Risk and exposure assessment for agricultural workers during treatment of cucumber with the fungicide fenarimol in greenhouses

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Abstract The exposure pattern and potential risk of fenarimol emulsifiable concentrate to agricultural workers were investigated during the preparation of the pesticide suspension and the application of the prepared suspension to the cucumber in a greenhouse environment. The dermal exposure to fenarimol was 0.17 ± 0.11 mg (0.001 ± 0.001% of prepared active ingredient) for mixing/loading and 0.22 ± 0.15 mg (0.003 ± 0.002% of applied active ingredient) for application, respectively. The most exposed part of body was the hand (100%) during mixing/loading, whereas the primary sites during application were the back and legs. In particular, 54.8% of dermal exposure occurred on the shins. The inhalation exposure to fenarimol was detected as 3.7 ± 1.0 μg for the applicator. In comparison with the exposure patterns to pesticides for agricultural workers in greenhouse reported in previous studies, lower dermal and inhalation exposures to fenarimol were observed during mixing/loading and application, respectively. The results of the risk assessment demonstrated that the possibility of risk to fenarimol exposure was lowest during mixing/loading and application in the greenhouse environment.

Keywords Cucumber · Exposure · Fenarimol · Greenhouse

Introduction

Pesticides are toxic compounds used to control many pests, diseases and weeds in the agricultural industry [1]. Agricultural workers are occupationally exposed to pesticides during the preparation of the spray suspension and the application to the crops through representative routes such as dermal deposition and inhalation [2–4]. As these exposures may be injurious to their health, it is necessary to determine whether the actual working conditions of the agricultural workers were sufficiently safe from pesticide intoxication.

In Korea, the risk possibility of pesticides has increased because farmers now use the pesticides at higher concentrations and for a longer time than previously to maintain or improve the crop production [5]. Moreover, the increase of greenhouse cultivation indicates that greater pesticide exposure may occur owing to the closed environment [6]. Many studies on pesticide exposure have been conducted for several decades. However, the studies were mainly focused on open fields, such as paddy fields and mandarin or apple orchards [2, 3, 7–12]. Only two cases of greenhouse exposure have been reported in Korea [7, 8]. Therefore, many studies and great investment are needed with regard to the pesticide exposure in a closed environment such as a greenhouse.

Fenarimol is a pyrimidine fungicide that acts against pests such as powdery mildews, scabs, rusts and leaf spots in pome fruit, stone fruit, vines and turf. It inhibits ergosterol biosynthesis in fungi, as systemic foliar fungicide and testosterone aromatase activity, which resulted in irreversible infertility in male rats [13]. The acute percutaneous LD50 for rabbits is > 2000 mg/kg, and the acceptable daily intake is 0.01 mg/kg b.w. [14]. Fenarimol

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was selected for this study because this was one of the pesticides mainly used for cultivation of cucumber and was formulated as emulsifiable concentrate (EC). Therefore, the effect of formulation on pesticide exposure could be evaluated by comparison with previous studies.

The present study was performed to assess the pesticide exposure of agricultural workers during the application of fenarimol EC to cucumbers in a greenhouse environment. The major body parts of exposure were identified, and the exposure pattern to fenarimol was compared with those of imidacloprid wettable powder (WP) and thiophanatemethyl WP in cucumber greenhouses as previously reported [7, 8].

Materials and methods

Reagents and materials

The pesticide used in the field study was the commercial fungicide Fenari EC 12.5% (fenarimol, Dongbu-hannong Chemical, Seoul, Republic of Korea) and obtained through a local vendor. The analytical standards of fenarimol (99.4%) were obtained from the manufacturing company (Dongbu-hannong Chemical) and Rural Development Administration, Korea. HPLC-grade acetone was used (Fisher Scientific Korea Ltd, Seoul, Republic of Korea).

Dermal exposure matrices and measurement

Dermal patches made from cellulose thin-layer chromatography paper (17CHR, 1-mm thickness, Whatman International Ltd, Maidstone, UK) were attached to a worker’s forehead, front of neck, back of neck, chest/abdomen, back, upper arms, forearms, thighs and shins of the protective garment to measure the dermal exposure [7, 15]. Hand, feet and facial exposures were monitored using cotton gloves, socks and a mask [16]. The exposure parts of the dermal patch and mask were 50 and 200 cm², respectively.

