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ScienceDirect
Ecotoxicology and Environmental Safety 72 (2009) 93–98
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Influence of bovine manure on dissipation of hexazinone in soil

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Received 30 June 2006; received in revised form 5 January 2008; accepted 19 January 2008
Available online 17 March 2008

Abstract

The effects of bovine manure (BM) on the degradation of hexazinone and formation of three of its major metabolites were investigated in sandy loam soil. The degradation half-life of hexazinone was 29.6 days in unamended soil, while it decreased to 21.8 days in BM-amended soil. The major metabolites formed in unamended soil were [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione] (metabolite A) and [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1, 3, 5H)-trione] (metabolite C), while metabolite B [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione] was not detected over the entire experimental period. However, in BM-amended soil, metabolite B was detected at 20 and 40 days after incubation, suggesting that BM contributed to formation of this metabolite. *N*-demethylation, removal of the dimethylamino group with formation of a carbonyl group at the 6-position of the triazine ring appeared to be the principal mechanisms involved in hexazinone metabolism in unamended soil. However, hydroxylation at the 4-positon of the cyclohexyl group as well as the above two modes were the principal pathways in BM-amended soil.

Keywords: Hexazinone; Bovine manure; Degradation; Metabolite; Forming pathway; Metabolism; Unamended; BM-amended; Soil; Degradation kinetics

1. Introduction

Hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-(1H,3H)-dione] is a non-selective herbicide in the triazine family, which is widely used to control a wide variety of broad leaf weeds, grasses and woody plants in forestry field nurseries, sugarcane and pineapple plantations, highway and railway grasses and industrial plant sites (Wu and Feng, 1997). High water solubility (33 g L^{-1} at 25 °C) and low soil absorption (Hornsby et al., 1995) allow hexazinone to move easily into the groundwater. Hexazinone is often detected in groundwater in the areas it is used, which raises great concerns about its safety to human health (Kubilius and Bushway, 1998). Previous work on environmental behavior of hexazinone mainly focused on residual analyses of the parent and its metabolites (Fischer and Michael, 1995); dissipation (Calderson et al., 2004); adsorption–desorption (Bouchard and Levy, 1985); leaching potential and mobility in soil (Bottni et al., 1996). The reported field dissipation half-life of hexazinone was 79 days, and the organic carbon distribution coefficient (K_{oc}) ranged from 34 to 74 (Toiber-Yasur et al., 1999). It is regarded to be not very susceptible to hydrolysis (Zhu et al., 1998) and photolysis (Neary et al., 1983), and thus residual activity may be expected to last several months. Hexazinone metabolites were first identified by Reiser et al. (1983) who separated five degradation products in plant seedling. Additionally, Kin and Kimball (1987) observed that [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione] was a major metabolite during the degradation of hexazinone in soils of lowbush blueberry fields in Nova Scotia, Canada.

Farm litter is commonly applied to soil as manure before application of pesticides. It contains a large number of microorganisms as well as nutrients (nitrates and phosphates), and thus can accelerate the bioremediation

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0147-6513/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ecoenv.2008.01.026

of soil contaminated by organic compounds. The addition of farm litter to soil alters biological and chemical conditions, both of which influence the rate and pathway of pesticide degradation in soils. Gupta and Baummer (1996) found that farm litter could enhance the biodegradation of atrazine in soil and Gan et al. (1998) observed that the addition of composted manure to the top 5-cm layer at 5% (w/w) reduced 1,3-dichloropropene emission by 47%. However, no data to date are available on the effects of farm litter on the degradation and metabolism of hexazinone in soil. Therefore, the main objective of this study was to assess the effect of a kind of farm litter (bovine manure) on environmental behavior of hexazinone in sandy loam soil. This information will be useful in determining the conditions needed for achieving optimal degradation and effective remediation for hexazinone-polluted soil.

