Study on Biological Monitoring of Fenpropathrin Exposure in Application by Utilizing Urinary 3-Phenoxybenzoic Acid Level

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Abstract

To estimate pesticide exposure faced by applicators, an investigation of exposure-absorption was conducted on two applicators under routine working conditions and using regular procedures during pesticide spraying of greenhouse strawberries with fenpropathrin. The authors hypothesized that 3-phenoxybenzoic acid (3-PBA) is a urinary excreted metabolite of fenpropathrin, a synthetic pyrethroid pesticide with 3-phenoxybenzyl moiety, and its determination would help to improve the assessment of fenpropathrin exposure-absorption in applicators. The extent of exposure-absorption was evaluated by the determination of urinary level of 3-PBA (biological monitoring), the amount of the chemical adhered to clothes and permeated to the skin surface, and its concentration in the air of the greenhouse. The results showed that the fenpropathrin concentration in the air was less than $0.1 \mu\text{g}/\text{m}^3$. The amount of adhesion was less than 0.001 to $10.25 \mu\text{g}/\text{cm}^2$, and there was very little permeation. However, the urinary 3-PBA concentration by biological monitoring appeared to be approximately twice that of pre-exposure levels in both applicators. This finding demonstrates that the applicators were exposed to fenpropathrin and absorbed it during spraying. We conclude that the fenpropathrin exposure-absorption in application could be estimated by the determination of urinary 3-PBA. This method of biological monitoring may be more useful indicator to accurately evaluate the working conditions in application.

Key words: Fenpropathrin application, Exposure-absorption, Urinary 3-phenoxybenzoic acid, Biological monitoring, Occupational health

Introduction

In Japan, health problems of farmers have been reported to be associated with spraying of agrochemicals¹⁻⁴⁾. Proper hygienic management of clothing, equipment (protective clothing, masks, etc.), working procedures, and working environment are necessary to prevent these problems. Basically, the management of labor hygiene of independent farmers doing farming in Japan is left to the individual farmers themselves. This leads to differences in the manner of management and its poor quality among many small-scale household farms. Under these circumstances, it is important to evaluate the extent of exposure to and absorption of agrochemicals by farmers during spraying, and to then improve working conditions

accordingly. Therefore, the authors investigated the exposure-absorption of fenpropathrin associated with its spraying over strawberries in greenhouses. Fenpropathrin (chemical name: α -cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropanecarboxylate) is a synthetic pyrethroid agrochemical being used in Japan as a pesticide against leaf mites.

The amount of the chemical adhered to and permeated various parts of the body, and the chemical concentration in greenhouse air were measured primarily for the assessment of dermal and inhalation route exposures, respectively. In addition to these conventional measurements, the authors considered it to be more effective to combine biological monitoring by using the urinary concentration of metabolites of the chemical as an indicator of exposure. Allowing direct evaluation of the actual extent of exposure to and absorption of the chemical during its spraying by ordinary equipment and procedures used by the subjects, biological monitoring is expected to allow comprehensive evaluation of clothing, equipment, and working procedures. This can also be instrumental for safety and

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hygiene education of farmers. However, there were no previous reports on biological monitoring of fenpropathrin after exposure. In fact, fenpropathrin has a 3-phenoxybenzyl moiety similar to permethrin and cypermethrin, which are synthetic pyrethroid agrochemicals⁹⁾ and are used as pesticides as is fenpropathrin. Permethrin and cypermethrin are known to be excreted in urine as 3-phenoxybenzoic acid (3-PBA)^{6,7)}. Indeed, the authors have also detected 3-PBA in urine of farmers after spraying permethrin over cabbages grown in open fields⁸⁾. The authors hypothesized that fenpropathrin with a 3-phenoxybenzyl moiety is also excreted in urine as 3-PBA and attempted to use urinary 3-PBA level as an indicator of exposure in biological monitoring of fenpropathrin exposure.

