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ASSESSMENT OF THE DERMAL EXPOSURE TO AZOXYSTROBIN AMONG WOMEN TENDING CUCUMBERS IN SELECTED POLISH GREENHOUSES AFTER RESTRICTED ENTRY INTERVALS EXPIRED — THE ROLE OF THE PROTECTIVE GLOVES

JOANNA JUREWICZ[1], WOJCIECH HANKE[1,2], WOJCIECH SOBALA[1], and DANUTA LIGOCKA[3]

[1] Nofer Institute of Occupational Medicine, Łódź, Poland
Department of Environmental Epidemiology
[2] Medical University, Łódź, Poland
Department of Informatics and Medical Statistics
[3] Nofer Institute of Occupational Medicine, Łódź, Poland
Department of Chemical Hazards

Abstract
Objectives: The purpose of our study was to determine the level of skin contamination by azoxystrobin in a group of women tending cucumbers in a vegetable-growing greenhouse after restricted entry intervals expired. **Methods:** Exposure samples were assessed on two days during the spring: first entry on the day after spraying of azoxystrobin and second entry six days later. Dermal exposure was measured by using patches on the outside of clothing and sampling gloves underneath regular working gloves. Pesticide deposited on clothing patches and gloves as a substitute for skin deposition was determined by liquid chromatography and mass spectrometry (LC-MS/MS). **Results:** The study has shown that workers in a Polish greenhouse are exposed to pesticides at re-entry into the greenhouse after pesticides were sprayed several days earlier. Azoxystrobin has been detected on hands, shoulders and chest. Higher levels of azoxystrobin were found on the cotton gloves of women tending the vegetables than on the patches. The levels decreased (by about 60%) on the patches and increased (by about 250%) on the cotton gloves between the two days of measurement. **Conclusions:** Women working in a vegetable-growing greenhouse and not directly engaged in the process of spraying experience a measurable dermal exposure to azoxystrobin. The protective gloves constitute a source of secondary exposure rather than protecting employees' hands from contact with the pesticide. More efficient personal protective gloves for proper protection of women working in vegetable greenhouses are needed.

Key words:
Greenhouse, Dermal exposure, Protective gloves, Patches

INTRODUCTION

Greenhouses produce a substantial quantity of vegetables and flowers, especially in autumn and winter. Work in greenhouses is performed in a warm microclimate during most time of the year. Working conditions in greenhouses pose significant health hazards, mainly due to high humidity, temperature and poor ventilation. The work may involve indirect exposure to pesticides resulting from contact with flowers and vegetables previously treated with pesticides. Flowers and vegetables can grow high and may be placed so closely that workers' skin at all levels of the body may be exposed to pesticides.

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Address reprint request to: J. Jurewicz, Department of Environmental Epidemiology, Nofer Institute of Occupational Medicine, św. Teresy 8, 91-348 Łódź, Poland (e-mail: joannaj@imp.lodz.pl).

Pesticides may be applied in greenhouses as a dust, mist, spray, or drench. The most often used pesticides are insecticides, fungicides, and growth regulators. Herbicides are not typically used in greenhouses [1]. Dermal exposure to pesticides is highly correlated with the manual contact with pesticide-treated plants and it is believed to be the major route of pesticide absorption during occupational use [2]. Exposure is suspected to be the greatest shortly after pesticides have been applied onto the plants. Dermal exposure is determined by the transfer of the pesticide residue from the surface of the foliage to the skin of the workers [3]. It depends on the amount available for transfer and the frequency and intensity of skin contact with the treated crops. Most insecticides, such as organophosphates, carbamates and organochlorines easily penetrate the skin [3]. Some pesticides are metabolised on the leaves to form toxic compounds with an even stronger penetration capacity through the skin than their parent compounds. Workers are exposed to pesticides via inhalation during application activities and to pesticide residues through the skin when they handle the treated plants.

The objective of the study was to measure the level of skin contamination by azoxystrobin in a group of women tending cucumbers in a vegetable-growing greenhouse after post-spraying restricted entry intervals expired. Dermal exposure was measured by using patches on the outside of clothing and sampling gloves underneath regular working gloves.

The active ingredient of azoxystrobin is a methoxyacrylate compound used as a preventive and curative systemic fungicide. Azoxystrobin is of low acute and chronic toxicity to humans, birds, mammals, and bees but is highly toxic to freshwater fish, freshwater invertebrates, and estuarine/marine fish, and very highly toxic to estuarine/marine invertebrates. Azoxystrobin has been classified as toxicity category IV for acute oral toxicity and skin irritation, and category III for acute dermal, inhalation and eye irritation. This compound is well absorbed through skin and completely metabolised in the rat. There are no significant plant metabolites that are not animal metabolites [4,5]. Nowadays azoxystrobin is one of the most often used pesticides in Polish greenhouses [6].

