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Typical Azole Biocides in Biosolid-Amended Soils and Plants Following Biosolid Applications

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Supporting Information

ABSTRACT: Biosolid application on agricultural land may contaminate soils with various household chemicals and personal care products. This study investigated the occurrence and dissipation of typical azole biocides climbazole, clotrimazole, and miconazole in biosolid-amended soils as well as the uptake of these biocides by plants. The field trial includes two treatment groups: old groups with biosolid application at rates of 5, 10, 20, and 40 t/ha every year within 5 years, and new groups with only one biosolid application. The results showed that climbazole, clotrimazole, and miconazole were detected in biosolid-amended soils, but not detected in control soils. These biocides were not found in the crop plants collected from the trial plots. The dissipation half-lives for climbazole, clotrimazole, and miconazole under the field conditions were 175−179, 244, and 130−248 days, respectively. High biosolid application rates and repeated biosolid applications could lead to higher persistence of the biocides in the agricultural soils. An exposure model could effectively predict the residual concentrations of climbazole and miconazole in the biosolid-amended soils of the old treatments with different biosolid application rates. Thus, the field trial demonstrated high persistence of these three biocides in the soil environments.

KEYWORDS: *biocides, sludge, biosolid application, soil, dissipation, plant*

INTRODUCTION

In recent years, azole biocides such as climbazole, clotrimazole, and miconazole have received increasing attention as emerging contaminants because they are widely used as active ingredients in pharmaceutical and personal care products (PPCPs) that treat fungal infections in humans.[1] The biological activity of azole biocides is based on their inhibition of cytochrome P450-dependent 14α-demethylase (encoded by the CYP51 gene) of steroidogenesis.[2] These azole biocides are usually administrated topically and orally. Removal from the skin by washing and through urinary excretion after application are probably the major entry pathways of azole biocides to wastewater. These azole biocides further reach the receiving environment primarily through discharge of effluent and disposal of sewage sludge due to incomplete removal during wastewater treatment.[3]

The occurrence of azole biocides has been reported in surface water, effluent, and sludge. For example, clotrimazole has been ubiquitously detected at concentrations of 3−34 ng/L in surface water in many countries including the United Kingdom, China, Germany, and Scotland.[4−7] The maximum concentrations of clotrimazole in wastewater treatment plant (WWTP) effluent and sludge were found up to 8650 ng/L and 2547 ng/g, respectively.[3,8] Climbazole and miconazole have been detected with maximum concentrations of 530 and 8 ng/L in surface water, 443 and 36 ng/L in WWTP effluent, and 1160 and 2069 ng/g in activated sludge.[3,9−11] The presence of biocides in the environment may cause potential adverse effects on nontarget organisms. Azole biocides can inhibit not only CYP51 but also other cytochrome P450 enzymes including aromatase (encoded by CYP19 gene).[2,12] It is reported that clotrimazole and miconazole can affect the aromatase in rainbow trout, *Xenopus tropicalis* frogs, and humans.[13−15] Therefore, it is essential to understand the distribution and fate of these biocides in the receiving environment.

Biosolid application on agricultural land is one of the pathways for these biocides to the environment. In some countries such as Australia, the United Kingdom, the United States, and Canada, sewage sludge (biosolid) with nutrient-rich organic materials is applied as fertilizer (biosolid) in agricultural fields to improve productive soils and stimulate plant growth.[16−19] However, biosolid application on agricultural land is still not allowed in China considering various contaminants in the biosolid.[17,20−24] Therefore, the Chinese Ministry of Agriculture started field trials of biosolid application in 2006 to address the concerns associated with biosolid application on agricultural land. Originally the field trial focused on inorganic contaminants,[25] but later organic contaminants including azole biocides in biosolid-amended soils were also assessed in the trial. Previous studies showed dissipation half-lives (DT_{50}) of miconazole of >300 days calculated from field tests, suggesting its highly persistent nature.[21,24] The fate of azole biocides such as clotrimazole was more persistent at low temperature (4 °C) in dry soils (moisture content = 4.5%) and in a loam under laboratory conditions.[26,27] However, the

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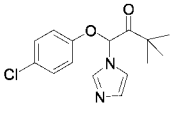
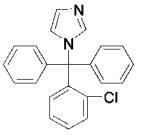
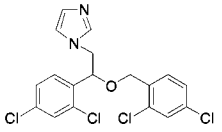
Table 1. Physicochemical Properties of the Target Biocides Investigated in This Study[#]

Compound	Climbazole	Clotrimazole	Miconazole
Structure			
Formula	$C_{15}H_{17}ClN_2$	$C_{22}H_{17}ClN_2$	$C_{18}H_{14}Cl_4N_2O$
CAS number	38083-17-9	23593-75-1	22916-47-8
Molecular weight	292.8	344.8	416.1
Water solubility (mg/L at 25 °C)	8.281 [a]	0.03 [a]	0.011 [a]
Log K_{ow}	3.76 [a]	4.1/6.26 [b]	6.25 [a]
pKa	7.5 [c]	6.12 [b]	6.65 [d]
Log K_{oc}	3.655 [a]	4.786 [a]	4.834 [a]

[#] [a]The log K_{ow} values were calculated by EPI suite model.[29] [b] Data from OSPAR.[30] [c] Calculated by ALOGPS 2.1.[31] [d] Data from the literature.[32]