Materials and Methods

Field Procedures: The survey was conducted in strawberry cultivating greenhouses at T-cho Kagawa, Japan in March 1994. The pesticide was a 2,000-fold dilution of Rody® (an agrochemical containing 10% fenpropathrin). The pesticide application was performed by two female applicators not wearing protective clothing for spraying. Applicator A (age: 62, body weight: 50kg, height: 146cm) wore a working costume which included long sleeve underwear, long sleeve thick blouse, trousers, apron, rubber boots, rubber gloves and a cotton hat. Except for sleeveless underwear and a thin blouse, the clothing of applicator B (age: 41, body weight: 48kg, height: 158cm) were the same as those of applicator A. The applicators have at least one health checkup a year, and were healthy according to the records. The present study was performed with the informed consent of the applicators. During the spraying, weather conditions in each greenhouse (air temperature, relative humidity, and globe temperature) were monitored. The concentration of fenpropathrin in the air and the respiratory exposure during application were measured at the center of the greenhouse (located ca. 1.5m above ground) and at the breathing zone (at the mouth level). Samples were taken using a charcoal silica gel tube sampler (Sibata Sci. Tec. Ltd.) equipped with a suction pump with airflow of 1 l/min. The dermal exposure was measured by using filter paper (Toyo, No.1034) sheets of sizes 3×4.2cm for adhesion (outside: on the clothes), and 6×4cm for permeation (inside: on the skin). To estimate the spatial variation of dermal exposure, filter paper was affixed to different parts of the body including the head, chest, abdomen, upper and lower back, inner and outer arms, and front and back legs. The described samples wrapped in tight fitting aluminum bags were put in a cooling box soon after sampling, and then delivered to the laboratory. Urine samples were collected at 0 (before application), 2 (after application), 8 (before sleeping), 17.5 (the following morning), 26 (1 day after application), 32.5 (before sleeping the following day), and 41.5 (the second morning) hours after the exposure. The urine samples were kept in a freezer during the collection and later stored at -18°C until analyzed.

Reagents and Standard Solutions: The standard of fenpropathrin used in this experiment was obtained from Sumitomo Chemical Company and 3-phenoxybenzoic acid (98%) was purchased from Aldrich Chemical Company, Inc. 1H, 1H-pentafluoropropanol was acquired from Tokyo Chemical Industry. Pentafluoropropionic anhydride, trifluoroacetic acid and other common reagents (acetone, concentrated sulfuric acid, chloroform, methanol, and ethyl acetate) were purchased from Wako Pure Chemical. All the reagents were used for the pesticide residue analysis. The standard solutions of fenpropathrin were prepared by dissolving in acetone,

and 3-phenoxybenzoic acid standard solutions were prepared by acylation after dissolving in acetone. The standard solutions were analyzed to establish a standard peak.

Analytical Procedures: The technique employed was basically the same as that described in our previous permethrin study⁸⁾, while some minor changes were adapted for convenience as follows: 1) The charcoal silicagel tube sampler was filtered through the sampler itself with 10ml of acetone to elute fenpropathrin. Two microliter-aliquots of this solution were then subjected to gas chromatograph-mass spectrometer/selected ion monitoring (GC-MS/SIM) analysis. 2) Fenpropathrin was extracted from the filter paper by sonicating for 30min with 10ml of acetone. From blank tests and recovery experiments using filter paper fortified with 1ml of 10ppm-fenpropathrin, the recovery rate of the filter paper extraction was found to be almost 100%. For subsequent GC-MS/SIM analysis, aliquots of extracts were transferred to autosampler vials. 3) Preparation of urinary metabolites: The creatinine level in the urine was measured with an automatic analyzer (Type 7050, Hitachi Co.) based on Jaffe's reaction. The urine samples were analyzed for the presence of the 3-PBA metabolite. The detailed procedures are as follows: (1) 1ml of conc. H₂SO₄ was added to 5ml of urine in a test tube (20×200mm). The unsealed tube was heated in a dry-bath (Taiyo) at 100°C for 2hours. After cooling, 10ml of distilled water was added to the residue. (2) For isolation and clean-up of sample with solid phase extraction, the acid hydrolyzed urine sample was poured into a pretreated Sep-Pak C₁₈ (Waters Associates) cartridge (the pretreatment involved the passage of 5ml of chloroform, then 5ml of methanol, and finally two volumes of 5ml distilled water through the cartridge). The cartridge was then washed with 4 volumes of 5ml-distilled water used for rinsing the sample tube. Finally, the cartridge was eluted with 5ml of chloroform, and the urinary metabolites of fenpropathrin were retrieved in a receiving bottle equipped with a glass funnel lined with a special filter paper (Advantec Toyo: No. 2S), which allows the elimination of water from the eluate. The eluate was evaporated to near dryness under a stream of nitrogen in Kuderna-Danish evaporative concentrator apparatus (Sibata Sci. Tec. Ltd). 3) To acylate the carboxylic acid groups, 200μl of pentafluoropropionic anhydride and 50μl of 1H, 1H-pentafluoropropanol was added to the residue. The residue vial was then tightly capped and heated to 90°C for 30min. After being cooled, 100μl of trifluoroacetic acid was added. The residue vial was tightly capped again and heated for 30min at 90°C to completely acylate the phenolic groups. After allowed to cool the residue was evaporated down to a suitable volume using a gentle stream of nitrogen, and re-dissolved in 150μl of ethyl acetate. This solution was then used as the sample for GC-MS/SIM analysis. The recovery rate of the metabolite (3-PBA) in urine was more than 90%.