METHODS

Study population

This study is a part of a bigger study which investigated whether the working conditions in 14 Polish greenhouses, mostly engaged in vegetable-growing production, impact on reproductive disorders among women working there [7,8]. The exposure to pesticides in that study was assessed only based on information received from person responsible for chemical protection on type of pesticides used during women's work. Thus, to confirm the exposure of women not directly engaged in the process of spraying, only one cucumber-growing greenhouse was chosen.

During the spring, 19 female workers working in a vegetable-growing greenhouse were asked to participate in the study. The greenhouse was involved only in long cucumbers growing. The operation continues from the beginning of March till the end of October. Technological break in the process of production and sale of the cucumbers lasts 2 weeks i.e. the last week of July and the first week of August.

Exposure to pesticide azoxystrobin was evaluated on one day and seven days after spraying, in group of tending and harvesting workers after restricted entry intervals had expired. Tending and harvesting workers are mostly engaged in cutting unnecessary leaves to let the sunlight reach the desired portions of the plant and this work require frequent contact with plant. In this study, the measurement of exposure was taken only during the time of tending the cucumbers. These working conditions are typical for vegetable-growing greenhouses in Poland.

All study participants completed a self-administered questionnaire, which contained information on age, height, weight, education level, the number of years they had worked for the vegetable-growing company, use of personal protective equipment, and whether or not they had received training on the safe use of pesticides. This training mainly involved the correct use of protective equipment, restricted entry intervals for pesticides and how to act during acute pesticide poisoning. Rubber or cotton gloves with a leather palm were used as the sole personal protection means by the workers. They worked in shirts and short pants. The protective gloves worn by workers were reused and the same protective gloves were used on the two days of measurement.

Potential dermal exposure measurements

Exposure samples were collected on two days after spraying of azoxystrobin in spring season on 19th and 25th of May. Dermal exposure was assessed by using 10×10 cm cotton patches attached to the outside of workers' t-shirts (left/right shoulder and chest — three patches). The patches were attached to clothing just before workers entered the greenhouse and were worn during the entire eight-hour workday. At the end of the workday, patches were removed from the workers' t-shirts, and each sample was separately stored for analysis on 19th and new patches were attached on 25th of May. The amount of azoxystrobin deposited during work on three cotton patches was added and the mean amount of chemical from cotton patches was taken. Mean amount per surface of patches (100 cm^2) was calculated.

The cotton gloves were used under workers' protective gloves (normally used by workers), just before workers entered the greenhouse, and were worn during the entire eight-hour workday on 19th and new cotton gloves were worn on 25th of May. To assess the exposure per square centimetre, the surface of cotton gloves was calculated in the way that the hand of the woman was drawn on an A4 paper sheet and scanned. In the graphical program the inside part of the hand was filled in with colour. The fraction of pixels of the colour of filling was calculated in relation to the pixels fraction in the whole picture. The surface of gloves was calculated as the fraction of scanned paper.

The exposure is mostly through workers hands, arms and chest, the exposure through the low part of the body is negligible [9]. Thus, the location of patches and cotton gloves coincides with most affected parts of the body. The cotton gloves (placed under the gloves normally worn by workers) and patches were changed between the days. New gloves and new patches were worn on each day of measurement to see the exposure of workers on a particular day.

Statistical analysis

The linear regression model was developed, using STATA 9 software, to assess differences between azoxystrobin level on cotton patches and gloves between one day and seven days after spraying in women tending and harvesting vegetables. Results were adjusted for the date of determination. The amount of azoxystrobin was transformed logarithmically. Separate models were fitted for gloves and patches.

Chemical analysis of samples

Pesticide exposure evaluation included deposition on clothing patches and gloves as a substitute for skin deposition and was performed according to the LC-MSMS methods developed, validated and used as Standard Operating Procedures (SOPs) of the Laboratory of Environmental Organic Pollutants, Nofer Institute of Occupational Medicine (NIOM) (SOPs: ZCZ-O-PB-07 for patches and ZCZ-O-PB-14 for determination of azoxystrobin in gloves). The product used in the cultivated area was Amistar 250 SC which consists of 250 g of azoxystrobin active ingredients in 1 litre of that product. This liquid was sprayed onto the cucumber culture.

Azoxystrobin (99.6%) was purchased from Riedel-de-Haen, methanol and water were of LC-MS grade (JT. Baker). A 5μm XTerra C18 MS column 2.1×150 mm (Waters, USA) was used. Stock pesticide solutions were prepared individually and gravimetrically using the formulated product and methanol.

