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Enantioselective bioaccumulation and toxic effects of metalaxyl in earthworm *Eisenia foetida*

Peng Xu [1], Jinling Diao [1], Donghui Liu, Zhiqiang Zhou *

Department of Applied Chemistry, China Agricultural University, Beijing 100193, PR China

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ABSTRACT

Knowledge about the enantioselective bioavailability of chiral pesticides in soil invertebrates facilitates more accurate interpretation of their environmental behaviors. In this study, the acute toxicities of R-metalaxyl and *rac*-metalaxyl to earthworm (*Eisenia foetida*) were assayed by filter paper contact test. After 48 h of exposure, the calculated LC_{50} values for R- and *rac*-metalaxyl were 0.052 and 0.022 mg cm^{-2}, respectively, resulting in a two fold difference in toxicity. For uptake experiment, earthworms were exposed in soil at two dose levels (10 and 50 mg kg^{-1}_{dwt}). The concentrations of two enantiomers in soil and earthworm were determined by high-performance liquid chromatography based on cellulose tri-(3,5-dimethylphenyl-carbamate) chiral stationary phase. The results showed that metalaxyl was taken up by earthworm rapidly, and the bioaccumulation of metalaxyl in earthworm was enantioselective with preferential accumulation of S-enantiomer. In addition, biota to soil accumulation factor (BSAF) used to express the bioaccumulation of metalaxyl enantiomers was investigated, and significant difference was observed between *rac*-metalaxyl and R-metalaxyl.

1. Introduction

Metalaxyl (*R,S*)-methyl-*N*-(2′-methoxyacetyl)-*N*-(2,6-xylyl)-DL-alaninate is an important phenylamide fungicide with protective and curative action, which is widely used to control diseases caused by air- and soil-borne Peronosporales on a wide range of temperate, subtropical and tropical crops (Tomlin, 2003). The compound is a chiral fungicide due to the presence of the stereogenic center in the alkyl moiety, and consists of a pair of enantiomers (Fig. 1) with R- and S-configuration showing levo- and dextrorotation, respectively (Hubele et al., 1988; Buser and Müeller, 1995). Metalaxyl was initially marketed as the racemic product, even though the R-enantiomer is about 3–10 times more fungicidally active than the S-enantiomer (Fisher and Hayes, 1985). In many countries, *rac*-metalaxyl has been replaced by metalaxyl-M, and the product enriched with R-enantiomer (trade name: metalaxyl-M, Mefenoxam; M = minus; laevorotatory; Nuninger et al., 1996), typically comprises 97.5% of the R-enantiomer and 2.5% of S-enantiomer and has the same biological activity as racemic metalaxyl at ≈50% of the application rate (Buser et al., 2002).

As many as 25% of all pesticide active ingredients are chiral, and the enantiomers of chiral pesticide usually differ in their biological activity, toxicity, effects on beneficial and non-target organisms, and environmental fate (Williams, 1996; Hegeman and Laane, 2002). These differences may lead to variations in microbial degradation rates and would mean that one enantiomer is more persistent in the environment than the others. This has led to increased research on the enantioselectivity of chiral compounds. Many reports have documented the enantioselective degradation in soil (Buser et al., 2002; Buerge et al., 2003; Monkiedje et al., 2007) and sunflower (Zadra et al., 2002) when *rac*-metalaxyl was applied. Qiu et al. (2005) reported the enantioselective degradation kinetics of metalaxyl in rabbits, and the S-enantiomer levels in plasma, liver, and kidney decreased more rapidly than the R-isomer. In addition, the differences in acute, chronic and sublethal toxicity between R-metalaxyl and *rac*-metalaxyl on aquatic organisms have been reported (Yao et al., 2009). However, other aspects such as enantioselective toxicity and bioaccumulation of metalaxyl in earthworm were not investigated in those studies.

Metalaxyl with log K_{ow} = 1.65 and log K_{oc} = 1.85 is moderately stable under normal environmental conditions. Under field conditions, it has a half-life of one to 8 weeks in soil, and its average half-life in soil is about 70 d (USEPA, 1994; Tomlin, 2003; http://www.syrres.com/esc/physdemo.htm). Until now there is little report about the transfer of metalaxyl from soil to earthworm and the effects of metalaxyl on toxicity in earthworm, much less the report about the enantioselective bioaccumulation of metalaxyl in earthworms from soil.

In this study, we selected the commonly used test species, *Eisenia foetida*, for bioavailability tests under laboratory conditions.

* Corresponding author. Address: Department of Applied Chemistry, China Agricultural University, Yuanmingyuan West Road 2, Beijing 100193, PR China. Tel./fax: +86 10 62733547.

E-mail address: zqzhou@cau.edu.cn (Z. Zhou).

[1] These authors contributed equally to this paper.

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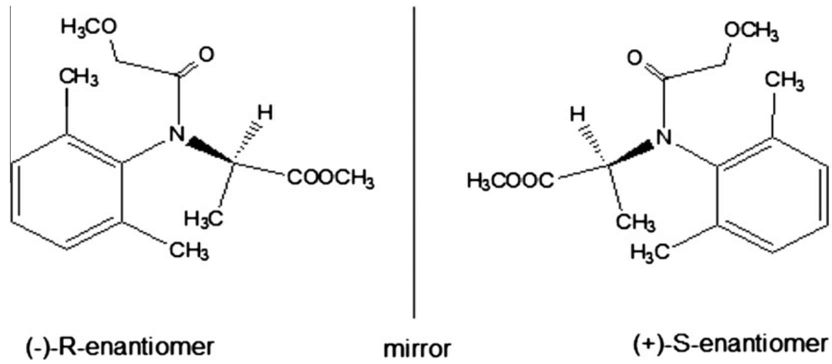

Fig. 1. Structures (absolute configurations) of R- and S-metalaxyl.