Table 2. Information of the Field Trial Sites and Treatments

treatment[a]	pH[b]	TOC[b,c] (%)	clay[b] (<0.002 mm) (%)	biosolid application (t/ha)	urea application (t/ha)
Old Group, First Application October 5, 2006					
CK1	7.7 ± 0.1	0.7 ± 0.0	23.6 ± 11.3	0	0
CK2	7.5 ± 0.1	0.7 ± 0.0	16.5 ± 3.6	0	5
OT1	7.7 ± 0.0	0.8 ± 0.1	23.3 ± 2.3	5, every year	5
OT2	7.7 ± 0.1	0.8 ± 0.1	30.9 ± 2.4	10, every year	5
OT3	7.7 ± 0.1	0.9 ± 0.1	28.6 ± 2.8	20, every year	5
OT4	7.6 ± 0.1	1.3 ± 0.2	28.2 ± 0.2	40, every year	5
New Group, First Application October 5, 2010					
CK3	7.7 ± 0.0	0.8 ± 0.1	31.8 ± 14.2	0	0
NT2	7.6 ± 0.1	0.8 ± 0.1	19.8 ± 3.0	10, once	0
NT3	7.6 ± 0.0	1.0 ± 0.1	20.7 ± 2.4	20, once	0
NT4	7.7 ± 0.1	0.7 ± 0.1	17.9 ± 0.9	40, once	0

[a]The field trial includes two treatment groups, the old group having six treatments including controls (CK1 and CK2) and treatments (OT1, OT2, OT3, and OT4) and the new group having four treatments including control (CK3) and treatments (NT2, NT3, and NT4). [b]Mean ± standard deviation (%) (n = 3 for OT and n = 2 for NT). All pH, TOC, and clay content values were detected in the samples collected in October 2010. [c]TOC, total organic carbon content.

dissipation behavior of organic contaminants under laboratory conditions can be very different from that under field conditions.[17] Uptakes of organic contaminants by plants and bioaccumulation in earthworms from soils following biosolid application have been demonstrated,[22,28] but studies on the dissipation behavior and uptake of these azole biocides by grain crops under field conditions are still very limited.[21]

This study aimed to investigate the occurrence and fate of three typical azole biocides, climbazole, clotrimazole, and miconazole, in biosolid-amended soils under field conditions. The field trials were performed in Shandong, China, with two different treatment groups: repeated biosolid application every year (old group, OT) and fresh biosolid application once a year (new group, NT). The field monitoring data were applied to construct an exposure modeling to predict the residual concentrations of the target compounds in the soils after repeated application of biosolid at various application rates. Following biosolid application at different rates, soils and grain crops grown in the treated plots as well as control plots with no biosolid application (CK) were collected for the assessment of contamination and dissipation of these three biocides.

MATERIALS AND METHODS

Chemicals and Materials. Chemical standards of climbazole, clotrimazole, and imazalil-D5 were obtained from Dr. Ehrenstorfer GmbH (Germany). Miconazole and clotrimazole-D5 were purchased from United States Pharmacopeia (USA) and Toronto Research Chemicals (Canada), respectively. The physicochemical properties of the three target biocides are shown in Table 1. All of the organic solvents were of HPLC grade and available from Merck Corp. (China), CNW Technologies (Germany), and Teida Co. (USA). HPLC grade water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Oasis HLB cartridges (200 mg, 6 mL) were supplied by Water Corp. (Milford, MA, USA). Individual stock solutions of the target compounds and internal standards were prepared at 100 mg/L in methanol and stored in amber glass bottles at −18 °C prior to use in the preparation of working solutions.

Field Trials. Field trials of biosolid application on agricultural land were carried out in Shandong, China. The biosolid applied at the Shandong site was dewatered sludge from a WWTP in Beijing and collected in May 2006. Meanwhile, the dried biosolid was stockpiled in a warehouse before use, and the same well-mixed biosolid was always applied in each treatment mentioned in this study. Biosolid samples were collected every year and stored in a refrigerator for chemical analysis. Field trial setup includes two treatment groups: old group and new group (Table 2). The old treatment group includes six

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treatments: control with no biosolid application (CK1), control with urea at a rate of 5 t/ha (CK2), and treatments (OT1, OT2, OT3, and OT4) with biosolid application rates of 5, 10, 20, and 40 t/ha and with the same urea application rate of 5 t/ha every year. Each old treatment had three replicate plots (8 × 5 m, each). For the old group, the biosolid was first applied on October 5, 2006, and then reapplied with the same rates on October 5 every year for 5 years. The new treatment group includes four treatments: control with no biosolid application (CK3) and treatment (NT2, NT3, and NT4) with one biosolid application at a rate of 10, 20, and 40 t/ha, respectively, on October 5, 2010. In each treated plot, the biosolid was spread randomly over the fields and then mixed well using a hoe with the soil of 0−20 cm depth immediately following application. During the trials, the crops including wheat (October−June) and corn (June−September) were planted in both old and new treatment plots.