2. Materials and methods

2.1. Chemicals

Hexazinone (99.7% purity) was purchased from Shenyang Chemical Engineering Institute, Shenyang, China. Metabolite A [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione], metabolite B [3-(4-hydroxycyclohexyl)-6-(dimethylamino)-1-methyl-1,3,5-triazine- 2,4(1, 3H)-dione] and metabolite C [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1, 3, 5H)-trione] were gifts from DuPont de Nemours (Experimental station, Wilmington, DE, USA). The purity of the former three metabolites was 99.0%. Working standards of hexazinone and its metabolites in methanol were prepared freshly every 2 weeks and stored in dark bottles at −20 °C until use. Analytical grade reagents and solvents locally procured were purified and redistilled before use. The water used in this experiment was purified with a Mill-Q-Plus system (Millipore, Molsheim, France).

2.2. Soil and bovine manure

The soil was collected from the 0 to 20 cm soil profile of an experimental plot located at Pinghu, Zhejiang province, Southeast China. To our knowledge, the plot had never been applied with hexazinone before sampling. The texture of the soil was classified as sandy loam soil with 56.7% sand, 24.4% silt and 18.9% clay. The other physical–chemical properties of this soil are listed in Table 1. The collected soil was air-dried, ground and passed through a 2-mm sieve before use.

Bovine manure (BM) was collected from a farmhouse, located at the suburb of Zhengzhou, China. This manure was composted and matured for 6 months. The composted BM was air-dried at room temperature, homogenized and crushed to pass a 2-mm sieve. BM had a pH of 7.8 and an organic carbon content of 298.3 g kg^{-1} on a dry weight basis. The cation exchange capacity, total nitrogen and maximum water-holding capacity were 48.9 $cmol_{(+)}\,kg^{-1}$, 28.4 g kg^{-1}, and 49.8%, respectively, on a dry weight basis (Table 1).

Table 1
The basic physico-chemical properties of soil and farm litter used

Type of materials	pH	Organic carbon content (g/kg)	CEC ($cmol_{(+)}$/kg)	TN (g/kg)	WHC_{max} (%)
Sandy loam soil	7.3	14.7	11.6	3.6	58.7
Bovine manure (BM)	7.8	298.3	48.9	28.4	49.8

CEC: cation exchange capacity; TN: total nitrogen; WHC_{max}: maximum water-holding capacity.

Microorganisms in soil and BM were enumerated by plate counting on nutrient agar for bacteria and Rose Bengal agar for fungi (Houot et al., 1998). The bacterial and fungal numbers were 5.6×10^6 and 3.1×10^4 CFU g^{-1} (colony-forming units) in soil, and 75.3×10^6 and 21.2×10^4 CFU g^{-1} in BM, respectively.

2.3. Hexazinone degradation in the sandy loam soil

The soil sample (10 g) was spiked with stock solution of hexazinone to obtain a final concentration of 10 μg g^{-1} on a dry weight basis, adjusted to 60% of maximum water-holding capacity (WHC_{max}), and introduced into a 100-mL flask for each set. Each flask containing soil was weighed and incubated at 30 ± 1 °C in the dark. The weight loss due to evaporation of soil moisture was maintained by periodical addition of sterilized deionized water at intervals of 2 days over the entire incubation period. Each set was conducted in triplicate and processed as described below for residual analysis of hexazinone at intervals of 0 (2 h after spiking), 5, 10, 20, 40, and 60 days after treatment (DAT).

2.4. Effect of BM on degradation of hexazinone and metabolite formation

To determine if BM had a significant impact on hexazinone degradation, BM was added into the soil at a concentration of 5% (w/w). The amended soil was mixed thoroughly, and then spiked with stock solution of hexazinone to obtain a final concentration of 10 μg g^{-1} on a dry weight basis. The soil was then prepared as described in Section 2.3 to compare the differences of hexazinone degradation under unamended and BM-amended conditions. At regular intervals, triplicate samples were removed for each set and processed for analyses of hexazinone residue. If the samples were not analyzed immediately, they were stored at −20 °C until analyzed.