Inhalation exposure matrices and measurement

The XAD-2 resin (ORBOTM 609 Amberlite XAD-2, 400/200 mg, Supelco, Bellefonte, PA, USA) was used for the measurement of inhalation exposure, which was connected to an air pump (GilAir-3, Sensidyne, Clearwater, FL, USA) and 37-mm open-faced cassettes equipped with a glass fiber filter (type AE, SKC, Eighty Four, PA, USA). The spray drift of the pesticides was collected by using the XAD-2 resin at flow rate of 2 L/min.

Field study

The cucumber greenhouse for field studies is located in Pyeongtaek, Gyeonggi, Republic of Korea. The cucumber was 200 cm high, in a fruiting stage, and planted at intervals of 20 × 130 cm. The temperature was measured with the thermometer at the start and end of each experiment, whereas the relative humidity was determined using a hygrometer. The temperature and the relative humidity in the field were 24–27 °C and 51–57%, respectively. The applicator was a man with a height of 168 cm and a weight of 70 kg. The spray suspension was prepared from Fenari 12.5% EC (100 mL, 1 bottle) and 400 L water for 2 min. The applicator sprayed 280 L of prepared solution with a power sprayer at flow rate of 4.7 L/min for 60 min in a cucumber field of 250 m². The total prepared and sprayed amounts contained 12.5 and 8.75 g a.i. (active ingredient), respectively. Each mixing/loading and application was repeated twice.

Extraction and analysis from exposure matrices

Pesticide residue on the exposure matrices were extracted with acetone by agitation at 200 rpm for 60 min on a shaker (Wooju Scientific, Gimpo, Republic of Korea). After concentration with a nitrogen evaporator (Reacti-Vap, Pierce, Rockford, IL, USA), fenarimol residue was determined with a gas chromatograph equipped with a nitrogen-phosphorous detector (HP 5890 series II plus, Hewlett-Packard Co., Avondale, PA, USA) and a 30 m × 0.53 mm, i.d., (di = 1.5 μm) HP-5 capillary column (Agilent Technologies, Inc., Santa Clara, CA, USA). The flow rates of the gases were 15 mL/min in splitless mode for nitrogen as the carrier gas and 105 and 3.6 mL/min for air and hydrogen, respectively. The injection volume was 1 μL. The injector and detector temperatures were set at 260 and 320 °C, respectively. The oven temperature was held at 210 °C for 1 min, raised to 280 °C at a rate of 10 °C/min and maintained for 2 min.

Method validation

The instrumental limit of detection (ILOD) and instrumental limit of quantitation (ILOQ) were determined through the analysis of 0.001–1.0 ppm standard solutions. The method limit of quantitation (MLOQ) was calculated through the consideration of ILOQ, injection volume and extraction solvent volume [3]. The integrated peak area after five repeated injections of 0.1 and 1 ppm of standard solution were compared for instrumental reproducibility. The linearity of the calibration curve in the range from the ILOQ to 10 ppm was checked on the day of preparation and 3 days later. The control exposure matrices were
fortified to MLOQ, 5 MLOQ and 10 MLOQ levels and analyzed three times to validate the matrix extraction efficiency (recovery). The trapping efficiency and breakthrough of XAD-2 resin was investigated by spiking on the bottom of a U-shaped glass tube (Daejung Chemical, Daejeon, Republic of Korea) at 70 °C and the 1st-part of the XAD-2 resin at 100 and 10 MLOQ, respectively. This test was conducted at 2 L/min for 4 h and repeated three times. The U-shaped glass tube was heated to 70 °C.

**Calculation of dermal and inhalation exposure**

Dermal exposure intensity (µg/cm²), dermal exposure amount per body part (µg) and inhalation exposure amount (ng) of the mixer/loader and applicator were determined based on the approach suggested by Choi et al. [7]. The area of the matrix was 50, 935, 1266 and 200 cm², for the patch, glove, sock and mask, respectively. The body surface area and the respiration rate (1270 L/h) used for the exposure assessment for an adult Korean in this study as suggested by Kim et al. [16].

**Risk assessment**

The potential dermal or inhalation exposures (PDE or PIE) were calculated through the extrapolation of the corresponding dermal exposure amount (µg) of the whole body or inhalation exposure amount (ng) per activity with five working activities per day [7]. The actual dermal exposure (ADE), the absorbable quantity of exposure (AQE) and the margin of safety (MOS) for the mixer/loader and applicator were determined based on the approach suggested by Choi et al. [7]. For exposure assessment, the actual exposure (AQE) was estimated from the ADE and PIE through dermal contact and inhalation, respectively. MOS values were determined for risk assessment through the comparison of AQE and acceptable operator exposure level (AOEL). The penetration rates of fenarimol through clothes were 1 and 10% for the mixer/loader and applicator, respectively [16], whereas the skin absorption rate was 2.6% [17]. The AOEL, reported as the relevant risk value, was 0.02 mg/kg/day for fenarimol [17], and the body weight for an average Korean adult male was 70 kg [7].

**Statistical analysis**

The significant differences between the exposure to fenarimol EC in this study and previously reported exposures to imidacloprid WP and thiophanate-methyl WP in cucumber greenhouses [7, 8] were determined through an independent t test (two-tailed) using SPSS version 23 software (SPSS, Chicago, IL, USA).

**Results and discussion**

**Method validation**

The ILOD and ILOQ for fenarimol were 0.01 and 0.05 ng, respectively, which indicated that the analytical sensitivity was sufficient for the analysis. The reproducibility of the instrumental analysis was good (CV < 3%). The linearity of the calibration curve for fenarimol was consistent in the range from the ILOQ to 10 mg/L over 3 days (R² > 0.9999). The matrix extraction efficiency (recovery) was 84–111% with a small relative standard deviation of 0.2–11.2%, which indicated that the extraction procedures were reasonable (Fig. 1). The trapping efficiency of the XAD-2 resin was reliable, with a mass balance of 94.9 ± 4.9% (Table 1). Fenarimol was not transferred to the 2nd-part of the resin in the breakthrough test (Table 1), which demonstrated that the XAD-2 resin has a powerful retention capacity for fenarimol.

**Dermal exposure during mixing/loading**

Cotton gloves were used to determine the hand exposure of the agricultural workers [16], because they are generally used for measurements of potential dermal exposure, even though overestimation may occur if the cotton gloves absorb more of the pesticide product through direct contact, especially that of liquid formulations, such as EC. The amount of dermal exposure to fenarimol EC was 0.17 ± 0.11 mg for mixing/loading, which corresponded to 0.001 ± 0.001% of the total prepared amount (fenarimol, 12.5 g a.i.). The ratio of dermal exposure to fenarimol EC was similar to that of imidaclopid WP (0.001 ± 0.001%) or thiophanate-methyl WP (0.001%), reported in previous studies [7, 8]. Dermal exposure only occurred through the hand owing to the lower scattering possibility of EC in contrast to WP. Hand exposure was reported to reach 92.5–99.9% of the dermal exposure during mixing/loading, especially when using the liquid formulation as a soluble liquid (SL) [7–9, 18, 19]. Therefore, the exposure level during mixing/loading remarkably decreased through the use of appropriate gloves as hand protection.

**Dermal exposure during application**

Generally, the applicator is exposed to pesticides mainly through dermal absorption. The dermal exposure amount of fenarimol EC was 0.22 ± 0.05 µg (0.003 ± 0.002% of the total applied a.i.) during the application in the greenhouse (Table 2). However, the higher exposure ratios of the dermal to the total applied amount were reported as
0.013 ± 0.003 and 0.016 ± 0.010% in the cucumber greenhouse for imidacloprid WP and thiophanate-methyl WP, respectively [7, 8]. The possibility of dermal exposure for WP compared with EC was approximately five times higher (P < 0.05). In contrast, in open fields such as an apple orchard, the dermal exposure of EC was higher than that of WP, which was higher than that of SL [9, 12]. These results demonstrated that the formulation type was one of the significant factors that affect the exposure level for applicators, which was in contrast with that of Vidal et al. [20]. However, owing to insufficient exposure cases, further studies are needed to clarify the effects of the formulations on pesticide exposure.