The field trials started in October 2006, but the sampling campaign for organic contaminants was conducted only from the beginning of October 2010 to October 2011. Initial field trials paid attention to inorganic contaminants in the biosolid-amended soils.[25] Soil samples were collected in 1 L glass jars from each field plot at the depth of 0−20 cm from five points in each plot and then combined into one composite sample. First sampling took place at the Shandong site on October 5, 2010, before the reapplication of biosolid for the old group and after the first application of biosolid for the new group, respectively. Moreover, the soil samples were sampled consecutively on the fifth of every month until October 2011. However, due to the frost period in Shandong, no soil samples were collected in January and February 2011. The collected soil samples and biosolid samples were freeze-dried, then sieved through a 0.90 mm mesh standard screen, and then stored in the dark at 4 °C prior to extraction. Plant samples were collected from each new treatment plot during harvesting periods in June 2011 for wheat and September 2011 for corn. Wheat plant samples were divided into wheat and wheat stalk, whereas corn plant samples were separated into three parts: corn, corn stalk, and corn cob. The collected plant samples were air-dried, then cut into pieces, and stored in the dark at 4 °C before extraction.

Site information including soil properties and application rates is given in Table 2. The soil type and soil texture was fluvo-aquic soil and clay loam, with a field moisture capacity of 23%. The average annual temperature was 12.9 °C, whereas the average annual rainfall was 522 mm. Soil pH was determined with 0.01 M $CaCl_2$ (soil to solution ratio of 1:5) using a pH meter, the total organic carbon content (TOC) of soil was measured by a LECO carbon and nitrogen analyzer, and soil particle size distribution was analyzed by using the pipet method.[33]

Chemical Analysis. Our previous method for biocides was adopted for the extraction of the three target compounds in solid samples by ultrasonic extraction and instrumental analysis by ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS).[34] Each lyophilized and homogenized solid sample (2 g for soil, 0.5 g for biosolid sample, and 2 g for plant sample) was weighed into a 30 mL glass tube, followed by addition of 100 μL of 1 mg/L mixed internal standard solutions (clotrimazole-D5 and imazalil-D5). Then the samples were mixed well and stored in a cold room (4 °C) overnight. Ten milliliters of methanol was added into each sample. Then the samples were mixed by a vortex mixer for 30 s, extracted in an ultrasonic bath for 15 min, and centrifuged at 2800 rpm for 10 min. The clear supernatant from each sample was collected into a 250 mL flat-bottom flask by a glass pipet. The extraction procedure was repeated twice using 10 mL of methanol and then 10 mL of methanol/0.1% (v/v) formic acid in Milli-Q water (5:5, v/v) as the extraction solvent, respectively. The supernatants for each sample were combined and diluted with Milli-Q water to a volume of 300 mL.

A cleanup step with solid-phase extraction (SPE) cartridges was used to purify the aqueous solid extracts. Prior to the SPE cleanup, 4 M H_2SO_4 was used to adjust the pH value of each aqueous extract to 3. Each Oasis HLB cartridge (200 mg, 6 mL) was preconditioned successively with 10 mL of methanol and 10 mL of Milli-Q water before use. The aqueous extract was loaded onto the cartridge at a flow rate of 5−10 mL/min. Each sample bottle was rinsed twice with two aliquots of 50 mL of 5% (v/v) methanol in Milli-Q water, followed by passing through the cartridge after sample loading. The cartridge was dried under vacuum for 3 h, and the target compounds were eluted with 3 × 4 mL of ethyl acetate. The eluate was dried under a gentle nitrogen stream, redissolved in 1 mL of methanol, then filtered through a 0.22 μm membrane filter (Anpel, Shanghai, China) into a 2 mL amber glass vial (Agilent, Santa Clara, CA, USA), and stored at −18 °C until UHPLC-MS/MS analysis. Prior to the UHPLC-MS/MS analysis, a 100 μL aliquot of each sample extract solution was dried and reconstituted in a mixed solvent (50% methanol in Milli-Q water, v/v).

The target compounds were determined using an Agilent 1200 series ultrahigh-performance liquid chromatography (Agilent) coupled to an Agilent 6460 triple-quadrupole mass spectrometry with electrospray ionization (ESI) in positive ionization mode (UHPLC-MS/MS). A Zorbax SB-C18 (100 mm × 3 mm, 1.8 μm particle size) column was used as the chromatographic column, with its corresponding precolumn filter (2.1 mm, 0.2 μm) from Agilent Technologies for chromatographic separation of these target compounds. Detailed information about LC operating parameters can be found in Table S1 of the Supporting Information. The source parameters and the mass spectrometric operating parameters including fragmentor voltage, collision energy (CE), precursor ion, and product ions for each compound can be found in Tables S1 and S2 of the Supporting Information. Quantification of the target compounds was performed in multiple reaction monitoring (MRM) mode. The identification of the target compounds was based on their retention times (within 2%) and the ratios of the two selected precursor−product ion transitions (within 20%) in comparison with the corresponding standards.

Recovery tests of the target compounds were conducted by spiking known concentrations of the target standards (40, 100, and 200 ng/g for biosolids, 20, 50, and 100 ng/g for soils and 50 ng/g for plants) into biosolid, soil, and wheat samples in three replicates. The analytical method for the three biocides showed satisfactory performance with their recoveries of 79.9−102, 73.4−103, and 60.7−123% from the biosolids, soils, and plants, as shown in Table S3 of the Supporting Information. The limits of detection (LOD) and quantitation (LOQ) were defined as 3 and 10 times the signal-to-noise (S/N) ratio under the lowest spiked concentration in those biosolid and soil samples. The LOQs of the target compounds were in the range of 0.13−0.38 ng/g for biosolid samples, 0.02 ng/g for soil samples, and 2.55−12.9 ng/g for plant samples, respectively (Table S3).