In unamended and BM-amended (5% by w/w) soil, the major metabolite formation was studied in parallel with residual analyses of hexazinone. The major metabolites of hexazinone were identified and quantified by co-chromatography using authentic standards. A few minor metabolites were not taken into account due to lack of authentic standards in this experiment.

2.5. Extraction and clean-up of soil samples

Each soil sample (10 g) was extracted with 20 mL of methanol, shaken vigorously for 2 h on a mechanical shaker and filtered through a Buchner funnel. The soil was extracted three times, and the filtered phases were combined and mixed in a flask, evaporated to dryness on a rotary evaporator, and the residue was redissolved in methanol (5 mL). Aliquots (2 mL) of the methanol portion were further purified by passing through a 0.2-μm filter to remove any soil particles, and then determined for hexazinone and its metabolite residues by HPLC.

2.6. Analyses of hexazinone and its metabolites by HPLC

The residues of hexazinone and its major metabolites (A–C) were determined using an Agilent 1100 model HPLC equipped with diodearray detector. The operation was run under the following conditions: cartridge column, Nova-Pak C_{18} (4.6 mm × 150 mm i.d., 5 μm particle size); flow rate, 1 mL min^{-1}; detection wavelength, 247 nm and injection volume, 20 μL. The mobile phase consisted of methanol and water acidified to pH 5 with formic acid, and the chromatograph started with 40/60 methanol–water (v/v) for 7 min and then 80/20 for 22 min. An external standard method was used for calibration. The retention times of hexazinone,

metabolites A–C were about 16.3, 13.2, 4.8 and 19.5 min, respectively. The detection limits for hexazinone and its three metaboites were 0.05 μg g^{-1}.

2.7. Recovery study

To estimate residual recoveries of hexazinone along with its three metabolites, a recovery study was performed by fortifying unamended or BM-amended soil (5% by w/w) at a series of concentrations (0.2–10 μg g^{-1}) of each chemical. The spiked soil was extracted and analyzed following the method described above. The average recoveries ranged from 84.9% to 96.8% for hexazinone, and from 80.3% to 96.4% for three metabolites in unamended or BM-amended soil, respectively (data not shown). The coefficients of variance varied between 1.4% and 3.6% for hexazinone, and between 3.8% and 8.5% for three metabolites, respectively. As a result, the method adopted for residual analyses of hexazinone and its three metabolites was satisfactory.

3. Results

3.1. Hexazinone degradation in unamended soil

As shown in Fig. 1, hexazinone residues decreased gradually with time in unamended soil. The initial residue of 9.56 μg g^{-1} dissipated to 8.78, 7.39 and 6.57 μg g^{-1} after 5, 10, and 20 days of incubation, respectively. At 20 DAT, the degradation percentage was 31.3% in comparison with the initial concentration (9.56 μg g^{-1}), followed by 63.6% at 40 DAT. At the end of incubation (60 days), an approximate 74.0% of hexazinone was degraded. The degradation rate of hexazinone was found to follow the first-order kinetics ($R^2 = 0.9840$) with a half-life of 29.6 days (Table 2).

3.2. Effect of BM on degradation of hexazinone

Amendment of organic carbon in the form of manure, distillery effluent or sewage sludge is a recommended agricultural practice to increase soil fertility. The different kinds of amendments have different physical–chemical and biological characteristics, which will lead to different effects on pesticide degradation and metabolism (Singh, 2005). Therefore, hexazinone dissipation was compared in BM-amended (5% by w/w) and unamended soil. Hexazinone residues with the elapse of time (0–60 days) in BM-amended soil are shown in Fig. 2. The residue at 60 DAT was only 1.33 μg g^{-1} showing an 86.4% loss of hexazinone with a half-life of 21.8 days based on the first-order kinetics equation, while only 73.9% disappearance occurred in unamended soil with a half-life of 29.6 days. In general, the degradation rate constant (k) of hexazinone in BM-amended soil approximately increased by 35.9% over that in unamended soil. The bacterial and fungal populations of this manure (BM) were about 14 and 7 times, respectively, more than that of the investigated soil. Higher microbial densities possibly introduced more hexazinone-degraders, which enhanced the degradation rate of hexazinone.

