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Residue and intake risk assessment of prothioconazole and its metabolite prothioconazole-desthio in wheat field

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Abstract In the environment, plants and animals in vivo, pesticides can be degraded or metabolized to form transformation products (TPs) or metabolites, which are even more toxic than parent pesticides. Hence, it was necessary to evaluate residue and risk of pesticides and their TPs (or metabolites). Here, a rapid, simple, and reliable method using QuEChERS and LC-MS/MS had been developed for simultaneous analysis of prothioconazole and its toxic metabolite, prothioconazole-desthio, in soil, wheat plant, straw, and grain. The average recoveries of prothioconazole and prothioconazole-desthio in four matrices ranged from 86 to 108% with relative standard deviations (RSDs) of 0.53–11.87% at three spiking levels. The method was successfully applied to investigate the dissipation and terminal residues of the two compounds in wheat field. It was shown that prothioconazole was rapidly degraded to prothioconazole-desthio, with half-lives below 5.82 days. Prothioconazole-desthio was slowly dissipated in soil and plant. The terminal residues of prothioconazole in wheat grain with a pre-harvest interval (PHI) of 21 or 28 days were below the maximum residue limits (MRLs) (0.1 mg/kg, Codex Alimentarius Commission (CAC)). We also evaluated the intake risk of prothioconazole-desthio residues in wheat grain in China. For long-term intake assessment, the hazard quotients (HQ) ranged from 1.30 to 5.95%. For short-term intake assessment, the acute hazard indexes (aHI) ranged from 1.94 to 18.2%. It indicated that the intake risk of prothioconazole-desthio in wheat consumption was acceptable. Thus, the prothioconazole application on wheat with the scientific practices would not pose public health risk.

Keywords Prothioconazole · Prothioconazole-desthio · Wheat field · Residue · Intake risk

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Introduction

Prothioconazole, a broad-spectrum systemic fungicide of the triazolinthione family (Zhou et al. 2015), has been widely used in agriculture fields such as cereals (Audenaert et al. 2010; Edwards and Godley 2010), beans (Xavier et al. 2013), and various root crops (EFSA 2010). In the environment, plant and animal in vivo, prothioconazole can rapidly be degraded or metabolized to prothioconazole-desthio (Fig. 1). Parker et al. (2013) had conceived that the high potency of prothioconazole as a fungicide could be enhanced due to the highly active prothioconazole-desthio. The toxicity data showed that the oral lethal dose (LD_{50}) of prothioconazole-desthio was approximately 2200 mg/kg body weight (bw), when the LD_{50} of prothioconazole was higher than 6200 mg/kg·bw in rats (WHO 2008). The metabolite was more toxic than the parent compound. In addition, prothioconazole-desthio and

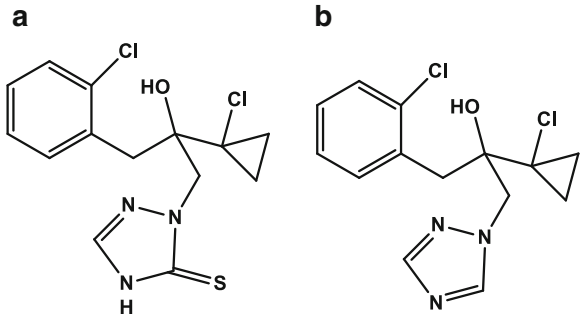

Fig. 1 Chemical structures of **a** prothioconazole and **b** prothioconazole-desthio

prothioconazole were the major residue components in plants. Therefore, it is of great significance to assess the residues of metabolites in crops. And the residue definition of prothioconazole in crops was its metabolite, prothioconazole-desthio.

Wheat is a widely grown food crop all over the world, and China is one of the top 5 producers (Zafar Iqbal Khan et al. 2016). Powdery mildew is a devastating disease of wheat worldwide (Huang and Röder 2004). Among many fungicides, prothioconazole was widely applied on wheat crop for the prevention and control of powdery mildew. Hence, it was necessary to pay attention to the potential environmental hazard and human health risks of prothioconazole and prothioconazole-desthio. However, to our best knowledge, no literatures were reported on the residues and risk assessment of prothioconazole and prothioconazole-desthio in wheat.