A GCMS-QP2000G (Shimadzu) was used. The GC MS/SIM analysis conditions derived for fenpropathrin were as follows: As a strong peak was found at ion mass number (m/z) 141 and 181 from the mass spectrum's peak retention time (t_R) of 9.97 minutes in the total ion chromatogram obtained by GC-MS analysis with the standard of fenpropathrin, the authors then set the m/z to 141, 181 and obtained measurements for fenpropathrin by SIM method. The SIM analysis conditions of 3-PBA were obtained by the same method used in our previous permethrin study⁸⁾. In the present study, however, only m/z 346 was used in their m/z 346 and 197 because m/z 197 was unstable and not proportional for 3-PBA from fenpropathrin. The detailed GC-MS analysis conditions are given in Table 1.

Table 1 GC-MS/SIM analysis conditions.

	For fenpropathrin detection	For 3-PBA detection
Column	DB-5MS (J&W) 30m ×0.32mm I.D. 0.25 μ m film thickness	DB-5MS (J&W) 30m ×0.32mm I.D. 0.25 μ m film thickness
Carrier gas	He 10ml/min	He 10ml/min
Oven	200°C (2min) to 260°C at 8°C/min and 260°C (8min)	140°C (2min) to 200°C at 8°C/min 200°C to 260°C at 15°C/min and 260°C (4min)
Injection	Splitless, 260°C	Splitless, 200°C
Ion mass number	m/z: 181,141	m/z: 346

Results

The exposure survey was carried out in three greenhouses 33m(L)×5.4m(W)×3.1m(H), with an area of 540m². The pesticide application was conducted with a hand driven knapsack sprayer equipped with a single nozzle. The solution (2,000-fold dilution of Rody® containing 10% fenpropathrin) was sprayed to both sides as the applicators moved down the aisle between the rows of strawberries. Between the two applicators, a total of 100 liters of solution was applied in a 50min period. During this period (14:10-15:00), average air temperature, relative humidity, and globe temperature of all those applications were 11.2°C, 49.5%, and 13.1°C respectively.

Fig. 1 shows an example of the SIM chromatogram of fenpropathrin for the standard and the filter paper used for dermal exposure measurement. The mass spectrum was derived from the total ion chromatogram obtained with GC-MS for the fenpropathrin standard. The retention time of the highest peak on the total ion chromatogram was 9.97 minutes. After evaluating the mass spectrum, the authors set the ion mass numbers (m/z) at 141 and 181, and obtained measurements by the SIM method to detect fenpropathrin. Subsequently, fenpropathrin from the silicagel tube samplers used for the air sampling was measured with the GC-MS/SIM analysis and the same clear peaks were obtained as in the standard. The fenpropathrin concentrations in the air at both the breathing zones of the applicators and the centers of the greenhouses were less than 0.1 μ g/m³, which is the detection limit. Under the same conditions of analysis, the filter paper samples

used to estimate dermal exposure were also analyzed.

