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Dissipation and Residues of Chlormequat in Wheat and Soil

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Abstract A specific, sensitive method was developed for the analysis of chlormequat in wheat and soil by high performance chromatography/mass spectrometry. The fortified recoveries of soil were from 75.08% to 96.55%, with RSD 3.34%–15.18%, the limit of detection of the analytical method was 0.05 ng at a signal-to-noise ratio of 3, and the limit of quantification was 0.05, 0.1, 0.5 mg/kg for soil, wheat plants and wheat grain, respectively. The degradation dynamics and final residues of chlormequat in Beijing and Changchun were investigated. The half-life of chlormequat in wheat plants were 3.15 days in Beijing and 4.56 days in Changchun, while the half-life in soil was 3.88 days in Beijing and 4.51 days in Changchun. The final residues of chlormequat in soil were not detectable, and the final residues of chlormequat in wheat grain were below 0.50 mg/kg except for 3.51 mg/kg from high dosage plot of Changchun. The fact that all the final residues were below 5 mg/kg (GB2763 in National standards of the People's Republic of China, maximum residue limits for pesticide in food, Beijing, 2005) suggested that chlormequat could be safely used in wheat crops with the suitable dosage and application.

Keywords Chlormequat · Wheat · Soil · Residues · Dissipation · HPLC–MS

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Chlormequat, 2-chloroethyltrimethyl ammonium, is a plant growth regulator belonging to the quaternary ammonium pesticides family, typically applied as the salt chlormequat chloride. It is used as a stalk stabilizer in cereals and as a growth regulator and flowering promoter in fruits and vegetables. Chlormequat shows relatively low toxicity to human, animals and microorganism in the soil (Zhu et al. 2006). It has been classified as slightly toxic by WHO. Since its introduction to the market in 1966 (Castro et al. 2001), Chlormequat has been applied in many countries to some corps, including cotton, wheat, rye, fruits and vegetables (Vahl et al. 1998).

Wheat is a popular food crop in China, which is produced in two or three growth seasons,and lodging is a serious problem in the late-mid growth and development stages of wheat. Recent studies have shown that chlormequat could be a valuable pesticide against lodging when sprayed in the jointing stage of wheat (Zhu et al. 2006). Also chlormequat can increase wheat flowering and thus fruit set which results in improved yields under dry conditions (Allender 1992).

Previous studies on the environmental fate of chlormequat focused on the establishing testing method of chlormequat in some crops, such as pear (Zhao et al. 2000; Hau et al. 2000; Castro et al. 2001; Riedikera et al. 2002), tomato (Careri et al. 2002), wheat grain (Vahl et al. 1998; Juhler and Vahl 1999). But the analytical method of residues of chlormequat in soil has not been published, and no one has studied the dissipation and the ultimate residue of the chlormequat in crops.

The purpose of our work was to study the residual dissipation and ultimate residue of chlormequat in the wheat crops and the soil, and thereby provide an evaluation for scientific, safe use of chlormequat.

Materials and Methods

The Chlormequat chloride standards (99% purity) and formulations (80% WP) were obtained from Quanfeng Agriculture Chemical Co., Ltd (Anyang, China). HPLC-grade methanol was supplied by Honeywell (Burdick & Jackson, US). Ultrapure water was purchased from Wahaha company. Analytical-grade methanol, acetic acid, ammonium acetate, *n*-hexane, petroleum ether, ethyl acetate were purchased from the Beijing Reagent Company (Beijing, China).

All analysis was conducted with an Agilent 1100 HPLC–MS equipped with G1314AVWD,G1316A COLCOM,G1313AALS,G1311A Quatpump,G1379A Degasser and Ion Trap MSD. (Agilent, USA). A reverse-phase C_{18} HPLC column (150 mm × 4.6 mm i.d.,5 μm particle size) was used as the separation column and was maintained at 25°C. The mobile phase consisted of methanol/water (10/90 by volume) containing 50 mmol/L aqueous ammonium acetate, with a flow rate of 0.6 mL/min. The injection volume was 5μL. The retention time for chlormequat was 3.2 min.

In the field trials, the use of chlormequat chloride is for winter wheat in Beijing and spring wheat in Changchun. The field trials, including the dissipation experiment and ultimate residue experiment, were carried out in 2007 and 2008. Each experiment field consisted of three replicate plots with an area of 30 m^2 for each plots and was separated by irrigation channels (Zhao et al. 2008).