In this paper, we had established a simple QuEChERS and LC-MS/MS method for simultaneous analysis of prothioconazole and its toxic metabolite, prothioconazole-desthio, in wheat grain, wheat plant, wheat straw, and soil. The method was applied to investigate the dissipation behavior and terminal residues of the two compounds in wheat field in Shandong and Anhui provinces in 2015 and 2016. Meanwhile, we also evaluated the intake risk of prothioconazole-desthio residues in wheat in China. Thus, this study would provide scientific information for relevant safety evaluation of prothioconazole in wheat consumption.

Materials and methods

Standard solutions and chemicals

Prothioconazole standard (purity 98.5%) was provided by Henan Jinxiu Zhixing Crop Protection Ltd. (Zhengzhou, China). Prothioconazole-desthio (purity 99.5%) was purchased from Sigma-Aldrich Laborchemikalien GmbH (Seelze, Germany). The stock solutions of prothioconazole (504 mg/L) and prothioconazole-desthio (402 mg/L) were prepared in acetonitrile and kept at −20 °C in the dark. A Milli-Q water purification system (MA, USA) was applied to prepare ultra-pure water. Other used reagents such as sodium chloride (NaCl), anhydrous magnesium sulfate ($MgSO_4$), and acetonitrile were of analytical grade and provided by Beijing Chemical Works (Beijing, China). Formic acid was of HPLC grade and provided by Thermo Fisher Co. Ltd. (MA, USA). The organic syringe filter (polytetrafluoroethylene, PTFE; 0.22 μm) and sorbent materials such as octadecylsilane (C18, 50 μm, 60 Å) and graphitized carbon black (GCB, 120–400 mesh) were provided by Bonna-Agela Technologies (Tianjin, China).

LC-MS/MS analysis

The quantitative analysis of prothioconazole and prothioconazole-desthio residues was performed with Agilent Technologies and consisted of a 1260 autosampler, a 1260 Infinity Binary pump, and a 6420 triple quadrupole mass spectrometer (Agilent, CA, USA). A Poroshell 120 EC-C18 column (50 mm × 3.0 mm i.d., 2.7 μm; Agilent, CA, USA) was used for separation at 30 °C. The mobile phase in isocratic conditions, the mixture of acetonitrile and water (0.2% formic acid) (*v*:*v* 60:40), was performed at a flow rate of 0.4 mL/min. The injection volume was 5 μL. The retention times of prothioconazole and prothioconazole-desthio were 2.4 and 2.0 min, respectively.

The MS/MS analysis was operated in positive ESI mode. The drying gas temperature was set at 350 °C, and the nebulizer gas (N_2) pressure was 35 psi. The capillary voltage was 4000 V with a heater temperature of MS 1 and MS 2 at 100 °C. Multi-reaction monitoring (MRM) was applied to the detection of the target compounds. The m/z 344 → m/z 189 and m/z 312 → m/z 125 were used for identification of prothioconazole and prothioconazole-desthio, while the m/z 344 → m/z 326 and m/z 312 → m/z 70 were used for quantification, respectively. The fragmentors of prothioconazole and prothioconazole-desthio were 100 and 130 V, respectively.

Field trials

The field trials were conducted from April to July 2015 and 2016 at two sites: Laiyang (120.99 E, 36.97 N, Shandong, east of China) and Xiaoxian (116.93 E, 34.19 N, Anhui, south of China). The two sites have the following characteristic properties of soil: the pH of Laiyang and Xiaoxian were 7.32 and 6.79, and the corresponding values of organic matter were 3.89 and 1.71%, respectively. The average temperatures of Laiyang and Xiaoxian were 21 and 22 °C in 2015, respectively, and the corresponding values were 21 and 23 °C in 2016, respectively.

To investigate the dissipation of prothioconazole in soil and plant, 25% suspension concentrate (SC) of prothioconazole was sprayed once at 1.5 times of the recommended high dosage (337.5 g active ingredient per hectare, g a.i./ha) on the surface of bare soil and plants. The plot of the blank control was sprayed with water. The representative samples including soil (1 kg) and plant (1 kg) were collected from each plot for periodic analysis (2 h; 1, 2, 3, 5, 7, 10, 14, 21 and 30 days) after application and kept at −20 °C.