All data obtained from the analysis were under strict quality control procedures. For each batch of samples to be analyzed, a solvent blank, a standard solution (100 μg/L), and a method blank were run in sequence to check for background contamination and instrument performance.

Data Analysis. Measured concentration data for the three biocides in soils were expressed by mean (ng/g) ± standard deviation (n = 3, replicate samples at the same time). A one-way ANOVA and paired samples statistics were performed to determine significant differences ($p < 0.05$) between the concentration data of the three biocides in different treatments. Prior to all nonlinear regression fitting, the concentration data from the field trials were converted to a ratio of the initial concentration (C/C_0). C_0 represented the average concentrations of each biocide in the biosolid-amended soils included soil and biosolid in October 2010. Nonlinear regressions were used to determine the dissipation patterns of each compound. A standard first-order exponential decay model with two parameters was applied to fit the concentration data for determination of the dissipation patterns of each compound. The time to dissipate 50% of a chemical (DT50) was calculated on the basis of the first reaction kinetic. Linear regression analysis was performed to determine the relationships between the biocide concentrations and soil properties including clay content and TOC (%). Statistical analysis and dynamic curve fitting were conducted using the software SPSS 13.0 and Sigma Plot 10.0, respectively.

On the basis of the measured concentrations of biocides in the biosolid-amended soils, an exposure model was constructed to predict

residual concentrations of the target compounds in the soils for repeated biosolid application, with detailed equations giving as follows.

The total biosolid application amounts and the reciprocals of the estimated dissipation rate constant values of each compound were fitted to obtain a first-order exponential growth model with two parameters. Then an exposure modeling approach was derived from the biosolid application rate and application time to predict residual concentrations of target compounds in the biosolid-amended soils. The concentration data from March to October were used in the prediction, as dissipation occurred only in this time interval. The concentration data of NT from March 2011 and October 2011 were used as the first-year concentration data for prediction of OT. We assume that the degradation of azole biocides in the biosolid was negligible due to their persistence.[3,35] On the basis of the first-order exponential growth model, the dissipation rate constants of each compound with different application amounts of biosolid were calculated using eq 1

$$k_a = \frac{1}{b \times e^{(c \times a)}} \quad (1)$$

where k_a is the dissipation rate constant of biocide (month^{-1}) with different application amount of biosolid, a is the application amount of biosolid (t/ha), and b and c are the two parameters from the first-order exponential growth model.

The concentration ratio of each compound after 7 months (March−October) was determined by eqs 2 and eq 3

$$P_n = \frac{C_n^{\mathrm{Oct}}}{C_n^{\mathrm{March}}} = \frac{d \times e^{[-(m+7)\times k_a]}}{d \times e^{[-(m)\times k_a]}} = e^{(-7k_a)} \quad (2)$$

$$a = n \times f \quad (3)$$

where P_n is the concentration ratio of a biocide in biosolid-amended soils to which the biosolid had been applied n times and f is the application rate of biosolid per time (t/ha).

Equations 1, 2, and 3 may be combined to obtain the following equation (eq 4):

$$P_{(n,f)} = e^{-7/b \times e^{(c \times n \times f)}} \quad (4)$$

Exposure concentrations in March and October every year can be predicted on the basis of eqs 5 and 6 ($n \geq 3$)

$$C_{(n,f)}^{\mathrm{March}} = [(P_{(2,f)} \times P_{(3,f)} \times \cdots \times P_{(n-1,f)} + P_{(3,f)} \times \cdots \times P_{(n-1,f)} + \cdots + P_{(n-1,f)}) + 1] \times C_{(1,f)}^{\mathrm{March}} + (P_{(2,f)} \times P_{(3,f)} \times \cdots \times P_{(n-1,f)}) \times C_{(1,f)}^{\mathrm{Oct}} \quad (5)$$

$$C_{(n,f)}^{\mathrm{Oct}} = C_{(n,f)}^{\mathrm{March}} \times P_{(n,f)} \quad (6)$$

where $C_{(n,f)}^{\mathrm{March}}$ and $C_{(n,f)}^{\mathrm{Oct}}$ are the predicted concentrations in the soil after n times of biosolid application at the application rate of f in March and October.

RESULTS AND DISCUSSION

Occurrence of the Biocides in the Biosolid and Biosolid-Amended Soils. The three target compounds, climbazole, clotrimazole, and miconazole, were detected in the biosolid from Beijing WWTP at concentrations of 165 ± 6, 492 ± 21, and 427 ± 25 ng/g, respectively (three replicate samples). No significant losses were found during storage. Similar concentration ranges have been reported in dewatered sludge samples from other WWTPs. For example, the concentration of climbazole in a dewatered sludge sample from a Chinese WWTP was 152 ng/g;[34] clotrimazole were found at the concentrations in the range of 30−2547 ng/g, whereas miconazole was in the range of 150−2069 ng/g in dewatered sludge samples.[3,34,35] The accumulation of the three

