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Dissipation of four fungicides on greenhouse strawberries and an assessment of their risks

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ABSTRACT

Risk assessments of the fungicides pyraclostrobin, iprodione, tebuconazole and cyprodinil were carried after determining their persistence on strawberries following two applications by spraying in a greenhouse. The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method was used for sample preparation, and high-performance liquid chromatography-tandem mass spectrometry and gas chromatography-tandem mass spectrometry were used for sample analysis. The range for the average recoveries of the four fungicides was 86.2–105.4 %, and the relative standard deviation range was 2.7–6.1 %. The half-lives of pyraclostrobin, iprodione, tebuconazole and cyprodinil after a single application were 3.7, 3.6, 3.3 and 2.8 d, respectively. Compared with a single application, a second application of the fungicides increased the average concentrations of residues left on the strawberries. Risk assessments of the four fungicides were conducted by comparing national estimated daily intakes against acceptable daily intakes under good agricultural practice conditions. With application at the recommended dosage, strawberries sprayed with pyraclostrobin, iprodione and tebuconazole were safe for consumption after two applications. By contrast, while the first application of cyprodinil was safe, the second presented a high health risk. Even at the recommended dosage (720 a. i. g ha-1), repeat applications of cyprodinil should be banned.

1. Introduction

Strawberries are widely cultivated worldwide for their attractive fragrance, sweet taste and high economic value. While open field cultivation is common in Europe and North America, strawberries in China are often cultivated in greenhouses. Greenhouses provide a closed environment with high-temperature and -humidity, which can result in fungal diseases on crops.

Gray mold (*Botrytis cinerea*) is a common fungal disease in greenhouse strawberries, and can reduce production yields by as much as 30–60%. In agriculture, many fungicides have been used to treat and prevent growth of *B. cinerea*, including pyraclostrobin, iprodione, tebuconazole and cyprodinil. Pyraclostrobin is a new methoxyacrylate strobilurin (You, liu, liu, liu, & dong, 2012) that acts by inhibiting electron transfer in mitochondria (Joshi, Sharma, & Guruprasad, 2014; Zhang et al., 2012). The broad-spectrum dicarboximide contact fungicide iprodione (Omirou, Vryzas, Papadopoulou-Mourkidou, & Economou, 2009; Vanni, Gamberini, Calabria, & Nappi, 2000, Vanni, Gamberini, Calabria, & Pellegrino, 2000), triazole fungicide tebuconazole (Patyal, Sharma, Chandel, & Dubey, 2013), and systemic fungicide cyprodinil (Pose-Juan, Cancho-Grande, Rial-Otero, & Simal-Gándara, 2006) are also used to control gray mold growth in greenhouses. Because strawberries are harvested over a long period of 5 months, repeat applications of these fungicides are common. Improper use of these fungicides may result in contamination of strawberries with chemical residues.

Previous studies have reported the presence of pyraclostrobin, iprodione, tebuconazole and cyprodinil residues in major crops, such as maize, apple, cabbage and tomatoes (Liu et al., 2014; Omirou et al., 2009; Patyal et al., 2013; You et al., 2012). However, very few reports have established maximum residue limits (MRLs)

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and guidelines for the use of agrochemicals in strawberry production because it is a relatively minor crop. No MRLs are available for pyraclostrobin, iprodione and tebuconazole on strawberries in China (GB-2763 2014, China). There are some MRLs for the four fungicides on strawberries grown in the USA (15 mg kg^{-1} for iprodione) and Japan (2 mg kg^{-1} for Pyraclostrobin and 20 mg kg^{-1} for iprodione).

Risk assessments (Knežević, Serdar, & Ahel, 2012) and residue experiments for these fungicides on strawberries are required. The acceptable daily intake (ADI) is an important parameter in assessing the health risk of fungicides. The ADI is obtained by dividing the concentration that results in no adverse effects in long-term studies by a safety factor of 10 or 100. The ADIs of pyraclostrobin, iprodione, tebuconazole and cyprodinil are 0.03, 0.06, 0.03 and 0.03 mg per kilogram of body weight (mg kg^{-1} bw), respectively.

In this article, the presence of residues of these fungicides on strawberries was determined by gas chromatography-tandem mass spectrometry (GC–MS/MS) or high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS) after treatment of the samples by the QuEChERS method. The main goals of this study were to (i) quantify the degradation kinetics of the four fungicides after repeat applications to strawberries, and to (ii) assess the health risks of these fungicides under good agricultural practice (GAP) conditions.

2. Material and methods

2.1. Chemicals and reagents

Analytical standards of pyraclostrobin, iprodione, tebuconazole and cyprodinil (purity>98.0%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Commercially available fungicides were obtained as follows: pyraclostrobin (25% emulsifiable concentrate), BASF Company (Ludwigshafen, Germany); iprodione (225 g L^{-1} aqueous suspension concentrate), Noposion Agrochemicals Co., Ltd (Shenyang, China); tebuconazole (250 g L^{-1} oil-in-water emulsion), KYX chemical Co., Ltd (Qingdao, China); and cyprodinil (50% water dispersible granules), Nonghua Biological Technology Co., Ltd (Anhui, China). Acetonitrile (mass spectrometry grade) was purchased from Merck KGaA (Darmstadt, Germany). Materials for the QuEChERS method, including sodium acetate, anhydrous magnesium sulfate, graphitized carbon black, primary secondary amine, and C18, were obtained from Agela Technologies (Tianjin, China).