Standard preparation

Stock solutions of pesticide were prepared in methanol with aliquots taken to prepare mixtures/commodity combinations at concentrations ranging between 50 and 20 ng/ml. Serial dilutions using methanol produced a range of standard mixture solutions with pesticide concentrations ranging between 0.5 and 50 ng/ml. Aliquots of those solutions were subsequently admixed with blank matrix solution (obtained following extraction of blank patches or gloves) to produce a range of matrix-matched standards.

Sample preparation

The pesticide residues were extracted from patches or gloves by adding methanol (20 ml for patches and 100 ml for gloves extracted and analysed individually) and sonicated for 15 min. A 1 ml of methanol extract was then filtered into an autosampler vial using a disposable 1-ml syringe and a PTFE syringe filter.

Azoxystrobin was analysed by liquid chromatography (LC). The LC mobile phase was: methanol (80%) and 1 mM ammonium acetate (20%). A Waters XTerra C18 MS column, 5 μm; 2.1×150 mm was used. The flow rate was set at 0.20 ml/min at 30°C.

Mass spectrometry

ESI-MS/MS detection was achieved using the Quattro Micro API tandem mass spectrometer (Micromass/Waters, Manchester, UK). The instrument was operated in positive ion electrospray mode using multiple reaction monitoring (MRM) with 2 MS–MS transitions monitored: 404.1 → 372.2 (quantitative) and 404.1 → 344.2 (qualitative). A dwell time of 0.3 s and span to 0.2 Dalton were used. Argon of 99.9% purity (Multax, Poland) was used as a collision gas. A nitrogen generator (Parker, The Netherlands) was used to supply nitrogen as the desolvation gas. The optimum cone voltage was 26 V and collision energy values 13.

The optimum gas pressures were set at universally applied values of 200 l/h for desolvation gas flow, 10 l/h for cone and 4.5×10^{-3} mbar collision chamber pressure. The ion source operated at 150°C, the desolvation temperature was 280°C and capillary voltage was maintained at 3.8 kV. The recovery of azoxystrobin was above 75% (controlled in every serial of analysis), limits of detection and quantification were 0.8 ng/patch and 2.4 ng/patch, precision 10.5% and range of linearity was 2.4–10 000 ng/patch.

RESULTS

Study population characteristics

Exposure of the tending and harvesting workers (N = 19) was measured on each of the two sampling days. Characteristics of study participants in Table 1 show that workers mainly had vocational or secondary (incompleted/completed) education. The mean age of workers was about 41 years. Mean height 164±4.1 and weight 64±7.2. The number of years worked in the greenhouse was 14.1 years ±8.4. Only 26% of workers involved in tending and harvesting had received training on safe use of pesticides.

Table 1. Characteristics of study participants

Variable	Women tending and harvesting (N = 19)	
	N (%)	Mean±SD
Education		
Primary	2 (10.5)	
Vocational	9 (47.4)	
Secondary or higher education	8 (42.1)	
Height (cm)		164.1±4.1
Weight (kg)		64.2±7.2
Age (years)		40.7±7.4
Years worked in greenhouse		14.0±8.4
Received training on safe use of pesticides	5 (26.3)	

N — number of workers.
SD — standard deviation.

Dermal exposure to azoxystrobin on cotton patches and gloves

Arithmetic mean azoxystrobin contamination levels found on patches of women responsible for tending and harvesting vegetables was 0.14 ng/cm^2 (median: 0.13 ng/cm^2; interquartile range: 0.08 ng/cm^2) one day after azoxystrobin spraying and 0.08 ng/cm^2 (median: 0.06 ng/cm^2; interquartile range: 0.04 ng/cm^2) seven days after spraying (Table 2).

In study women, arithmetic mean contamination level found on cotton gloves was 16.5 ng/cm^2 (median 5.9 ng/cm^2; interquartile range 19.3 ng/cm^2) one day after spraying and 89.3 ng/cm^2 (median 72.4 ng/cm^2; interquartile range 88.3 ng/cm^2) six days later (seventh day after spraying) (Table 2).