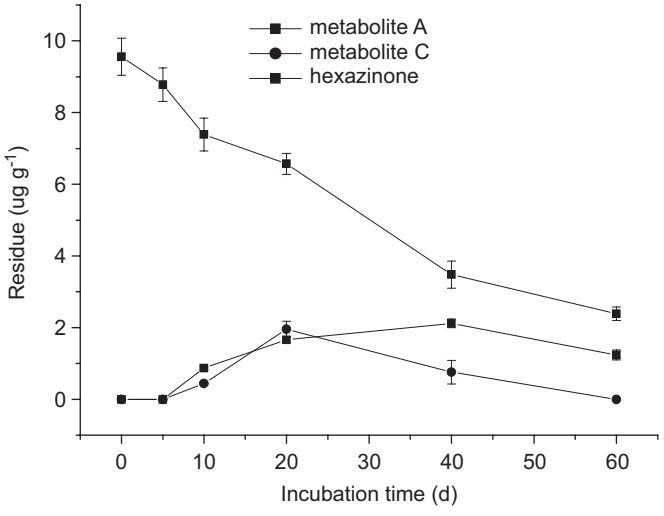

Fig. 1. Hexazinone degradation and metabolite formation in unamended soil.

Table 2
The degradation kinetic parameters of hexazinone in soil under different conditions

Different treatments	Degradation rate constants ($k \pm$SD) $\times 10^{-2}$ (d^{-1})	Half-lives $t_{1/2} \pm$SD (d)	Correlation coefficients (R^2)
Unamended soil	2.34	29.6	0.9840
BM-amended soil (5%)	3.18	21.8	0.9392

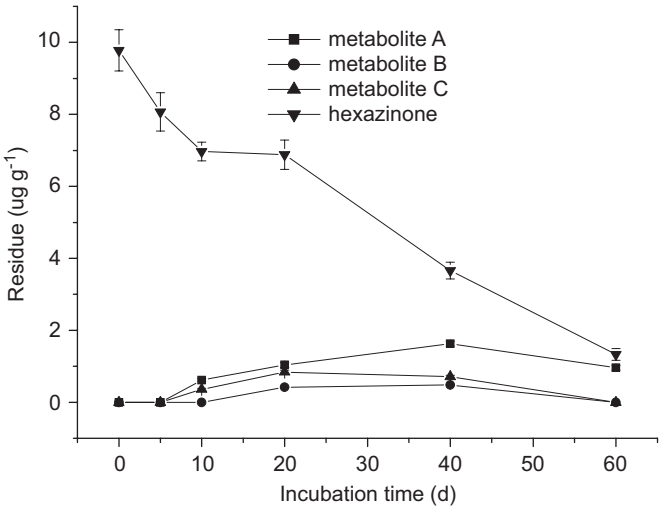

Fig. 2. Hexazinone degradation and metabolite formation in BM-amended soil.

3.3. Hexazinone metabolism in unamended soil

The major metabolites were quantitatively and qualitatively estimated in soil at an initial hexazinone concentration of 10 μg g^{-1}. The metabolites formed were identified by co-chromatography technique, i.e. comparing the retention time of metabolite with that of the authentic standard. As shown in Fig. 1 and Table 3, two major

Table 3
The metabolite formation of hexazinone at different incubation time

Incubation conditions	Metabolites	Residues ($\mu g\,g^{-1}$) at days after treatment					
		0	5	10	20	40	60
Unamended	A	BDL	BDL	0.87±0.04	1.66±0.07	2.12±0.12	1.24±0.14
	B	BDL	BDL	BDL	BDL	BDL	BDL
	C	BDL	BDL	0.44±0.02	1.96±0.22	0.76±0.33	BDL
BM-amended	A	BDL	BDL	0.62±0.03	1.04±0.07	1.63±0.07	0.96±0.07
	B	BDL	BDL	BDL	0.42±0.01	0.48±0.01	BDL
	C	BDL	BDL	0.36±0.03	0.84±0.05	0.71±0.04	BDL

BDL indicates below detectable level (&lt ;0.05 $\mu g\,g^{-1}$).