The terminal residue trials were carried out with a recommended high dosage (225 g a.i./ha) and 1.5 times of the recommended high dosage (337.5 g a.i./ha). Both dosage levels were designed to be sprayed two to three times with four pre-harvest intervals (PHI): 7, 14, 21, and 28 days in separate plots. The application interval was 7 days. A plot with the same size but no prothioconazole application was used as the control. Samples of wheat grain (2 kg), straw (2 kg), and soil (2 kg) were randomly collected with PHI of 7, 14, 21, and 28 days after the last application from different plots. All of the samples were kept at −20 °C prior to further analysis.

Each treatment had one control plot (no prothioconazole, water only) and three replicate plots. The area of each plot was 30 m^2. Different plots were separated by a buffer zone.

Extraction and purification procedure

5.0 g soil, 5.0 g wheat grain, 2.0 g plant, and 1.0 g straw samples were accurately weighed into 50-mL PTFE centrifuge tubes, respectively. The soil and wheat grain samples were extracted with 10 mL acetonitrile and 5 mL distilled water by vortexing for 1 min. After the addition of 2 g anhydrous $MgSO_4$ and 1 g NaCl, the sample tubes were vortexed for approximately 30 s. Then, the tubes were vigorously centrifuged at 4000 rpm for 3 min. One milliliter of supernatant layer was rapidly transferred into a 5-mL PTFE tube containing 60 mg of C18 and 150 mg of anhydrous $MgSO_4$. The tubes were vigorously centrifuged at 10,000 rpm for 3 min after vortexing for approximately 1 min.

The wheat plant and straw samples were extracted with the mixture of 1 mL distilled water and 10 mL acetonitrile by vigorously vortexing for 1 min. Then, 1 g NaCl and 2 g anhydrous $MgSO_4$ were added into the tubes. The extracts were centrifuged at 4000 rpm for 3 min after vortexing for 30 s. For plant samples, 1.5 mL of the acetonitrile layer was rapidly transferred into a 5-mL PTFE tube containing 5 mg of GCB and 200 mg of anhydrous $MgSO_4$ and then vortexed for 1 min. For straw samples, 1.5 mL of the supernatant layer was cleaned up by 60 mg C18 sorbent and 200 mg anhydrous $MgSO_4$ by vortexing for 1 min. Then, the tubes were centrifuged at 10,000 rpm for 3 min.

Ultimately, the upper layer (about 600 μL) was filtered via a 0.22-μm organic syringe filter to an autosampler vial prior to LC-MS/MS analysis.

Recovery experiments

For recovery experiments, prothioconazole and prothioconazole-desthio were quantificationally added to the blank samples at three different spiking levels each with five repetitions. The fortification levels were presented as follows: 0.02, 0.1, and 0.5 mg/kg for soil and wheat grain samples; 0.05, 0.1, and 0.5 mg/kg for wheat plant samples; and 0.1, 0.5, and 1 mg/kg for wheat straw samples, respectively.

Intake risk assessment

In order to evaluate the intake risks by consumption of prothioconazole-desthio-contaminated wheat, we calculated the long-term and short-term consumer health risk by the following equations:

$$\mathrm{EDI} = \mathrm{STMR} \times \mathrm{Fi}/\mathrm{bw} \quad (1)$$

$$\mathrm{HQ} = \mathrm{EDI}/\mathrm{ADI} \times 100\% \quad (2)$$

$$\mathrm{ESTI} = \mathrm{HR} \times \mathrm{LP}/\mathrm{bw} \quad (3)$$

$$\mathrm{aHI} = \mathrm{ESTI}/\mathrm{ARfD} \times 100\% \quad (4)$$

Table 1 Recoveries of prothioconazole and prothioconazole-desthio (n = 5) and LOQs