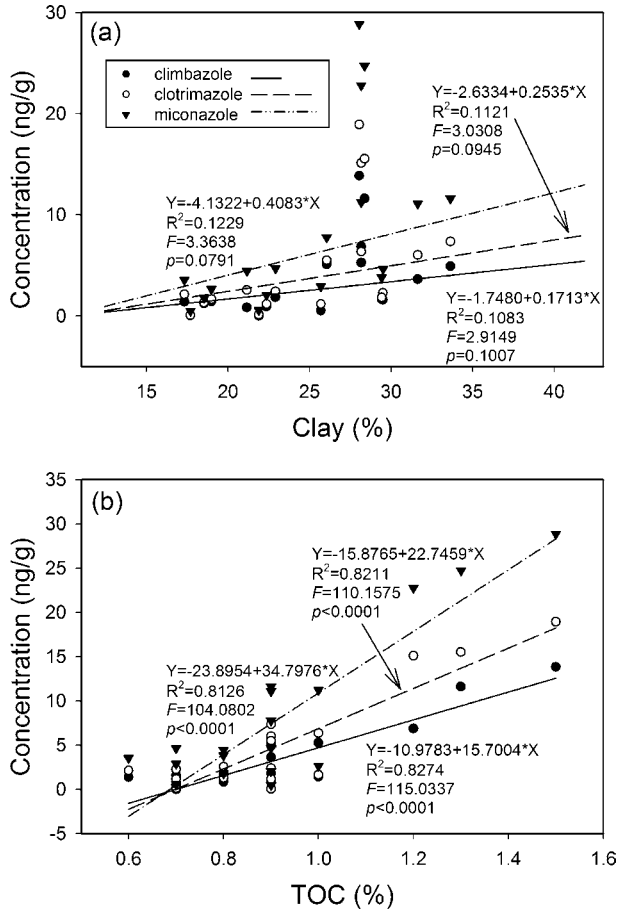

Figure 1. Correlation analysis between the concentrations of the three biocides and (a) the content (%) and (b) the total organic carbon content (TOC, %) of the biosolid-amended soils from both old and new treatments in October 2010 (n = 18).

azole biocides in biosolids could be attributed to their high log K_{ow} values (3.76−6.26) (Table 1). Because they are not easily digested and biotransformed in sewage sludge processes,[3,35] this also indicates that these azole biocides are very persistent in dewatered sludge.

Climbazole, clotrimazole, and miconazole were detected in all of the biosolid-amended soil samples collected from the treated plots (OT1, OT2, OT3, OT4, NT2, NT3, and NT4) at the Shandong site, but they were not found in the soil samples (CK1, CK2, and CK3) from the control plots without biosolid amendment (Tables S4 and S5 of the Supporting Information). For the old treatment group, the concentrations of climbazole, clotrimazole, and miconazole were 1.1−25.3, 1.6−39.0, and 1.9−46.3 ng/g with the following order: OT4 > OT3 > OT2 > OT1 > CK (CK1 or CK2) (significant difference, Duncan's multiple-range tests, $p < 0.05$) (Table S4). For the new treatment group, the concentrations of climbazole, clotrimazole, and miconazole were 0−12.7, 0.1−10.7, and 0.5−13.6 ng/g in the following order: NT4 > NT3 > NT2 > CK3. This is consistent with the biosolid application rates in both old and new treatments. Previous studies showed the presence of various PPCPs in agricultural soils with biosolid amendment.[17,22] Miconazole was found in biosolid-amended soils at concentrations of approximately 30−90 ng/g in the United States[24] and 150−340 ng/g in Canada.[21] Therefore, biosolid

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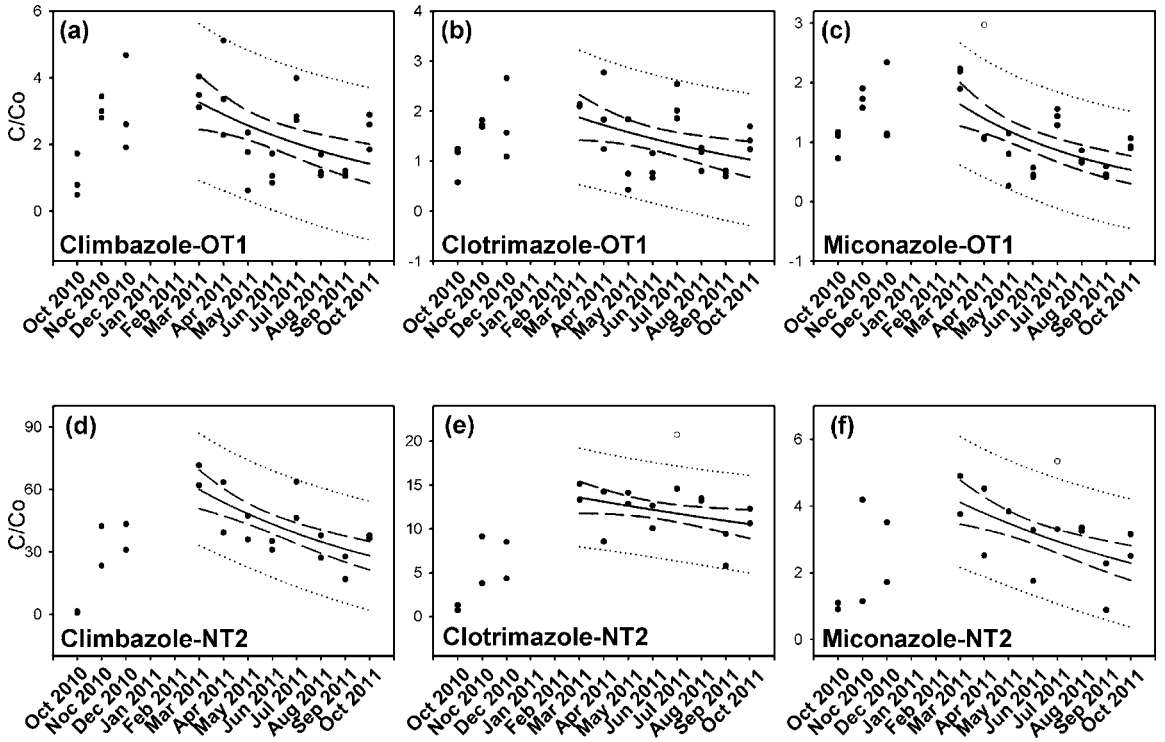

Figure 2. Field dissipation of climbazole, clotrimazole, and miconazole in the biosolid-amended soils within one year (October 2010−October 2011): (a) climbazole for OT1; (b) clotrimazole for OT1; (c) miconazole for OT1; (d) climbazole for NT2; (e) clotrimazole for NT2; (f) miconazole for NT2. All concentration data are normalized as a ratio of the concentration at each sampling time to the initial concentration (C/C_0). C_0 means the average concentrations of each biocide in the biosolid-amended soils in October 2010. Data points with empty symbols are treated as outliers during the first data fitting because the points are not included between the two 95% prediction bands. The nonlinear regression fits for the first-order model, 95% confidence band, and 95% prediction band are represented by the solid line, dashed line, and dotted line, respectively.

application on agricultural land is a pollution pathway for these azole biocides to the terrestrial environment.

Following application of biosolid on soil, the distribution of organic contaminants in soils would be influenced by soil properties such as clay content and TOC.[36,37] The present study showed significant correlations between the concentrations of the three biocides (climbazole, clotrimazole, and miconazole) and TOC in the biosolid-amended soils ($R^2 > 0.8$, $p < 0.05$) but no significant relationships with the clay contents ($R^2 < 0.2$ and $p > 0.05$) (Figure 1).

Field Dissipation of the Biocides in Soil. For both old and new treatment groups, big variations in the soil concentrations of the three biocides were observed for each treatment during the one year monitoring period (Tables S4 and S5), with the concentrations of each biocide increasing slightly from October 2010 to March 2011 (frost period) and then showing a decreasing trend from March 2011 to October 2011. This phenomenon has been observed before[38] and may also be partly due to the rapid carbon turnover, release of the three relatively hydrophobic biocides, or inhomogeneity of the collected field soil samples.[17] Therefore, nonlinear regression analysis was performed for the concentration data from March 2011 to October 2011 (Figure 2). The concentration data from March to October were fitted to the first-order kinetic model. Despite the poor fit, the modeling results were still acceptable considering the uncontrollable field conditions (Table 3). No significant dissipation was found for most treatments except for OT1 and NT2 with the lowest biosolid application rates showing significant dissipation ($p < 0.05$) (Table 3; Figures S1 and S2).

For OT1, the average concentrations during March 2011−October 2011 decreased from 3.8 to 2.6 ng/g for climbazole, from 4.3 to 3.0 ng/g for clotrimazole, and from 8.4 to 3.9 ng/g for miconazole, corresponding to losses of 32, 30, and 54%, respectively (Table S4). On the basis of the first-order model, the dissipation half-lives for climbazole, clotrimazole, and miconazole were 175 ± 64, 244 ± 117, and 130 ± 36 days, respectively (Table 3). Clotrimazole seemed slightly more persistent in the biosolid-amended soils than the other two biocides, climbazole and miconazole, as demonstrated by their half-lives.

For NT2, the average concentrations during March 2011−October 2011 decreased from 2.5 to 1.4 ng/g for climbazole, from 1.6 to 1.3 ng/g for clotrimazole, and from 2.3 to 1.5 ng/g for miconazole, corresponding to losses of 44, 19, and 35%, respectively (Table S5). However, no significant dissipation was found for clotrimazole ($p > 0.05$), but significant dissipation was found for climbazole and miconazole with dissipation half-lives calculated to be 192 ± 42 and 248 ± 69 days, respectively (Table 3).

For both old and new treatments, no significant dissipation was observed for the treatments with higher application rates ($p > 0.05$) (Table 3). Like antibiotics, these biocides with higher concentrations in those treatments with higher application rates may inhibit soil microbial activity due to their good bacteriostasis and antisepsis ability,[39−41] resulting in their persistence in the soil environment. Under the same biosolid application rate, OT2 had no significant dissipation for the three biocides, whereas NT2 showed significant dissipation.

Table 3. Summary of the Dissipation Information in Biosolid-Amended Soils Based on the First-Order Model for the Three Biocides in the Shandong Site

calculation	OT1	OT2	OT3	OT4	NT2	NT3	NT4
Climbazole							
fitting formula	$Y = 6.6763 \times e^{(-0.1190X)}$	$Y = 3.2293 \times e^{(-0.0605X)}$	$Y = 2.7187 \times e^{(-0.0232X)}$	$Y = 1.8578\times e^{(-0.0023X)}$	$Y = 114.6453\times e^{(-0.1080X)}$	$Y = 4.8014\times e^{(-0.0544X)}$	$Y = 4.9531\times e^{(-0.0190X)}$
R^{2a}	0.2314	0.0768	0.0157	0.0002	0.4567	0.1151	0.0248
p value^b	0.0173	0.2006	0.5688	0.9524	0.0041	0.2353	0.5905
k (error)^c	0.1190 (0.0387)	0.0605 (0.0371)	0.0232 (0.0314)	0.0023 (0.0295)	0.1080 (0.0224)	0.0544 (0.0301)	0.0190 (0.0221)
DT50 (error)^d	175 (64)	344 (338)	896 (1078)	9041 (8332)	192 (42)	383 (305)	1094 (3101)
Clotrimazole							
fitting formula	$Y = 3.1227 \times e^{(-0.0852X)}$	$Y = 2.2127 \times e^{(-0.0455X)}$	$Y = 2.4407 \times e^{(-0.0192X)}$	$Y = 2.5891 \times e^{(-0.0531X)}$	$Y = 16.8776 \times e^{(-0.0364X)}$	$Y = 3.2616 \times e^{(-0.0475X)}$	$Y = 3.5551 \times e^{(-0.0193X)}$
R^{2a}	0.1669	0.0535	0.0116	0.0691	0.1688	0.0752	0.0227
p value^b	0.0475	0.3003	0.6251	0.2256	0.1281	0.3429	0.6068
k (error)^c	0.0852 (0.0342)	0.0455 (0.0341)	0.0192 (0.0318)	0.0531 (0.0343)	0.0364 (0.0159)	0.0475 (0.0337)	0.0193 (0.0237)
DT50 (error)^d	244 (117)	457 (781)	1083 (621)	392 (434)	571 (308)	438 (625)	1077 (2121)
Miconazole							
fitting formula	$Y = 4.2908 \times e^{(-0.1604X)}$	$Y = 1.6909 \times e^{(-0.0386X)}$	$Y = 1.6649 \times e^{(-0.0093X)}$	$Y = 1.2385 \times e^{(-0.0022X)}$	$Y = 6.8012 \times e^{(-0.0837X)}$	$Y = 3.8029 \times e^{(-0.0841X)}$	$Y = 2.9699 \times e^{(-0.0206X)}$
R^{2a}	0.3452	0.0334	0.0026	0.0001	0.3670	0.2246	0.0224
p value^b	0.0032	0.4043	0.8234	0.9581	0.0167	0.0743	0.6095
k (error)^c	0.1604 (0.0389)	0.0386 (0.0362)	0.0093 (0.0324)	0.0022 (0.0324)	0.0837 (0.0217)	0.0841 (0.0308)	0.0206 (0.0256)
DT50 (error)^d	130 (33)	539 (4193)	2236 (1536)	9452 (8807)	248 (69)	247 (104)	1009 (1854)

^{a}The correlation coefficient of the first-order reaction kinetic model. ^{b}Significance of the first-order reaction kinetic model. ^{c}Rate constant of the first-order reaction kinetic model. ^{d}The dissipation half-life (days) determined using the first-order reaction kinetic model.

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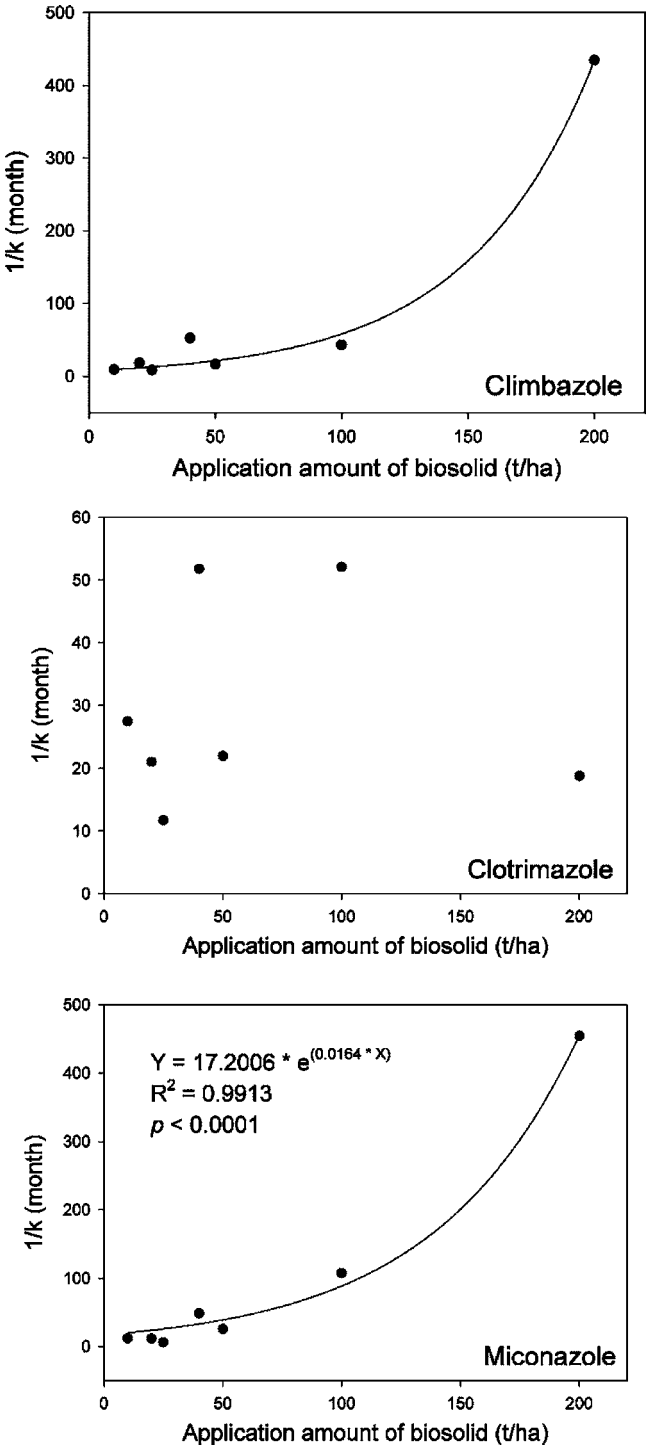

Figure 3. Relationships between the total biosolid application amounts and the reciprocals of the calculated dissipation rates ($1/k$) from each azole biocide in the biosolid-amended soils.

