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The fate of herbicide acetochlor and its toxicity to *Eisenia fetida* under laboratory conditions

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Abstract

To assess the toxic effects of the herbicide acetochlor on earthworms, we exposed *Eisenia fetida* (Savigny) to artificial soils (OECD soil) supplemented with different concentrations (5, 10, 20, 40 and 80 mg kg^{-1} soil) of acetochlor. The residues of acetochlor in soil and the effect of the herbicide on growth, reproduction, glutathione-S-transferases (GST) activity and cellulase activity of earthworms were determined. The degradation half-life of acetochlor in soil of acetochlor was between 9.3 and 15.6 days under laboratory condition; the degradation rate with low concentrations was faster than it was with higher concentrations. At 5 and 10 mg kg^{-1}, acetochlor had not significant effect on growth of *E. fetida* except after 15 and 30 days of exposure. When concentration >20 mg kg^{-1}, growth rates and numbers of juveniles per cocoon decreased significantly compared to the control in all treatments. However, cellulase activity decreased significantly in all treatments (5–80 mg kg^{-1}). This study showed that acetochlor had no long-term effect on the growth and reproduction of *E. fetida* at field dose (5–10 mg kg^{-1}). At higher concentrations of acetochlor (20–80 mg kg^{-1}), acetochlor revealed sublethal toxicity to *E. fetida*. Growth, numbers of juveniles per cocoon and cellulase activity can be regarded as sensitive parameters to evaluate the toxicity of acetochlor on earthworms.

Keywords: Acetochlor; Earthworm; *Eisenia fetida*; Residue; Growth rate; Sublethal toxicity; Biochemical toxicity

1. Introduction

The herbicide acetochlor has been widely used throughout the world, particularly in China, which consumes more than 10^4 t per year (Ye, 2003). As a member of the chloroacetanilide class of broad leaf herbicides, it is applied to the soil as a pre- and post-emergence treatment. It is mainly absorbed by the roots and leaves, inhibiting photosynthetic electron transport of the host (Nemeth-Konda et al., 2002).

Earthworms are one of the important components in decomposer communities and contribute significantly to the organic matter decomposition, nutrient cycling (Coleman and Ingham, 1988) and soil formation (Reinecke and Reinecke, 1998). Continuous application of pesticides may present risks to lead to soil pollution and affect soil fauna (Booth et al., 2000). *Eisenia fetida*, because of its low cost, easy handling and the standardization of the acute and subchronic ecotoxicological

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tests, is considered as a suitable biomonitor model species to determine the ecological hazard of heavy metals and pesticide contaminated soil (OECD, 1984).

Acetochlor showed significant carcinogenicity to the rat nasal epithelium (Green et al., 2000). The supramaximum tolerated dose (MTD) (2000 mg kg^{-1}) of acetochlor increased head abnormalities in *Epididymal cauda* sperm and reduced the fertility of the male rats (Ashby et al., 1997). Acetochlor increased the chromatid exchange frequency (SCE) of human lymphocytes (Hill et al., 1997). It also decreased soil microbial community diversity (Luo et al., 2004). However, only little is known about the effect of acetochlor on soil non-target animals of earthworms. LD50 of acetochlor to *E. fetida* is 0.307 mg kg^{-1} determined by filter paper test (Liang and Zhou, 2003a), and it is more toxic than methamidophos and Cu (LD50 is 0.708 and 118.70 mg kg^{-1}, respectively; Liang and Zhou, 2003a). However, it is less toxic than methamidophos, but more toxic than Cu in 14-day black soil toxicity test (Liang and Zhou, 2003b).

Whether the acetochlor has sublethal toxicity to earthworms at field application rates or whether acetochlor is non-toxic to no-target soil animals is not known. The aim of this study was (1) to investigate the degradation of acetochlor under laboratory conditions; and (2) to determine the acute toxicity, the sublethal toxicity and biochemical effects for *E. fetida* of acetochlor.

2. Materials and methods

2.1. Earthworms

The adult earthworms *E. fetida* with well developed clitellum (about 300 mg wet weight) were obtained from a synchronized culture in our laboratory and selected for this test. Animals were acclimated for 7 d to artificial soil substrates before use.

2.2. Chemical

The acetochlor [2′-ethyl-6-methyl-*N*-(ethoxymethyl)-2-chloroacetanilide] used was 90% miscible oil reagent and it was obtained from WU county chemistry factory, Zhejiang Province, China.

2.3. Soil preparation

The artificial soil was prepared according to OECD guideline 207 (1984), which comprised (by dry weight) 10% finely ground sphagnum peat, 20% kaolin clay, 70% industrial fine sand, with pH adjusted to 6.5 by addition of calcium carbonate. Acetochlor was dissolved in 5 ml acetone and mixed into a small quantity of fine quartz sand. The sand was mixed for at least 1 h to evaporate the acetone and then mixed thoroughly with the premoistened artificial soil. The final water content was adjusted to 50% of the maximum water holding capacity. 500 g of substrate was placed in individual polyethylene plastic containers (16 × 10.5 × 5 cm). Controls were prepared similarly but only with 5 ml acetone and no acetochlor. Groups of ten worms were weighed individually and placed in each test container and placed in a climate chamber (20 ± 1 °C, 12D/12L photoperiod and 400–800 lux). Five replicates were used for each treatment and control. The container lids were perforated to allow aeration and prevent the worms from escaping.

2.4. Acute toxicity

The LC50 of acetochlor was determined with the method recommended by ISO 11268-1 (1993). A range finding test was performed to determine the appropriate concentrations. Five concentration levels of 50, 100, 200, 300, 400 mg kg^{-1} soil (dry weight) were selected for the test. The mortality was determined at 7 and 14 days. Earthworms were sorted by hands and considered to be dead if they did not respond to gentle mechanical stimuli to the anterior region.

2.5. Sublethal toxicity

The experimental procedure was followed as described by ISO 11268-2 (1998). The low volume concentration recommended for the agricultural application (1500–3000 ml ha^{-1}) was converted for the surface area of the test containers (surface area 168 cm^2, soil content 500 g dry mass; Ma et al., 2004). This is equivalent to 5–10 mg kg^{-1} dry soil. The concentration levels of 5, 10, 20, 40 and 80 mg kg^{-1} dry soil were used for the reproduction test and biochemical assay. Earthworms were fed 5 g finely ground, urine-free cow dung added per week on the soil surface of each container. After 7, 15, 30, 45 and 60 days of exposure, earthworms were removed from soil, washed in distilled water and dried on paper towels. Survival rates and weights were recorded.

Cocoon production of the worms was determined after 28 days of exposure. Cocoons were collected by hand sorting and weighed, and then incubated for four additional weeks as described by Maboeta et al. (1999). Cocoons were cultured in Petri dishes at 25 ± 1 °C covered with three moist filter papers. The filter papers in these dishes were changed every three days to prevent bacterial growth. At the end of the test (28 days), the number of hatched cocoons and the number of juveniles produced over the eight-week test period were determined.

The growth rates were determined using the equation of Martin (1986):

$$\text{Relative growth rate} = \frac{W_t}{W_0} \times 100,$$