Sample	Fortified level (mg/kg)	Prothioconazole			Prothioconazole-desthio		
		Average recovery (%)	RSDs (%)	LOQs (mg/kg)	Average recovery (%)	RSDs (%)	LOQs (mg/kg)
Soil	0.02	86	3.56	0.02	108	2.16	0.02
	0.1	90	5.64		106	4.86	
	0.5	103	3.32		102	2.34	
Wheat grain	0.02	88	4.60	0.02	108	1.22	0.02
	0.1	101	5.48		108	2.11	
	0.5	101	5.28		103	5.09	
Wheat plant	0.05	87	6.87	0.05	88	2.39	0.05
	0.1	90	5.24		94	0.53	
	0.5	101	6.22		97	2.29	
Wheat straw	0.1	92	11.01	0.1	93	5.31	0.1
	0.5	93	9.84		96	4.52	
	1	100	5.80		92	11.87	

where EDI is the estimated daily intake (mg/kg bw day), STMR is the supervised trials median residue level (mg/kg), Fi is the food consumption (kg), bw is the average body weight (kg), ADI is the acceptable daily intake (mg/kg bw day), ESTI is the estimated short-term intake (mg/kg bw day), ARfD is the acute reference dose (mg/kg·bw), HR is the highest residue level (mg/kg), and LP is the highest large portion of food (kg/day, 97.5th percentile of eaters).

Conventionally, HQ (hazard quotient) or aHI (acute hazard index) less than 100% indicates an acceptable risk for common consumers.

Results and discussions

Method validation

In LC-MS/MS analysis, the matrix effects which always influences chromatographic response have been widely reported (Souverain et al. 2004). The slopes of calibration curves obtained from the matrix-matched standards were compared with those from pure acetonitrile standards. The matrix effects were ignored when the slope ratios ranged from 0.90 to 1.1 (Chen et al. 2015). Matrix signal suppression effects of prothioconazole and prothioconazole-desthio were observed in soil, wheat plant, straw, and grain

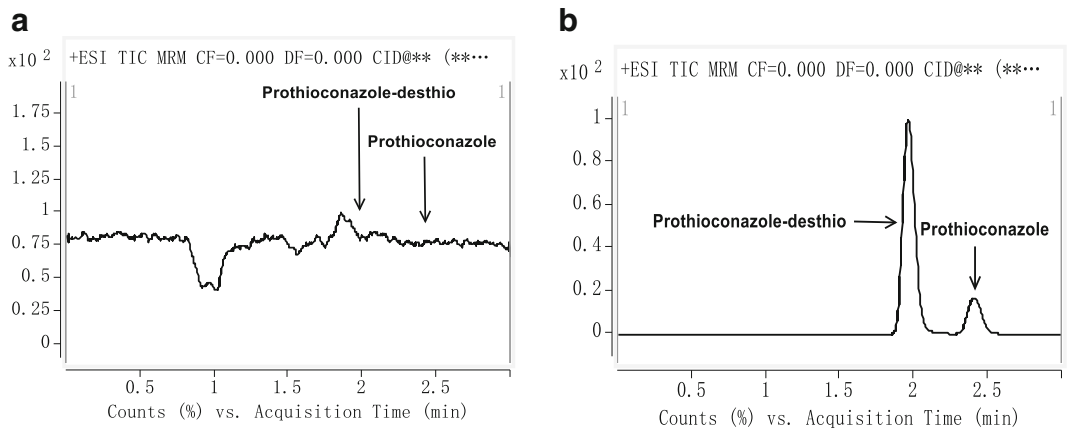

Fig. 2 The representative LC-MS/MS chromatograms. **a** Soil blank sample and **b** soil-spiked sample (0.1 mg/kg)

Table 2 Residues of prothioconazole and prothioconazole-desthio in soil at different time intervals in Shandong and Anhui

Interval (day)	2015				2016			
	Shandong		Anhui		Shandong		Anhui	
	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)
0	0.972	0.540	0.180	0.216	2.34	0.464	0.142	0.164
1	0.313	1.52	0.0936	0.190	1.03	2.16	0.117	0.172
3	0.130	1.54	0.0343	0.200	0.498	1.91	0.140	0.223
5	ND	0.598	0.0210	0.238	0.197	1.21	0.0906	0.197
7	ND	0.509	ND	0.256	0.150	1.40	0.0699	0.224
10	ND	0.508	ND	0.208	0.0255	0.965	0.0661	0.280
14	ND	0.425	ND	0.178	ND	0.859	0.0229	0.218
21	ND	0.379	ND	0.158	ND	0.594	ND	0.220
30	ND	0.303	ND	0.118	ND	0.439	ND	0.201

