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Toxicological effects of soil contaminated with spirotetramat to the earthworm *Eisenia fetida*

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HIGHLIGHTS

- Multiple biomarkers were used to evaluate the toxic effects of spirotetramat on *Eisenia fetida*.
- There were potentially toxic effects of spirotetramat to *E. fetida*.
- Spirotetramat can induce DNA damage in *E. fetida*.
- The comet assay is a sensitive method for detecting DNA damage in earthworms.

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ABSTRACT

The aim of this study was to evaluate the potential toxicity of spirotetramat to the earthworm *Eisenia fetida* in a natural soil environment. Many biochemical markers, viz., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione S-transferase (GST), cellulase, and malondialdehyde (MDA) contents were measured after exposure to 0.25, 1.25, and 2.5 mg kg^{-1} for 2, 7, 14, 21, and 28 days. In addition, the comet assay was performed on earthworm coelomocytes to assess the level of genetic damage. The results demonstrate that the SOD activity and MDA content were significantly stimulated by the highest dose (2.5 mg kg^{-1}) of spirotetramat for the entire period of exposure. The activities of CAT and POD increased significantly by 2 d and 21 d, respectively, but the activities of both were significantly inhibited after prolonged exposure (28 d). After an initial increase on the 2nd day, the cellulase activity in the high-dose treatment group was significantly inhibited for the entire remaining exposure period. The comet assay results demonstrate that spirotetramat ($\leqslant$2.5 mg kg^{-1}) can induce low and intermediate degrees of DNA damage in earthworm coelomocytes. The results indicate that spirotetramat may pose potential biochemical and genetic toxicity to earthworms (*E. fetida*), and this information is helpful for understanding the ecological toxicity of spirotetramat on soil invertebrate organisms.

1. Introduction

Worldwide, there is an increasing concern over soil contamination because of the widespread agricultural use of pesticides. Thus, an increasing number of studies have focused on pesticide toxicity to the soil eco-environment and have reported useful information. Among the soil eco-toxicological studies, the use earthworms as model organisms to assess the toxicity of pesticides has been one of the primary techniques implemented to predict the potential effects of soil contamination by pesticides (Song et al., 2009; Alves et al., 2013).

Spirotetramat {*cis*-3-(2,5-dimethylphenyl)-8-methoxy-2-oxo-1-azaspiro[4.5]dec-3-en-4-yl ethyl carbonate}, an innovative, ambi-mobile insecticide, was developed by Bayer Crop Science (Germany) for the control of whiteflies, aphids, scales and other sucking insect pests of agricultural crops (Brück et al., 2009; Ouyang et al., 2012). At present, spirotetramat is successfully registered and widely used in several countries, including the United States, China, Brazil, and Mexico, among others (Yin et al., 2014). Therefore, several studies have investigated the effects of

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spirotetramat on the environment and on non-target organisms. Liu (2011) reported that rats decreased in weight and suffered from damage to the liver and the genitals when orally administered with spirotetramat (100 mg kg^{-1} d^{-1}) for seven days. Furthermore, Wu et al. (2012) demonstrated that spirotetramat can be absorbed and transformed into metabolites in rats and that the contents of these metabolite residues vary significantly among organs and tissues. In addition, a number of adverse effects of spirotetramat on aquatic organisms have been reported in previous studies. For example, Chen and Stark (2010) reported that the population size of the cladoceran *Ceriodaphnia dubia* is significantly decreased by exposure to a range of spirotetramat concentrations (0–40 mg L^{-1}). Agbohessi et al. (2013) observed that spirotetramat significantly inhibits the hatching rates of the eggs of the African catfish, *Clarias gariepinus*. Yin et al. (2014) reported that sub-lethal doses of spirotetramat cause oxidative stress and lipid peroxidation in toad (*Bufo bufo gargarizans*) tadpoles. However, to our knowledge, few studies that have examined the effects of spirotetramat on terrestrial earthworms have been published to date.

