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Dissipation of Oxaziclomefone and Residue Analysis in Rice, Soil and Water Under Field Conditions

Hongwu Liang · Li Li · Ran Ji · Dongmei Qin · Fengmao Liu

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Abstract The analytical method for the residue analysis of a novel herbicide, oxaziclomefone, and its dissipation in soil, water, rice plants and husked rice in rice fields at GAP (Good Agricultural Practices) conditions were studied. Oxaziclomefone residues were determined by GC-NPD. Mean recoveries ranged from 84.4 to 108.2% with relative standard deviations (RSDs) of 2.4 to 17.2% at three different spiking levels for each different matrix. The limits of quantification (LOQ) were found to be 0.01 mg/kg in soil, water, rice plants and husked rice and 0.02 mg/kg in rice hull. The mean half-lives of oxaziclomefone residues in water, soil and rice plants were 11.3, 37 and 4.4 days, respectively. At harvest, soil, straw, rice hull and husked rice samples were found to contain oxaziclomefone below the maximum residue level (0.1 mg/kg) set by Japan and Korea. Following the recommended application method, this herbicide is therefore safe to apply to rice fields.

H. Liang · F. Liu (✉)
College of Science, China Agricultural University, 100193 Beijing, People's Republic of China
e-mail: liufengmao@yahoo.com

L. Li
State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, People's Republic of China

R. Ji
Beijing Research Institute of Chemical Industry, SINOPEC, 100013 Beijing, People's Republic of China

D. Qin
Institute for the Control of Agrochemicals, Ministry of Agriculture, 100026 Beijing, People's Republic of China

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Oxaziclomefone,3-[1-(3,5-dichlorophenyl)-1-methylethyl)-3,4-dihydro-6-methyl-5-phenyl-2H-1,3-oxazin-4-one, previously called MY-100, is a novel herbicide developed by Bayer CropScience. It is effective at controlling cockspur (*Echinochloa crus-galli*), other grasses and annual sedges that can substantially reduce the yield of rice in paddy fields O'Looney and Fry (2005a, b). Oxaziclomefone inhibits meristem growth in a manner unlike any known herbicides. After post-emergence application in flooded paddies, it is primarily taken up by plant roots. Initial symptoms in *Echinochloa crus-galli* were observed in the fresh leaves, followed by necrosis and plant death (O'Looney and Fry 2005b; Suzuki et al. 2003). Oxaziclomefone has not been shown to diminish turgor pressure, so its ability to inhibit cell expansion must depend on either changes in wall extensibility (e.g., decreased wall extensibility) without influencing the synthesis or post-synthetic modification of major architectural wall components or the redox environment of the apoplast (O'Looney and Fry 2005a). Figure 1 shows the structure and basic information for oxaziclomefone.

Mode of action and environmental fate studies for oxaziclomefone have been undertaken by several researchers. Jiang (2006) developed a method for determination of oxaziclomefone by HPLC. Kawata et al. (2005) studied runoff of oxaziclomefone from a paddy field using LC/MS, which revealed that concentrations of clomeprop and oxaziclomefone decreased by 90% within a week of application to the paddy water. To our knowledge, neither analysis of oxaziclomefone by GC nor residue dissipation in rice under field conditions has ever been evaluated. Because there is

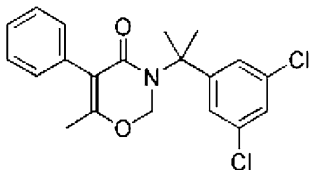

Fig. 1 Chemical structure and characteristics of oxaziclomefone. Molecular weight = 376.3; log P= 4.01; solubility in water at 25°C = 0.18 mg/L

currently no residue limit for oxaziclomefone in China, the results of this work on the analytical method development and evaluation of residue levels in the field will be useful in establishing a maximum residue limit (MRL) and keeping agricultural products safe.

Materials and Methods

Reference standards of oxaziclomefone were purchased at purities of 97.9% and 1% SC (suspension concentrate). Acetone, petroleum ether (60–90°C), ethyl acetate, dichloromethane, chloride sodium, anhydrous sodium sulfate were of analytical grade. Silica gel (60–80 mesh), purchased from Qing Dao Hai Yang Chemical Co., Ltd., was heated at 140°C for 4 h, then deactivated with 5% water (w:v) prior to use. Petroleum ether was distilled. A rotary evaporator (Institute of Biophysics, Chinese Academy of Sciences Kelong Co., Ltd.), glass column (6 mm i.d. × 420 mm) and air bath vibrator (ZD-2, Jintan City, Jiangsu Province Jincheng Guo-Sheng Experimental Co., Ltd.) were also used in the study.

Oxaziclomefone concentrations were determined on an Agilent 6890 N GC, equipped with an NPD detector and a HP-1 (30 m × 0.32 mm × 0.25 μm) capillary column. The injector was operated at 290°C with an injection volume of 1 μl. Oven temperature was programmed as follows: 120°C for 1 min, rising to 180°C (4°C/min) for 1 min, and rising to 280°C (50°C/min) for 1 min. Nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The NPD detector was operated at 300°C with detector gas flows at 3.0 and 60.0 mL/min for H_2 and air, respectively. The approximate retention time of oxaziclomefone was 13 min.

Field trials were carried out in Beijing and Kunming, China, over two consecutive years, according to the Guidelines for Pesticide Residue Field Trials (NY/T 788-2004), issued by the Ministry of Agriculture, the People's Republic of China. Each experimental treatment consisted of three replicate plots and a control plot that were separated by irrigation channels; the area of each plot was 30 m^2.

To study the dissipation of oxaziclomefone from water and soil, oxaziclomefone (1% SC) was applied to the flooded rice plots 7 days after transplanting rice seedlings dissolved in water at 1,500 g a.i.ha^{-1} (30 times the recommended dosage). Water samples were collected in bottles at 10 randomly selected points from each plot. Soil samples (0–10 cm) were collected at 8 randomly selected sampling points in each plot using a soil auger. Water and soil samples were collected at different intervals during the 120 days following application. All samples were stored at −20°C until further analysis.

In an additional three plots, growing plants were sprayed with 150 g a.i.ha^{-1} (3 times of recommended dosage) 7 days after transplanting the rice seedlings. Plant samples were collected at 0 (2 h after spraying), 1, 3, 7, 10, 14, 21, 30, and 45 days after spraying and stored at −20°C until further analysis.

To investigate the residue of oxaziclomefone remaining in plants and soil, both the recommended dose (50 g a.i.ha^{-1}) and 2 times that (100 g a.i.ha^{-1}) were applied to separate plots. At harvest, soil, straw and rice samples were collected for terminal residue analysis. Rice was air-dried at room temperature and shelled into the hull and husked rice. The husked rice was then ground into a powder. All samples were stored at −20°C until analysis.

Fifty milliliters of each water sample was added to a 250-mL separatory funnel containing 20 mL of petroleum ether. The sample was shaken vigorously for 1 min. The organic layer was collected, and the aqueous layer was re-extracted. The organic portions were combined and filtered through 10 g of anhydrous sodium sulfate, then concentrated to dryness on a rotary evaporator under vacuum at 35°C. The dried extracts were redissolved in 5 mL of acetone for GC-NPD analysis.

A portion (20 g) of each homogenized soil sample was extracted twice with 50 mL of acetone on a shaker table for 30 min. Extracts were filtered through a Buchner funnel using acetone (20 mL) as the wash solvent. The combined filtrate was evaporated to near dryness on a vacuum rotary evaporator at 35°C. The extract was transferred to a 250-mL separatory funnel containing 20 mL of 10% NaCl solution. The sample solution was then extracted twice by liquid–liquid partitioning with 20 mL of petroleum ether. The organic portions were combined and filtered through 10 g of anhydrous sodium sulfate, then concentrated to dryness on a rotary evaporator under vacuum at 35°C. The final extracts were brought up to 5 mL in acetone for GC-NPD analysis.

Samples (10 g) of rice hull and husked rice (rice plant) were ultrasoniated twice with 80 and 50 mL of acetone for 5 min (Rice plant samples were extracted twice with 50 mL of acetone on a shaker table for 30 min). The samples were then filtered through a Buchner funnel to a 250-mL round bottom flask with acetone (40 mL) as a wash solvent. Filtrates for each type of sample were combined and rotary evaporated to near dryness at 35°C. The extracts were transferred to a 250-mL separatory

funnel containing 50 mL of 10% NaCl solution and extracted three times by liquid–liquid partitioning with 30 mL of dichloromethane. Rice plant samples were treated in the same way but partitioned with petroleum ether. The organic portions were combined and filtered through 10 g of anhydrous sodium sulfate, then concentrated to dryness on a rotary evaporator under vacuum at 35°C for further clean-up.

A glass column was packed with 2.5 g of silica-gel deactivated with 5% water (w/v) between two 1-cm layers of anhydrous sodium sulfate on top of a glass wool plug. The column was prewashed with 10 mL of petroleum ether to remove any impurities. Concentrated extracts were poured on top of the column, washed with 20 mL of petroleum ether and eluted with 40 mL of petroleum ether/ethyl acetate = 92:8 (v/v). The first 15 mL of eluate was discarded. The remaining eluate was concentrated to dryness on a rotary evaporator under vacuum at 35°C and brought up to 5 mL in acetone for GC-NPD analysis.

Results and Discussion

Residue concentration and half-life of oxaziclomefone were calculated by the first-order kinetics equations, $C_t = C_0 e^{-kt}$ and $t_{1/2} = \ln 2/k$, respectively. The variables are defined as follows: C_t denotes the concentration of the pesticide residue at time (t), C_0 denotes the initial concentration, k is the rate constant, and $t_{1/2}$ is the half-life (Li et al. 2008).

Recoveries were determined at three levels of fortification (Table 1). The mean recoveries from six replicates of fortified samples for each matrix ranged from 84.4 to 108.2% with relative standard deviations (RSDs) of 2.4 to 17.2%, which is within the acceptable limits for routine analysis of oxaziclomefone residues. Samples were quantified using external standards, with a positive linear range of 0.002–2.0 mg/L ($y = 32.532x + 0.4084$, $R^2 = 0.9997$). Limits of quantification (LOQ), defined as the minimum fortified level of recovery, were 0.01 mg/kg in soil, water, rice plants, and husked rice and 0.02 mg/kg in rice hull. The limit of detection (LOD) was 0.0005 mg/kg in water and 0.002 mg/kg in soil, rice plants, husked rice and rice hull at a signal-to-noise ratio of 3.

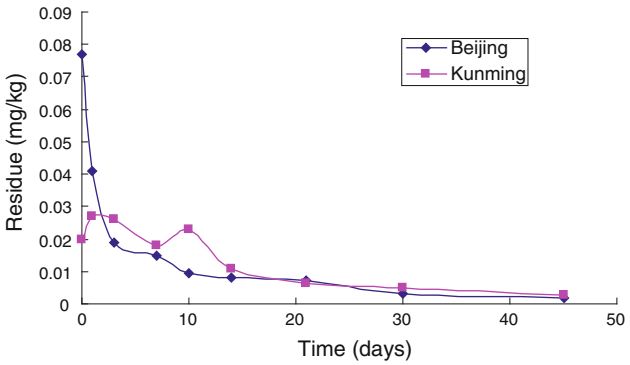

Fig. 2 Dissipation of oxaziclomefone residues in water samples under natural field conditions in Beijing and Kunming

Figure 2 shows the dissipation curve of oxaziclomefone in water under field conditions. The initial concentrations in the Beijing and Kunming samples were 0.078 and 0.027 mg/L with half-lives of 9.6 and 13 days, respectively. As shown in the figure, the decline in oxaziclomefone concentration was gradual and continuous after application. Concentrations were reduced by more than 90% 14 days after application in Beijing and 45 days in Kunming. Half-life ($t_{1/2}$) and other statistical parameters of dissipation were calculated from the experimental data and are summarized in Table 2.

Figure 3 shows the dissipation curve of oxaziclomefone in rice plants under field conditions. The initial concentrations in the Beijing and Kunming samples were 7.73 and 3.0 mg/kg at high concentration with half-lives of 4.4 and 4.4 days, respectively. There was a steady decrease in residue content, and, by day 30, concentrations had dropped to 0.088 and 0.032 mg/kg. Fourteen days after application, oxaziclomefone was more than 90% degraded in the plant samples at both locations. Half-life ($t_{1/2}$) and other statistical parameters of dissipation were calculated from the experimental data and are summarized in Table 2.

Figure 4 shows the dissipation curve of oxaziclomefone in the soil under field conditions. Initial concentrations were 1.22 and 1.41 mg/kg in Beijing and Kunming with half-lives of 36.9 and 37.1 days, respectively. The dissipation rate was slower than in water and rice plants, with

Table 1 Average recoveries and relative standard deviation (± RSD) of fortified samples

Fortification level (mg/kg)	Soil (%)	Water (%)	Rice plant (%)	Rice hull (%)	Husked rice (%)
0.01	105.2 ± 17.2	108.2 ± 5.3	87.1 ± 12.4	–	97.4 ± 2.8
0.02	–	–	–	92.7 ± 6.7	–
0.1	85.2 ± 4.4	90.5 ± 4.8	84.4 ± 4.0	97.5 ± 4.0	99.2 ± 4.1
1.0	91.0 ± 2.4	92.7 ± 4.9	87.1 ± 2.5	94.3 ± 2.9	91.1 ± 3.8

Values are the average of six replicates

Table 2 Half-life and other statistical parameters for oxaziclomefone dissipation in the rice field conditions

Matrix	Sample Location	Regression equation	Correlation coefficient (R^2)	Degradation rate constant, k, (days^{-1})	Half-life (days)
Water	Beijing	$Y = 0.0321e^{-0.0719x}$	0.86	0.0719	9.6
	Kunming	$Y = 0.0262e^{-0.0533x}$	0.93	0.0533	13
Rice plant	Beijing	$Y = 5.2397e^{-0.1584x}$	0.94	0.1584	4.4
	Kunming	$Y = 2.5421e^{-0.1559x}$	0.98	0.1559	4.4
Soil	Beijing	$Y = 0.925e^{-0.0188x}$	0.94	0.0188	36.9
	Kunming	$Y = 1.3654e^{-0.0187x}$	0.94	0.0187	37.1

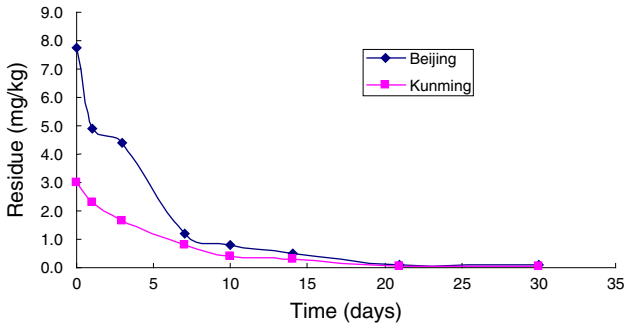

Fig. 3 Dissipation of oxaziclomefone residues in rice plant samples under natural field conditions in Beijing and Kunming

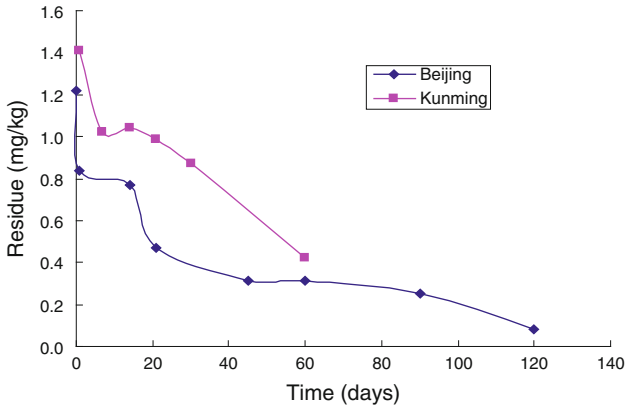

Fig. 4 Dissipation of oxaziclomefone residues in soil samples under natural field conditions in Beijing and Kunming

residues declining 93.3% after 120 days in Beijing and 70.2% after 60 days in Kunming. Half-life ($t_{1/2}$) and other statistical parameters of dissipation were calculated from the experimental data and are summarized in Table 2.

When oxaziclomefone was applied at the recommended dosage and 2 recommended levels over one application, terminal residue levels in rice plants, soil, rice hull and husked rice were evaluated over 2 consecutive years. Residues were detectable in only some of the soil samples at harvest and were all below 0.08 mg/kg. The residue contents in rice plants, rice hull and husked rice at harvest were well below the LOQ.

From these results, it is evident that the dissipation of oxaziclomefone in rice plants was faster than in paddy water and soil. The mean half-lives were 4.4, 11.3 and 37 days, respectively. These differences suggest that dilution due to plant growth may have played a significant role in the decline of oxaziclomefone in the different matrices, regardless of the effect of physical and chemical factors like light, heat, pH and moisture (Tewary et al. 2005). Concentrations in paddy water decreased rapidly, compared to their slow decline in soil. This observation is similar to that reported by Kawata et al. (2005). A longer persistence under field conditions can be attributed to oxaziclomefone's low water solubility and high log p value.

While the FAO/WHO has not established maximum residue limits (MRLs) for oxaziclomefone, Japan and Korea's MRL for oxaziclomefone in rice is 0.1 mg/kg. At the time of harvest, no residue was detectable in the rice hull, husked rice and straw final products, and the highest residue in soil at this time was still below 0.08 mg/kg. These results suggest that it is safe to use oxaziclomefone on crops according to the recommended dosages without posing significant harm to humans.

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