This suggests that repeated biosolid application could lead to more persistence of the biocides in agricultural land.

Limited previous field studies also demonstrated high persistence of clotrimazole and miconazole in sludge-amended soils, with dissipation half-lives of 347, 1386, and 440 days for miconazole in North America and China[21,24,38] and 365 days for clotrimazole in China.[38] Compared to the field studies, the dissipation half-life from a laboratory study was 55 days for clotrimazole in a clay loam soil, which is much lower than the 244 days from the present field.[27] This suggests that laboratory experimental data for organic contaminants may overestimate field dissipation rates and inaccurately predict the field dissipation patterns.[17] Soil properties could also affect the dissipation behavior; for example, clotrimazole is known to be more persistent in a loam than in a clay loam under laboratory conditions.[27]

Prediction of Residual Concentrations in Biosolid-Amended Soils. On the basis of the bioslid application rates and measured concentrations from NT, residual concentrations in the soils in March and October of every year could be predicted by the exposure model. Good correlations between the biosolid application amounts and dissipation rates were found for climbazole and miconazole ($R^2 > 0.98$ and $p < 0.0001$) but not for clotrimazole (Figure 3). In fact, clotrimazole was the most persistent among the three compounds in the present study, and no significant dissipation was observed (Table 3). The functions for the two biocides climbazole and miconazole also proved that higher biosolid application rates and repeated biosolid application could lead to higher persistence of these two compounds in the agricultural soils. The two parameters from the first-order exponential growth model were then used for the prediction of residual concentrations of the two compounds in the soils.

The predicted concentrations from the exposure model were compared to the measured concentrations for the two compounds (Table 4). The relative errors between the predicted and measured concentrations ranged from 3.7 to 25.2% for climbazole and from −17.5 to 35.4% for miconazole. Despite some deviations from measured concentrations, the predicted concentrations for the two biocides could be used in the risk assessment.

Bioaccumulation of the Biocides in the Crop Plants. None of the target biocides were found in the crop plant samples (wheat, wheat stalk, corn, corn stalk, and corn cob) collected from the trial plots. Although previous studies showed bioaccumulation of organic contaminants such as some PPCPs in various plant species (carrot, lettuce, and soybean),[28,42] no uptake or bioaccumulation of the three target biocides was observed in the present study. This is consistent with another previous finding that miconazole was not found bioaccumulated in wheat in biosolid-applied plots with a biosolid application rate of 22 t/ha.[21] This could be explained by some factors such as chemical properties and experimental conditions. Reported bioaccumulation data are mostly from spiked nutrient solutions or very high application rates.[28,42] High adsorption capabilities of the three biocides (Table 1) are also a limiting factor for them to be taken up by plants. In Canada, the maximum allowed dewatered biosolid application rate is 8 t/ha per 5 years.[18] If we consider only human safety from the three biocides climbazole, clotrimazole, and miconazole in biosolid, application rates of 5−40 t/ha every year would be acceptable.

However, considering their persistence in soil environments, the three biocides may pose potential ecological risks to soil organisms. Due to the lack of sufficient terrestrial toxicological data, proper risk assessment could not be performed at the current stage. Fungicide resistance is also a concern for modern agriculture.[43] Therefore, further studies are required to investigate potential environmental risks from those biocides in biosolids.

Table 4. Concentrations Predicted by the Exposure Model in Comparison with the Measured Concentrations for Climbazole and Miconazole of Different Old Treatments in Biosolid-Amended Soils in 2011

				climbazole (ng/g)			niconazole (ng/g)		
treatment	n^a	f^b(t/h)	time	predicted	measured	relative error[c] (%)	predicted	measured	relative error (%)
OT2	5	10	March 2011	6.1	7.0	12.9	7.4	11.3	34.5
	5	10	Oct 2011	4.4	5.3	17.0	6.2	9.4	34.0
OT3	5	20	March 2011	11.4	12.9	11.6	18.1	15.4	−17.5
	5	20	Oct 2011	10.1	13.5	25.2	16.8	19.7	14.7
OT4	5	40	March 2011	23.2	24.1	3.7	24.5	29.9	18.1
	5	40	Oct 2011	22.8	25.3	9.9	24.1	37.3	35.4

[a] n, number of biosolid applications. [b] f, biosolid application rate per year. [c] Relative error = (measured − predicted)/measured × 100.

■ ASSOCIATED CONTENT

Supporting Information

Detailed LC-MS/MS instrumental conditions and mass transitions, recoveries and detection limits for the three target compounds, field monitoring concentration data, and dissipation plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CK, control plot without biosolid application; OT, old groups; NT, new group; DT_{50}, dissipation half-lives; WWTP, wastewater treatment plant; TOC, total organic carbon content; UHPLC-MS/MS, ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantitation; PPCPs, pharmaceutical and personal care products

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