In earthworm, biochemical responses against environmental stress are sensitive, informative, reproducible, and can indicate the potential toxicity of a chemical at concentrations or time points prior to levels of toxicity with potential to cause tissue damage, increase disease susceptibility, or induce death. Therefore, biochemical responses of earthworms to toxic substances are regarded as early warning indices of pollution in a soil environment. Previous studies have demonstrated that reactive oxygen species (ROS) can be generated in earthworms exposed to the stresses of environmental contaminants (Zhang et al., 2013, 2014b). The overproduction of ROS can lead to oxidative damage to macromolecules such as nucleic acids, proteins, and lipids, eventually resulting in damage to the cell (Sabatini et al., 2009). To prevent oxidative damage, there are a number of antioxidant defense mechanism, such as the production of superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD), and glutathione S-transferase (GST) as well as common non-enzymatic mechanisms for scavenging excess ROS and thus alleviate the deleterious effects of ROS in earthworms (Maity et al., 2008; Sanchez-Hernandez et al., 2014). These antioxidant enzymes are regarded as good biomarkers of the toxic effects of contaminants on earthworms (Markad et al., 2012; Cao et al., 2014). In addition, the extent of lipid peroxidation and the activity of cellulase have also been used as biomarkers to evaluate the toxicity of contaminants on earthworms. Malondialdehyde (MDA), a product of lipid peroxidation, has been measured for numerous years as a convenient biomarker for assessing the oxidative stress of earthworms (Lin et al., 2010). Cellulase, an important digestive enzyme in earthworm, plays a crucial role in organic matter decomposition in soil (Aira et al., 2006). Cellulase has been used effectively as a biomarker for the assessment of earthworms exposed to contaminants in previous studies (Hu et al., 2010; Tejada et al., 2010; Zhang et al., 2014a). In previous earthworm studies, genotoxicity has usually been assessed by evaluating the extent of DNA damage. The comet assay [alkaline single-cell gel electrophoresis (SCGE)], is a simple, reliable, and sensitive method to detect DNA damage caused by environmental genotoxins that has been widely used within various scientific disciplines to evaluate the genotoxicity of pollutants to earthworms (Button et al., 2010; Markad et al., 2012; Cao et al., 2014).

In this study, we investigated the biochemical response of and the extent of DNA damage to the earthworm *Eisenia fetida* caused by spirotetramat under standard laboratory conditions. The activities of several antioxidant enzymes, including SOD, CAT, POD, and GST, were investigated to evaluate the level of antioxidant protection. The cellulase activity and the extent of lipid peroxidation were assayed to assess the effect of spirotetramat on the digestive system and the level of membrane damage, respectively. The extent of DNA damage in the coelomocytes of earthworms was determined by the comet assay. The purpose of this study was to obtain fundamental data to support a comprehensive understanding of the effects of spirotetramat on terrestrial earthworms and to provide useful information on the potential ecological risks of spirotetramat to the soil ecosystem.

2. Materials and methods

2.1. Chemicals and reagents

Spirotetramat (CAS No. 203313-25-1, purity 98.5%) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Coomassie brilliant blue G-250, nitro blue tetrazolium (NBT), guaiacol, L-methionine, thiobarbituric acid (TBA), glutathione (GSH), and phenylmethanesulfonyl fluoride (PMSF) were purchased from Sigma–Aldrich Shanghai Trading Co. Ltd., China. All other chemicals used in the study were of analytical grade.

2.2. Earthworm and soil

Earthworms (*E. fetida*) were obtained from an earthworm-culturing farm located in Qingdao, China, and maintained in the same loam soil at 20 ± 1 °C for at least 2 weeks prior to use. Earthworms were fed on cattle manure and the moisture content was adjusted to 35% with distilled water. Healthy adults with well-developed clitella and weighing approximately 350–450 mg were selected for the toxicity test. Before the exposure experiment, the earthworms were removed from the culture, rinsed with distilled water, and maintained on damp filter paper in the dark at 20 ± 1 °C for 24 h to allow for the voiding of gut contents.

The natural soil that was used in the present experiment was collected from the surface layer (0–20 cm) in Peony Garden of Qingdao Agricultural University, China. The physical and chemical properties were as follows: pH, 6.8; organic matter content, 25.3 g kg^{-1}; available *N*, 143 mg kg^{-1}; available *P*, 32 mg kg^{-1}; and available *K*, 168 mg kg^{-1}. Prior to spiking the soil with spirotetramat, the soil was air-dried and sieved through a 2 mm mesh screen.

2.3. Toxicological assay

The concentrations of spirotetramat in soil were established according to the guidelines of the Organization for Economic Co-operation and Development (OECD, 2000). The highest predicted environmental concentration (PEC) of spirotetramat for soil, for an application dosage of 180 g a.i. ha^{-1}, was listed as 0.25 mg kg^{-1} dry weight. Therefore, the earthworms were exposed to the following spirotetramat concentrations: 0 mg kg^{-1} (control), 0.25 mg kg^{-1} (1 × PEC), 1.25 mg kg^{-1} (5 × PEC), and 2.5 mg kg^{-1} dry weight (10 × PEC). The experimental exposure was conducted in clean 2 L glass beakers (diameter, 138 mm) containing 1000 g of dry soil. Spirotetramat was spiked into the soil at the appropriate concentrations to achieve the nominal concentrations in equal volumes of acetone. The soil treatments were then vented for 24 h in a fume hood to remove all the acetone. The moisture contents of all of the soil samples were adjusted to 35% of the final weight with distilled water and then stored in the dark overnight prior to the additions of earthworms. Twenty mature *E. fetida* were added into each soil treatment. Each treatment was replicated three times. All of the treatments were maintained under a 12/12 light–dark cycle at 20 ± 1 °C for 28 days. Ten grams of wetted cattle manure was