ND below LOQ

matrices with the slope ratios ranging from 0.739 to 0.894 (Table S1 in the "Supplementary material"). To eliminate matrix effects, prothioconazole and prothioconazole-desthio were quantified with calibrations of the external matrix-matched standard. Satisfactory linearities of target compounds were achieved in the range of 0.01–5 mg/L with the correlation coefficient ($R^2 > 0.99$). Good validation parameters like accuracy, repeatability, and precision in terms of recovery were obtained, as shown in Table 1. The mean recoveries of prothioconazole and prothioconazole-desthio at three different fortification levels ranged from 86 to 101% and 88 to 108%, with the RSDs of 3.32–11.01% and 0.53–11.87%, respectively. The limits of detection (LODs) at a signal-to-noise ratio (S/N) of three were evaluated to be 6.0×10^{-4}, 3.2×10^{-4}, 5.2×10^{-4}, and 4.2×10^{-4} mg/L for prothioconazole and 3.0×10^{-5}, 5.6×10^{-5}, 9.4×10^{-5}, and 1.6×10^{-5} mg/L for prothioconazole-desthio in soil, plant, straw, and grain,

Table 3 Residues of prothioconazole and prothioconazole-desthio in wheat plants at different time intervals in Shandong and Anhui

Interval (day)	2015				2016			
	Shandong		Anhui		Shandong		Anhui	
	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)	Parent residues (mg/kg)	Metabolite residues (mg/kg)
0	0.143	3.10	ND	3.00	2.00	1.34	0.367	0.326
1	0.0981	2.54	ND	1.93	1.48	1.45	0.349	0.690
3	ND	1.83	ND	0.921	0.507	1.14	ND	0.355
5	ND	0.668	ND	0.773	0.546	1.36	0.204	0.397
7	ND	0.211	ND	0.332	0.192	1.48	ND	0.188
10	ND	0.185	ND	0.277	0.0720	1.02	ND	0.0616
14	ND	0.101	ND	0.175	ND	0.394	ND	ND
21	ND	0.0904	ND	0.113	ND	0.355	ND	ND
30	ND	ND	ND	ND	ND	0.231	ND	ND