In the jointing stage, the spraying of chlormequat chloride (80%WP) was carried out on the wheat crop, and the applied dose was 3,000 mg/kg (750 kg water/ha). About 200 g wheat plant and soil samples were collected at 2 h, 1, 2, 3, 5, 7, 14, 21 and 28 days after spraying. The collected wheat plant samples were cut into small pieces (about 0.5 cm × 0.5 cm). To investigate the dissipation of chlormequat in soil, blank soil which no plants were grown in was sprayed at the dosage of 3,000 mg/kg. The sampling dates were the same as those for the plant materials. Soil was sampled to a depth of 0–10 cm at randomly selected sampling points in each plot. All collected samples were stored in a freezer at −20°C for further analysis.

The ultimate residue experiment was performed at two dosage levels: lower dosage of 2,000 mg/kg (the recommended dosage), and a higher dosage level of 3,000 mg/kg, respectively. In the jointing stage, the high and low dosage treatment groups were sprayed one time. Wheat grain and soil samples were collected on harvest days after the application of the chlormequat chloride (80% WP).

Soil samples (10 g) were put in a flask, and 50 mL methanol-ammonium acetate (100 mM, pH = 3, 10/90) solution were added. The soil was marinated in the solution about 12 h, and then the samples were shaken in a reciprocating shaker for 1 h. After shaking extraction, the samples were subjected to ultrasonic extraction in an ultrasonic cleaner for 10 min. The mixture were transferred to a 100 mL centrifuge tube, and centrifuged at 4,000 r/min for 5 min. 10.0 mL of the supernatant was transferred to an evaporating flask. Then it was concentrated and evaporated to near dryness on a rotary vacuum flash evaporator at 70°C. The residues was dissolved in 1.0 mL of methanol (HPLC grade), and the solution was transferred to a HPLC sample vial for instrumental analysis after filtered through the 0.22 μm aqueous filter.

Samples (5 g) of wheat powder or wheat plants were put in flasks, and 50 mL methanol–water (pH = 3, 50/50) solution were added, then the following step is the same as the soil until the centrifugation. After the extracts were centrifuged, all the supernatant was transferred to a separatory funnel for the liquid–liquid extraction. A 50-mL aliquot of light petroleum was added to funnel. The separatory funnel was shaken vigorously for about 30 s, and stayed to separate for 5 min. The organic layer (upper layer) was discarded, and the aqueous layer was washed with 50-mL light petroleum again. The aqueous layer was collected in a 100-mL evaporating flask, and was evaporated to dryness using a rotary vacuum evaporator over a water bath at 70°C. The residues was dissolved in 1.0 mL of methanol (HPLC grade), and the solution was transferred to a HPLC sample vial for instrumental analysis after filtered through the 0.22 μm aqueous filter.

Results and Discussion

The recovery levels, precision and limits of detection of the analytical method were investigated. Chlormequat was added to the untreated wheat, wheat plants and soil blank samples at three concentration levels with five repetitions. The recovery results were shown in Table 1. The limit of detection (LOD) of chlormequat was 0.05 ng at a signal-to-noise ratio of 3, and the limit of quantification (LOQ) was 0.05, 0.1, 0.5 mg/kg for soil, wheat plants, and wheat powder, respectively. The precision of the method in terms of relative standard deviations (RSD) was from 3.34% to 12.09%. The recovery and precision results showed that it was a reliable method for determination of chlormequat in wheat and soil samples.

The results of dissipation data in wheat plants were shown in Fig. 1. The initial concentration of chlormequat in wheat plants 2 h after treatment was 36.497 mg/kg in Beijing and 19.933 mg/kg in Changchun. Chlormequat dissipated rapidly in 5 days after application. From Fig. 1, we can know that above 90% of the residue in wheat plants had dissipated 21 days after treatment. The dissipation dynamics of chlormequat in Beijing and

Table 1 Recovery data of chlormequat in wheat powder, wheat plants and soil sample

