern was the same as with the spreader.

The results of the measurement of permethrin in the air are shown in Table 2.

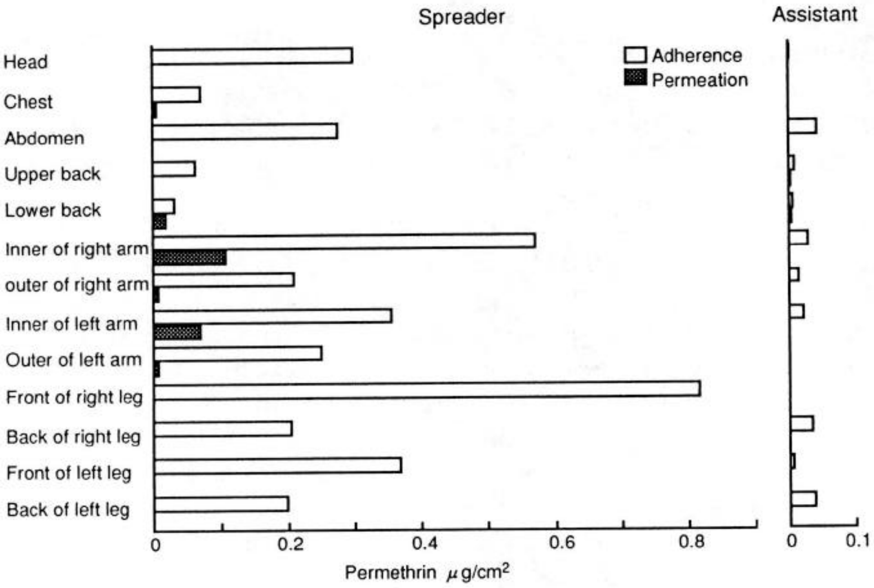

Figure 4. Amounts of adherence and permiation of permethrin to different parts of body
adherence;deposition as measured on filter paper applied to the surface of clothing
permeation (through clothing); deposition as measured on filter paper applied to the inside of clothing (surface of the skin)

Table 2. Permethrin concentration in the air of applying area

| Spreader's mouth area | Fixed point 1* | Fixed point 2** |
|---|---|---|
| 14.6 μg/m$^3$ | N.D. | 4.2 μg/m$^3$ |

*; windward side in the field, **; leeward side in the field
N.D.; Not detected at the detection limit of 0.1 μg/m$^3$

It was 14.6 μg/m$^3$ around the area of the spreader's mouth and N.D. (< 0.1 μg /m$^3$) and 4.2 μg/m$^3$ at the fixed points in the field.

Figure 4 shows the amounts of adherence and permeation of permethrin to different parts of the body. For the spreader, the amount of adherence was 0.03 (lower back area) - 0.82 μg/cm$^2$ (surface of front of right leg). There was a tendency for the values to be greater on the frontal surface of the legs and on the inner part of the arms. These findings indicate that permethrin had filtered through the clothes and onto the inner part of the arms; about 20% of the amount of adherence was due to permeation. However, this did not appear to be so with regards to the legs. In spite of the amount of adherence, pesticide to the frontal surface of the spreader’s legs being high, it did not appear that it was due to permeation (through clothing) because the spreader had been wearing waterproof trousers. As for the assistant, she had a fairly low amount of adherence compared to the spreader. Even so, the pattern was the same as with the spreader.

The present study revealed 3PBA in the urine of the spreader, following application of pesticide to crops, and exposure to and absorption of permethrin during application operations. The spreader made an attempt to avoid direct contact with the pesticide and used a pole with the spray spouts located 240 cm away from the hand-hold. However, since he was advancing forward while moving the pole back and forth 180°, added to fact that the wind changed direction, and in spite of the fact that cabbage is a crop that grows close to the ground and the pesticide is spread low, the concentration of airborne permethrin in the air around the spreader’s mouth was much higher than that around the fixed points in the field. A dust mask was worn, but it was rather simple, meant to be discarded after using once; furthermore, did not seem to fit the face well. Because of this, we can assume that the spreader suffered exposure to and absorption of permethrin by inhalation during the spreading operation. Since permethrin was found to have adhered to and permeated through clothing and there were also directly exposed-areas (such as the surface of the face), percutaneous exposure and absorption can also be assumed. The spreader who performed application operations in this survey was wearing a mask and waterproof trousers, so we thought that his apparel and equipment for applying pesticides were comparatively good. However, 3PBA, a metabolite of permethrin in urine, was clearly detected after the application of the pesticide. This showed that there is need for improvement in the apparel and equipment used for application of pesticide. In particular, investigation of ways to improve the effectiveness of masks and to better protect the upper body is suggested. In addition, although the assistant was not exposed as much as the spreader, 3PBA was detected in her urine which suggests the importance of protective measures in her case as well.

We examined the state of exposure to pesticide in agricultural workers who apply permethrin to cabbage being cultivated in open fields. First, we investigated a method of analyzing 3PBA, a metabolite of permethrin in urine and developed a simple and rapid SPE-GCMS analysis, using SPE in pre-treatment. With this SPE-GCMS analysis, 3PBA was clearly detected in the urine of agricultural workers after the application of permethrin, and exposure and absorption was confirmed. At the same time, we examined the levels of permethrin that deposited on and permeated through clothing to skin on different parts of the body as well as the concentration of airborne permethrin, and were able to clarify the conditions under which agricultural workers are exposed to pesticide in the course of application operations, as well as some problems related to apparel and equipment used in these operations. In Japan, pesticide application using a stationary power-driven sprayer and performed by a spreader and an assistant, as in the present study, is widespread. Accordingly, these results highlight many of the problems common to pesticide application in Japan. Our study provides basic data on health protection measures for agricultural workers.

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