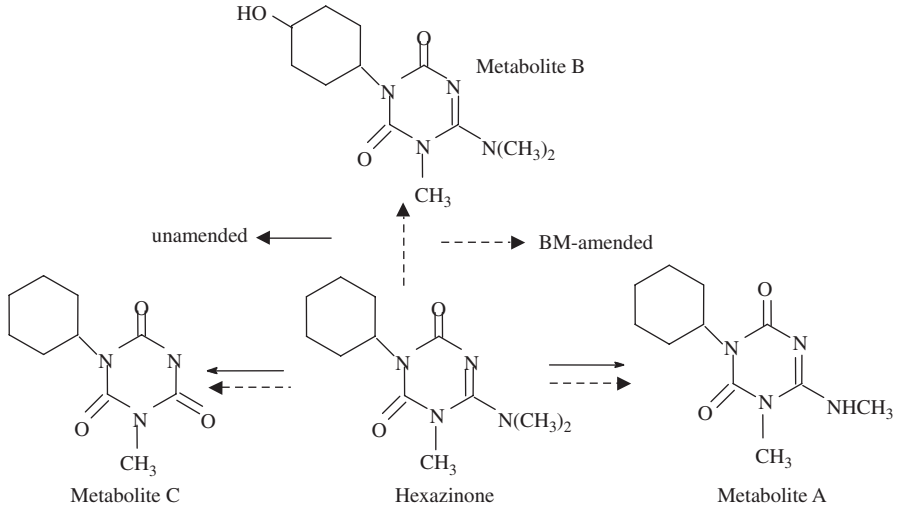

Fig. 3. The major metabolites of hexazinone in sandy loam soil under different conditions.

metabolites formed in unamended soil were [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione] (metabolite A) and [3-cyclohexyl-1-methyl-1,3,5-triazine-2,4,6(1, 3, 5H)-trione] (metabolite C). Metabolite A resulted from loss of methyl group at the 6-position of dimethylamino group. The dimethylamino group detached and the carbonyl group formed at the 6-position of triazine ring, yielding metabolite C. Both metabolite A ($0.87\,\mu g\,g^{-1}$) and C ($0.44\,\mu g\,g^{-1}$) started forming from the 10th day of incubation. The concentration of metabolite A reached its maximum level ($2.12\,\mu g\,g^{-1}$) at 40 DAT, and then declined to $1.24\,\mu g\,g^{-1}$ on the 60th day. In comparison with metabolite A, the maximum residue of metabolite C was also observed at 40 DAT, but below detectable level ($<0.05\,\mu g\,g^{-1}$) was found at the termination of experiment (60 days), which proved the further degradation of this metabolite. However, metabolite B was not detected over the whole experimental period in unamended soil.

3.4. Effect of BM on hexazinone metabolism

Application of BM to the sandy loam soil affected formation of three of hexazinone metabolites. The maximum monitoring concentrations for metabolite A and C were 2.12 and $1.96\,\mu g\,g^{-1}$ in unamended soil, while it decreased to 1.63 and $0.84\,\mu g\,g^{-1}$, respectively, in BM-amended soil (Table 3). Additionally, metabolite B was not detected in unamended soil while it was detected to be 0.42 and $0.48\,\mu g\,g^{-1}$ at 20 and 40 DAT (Fig. 2), which demonstrated that addition of BM promoted formation of metabolite B. Metabolite B, bearing a hydroxyl group at 4-position of cyclohexyl group, is a reducing form of hexazinone and therefore reducing conditions contribute to formation of this metabolite.