2.2. Field experiment design

A field experiment was designed according to the "Guidelines on pesticide residue trials NY/T 788-2004 (China)". The experiment was conducted during the harvest period for greenhouse strawberries from September 2012 to April 2013. The greenhouse was located in Hangzhou (E119.16, N29.29), China, and was 300 m^2 (50 m $\times$ 6 m) with a height of 2.5 m. The greenhouse was divided into a blank control plot and four test plots, each of 54 m^2 (9 m $\times$ 6 m). A buffer area (1 m $\times$ 6 m) separated adjacent plots.

The four test plots were sprayed with recommended dosages of 150 g ha^{-1} active ingredient (g a. i. ha^{-1}) of pyraclostrobin, 360 a. i. g ha^{-1} iprodione, 112.5 a. i. g ha^{-1} tebuconazole and 720 a. i. g ha^{-1} cyprodinil, respectively. The blank control plot was sprayed with the same volume of pure water as that used for the diluent fungicides. After 7 d, each spraying was repeated. Representative strawberry samples were collected according to the diagonal principle at intervals of 2 h, and 1, 3, 5 and 7 d after the first spraying, and at intervals of 1, 3, 5 and 7 d after the second spraying. Each sample, which weighed at least 1 kg, was stored at −20 °C after homogenization in a blender. The temperature in the greenhouse ranged from 15 to 28 °C, while the relative humidity ranged from 80 to 100%.

2.3. Pretreatment method

Before analysis, each sample of strawberries was thawed at room temperature. The fungicide residues on the strawberry samples were extracted and purified by the QuEChERS method as detailed below. Approximately 10 g of each thawed homogenized sample was weighed into a 50 mL centrifuge tube, and 10 mL of acetonitrile was added. After 2 min on a vortex mixer (Talboys, Troemner, Thorofare, NJ) at a relative centrifugal force (RCF) of 44.72 $\times$ g, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate were added to the tube. The mixture was shaken and then left to stand for 15 min. After this, the tube was centrifuged at an RCF of 1006 $\times$ g for 3 min, and 1.5 mL of the supernatant was transferred to a 2 mL centrifuge tube that contained 50 mg of primary secondary amine, 50 mg of C18, and 150 mg of $MgSO_4$. After shaking, the mixture was centrifuged at an RCF of 7155 $\times$ g for 3 min. To prepare the iprodione, tebuconazole and cyprodinil samples for GC–MS/MS analysis, the supernatant was filtered through a 0.22 μm membrane. For the pyraclostrobin sample, the supernatant was diluted with Grade 1 purified water (conductivity≤0.01 mS/m at 25 °C) to (1:1, v:v), and then filtered through a 0.22 μm membrane for HPLC-MS/MS analysis.

2.4. GC–MS/MS and HPLC-MS/MS

GC–MS/MS was conducted using a TSQ Quantum GC (Thermo Fisher Scientific, Waltham, MA) equipped with a triple-quadrupole mass detector in ESI mode. A capillary fused silica column VF-17 MS (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm, Agilent Technologies, Santa Clara, CA) combined with a deactivated pre-column (1 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm, Agilent, Technologies) at the inlet side was used. The temperature program started at 50 °C (held for 1 min), increased to 125 °C at 25 °C min^{-1}, increased to 300 °C at 20 °C min^{-1}, and finally held for 5 min. The injection volume was 1 μL and the total run time was 16.5 min. Two transitions were used for quantification and qualification as follows: m/z 187 > 159 (5 eV) and 187 > 124 (20 eV) for iprodione, m/z 250 > 125 (20 eV) and 252 > 127 (20 eV) for tebuconazole, and m/z 224 > 108 (20 eV) and 225 > 210 (18 eV) for cyprodinil.

HPLC-MS/MS detection was conducted using a TSQ Quantum Discovery mass spectrometer system (Thermo Fisher Scientific) equipped with a triple-quadrupole mass detector operated in multiple reaction monitoring mode with positive electron ionization. A Luna C18 HPLC column (150 mm $\times$ 4.6 mm, 3 μm, Phenomenex, Torrance, CA) was used for the separation. The mobile phase was 90% acetonitrile and 10% water (v/v), and contained 0.1% (volume fraction) formic acid. The injection volume was 5 μL and the total run time was 7 min. Two transitions of m/z 388 > 163 (29 eV) and 388 > 194 (19 eV) were used for quantification and qualification for pyraclostrobin.

Typical results from standard solutions for pyraclostrobin (HPLC-MS/MS) and iprodione, tebuconazole and cyprodinil (GC–MS/MS) are shown in Fig. 1. The retention times of pyraclostrobin, iprodione, tebuconazole and cyprodinil were 2.78, 9.26, 10.57 and 10.91 min, respectively.

2.5. Model calculation

Degradation of the fungicides over time after a single application is often described according to first-order kinetics as follows: