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Dissipation and Residues of *N*-(2-Bromophenyl)-2-(4,6-Dimethoxypyrimidin-2-Yloxy)Benzylamine Residues in Rape and Soil Under Field Conditions

Dan Liu · Dongmei Qin · Ran Ji

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Abstract *N*-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamine, compound A, is a highly active herbicide. Field trials indicate that it controls major weeds with a good tolerance on rape by postemergence application. In the present study, it was studied the dissipation behavior of compound A residues in rapes and soils. Analyses were carried out using high performance-liquid chromatography with diode array detection. The limits of detections (LOD) of compound A in rape plant and rape seed samples were 0.03 mg kg^{-1} and its in soil was 0.02 mg kg^{-1}. At three different spiking levels mean recoveries and relative standard deviation (RSD) from spiked samples in five replicated experiment for each matrix were in the range 87.3%–96.4% and 2.9%–8.7%, respectively. The dissipation rate was found to vary with the nature of the studied sample. In soil, half-live were 33 and 25 days in Yunnan and Zhejiang, respectively, while in rape, it was 7.2 and 8.5 days. The residual amounts of compound A in rape and soil samples were below LOD after the recommended dosage and two times dosage. According to the calculation of the ADI of compound A and other data, the result suggest that the MRL for compound A in rape is 1 mg kg^{-1}.

Keywords *N*-(2-Bromophenyl)-2-(4,6-dimethoxy pyrimidin-2-yloxy)benzylamine · Pesticide · Rape · Soil · HPLC–DAD

D. Liu (✉)
Department of Applied Chemistry, College of Science, China Agricultural University, Beijing 100193, China
e-mail: dliu@cau.edu.cn; ldjiaf@163.com

D. Qin
Institute for the Control of Agrochemicals, Ministry of Agriculture, Beijing 100026, China

R. Ji
Beijing Research Institute of Chemical Industry, SINOPEC, Beijing 100013, China

N-(2-Bromophenyl)-2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamine, compound A (Fig. 1), is a highly active herbicide, which belongs to a novel class of chemistry. Its herbicidal activity is assessed under greenhouse conditions. It is effective against many grass weed species, as well as broadleaf weeds, under greenhouse conditions. Field trials indicate that it controls major weeds with a good tolerance on rape by postemergence application (Wu et al. 2006; He et al. 2005). Their study mainly describes a synthesis procedure for compound A, characterize its herbicidal activity, and evaluate its toxicological level. Because compound A was a new selective postemergent herbicide for weed control, there were few relative reports about compound A at present. To the best of our knowledge, Xia (2007) studied quantitative analysis of herbicide compound A by HPLC, which was a macro analysis. No analysis method and data have been published on the determination of compound A residues in rape.

The present work was carried out to establish the simple, efficient analytical method for determination of compound A residues in rape and soil samples. The maximum residue limit (MRL) of compound A was established, as 1 mg kg^{-1} in rape. Compound A was applied in this crop to evaluate its dissipation behavior and residue levels under field conditions, and afford evidence for registration in China.

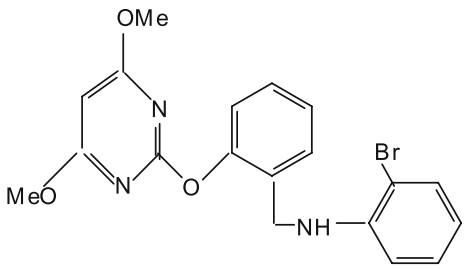

Fig. 1 The structure of compound A

Materials and Methods

An analytical standard (99.97%) and commercial grade (20% Suspending Agent) of compound A were supplied by Zhejiang Chemical Industry Research Institute. Acetonitrile, methanol, dichloromethane, acetone, petroleum ether, hexane, sodium sulfate anhydrous, sodium chloride were analytical grade reagents (Beijing Chemical Reagents company, China). Silica gel (80–100 mesh) was purchased from Qing dao Hai yang chemical Co., Ltd and heated at 140°C for 4 h and added with 5% water (W:V) before use.

Compound A was determined by a Waters Alliance 2695 with photodiode array detector 2996, attached with a Waters Empower ChemStation for data acquisition. The capillary column was Agilent Zorbax SB-C18 (250 mm length, 4.6 mm i.d). The mobile phase was Methanol:water (85:15, v/v) with 0.80 mL/min flow rate. The column temperature was 30°C. The slitwidth was 1.2 nm. Detection was at 246 nm with an injection volume of 20 μL. The approximate retention time of compound A in the upper instrument conditions was 9.8 min.

The field trials were carried out in Zhejiang Hangzhou and Yunnan Kunming including the dissipation experiment and the final residue experiment. Each experiment field consisted of three replicate plots with an area of 30 m^2 (5 × 6 m) and maintained 1 m distance between the plots. Another three untreated plots were sprayed with water without any herbicides, were maintained as controls.

In order to study the dissipation trends of compound A in rape residues, it was applied at 120 g.a.i./ha (grams of active ingredient (ai) per hectare; two times recommended dosage). The applied dose in soil with no herbicide was 1,000 g.a.i./ha. Compound A was dissolved in water and sprayed in the growing rape and soil using a knapsack sprayer. The ultimate residue experiment was designed as two dosages. The applied doses were 60 g.a.i.ha^{-1} (recommended dosage) and 120 g.a.i./ha (2 times recommended dosage). After the rape had reached the 4-leaf stage, the spraying treatment was conducted at different dosages by diluting the formulation of Compound A with water.

For studying the dissipation of compound A in rape, samples were collected randomly at 0, 1, 3, 6, 10, 14, 21, 30, 45 and 60 days after the application. Soil samples were collected from each plot using a soil sampler from the surface to a depth of 15 cm. The intervals of collecting soil were 0, 1, 3, 6, 10, 14, 21, 30, 45, 60, 90 and 120 days in Zhejiang Hangzhou and Yunnan Kunming. At harvest time, from each ultimate residue field, 500 g rape and soil samples were collected randomly for residue analysis. The samples blank were collected, at time. The rape samples were homogenized and 200 g were taken in plastic boxes. Little stones and other unwanted materials were removed from soil samples (500 g) and then dried at room temperature and screened through 40-mesh sieves. All collected samples were stored in a deep freezer at −20°C until analysis.

Extraction Method in Rape Sample was Different from that in Soil Sample

A 10 g rape sample was extracted with an amount of 80 mL acetonitrile with mechanical shaking for 30 min. Extracts were transferred to a 500 mL separating funnel, the flasks were washed twice with 20 mL acetonitrile. The volume of acetonitrile was 100 mL. Then the sample solution was extracted by liquid–liquid partition with petroleum ether twice with 2 × 32 mL. The volume rate of acetonitrile and petroleum ether was 15:4. The petroleum ether portion was discarded, the acetonitrile portion was combined and filtered through the funnel with anhydrous sodium sulphate and evaporated to near dryness in a rotary evaporator at 40°C and then dried with a weak nitrogen stream without disturbing the surface of the solution. The residue was dissolved in about 2 mL of dichloromethane/Hexane/Methanol (20:80:1, v/v/v) for further clean-up.

A 20 g soil sample was extracted with an amount of 100 mL actone/water (4:1, v/v) with mechanical shaking for 30 min. Extracted was filtered through a Buchner funnel. The filter residue and the flask were washed twice with 2 × 20 mL acetone. The mixtures were combined and evaporated with the vacuum rotary evaporator at 40°C in order to remove acetone. These concentrated extract was transferred into a 250 mL separating funnel, 100 mL 10% NaCl (w/v)solution was added and then the sample solution was extracted with dichloromethane (30 mL × 2). The organic portions were combined and filtered through the funnel with anhydrous sodium sulphate and evaporated to near dryness. The residue was dissolved in about 2 mL of dichloromethane/Hexane/Methanol (20:80:1, v/v/v) for further clean-up.

A glass column (400 × 6 mm i.d.), which was packed with a plug of glass wool and 2 g silica-gel of 5% water (w/v) between two layers of 1 cm of anhydrous sodium

sulphate, was prewashed with 10 mL dichloromethane/Hexane/Methanol (20:80:1, v/v/v) in order to remove impurity of silica-gel. The concentrated extract was transferred to this column and eluted with 45 mL petroleum ether/ethyl acetate (4:1, v/v). The first 15 mL of eluate was discarded. The remaining eluate was collected and evaporated under vacuum at 40°C to dryness and made up to 2 mL in methanol for quantitative analysis by HPLC–DAD.

Results and Discussion

The linearity, the recovery and the limits of detection (LOD) of Compound A determination-method validation were carried out.

For most chromatographic procedures a linear relation could be observed between detector response (y) and analyte concentration (x). The linear relationship for compound A could be expressed as a linear regression equation: $y = 3{,}525.4x - 257.93$, $R = 0.9997$, where y = peak area, x = compound A concentration (ng), and R = correlation coefficient. Good linearity was achieved in methanol at the range of 2–40 ng.

The efficiency of the residue analytical method has been evaluated by spiking rapeseed, soil and rape samples five replications at different spike levels (0.03, 0.2, 2.0 mg kg^{-1}; Table 1). The fortified recoveries of compound A in samples ranged from 82.5% to 103.3%. The relative standard deviation (RSD) ranged from 2.9% to 8.7%. The recovery and accuracy of the results were acceptable according to Guideline on pesticide residue trials issued by the Ministry of Agriculture of the People's Republic of China, 2004.

The LOD were determined at a value 3 times the background noise obtained for blank samples, whereas the limits of quantification (LOQ) were determined at a value 10 times the background noise. The LODs of compound A in rape and rape seed samples were 0.03 mg kg^{-1}. In soil it was 0.02 mg kg^{-1}.

The results of dissipation data of compound A in rape in Zhejing hangzhou and Yunnan Kunming were showed in Fig. 2. As shown in the figure, compound A dissipated with gradual and continuous deterioration after application. The dissipation trend of Compound A in rapes followed first order kinetics. The half-lives were 7.2 and 8.5 days in Kunming and in Hangzhou.

Figure 3 showed the dissipation for compound A in the soil samples. The initial concentration of compound A in

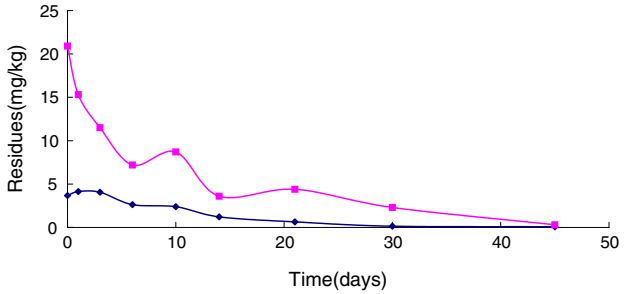

Fig. 2 Dissipation curve of compound in rape in Hangzhou and Kunming: *top line*, Zhejiang Hangzhou; *bottom line*, Yunnan Kunming

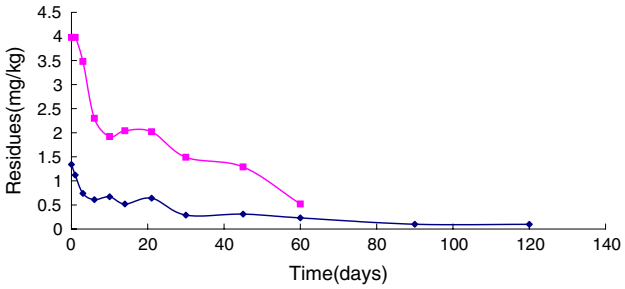

Fig. 3 Dissipation curve of compound in soil in Hangzhou and Kunming: *top line*, Zhejiang; *bottom line*, Yunnan

Table 1 Recovery and RSD of compound A in samples spiked at different levels

Sample	Spiked levels mg/kg	Recovery, %						RSD %
		1	2	3	4	5	Average	
Rape seed	0.03	92.3	95.7	91.4	84.6	101.5	93.1	6.6
	0.2	98.9	90.2	86.4	94.4	92.9	92.6	5.0
	2.0	89.2	85.5	86.5	90.1	92.7	88.8	3.2
Soil	0.02	99.5	96.2	102.1	88.0	82.6	93.7	8.7
	0.2	82.5	94.5	86.8	90.9	97.3	90.4	6.5
	2.0	84.5	86.5	87.6	85.7	92.2	87.3	3.4
Rape plant	0.03	89.9	103.3	94.3	97.3	92.6	95.5	5.4
	0.2	100.3	96.6	98.9	92.4	94.0	96.4	3.4
	2.0	92.5	93.9	94.3	95.1	88.3	92.8	2.9

Table 2 Half life and other statistical parameters for compound A dissipation in different samples under field conditions

Sample	Locality	Regression equation	Determination Coefficient (R^2)	Half-life $t_{1/2}$ (days)
Rape	Yunnan Kunming	$\ln C_t = -0.0958t + 4.61$	0.9684	7.2
	Zhejiang Hangzhou	$\ln C_t = -0.0817t + 16.736$	0.9347	8.5
Soil	Yunnan Kunming	$\ln C_t = -0.021t + 0.8448$	0.9018	33
	Zhejiang Hangzhou	$\ln C_t = -0.0275t + 3.2957$	0.8913	25

soil was lower than in rape. Dissipation time of compound A in soil was slower than in the rape. The dissipation trend of Compound A in soils followed first order kinetics. Half-lives ($t1/2$) and other statistical parameters of Compound A residue dissipation in rape were calculated from the experimental data in Table 2. The half-lives were 33 and 25 days in Kunming and in Hangzhou. Compound A had lower degradation rates in the Yunnan soil than in the Zhejiang soil. The behavior of pesticides in soils is governed by a variety of complex dynamic physical, chemical and biological processes, including sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off, and leaching. These processes directly control the transport of pesticides within the soil. The relative importance of these processes varies with the chemical nature of the pesticides and the properties of the soil (Arias-Estévez et al. 2008). In this study, different soil type, pH and organic matter content etc. in Zhejiang and Yunnan get different half lives of compound A in soils. These between-site differences suggest that local soil characteristics and climate affect the dissipation of compound A.

The residue content of compound A in rapeseeds and soils at the harvests was below the LOD (0.03 mg/kg) following the recommended dosage and two times dosage in Zhejiang and Yunnan.

The MRL for Compound A in rape. The World Health Organization (WHO), Food and Agricultural Organization (FAO) or other governmental agencies have not established available MRL for Compound A in rape. An MRL is the maximum concentration of residue following administration of a veterinary medicine which is legally permitted or acceptable in food. The calculation of the MRL value is based on the acceptable daily intake (ADI) for the drug in question. The calculation of the ADI includes an extremely large safety factor. In addition, the MRL calculation assumes an average intake per person of total dietary daily.

According to 90 days toxicity tests of compound A in Zhejiang Chemical Industry Research Academy, the NOAEL of compound A in male rats was 100 mg/kg day and in female rats was 100 mg/kg day. The NOAEL of compound A was 100 mg/kg day. Safety factor was 100. The calculation of the ADI from the NOAEL of compound A and Safety factor was 1.0 mg/kg day. In China, the human body standard weight is 60 kg, an average intake per person is 1.13 kg total dietary daily, the intake of edible oils accounts for 2.21% of the total dietary intake. Rape seed oil yield rate was 50%. From the animal to the human, a safety factor was 1,000. The calculation of the final MRL for compound A in rape was 1 mg kg^{-1} from the upper data. Suggesting that the MRL for compound A in rape was 1 mg kg^{-1}.

Therefore, compound A could be considered as safe at the recommend dosage in the rape crop. Compound A is a safe herbicide with regard to human health.

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References

Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara J, Mejuto J-C, García-Río L (2008) The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric Ecosyst Environ 123:247–260. doi:10.1016/j.agee.2007.07.011

He Q, Wu J, Chen J (2005) Study on synthesis and biological activity of the new herbicide ZJ0777. J Zhejiang Univ Sci Ed 32:567–569

Wu J, Cheng J, Lu L (2006) *N*-(2-Bromophenyl)-2-(4, 6-dimethoxypyrimidin-2-yloxy)benzylamine, a new selective postemergent herbicide for weed control in winter oilseed rape. J Agric Food Chem 54:5954–5957. doi:10.1021/jf061063p

Xia Y (2007) Quantitative analysis of herbicide ZJ0777 by HPLC. Pestic Sci Admin 25:5–7