Meanwhile, the patterns of exposure distribution of the pesticide formulations were similar. The dermal exposure on the back and shins, the primary sites of applicator contamination, was 0.12 ± 0.05 (0.06 ± 0.02 μg/cm²) and 0.06 ± 0.09 mg (0.02 ± 0.03 μg/cm²), respectively, which showed the large fluctuation of dermal exposure in the working environment, as described in previous studies (Table 2) [21, 22]. As shown in Fig. 2, the contribution ratio of the legs was 63.1% (shin, 54.8%), as a result of indirect contamination through contact with the sprayed plants [21, 23]. Other studies with imidacloprid (legs, 51.2%) and thiophanate-methyl (legs, 52.4%) reported similar exposure patterns, despite the different formulations [7, 8].

**Inhalation exposure**

The airborne spay drift resulted in health concerns in the working environment. The inhalation exposure to fenarimol for mixing/loading was not observed because the EC was not dispersed. During application, the inhalation
exposure of fenarimol was determined as 3.7 ± 1.0 µg, which corresponded to 0.00004 ± 0.00001% of the total applied amount. Generally, 1% dermal exposure was assumed for respiratory exposure [24]. In this study, inhalation exposure to fenarimol was detected as average 2.3% of the dermal exposure, because of increased inhalation exposure and reduced dermal exposure, which was higher than that reported in previous studies on imidacloprid and thiophanate-methyl [7, 8]. However, owing to the very high variation as standard deviation of 2.0% between repetitions, statistically significant differences at the 0.05 level in the levels of inhalation exposure or ratios of the inhalation exposure to the total dermal exposure during application were not observed among the pesticides (fenarimol, imidacloprid and thiophanate-methyl) or between formulation types (EC and WP).

**Risk assessment**

In the exposure assessment, agricultural workers were only exposed to fenarimol through dermal contact during mixing/loading. However, they were exposed mainly through the inhalation during application (Table 3). The PDEs were similar between the mixer/loader and the applicator, but the ADE for the applicator was approximately 10 times higher, owing to the different rates of penetration through the clothes of 1 and 10% for mixer/loader and applicators, respectively. The ratio of PIE to AQE during application was 86.4%, which indicated that in a closed environment, such as a greenhouse, the applicator was mainly exposed to pesticides through inhalation. Whereas 6.4–10.4% of AQE were reported to occur through inhalation in the previous studies with imidacloprid and thiophanate-methyl, owing to reduced inhalation exposure and increased dermal exposure in comparison with fenarimol exposure [7, 8]. The MOS values for the risk assessment were > 1, which resulted from the low exposure possibility, the low toxicity (AOEL, 0.02 mg/kg) and the low skin absorption (2.6%) of fenarimol [17]. These results demonstrated that the agricultural workers in the cucumber greenhouse were safe from fenarimol toxicity during mixing/loading and application.

**Table 3 Risk to agricultural workers health during treatment of fenarimol in cucumber greenhouse**

| Worker       | Mixer/loader | Applicator |
|--------------|--------------|------------|
| PDEa (mg/day)| 0.9 ± 0.6f   | 1.1 ± 0.7  |
| ADEb (mg/day)| 0.0002 ± 0.0001 | 0.003 ± 0.002 |
| PIEa (mg/day)| N.D.         | 0.02 ± 0.00 |
| AQEc (mg/day)| 0.0002 ± 0.0001 | 0.02 ± 0.00 |
| MOSd        | 7865.7 ± 5043.9 | 66.9 ± 9.6 |

*N.D. not detected
*a Potential dermal exposure and potential inhalation exposure on the assumption of five working activities per day
*b Actual dermal exposure = [PDE × 1% (mix/loader) or 10% (ap- plicator) of penetration rate through clothes] × 2.6% of skin absorption
*c Absorbable quantity of exposure, the sum of the ADE and PIE
*d Margin of safety = AOEL (0.02 mg/kg b.w./day) × 70 kg b.w./AQE
*f Mean ± SD, n = 2

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