ND below LOQ

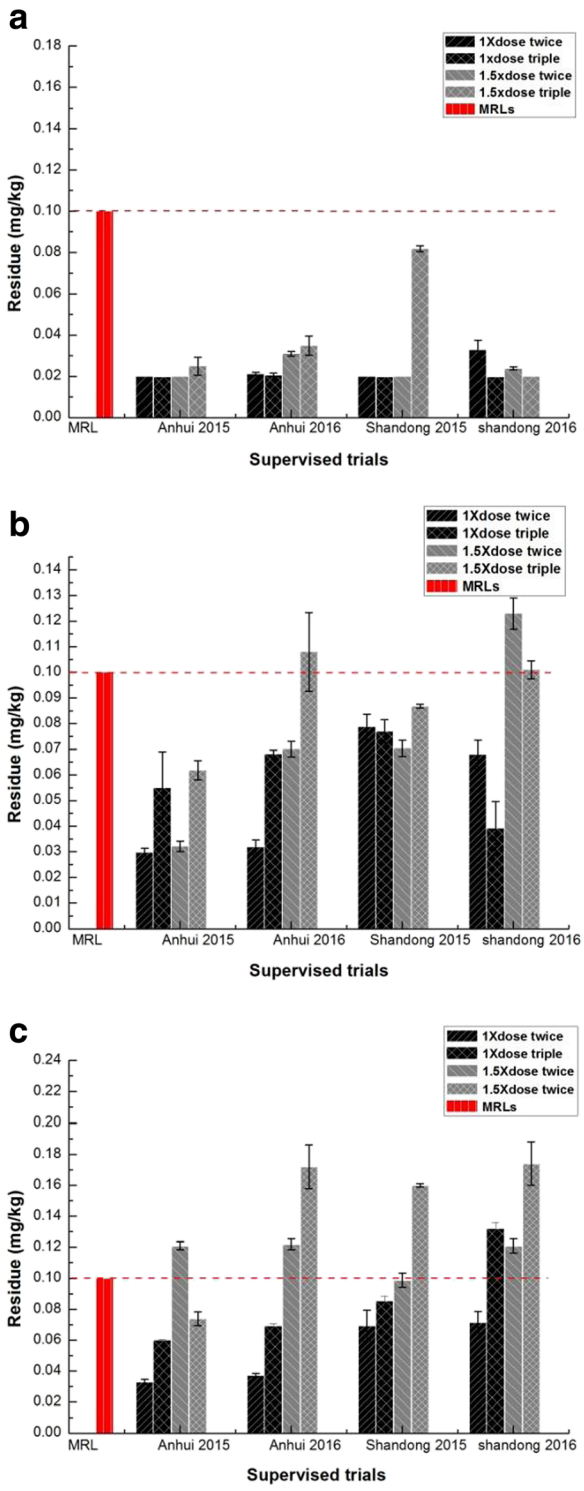

Fig. 3 Prothioconazole-desthio residues in wheat grain with PHI of 21 (**a**), 14 (**b**), and 7 days (**c**) from the supervised field trials

respectively. The limits of quantification (LOQs) of the two compounds were 0.02, 0.02, 0.05, and 0.1 mg/kg for soil, wheat grain, plant, and straw matrices, respectively. The results showed that the method was reliable for simultaneous determination of prothioconazole and prothioconazole-desthio in four matrices. The representative LC-MS/MS chromatograms of the two compounds in soil sample matrix were shown in Fig. 2.

Dissipation of parent prothioconazole and prothioconazole-desthio in soil and wheat plant

The residues of prothioconazole and prothioconazole-desthio in soil and wheat plant from Shandong and Anhui were analyzed as shown in Table 2 and Table 3. For soil samples, the initial residues of prothioconazole ranged from 0.142 to 2.34 mg/kg. Prothioconazole was rapidly degraded in soil samples with half-lives below 5.82 days. Prothioconazole residues were below LOQs at 5 and 7 days from Shandong and Anhui in 2015, respectively. The dissipation of prothioconazole complied with first-order kinetics in 2016, and the dissipation dynamics equations were as follows: $C = 1.8992e^{-0.4176t}$ (Shandong, $R = 0.9887$, $T_{1/2} = 1.66$ days) and $C = 0.1594e^{-0.1191t}$ (Anhui, $R = 0.9419$, $T_{1/2} = 5.82$ days). Usually, the degradation of pesticide in soil maybe affected by factors including climate, microorganisms, and chemical and physical properties of the soil (Liang et al. 2013). It was reported that prothioconazole was quite difficult to hydrolyze in buffer solution (FAO 2008), and its degradation in soil was not a result of photolysis (APVMA 2007). Therefore, the principal mechanism of prothioconazole degradation in soil might be microbially mediated. In general, more organic matter content could contribute to produce more microorganisms, and the amounts of organic matter content were higher in Shandong soil samples. So, it may be the reason why the dissipation was faster in soil from Shandong than that in Anhui.

For wheat plant samples, the initial concentrations of parent compound were below 2.00 mg/kg for samples from Shandong and Anhui. Prothioconazole residues were below LOQs at 3 days in Shandong and the initial concentrations in Anhui were below LOQs in 2 h after application in 2015. Prothioconazole residues were below LOQs at 14 and 7 days for Shandong and Anhui plant samples in 2016, respectively. The dissipation of prothioconazole in Shandong plant samples in 2016 could be calculated by the first-order kinetic equation as follows: $C = 1.9302e^{-0.3241t}$ (Shandong, $R = 0.9825$,

Table 4 Chronic intake assessment results evaluated by median residue level from the supervised field trials

PHI (day)	STMR (mg/kg)	Fi (kg/d)	EDI (mg/kg bw day)	HQ (%)	ADI (mg/kg bw day)
7	0.0912	0.4107	0.000595	5.95	0.01
14	0.0692		0.000451	4.51	
21	0.0200		0.000130	1.30	
28	0.0200		0.000130	1.30	

$T_{1/2}$ = 2.14 d). The data indicated the easy degradation of prothioconazole in wheat plant, with the half-lives below 2.14 days. The differences in dissipation rates might be affected by the environment factors such as sunlight and rainfall (Malhat et al. 2014; Ghani and Abdallah 2016).