Sample type	Added mg/kg	Recovery (%)						RSD (%)
		1	2	3	4	5	Average	
Soil	2.5	84.31	97.88	105.0	85.36	87.89	92.09	9.77
	1	74.04	74.69	75.78	71.20	79.69	75.08	4.11
	0.05	74.09	70.09	88.39	78.21	80.02	78.16	8.81
Wheat plants	1	86.65	78.10	87.67	79.56	89.96	84.39	6.21
	0.5	78.88	105.3	100.1	88.35	84.12	91.35	12.09
	0.1	96.00	97.50	99.37	98.63	91.25	96.55	3.34
Wheat grain	5	103.2	92.28	88.52	73.29	90.12	89.48	11.98
	1	73.92	71.91	71.95	81.27	87.35	77.28	8.82
	0.5	70.86	79.50	77.57	101.5	95.36	84.96	15.18

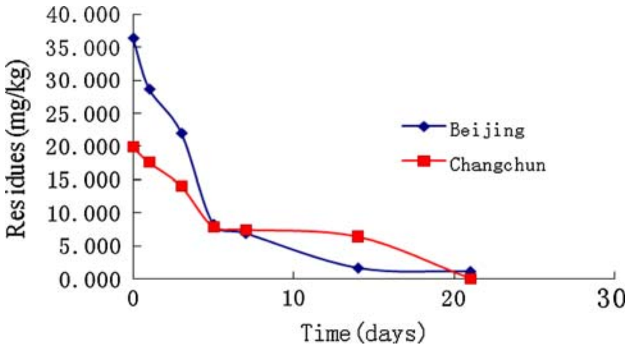

Fig. 1 Dissipation of chlormequat in wheat plants in Beijing and Changchun, 2007

Changchun could be described by the following equation: $C = 34.774e^{-0.2199t}$ and $C = 20.105e^{-0.1519t}$, respectively. The half-life time of chlormequat in wheat plant was 3.15 day in Beijing and 4.56 day in Changchun.

Figure 2 shows that the initial concentration level of chlormequat in soil was obviously lower than in the wheat plants. The concentration of chlormequat in the soil was 3.205 mg/kg in Beijing and 0.837 mg/kg in Changchun 2 h after application. The dissipation data showed that above 90% of the residue in soil had dissipated 21 days after treatment. An obvious decline of chlormequat can be observed in 5 days after treatment. The residual dynamics of chlormequat in Beijing and Changchun could be described by the equations $C = 7.0502\ e^{-0.1786t}$ and $C = 0.9613e^{-0.1538t}$, respectively, with a half-life of 3.88d in Beijing and 4.51d in Changchun.

The ultimate residue data were shown in Table 2. The concentration level of chlormequat in wheat powder and soil was determined after the application of chlormequat chloride (80% WP) in high dosage and low dosage. The concentration of chlormequat in wheat powder was below 0.5 mg/kg except that the final residue in Changchun 2007 was 3.51 mg/kg.

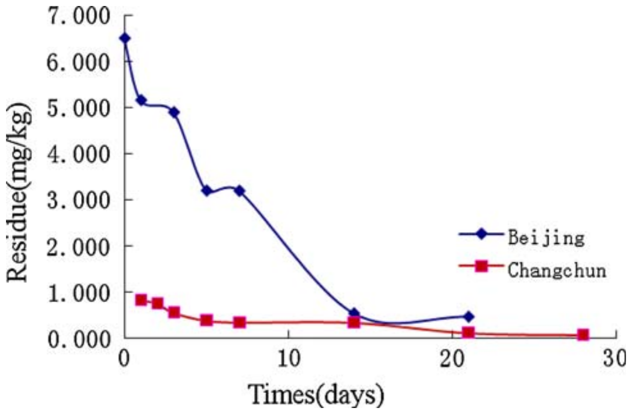

Fig. 2 Dissipation of chlormequat in soil in Beijing and Changchun, 2007

Table 2 Ultimate residue of chlormequat in wheat powder and soil

Sample type	Dosage (mg/kg)	Residue (mg/kg)			
		Beijing		Changchun	
		2007	2008	2007	2008
Wheat grain	High dosage	0.04	0.07	3.51	0.31
	Low dosage	ND	ND	0.50	0.06
Soil	High dosage	ND	ND	ND	ND
	Low dosage	ND	ND	ND	ND

ND: <LOD

In some countries, the Maximum residue limits (MRL) for chlormequat in some crops have been established. In China, the MRL of chlormequat in wheat was 5 mg/kg, and EU has also establish- ed the MRL of chlormequat in wheat as 2 mg/kg. Therefore, it is safe to spray chlormequat chloride (80% WP) at the recommended dosage due to its lower toxicity to biotic population, its short half-life and the final residue which below the MRL (GB2763 2005).

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