The objectives of this study were to (i) investigate the enantioselective bioaccumulation of metalaxyl in earhtworm from soil, (ii) evaluate the acute toxicity of *rac*-metalaxyl and R-metalaxyl to earthworm using filter paper contact test.

2. Materials and methods

2.1. Chemicals and reagents

The fungicides of *rac*-metalaxyl (>99.0% purity) and metalaxyl-M (>97.5% purity) were provided by Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). Water was purified by a Milli-Q system. *n*-hexane (HPLC grade) and 2-propanol (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals and solvents were analytical grade and purchased from commercial sources.

2.2. Earthworms

Mature redworms (*E. foetida*) purchased from northern suburbs farm, Beijing, were maintained in a wooden breeding box ($50 \times 50 \times 20\ cm^3$) containing a mixture of soil and cattle manure. The worms were active when being introduced in the experiment.

2.3. Acute toxicity test

According to the OECD guideline 207, a paper contact toxicity assay was used to test the acute toxicity of *rac*-metalaxyl and R-enantiomer to earthworms (OECD, 1984).

A range of known concentrations of test substances were prepared with acetone as the solvent. After the depuration period of 24 h on wet filter paper under dark conditions to evacuate the earthworms′ gut content, earthworms were rinsed in tap water and dried by absorbent paper cautiously. One milliliter of solutions was pipetted and added to the filter paper (5.5×11.5 cm) placed in flat-bottomed glass vial (3.6 cm in diameter, 8 cm in length). The concentrations of *rac*-metalaxyl on filter papers were 0.0126, 0.0190, 0.0253, 0.0316, 0.0379, 0.0443, and 0.0506 mg cm^{-2}. The concentrations of R-metalaxyl on filter papers were 0.0316, 0.0395, 0.0474, 0.0553, 0.0632, 0.0711, and 0.0791 mg cm^{-2}. After drying of the solvent under a stream of compressed air, 1 mL of deionized water was added to each vial. Controls were also run in parallel with the carrier solvent alone. Ten replicates for each treatment and each vial containing one worm were done. Each vial was sealed with plastic film with several ventilation holes. After that all the vials were placed in a room at 20 ± 2 °C, and mortality of earthworms was observed after incubation for 48 h. The LC_{50} values were determined from the survival data using a probit equation with SPSS 16.0.

2.4. Soil collection and earthworm exposures

The site to collect soil is a grassland area 20 km northwest of Beijing, China, which have not received any pesticide applications for 10 years at least. After removing the grasses and stones, the top soil (0–10 cm) was collected. The soils were sieved (2 mm) and air-dried at room temperature and stored in a dark dry place until a few days before they were used. Physicochemical properties of the soil were as follows: organic matter, 2.13 ± 0.12%; clay (<2 μm), 4.7 ± 0.1%; silt (<20–2 μm), 41.0 ± 1.2%; sand (2000–20 μm), 54.3 ± 1.4%; and pH (water, ratio 1:2.5), 7.6 ± 0.2.

We did the procedure in steps (dilution spike) to ensure that 250 g_{dwt} of medium was spiked homogeneously with *rac*-metalaxyl (Jager et al., 2003). First, 2.5 mg and 12.5 mg of metalaxyl were dissolved respectively in 10 mL of acetone, and then the acetone solution was slowly added to dry soil (50 g) while mixing continued for about 5 min. The spiked soil was left in a fume cupboard over-night and after that time the acetone had evaporated. Next, the contaminated dry soil (50 g_{dwt}) was mixed thoroughly with 200 g_{dwt} of uncontaminated medium, and then the contaminated soil (250 g_{dwt}) was transferred to a 500 mL glass jar. The final soil concentrations of metalaxyl were at 10 and 50 mg kg_{dwt}^{-1} soil, respectively. Ninety gram tap water was added to each jar to restore the 36% water content.

In this experiment, each earthworm weighed between 200 mg and 300 mg. Before the worms were introduced, they were allowed to live in that kind of uncontaminated soil for 1 week to acclimate. Five g_{wwt} earthworms were exposed to the chemical in each jar containing 340 g_{wwt} contaminated soil after evacuating their gut contents on moist filter paper for 3 h at 20 °C. The jars containing contaminated soil and worms were weighed, and the loss of water by evaporation was compensated by addition of tap water every 2 d. All the jars were placed in dark in environmental chamber controllable to 20 ± 2 °C.

For the uptake experiment, worms were collected after exposure periods (0.25, 0.5, 1, 3, 5, 7, 10, 14, 21 and 31 d), rinsed in tap water, and allowed to depurate most of their gut contents on moist filter paper for 3 h. Water on the surface of the worms was dried by absorbent paper cautiously, and then the worms were weighed and frozen at −20 °C (in 50 mL of polypropylene tubes). Soil samples (6.8 g_{wwt}) sampled from each jar were also stored at −20 °C. All the incubations were carried out in triplicate at each sample point.

2.5. Chemical analysis

To determine the residue of metalaxyl enantiomers, analytical methods were conducted for the treatments. Earthworms and soils were extracted and the extracts were further analyzed on high-performance liquid chromatography (HPLC).