The metabolite, prothioconazole-desthio, formed rapidly after application. Its initial concentrations in soil ranged from 0.164 to 0.540 mg/kg. Prothioconazole-desthio rapidly increased first and then continuously decreased during the experimental period, as the significant component of the residues. For wheat plant samples, prothioconazole-desthio initial residues were 0.326–3.10 mg/kg. It regularly declined during the field trial period both in Shandong and Anhui in 2015. However, prothioconazole-desthio residues reached maximum value at 7 and 1 day in Shandong and Anhui in 2016, respectively, and then reduced during the field trial period.

Terminal residues of prothioconazole-desthio in soil, wheat straw, and wheat grain

According to the terminal residue data, the residues of prothioconazole-desthio were below 1.02 mg/kg in soil from 7 to 28 days (Table S2 in the "Supplementary material"). In wheat straw, the terminal residues ranged from 0.228 to 15.5 mg/kg (Table S3 in the "Supplementary material").

The terminal residues of prothioconazole-desthio in wheat grain were summarized in Fig. 3. The MRLs of prothioconazole-desthio in wheat grain were 0.1 mg/kg (CAC 2016). Its residues in wheat grain were below LOQs with PHI of 28 days in all of the supervised field trials, and the residues were below MRLs (0.1 mg/kg, CAC) with PHI of 21 days. The residues were higher than MRLs in three treatments with PHI of 14 days. Prothioconazole-desthio residues exceed the MRLs in seven treatments with PHI of 7 days as shown in Fig. 3. The residues of prothioconazole-desthio in wheat grain reduced as the PHI extended. We have proposed a safe PHI of 21 days for prothioconazole formulation(25% SC)in wheat due to the terminal residue data.

Intake risk of prothioconazole-desthio

In this paper, we have evaluated the intake risks of prothioconazole-desthio in wheat grain as shown in Tables 4 and 5. The ADI value for prothioconazole-desthio is 0.01 mg/kg bw day (FAO 2008; EU 2017). Different ARfD values for prothioconazole-desthio have been provided by FAO (1 mg/kg·bw) and EU (0.01 mg/kg·bw). ARfD standards of EU (0.01 mg/kg·bw) have been used to assess the short-term risk considering maximization of the health risk. The average body weight is 63 kg for an average

Table 5 Acute intake assessment results evaluated by the highest pesticide residues from the supervised field trials

PHI (day)	HP (mg/kg)	LP (kg/d)	ESTI (mg/kg bw day)	aHI (%)	ARfD (mg/kg bw)
7	0.188	0.6107	0.00182	18.2	0.01
14	0.128		0.00124	12.4	
21	0.0832		0.000807	8.07	
28	0.0200		0.000194	1.94	

Chinese adult, and the daily food intake of grains and flours was 0.4107 kg/day, which comprises approximately 39.1% of total diet (1.030 kg/day) in China (Wang 2005). The highest large portion of wheat flour is 0.6107 kg/day for Chinese adults (WHO 2012). The residues were below LOQ with PHI of 28 days in wheat from Shandong and Anhui both in 2015 and 2016. In order to make a comprehensive intake risk assessment, we assumed the residues of prothioconazole-desthio 0.02 mg/kg with PHI of 28 days.

For long-term risk assessment, all the calculated EDI values of prothioconazole-desthio were much less than the ADI values as shown in Table 4. For short-term risk assessment, all the ESTI values were tiny and below ARfD values as shown in Table 5. The HQ or aHI was less than 100%, which indicated there was a negligible chronic term or acute risk with consumption of wheat grain.

Conclusions

In this study, a simple QuEChERS method coupled with LC-MS/MS was established and validated for simultaneous analysis of prothioconazole and its metabolite prothioconazole-desthio. The analytical method had presented good validation parameters with excellent recovery, specificity, and sensitivity. The LOQs of the method were 0.02, 0.02, 0.05, and 0.1 mg/kg for soil, wheat grain, plant, and straw samples, respectively. The parent prothioconazole was rapidly dissipated to the toxic metabolite, prothioconazole-desthio, with the half-lives below 5.82 days. However, the metabolite was slowly dissipated in soil and wheat plant. The terminal residues of prothioconazole-desthio in wheat grain samples were below 0.1 mg/kg (MRLs, CAC) with PHI of 21 and 28 days. All of the HQ or aHI do not exceed 100%, indicating the intake risk of prothioconazole-desthio in wheat was acceptable. Therefore, the prothioconazole applications on wheat with good agricultural practices did not cause public health risk.

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