The combined concentrations of metabolites A, B and C represented a maximum of about 31% of the initial concentration of hexazinone. Besides the former three major metabolites, more minor peaks were also found in BM-amended soil than in unamended soil. However, the identity of these minor metabolites could not be established by co-chromatography due to lack of authentic standards and a further study on them was not conducted. Overall, the parent molecule of hexazinone was detected to be the highest peak, followed by those containing its degradation products in unamended and BM-amended soil. Therefore, by combining the findings of this experiment under different conditions, a possible degradation pathway of hexazinone was deduced and is presented in Fig. 3.

4. Discussion

The degradation rate of hexazinone found in this study is in general agreement with the reported field half-lives varying from 24 to 74 days (Zhu and Li, 2002). Bottni et al. (1996) reported a comparable half-life of 30 days in a soil from the Medena province, Italy. The long half-life of hexazinone in soil may reflect its structural feature, which is recalcitrant to microbial and chemical degradation.

Dungan et al. (2001) also observed a similar phenomenon that 1,3-dichloropropene degradation was 2.3 and 3.3 times faster, respectively, in soil amended by composted steer and chicken manure than in unamended soil. The enhanced biodegradation of atrazine in soil using poultry litter was found in Gupta and Baummer (1996) and the identical conclusion on atrazine degradation in loamy soil after addition of organic amendments was also found in Houot et al. (1998). However, farm manure application can also slow the degradation of triadimefon in soil (Singh, 2005). Accordingly, different kinds of manures may exert different effects on pesticide degradation. On one hand, decreased bioavailability due to increased sorption to the additional organic matter of litter will retard degradation. On the other hand, co-metabolic biotransformation can be enhanced by the general increase in microbial populations and activities. Therefore, the combined effects on pesticide degradation occurred in amended soil by farm litter.

To date, many studies confirmed that hexazinone had seven primary metabolites in soil, which mainly derived from demethylation, removal of the dimethylamino group with formation of a carbonyl group at 6-position of triazine ring, and hydroxylation at 4-position of cyclohexyl group (Wang et al., 2005). Kin and Kimball (1987) observed that [3-cyclohexyl-6-(methylamino)-1-methyl-1,3,5-triazine-2,4(1, 3H)-dione] (metabolite A) was a major metabolite during the degradation of hexazinone in soils of lowbush blueberry fields in Nova Scotia, Canada and the trione derivative (metabolite C) appeared likely to be a primary metabolite under moist, warm conditions. The former conclusions were comparable with this observation in unamended soil, which further proved that the singly demethylated metabolite A and the deaminated metabolite C, existing almost entirely as the ketoneautomer (i.e. the 1,3,5-trione), were the most abundant among the metabolites of this herbicide.

Singh (2005) reported that reduction potential (Eh) in soil amended by composted manure increased faster than that in unamended soil, which hastened the onset and attainment of soil reducing conditions. Therefore, the application of BM to soil promoted formation of the reducing metabolite B. It could be concluded from this research that *N*-demethylation at 6-position of triazine ring, hydroxylation at the 4-positon of cyclohexyl group, and removal of the dimethylamino group with formation of a carbonyl group at 6-position of triazine ring appeared to be the principal mechanisms involved in hexazinone degradation in the investigated soil. Additionally, the application of BM in soil promoted the occurrence of metabolite B.

5. Conclusions

The degradation and metabolism of hexazinone were affected by application of BM in the sandy loam soil. The major metabolites formed were metabolite A and C in unamended soil. However, in BM-amended soil, metabolite B was observed at 20 DAT besides metabolites A and C, and the combined concentrations of three metabolites corresponded to a maximum of 31% conversion of the fortification dose. *N*-demethylation, removal of the dimethylamino group with formation of a carbonyl group at the 6-position of triazine ring appeared to be the principal mechanisms involved in hexazinone metabolism in unamended soil. However, hydroxylation at the 4-positon of cyclohexyl group as well as the above two modes were the principal pathways in BM-amended soil.

Acknowledgments

This work was funded by Wenzhou Medical College (5010 key project No. XNK07003). This work was also partially funded by Hubei Province Natural Science Foundation of China (Grant no. 2006ABA182). The authors are also grateful to the anonymous reviewers for their reading of the manuscript, and for their suggestions and critical comments.

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