Fig. 2 represents the amount of adhesion and permeation of fenpropathrin in relation to the different parts of the body. The adhesion observed in applicator A ranged from non-detectable level (ND; <0.001 μ g/cm²) to 10.25 μ g/cm². The leg areas, particularly the right front leg (10.25 μ g/cm²) showed strong adhesion. Signs of adhesion were also detected on the abdomen, lower back and left outer arm. No other sampling areas showed any signs of adhesion. Permeation was strongly detected on the upper back only. For applicator B, adhesion ranged from 0.01 μ g/cm² (right outer arm) to 0.05 μ g/cm² (right back and left front legs). Slight permeation was detected on the left inner arm, right back leg, and abdomen. In contrast with applicator A, the adhesion was detected in all sampling parts of the body in applicator B, as shown in Fig. 2.

The SIM chromatograms of 3-PBA obtained from the standard solution and a urine sample taken from a fenpropathrin applicator are given in Fig. 3. In our previous permethrin study⁶⁾ the same GC-MS/SIM analysis method was used and the same mass spectrum, 346 m/z and 197 m/z were obtained. However, in the current study only m/z 346 was used because m/z 197 was unstable and not proportional for use under the GC-MS/SIM condition for 3-PBA metabolized from fenpropathrin.

Fig. 4 depicts the excretion of urinary metabolite 3-PBA in each applicator, represented by the ratio of after-exposure to pre-exposure 3-PBA concentration. The values of metabolite 3-PBA in all urine samples were corrected for gram creatinine. At eight hours after application (before sleeping), the urinary 3-PBA concentration was about two times higher than the pre-exposure level

Fig. 1 Selected ion chromatograms of fenpropathrin from the standard and filter paper used for dermal exposure measurement.

Fig. 2 Amounts of adhesion and permeation of fenpropathrin in different parts of the body.

for both applicators. One day after application, the concentration started to decrease slowly toward the pre-exposure level.

Discussion

The synthetic pyrethroids having a 3-phenoxybenzyl moiety, such as permethrin and cypermethrin are known to excrete 3-PBA as one of the major urinary metabolites^{6,7}. Woollen et al.⁷ noted in a cypermethrin study that for the purpose of biological monitoring, the amount of phenoxybenzoic acid metabolites would best reflect the exposure-absorption of cypermethrin by all routes without under or overestimation. The chemical names are *m*-phenoxybenzyl ($\pm$)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate for permethrin, ($\pm$)- α -cyano-3-phenoxybenzyl ($\pm$)-*cis,trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate for cypermethrin, and α -cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropanecarboxylate for fenpropathrin. Anticipating that fenpropathrin, which has the same phenoxybenzyl moiety,

might be excreted as 3-PBA, and that its detection would help improve assessment of exposure-absorption into the body, the authors examined the applicators' urine samples for 3-PBA from fenpropathrin. As shown in Fig. 3, the SIM chromatograms of 3-PBA from fenpropathrin obtained by the GC-MS/SIM analysis was in agreement with the results for urinary 3-PBA in our previous permethrin study⁹. In addition, the authors also carried out some experiments on the metabolite 3-PBA from the urine sample of a person unexposed to any agrochemicals or household insecticides. No 3-PBA metabolite was detected, and similar results were already reported⁹. Since 3-PBA does not exist in the urine of ordinary people, the authors concluded that the 3-PBA concentration in urine would be useful as an indicator for biological monitoring of subjects exposed to fenpropathrin. This study was a trial for biological monitoring applied to assess the exposure-absorption in fenpropathrin users. To our knowledge, this is the first study on the determination of urinary 3-PBA for estimation of occupational exposure to fenpropathrin.

Fig. 3 Selected ion chromatograms of 3-PBA from the standard and urine sample of applicator exposed to fenpropathrin.

Fig. 4 3-PBA excretion against time in applicator exposed to fenpropathrin. Urinary 3-PBA was represented as the ratio, assuming that the 3-PBA concentration (ng/mg creatinine) before exposure is one.