The level of azoxystrobin was higher on cotton gloves of women responsible for tending and harvesting vegetables than on patches. The level of azoxystrobin decreased on

Table 2. Concentration of azoxystrobin on patches and gloves of women responsible for tending and harvesting vegetables (ng/cm^2)

Re-entry	Gloves		Patches	
	Arithmetic mean (SD)	Median (interquartile range)	Arithmetic mean (SD)	Median (interquartile range)
1 day after spraying	16.5 (21.5)	5.9 (19.3)	0.14 (0.07)	0.13 (0.08)
7 days after spraying	89.3 (74.2)	72.4 (88.3)	0.08 (0.06)	0.06 (0.04)

Table 3. Linear regression model for azoxystrobin concentration on cotton patches and gloves between the days of measurement in women responsible for tending and harvesting vegetables

Location of patches	Geometric mean ratio*	95% PU
Cotton chest and arm patches	0.61	0.47–0.79
Cotton gloves	2.47	1.40–4.39

* Ratio of geometric mean 7 to 1 day after spraying; adjusted for the date of determination.

patches and increased on cotton gloves between the two days of measurement.

The amount of chemical deposited during work on patches between the days of measurement was about 60% lower seven days after spraying compared to one day after spraying. On the other hand the amount of azoxystrobin was almost 2.5 times higher on cotton gloves seven days after spraying compared to one day after spraying. The results were adjusted for the date of determination (Table 3).

DISCUSSION

This study has shown that workers in a Polish greenhouse are indeed exposed to pesticides at re-entry into the greenhouse after spraying pesticides on previous days. Levels of dermal exposure to azoxystrobin on hands, shoulders and chest have been determined.

The main route of exposure is through hands as the higher levels of azoxystrobin were found on cotton gloves (used under workers protective gloves) than on patches which was associated with the work they performed in greenhouse: tending — cutting unnecessary leaves to let the sunlight reach the desired portions of the plant, and this work requires frequent contact with the plant. The exposure in this study was measured during the spring, when women were only engaged in cutting leaves of the plants. Exposure of the hands can be an important contributor to the total dermal exposure and has been shown to account for 50% to 90% of the total body exposure [10,11].

The level of azoxystrobin found on patches decrease between 1 and 7 days after spraying, whereas the gloves' level of azoxystrobin of workers responsible for tending and harvesting vegetables increased between the days of measurement.

The observed decrease of azoxystrobin level on patches after second re-entry agrees with decrease of residues of azoxystrobin on leaves after spraying [12]. In a study conducted by Caffarelli, slow decrease of azoxystrobin concentration was observed between 1 and 8 day after spraying. After about the 30 days the concentration decreased almost to zero.

The phenomenon of increased hand exposure in women responsible for tending and harvesting vegetables can be explained by secondary exposure from the protection gloves on which pesticide can accumulate in periods after pesticides have been applied.

So the protective gloves, instead of reducing the exposure, in fact are rather a source of additional exposure.

Lots of studies showed that, in practice, personal protective equipment is often not as effective as it should be as a risk reduction measure [13,14] and can in some cases actually increase the risk. The reasons why gloves are not the effective protection can be: the selection of completely wrong type of gloves: cotton gloves with a leather palm (such gloves were worn by some of the study participants) provides no protection against chemicals and may actually increase exposure as the chemical tends to be held in contact with the skin [15]. Contamination could also be transferred into the gloves from contaminated hands when the user has not donned or removed the gloves properly [16,17]. Contamination of the inside of gloves is common and brings the hazardous substances into intimate contact with the skin. Occlusion by gloves raises skin temperature and hydration, leading to a deterioration of its natural barrier properties [17]. Also physical damage, degradation (when the chemical actually attacks the material from which the gloves have been manufactured) and penetration (when the chemical is absorbed into the external glove surface, migrates through the glove at a molecular level, then emerges as a vapour from the internal surface) can increase the risk of higher exposure [15].

Potential exposure to pesticides evaluated as a measurements of deposits of azoxystrobin on sampling elements (patches, gloves) as a substitute for skin deposition confirmed that the women not directly engaged in the process of spraying may be exposed to pesticides during their work in greenhouse. On other hand, the protective gloves do not properly protect the workers and can be even the sources of considerable additional exposure. The workers do not change their gloves often enough and significant residues of pesticides can be found in them; sometimes the workers used wrong type of gloves or gloves with some physical damage. This is due to greenhouse operator practice of providing to the workers a lump sum of money each year for the purchase of suitable personal protective equipment for themselves. So, the decision how long the gloves can be used is left to workers. Therefore, more efficient personal protective gloves for proper protection of women working in vegetable greenhouses are needed.

CONCLUSIONS

1. The results of the presented study reveal that workers in Polish greenhouses are potentially exposed to pesticides at re-entry into the greenhouse after spraying pesticides on previous days.
2. The personal protective gloves do not properly protect the workers and can be even the sources of additional exposure.
3. Efficient personal protective gloves for proper protection of women working in vegetable greenhouses are needed.

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