Fig. 4 shows the change with time of urinary 3-PBA concentration obtained from biological monitoring. In urine sampled at eight hours after application, the urinary 3-PBA concentration appeared to be about twice the pre-exposure level. One day after application, the concentration started to decrease gradually toward the pre-exposure level. The applicators revealed to have a trace of 3-PBA (background level) in urine before the application while there was no excretion of 3-PBA in non-exposed ordinary people. This may be due to the relatively frequent contact with agrochemicals of agriculture workers, noting that the applicators were asked

to keep non-exposure condition only during the period of urine sampling. Moreover, the 3-PBA concentrations in urine samples of the second morning have been lower than those of pre-application may due to a fluctuation of background levels. The reason for existence of the 3-PBA-background level is still not clear. Further studies are desirable.

The urinary 3-PBA excretion pattern of fenpropathrin resembles the 3-PBA excretion pattern⁹⁾ after exposure to a fumigant (a kind of household insecticide containing permethrin). 3-PBA follows a two-compartment model, rapid and slow phases.

According to a previous report⁹⁾ the biological half-life of 3-PBA is about 10 hours in the rapid phase and several tens of hours in the slow phase. This 3-PBA-excretion pattern is also similar to that observed in the cabbage field survey using permethrin⁸⁾. These findings confirmed the exposure to and absorption of fenpropathrin during the pesticide spraying operation of greenhouse strawberries. Meanwhile, the authors discovered marked adhesion on the right front leg and permeation on the upper back in applicator A. In another exposure study¹⁰⁾ in a grape orchard, the fenpropathrin deposition on exposure pads fixed over the clothing of the applicator was the highest on the head. This discrepancy can be explained by the difference in target products (strawberries vs. grapes). In the present study, the exposure of the leg in applicator A was marked, particularly the right front leg ($10.25 \mu\text{g}/\text{cm}^2$) as shown in Fig. 2. This may be attributed to a possible leak in the sprayer nozzle, as well as the target product being strawberries. Consequently, when the spraying region is low, such as with strawberries, the leg parts should have more protection. Serious permeation in the upper back region in the absence of adhesion was probably due to a leak in the sprayer tank. Adhesion may not have been detected due to the disjointed nature of the adhesion and permeation sampling area. Moreover, even though the applicators wore thick clothing due to the chilly early spring season, permeation through clothes could occur since special protective clothing were not worn. This can be surmised from a previous study about the material of protective clothing¹¹⁾, which indicated that clothes, if not waterproof, have almost no protective capacity. To provide full protection, it is very important for the agrochemical applicators to wear protective gear. Equipment should be well maintained, and particular attention should be paid to the back of knapsack sprayers where leakage could seriously contaminate the user.

The biological monitoring revealed the urinary excretions of the 3-PBA metabolite were similar in both applicators. Despite two high degree exposure areas (the right front leg and the upper back) in applicator A, a low degree of adhesion was noted in all

sampling areas in applicator B (Fig. 2). This indicates that respiratory exposure may be more important as an absorption route than dermal exposure. The authors also found that respiratory absorption cannot be neglected, in light of the fact that applicators did not use masks and the air concentrations of fenpropathrin were below the limits of detection ($<0.1 \mu\text{g}/\text{m}^3$). Respiratory tract (inhalation) is also generally considered to be the main route of exposure to hazardous substances in the industrial workplace. Therefore, a mask should be worn for respiratory protection even when the spraying region is low or low-pressure equipment such as hand driven sprayers are used. In our experiments, the risk of poisoning was negligible. Further exposure accident has not been reported in Japan. However, the situation may differ with the working conditions. In the summer when applicators are lightly clothed, the possibility of poisoning is higher if the agrochemical used is more toxic. It is important for agrochemical users to maintain strict working habits and to make every effort to minimize the risk themselves. Nevertheless, according to our observations in the field survey^{8, 11)}, protective precautions are often not seriously taken. In light of these considerations, the biological monitoring will be helpful not only to convey safety and health concerns to agricultural workers but also to design a more secure working system.

In conclusion, the determination of urinary 3-PBA would be useful for biological monitoring of fenpropathrin exposure in application. This method of biological monitoring has the potential to be applied for estimation of the agrochemical exposure in agriculture field.

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