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Original Articles

# Mesotrione-induced oxidative stress and DNA damage in earthworms (*Eisenia fetida*)

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ABSTRACT

Mesotrione, a triketone herbicide, has been widely used in corn fields in recent years. The present study evaluated the toxicity of mesotrione to earthworms after exposing for 28 d at 0, 0.1, 1.0 and 2.5 mg/kg in a soil mixture (10% sphagnum peat, 20% kaolinite clay, and 70% sand by weight). The levels of reactive oxygen species (ROS), detoxifying enzyme glutathione S-transferase (GST), antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (POD)], lipid peroxidation and DNA damage in earthworms were determined. The ROS levels in mesotrione-exposed groups were higher than the control during the whole experiment, however, the SOD activities exhibited a downward trend. The MDA content, GST, CAT and POD activities were elevated at 7th day, but returned to control level after 28 d. The damage of DNA was increased greatly with increasing doses and time, which showed the relationship between dose-effect and time-effect. The results suggested herbicide mesotrione can cause ecological damage to soils as attested by oxidative stress and DNA damage in earthworms.

## 1. Introduction

In recent years, pesticides have become widely used in agricultural production. And as a large group of pesticides, more than 400 types of herbicides are widely used to control the harm of weed for increasing the output and quality of agricultural products. (Huang et al., 2016). However, the continuous application of herbicides had caused serious environmental pollution and poses threat to the ecosystem (Bellec et al., 2015; Jiang et al., 2018). Mesotrione with the formula of [2-[4-(methylsulfonyl)-2-nithobenzoyl]-1,3-cyclohexanedione] is a triketone herbicide invented by Syngenta Participations AG Switzerland (Bensalah et al., 2011). Mesotrione has been sold under the brand name of "callisto" in Europe since 2001 and widely applied in the European Union since 2002.

The pure substance of mesotrione is a pale yellow solid, with a molecular volume of 230.1 ± 3.0 $cm^3$, density of 1.5 ± 0.1 $g/cm^{-3}$, melting point of 148.7–152.5 °C (with breakdown), and boiling point of 643.3 ± 55.0 °C (under standard atmospheric pressure). The mesotrione solubility varied with pH in water, and the solubility become higher with high pH (Rouchaud et al., 2000).

Mesotrione is an HPPD inhibitor distinct from pyrazole herbicide that can inhibit the activity of hydroxypyruvate dioxygenase (HPPD) and ultimately affect the generation of carotenoids (Garcia et al., 2015; Nakka et al., 2017). Mesotrione can effectively prevent weeds, such as abutilon, cockle and amaranth from growing in corn fields.

However, recent studies showed that mesotrione caused toxicity to the environment and organisms. Joly et al. (2013) assessed the toxic effects of mesotrione, nicosulfuron, S-metolachlor, benoxacor and their mixtures on prokaryote *Vibrio fischeri*. They found that the mixture of mesotrione and nicosulfuron showed high toxic reaction to *V. fischeri* by obtaining original IC50 values. Bonnet et al. (2008) proved mesotrione is poisonous to the eukaryote *Tetrahymena pyriformis* and the prokaryote *Vibrio fischeri*. In addition, to understand the toxicity characteristics and maximal non-effect dose of mesotrione, Xie et al. (2010) conducted a 90-day toxicity test and found that the highest no-effect dose for rats was 25.0 mg/(kg d). Crouzet et al. (2010) studied that mesotrione could affected soil microbial communities, when the concentration exceeded the recommended dose in the field. Piancini et al. (2015) investigated the toxicity of mesotrione to two kinds of fishes (*Oreochorimis niloticus and Geophagus brasiliensis*) and indicated that mesotrione could cause oxidative damage in two kinds of fishes after acute exposure (96 h). Wang et al. (2017) stated that mesotrione was not poisonous to the common carp (*Cyprinus carpio*) at low dose (1.8 μg/L) and could induce oxidative damage on it at the highest dose

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(180 μg/L). However, there are few studies on the chronic toxicity of mesotrione on *Eisenia fetida*. In our manucript, the chronic toxicity of mesotrione to earthworms in soil was studied.

The earthworm (*Eisenia fetida*) is one of the most sensitive organisms to soil pollutants and is considered a model organism for toxicological experiments by the Organization for Economic Cooperation and Development (OECD 207, 1984). Many studies tested the effects of low dose of some pesticides on environmental safety using *Eisenia fetida* as indicators (Rico et al., 2016; Zhang et al., 2018). Therefore, our research used *Eisenia fetida* as testing organism to assess the ecological toxicity of mesotrione.

In this study, the toxicity of mesotrione in soils was evaluated based on both oxidative damage and genetic toxicity. We selected *Eisenia fetida* as testing organisms and the changes in reactive oxygen species (ROS), antioxidant enzymes (SOD, CAT and POD), glutathione-S-transferase (GST) and lipid peroxidation in earthworms were determined after exposing to different doses of mesotrione in the artificial soil for 28 d. In addition, DNA damage in earthworms was assessed using Single Cell Gel Electrophoresis (SCGE).

## 2. Materials and methods

### 2.1. Chemicals, earthworms and soil

The purity of Mesotrione was 99.0%, which was bought from the Beijing Chemical Co. (Beijing, China). Other reagents were bought from the Tianjin Kai Tong Chemical Co. (Tianjin, China), the Sigma Chemical Co. (St. Louis, MO, USA), and the Beijing Chemical Co. (Beijing, China).

The earthworms (*Eisenia fetida*) were bought from an earthworm farm at Shandong Agricultural University. The earthworms were fed in soil using cow manure at 20 ± 1 °C for two weeks before using them for the experiment. Prior to the experiment, healthy earthworms (weight of 300–600 mg) with obvious clitellum were selected to cultivate in a soil for a night.

The testing soil was prepared by the Organization for Economic Cooperation and Development (OECD 222, 2004), including 10% sphagnum peat, 20% kaolinite clay, and 70% sand by weight. The pH of the testing soil was adjusted to 6.0 ± 0.5 by adding chemically pure $CaCO_3$ (1% weight of the above-mentioned soil) (Edwards et al., 2009).

### 2.2. Toxicity experiment

The doses of mesotrione were 0, 0.1, 1.0 and 2.5 mg/kg dry soil, with six replicates for each concentration. To prepare different concentration of mesotrione-treated soils, the mesotrione was dissolved in acetone and a certain amount of mesotrione solution was added to 10 g artificial soil and mixed well until the acetone evaporated. Then, 490 g artificial soil were stirred just until mesotrione and soil were thoroughly combined. The soil moisture was kept at 35% with the addition of deionized water (Guo et al., 2016). Then, 500 g soil was transferred to 1-L container, with 10 earthworms being placed in each container. Each container was placed in an environment of 20 °C with 12 h light:12 h dark (Wang et al., 2016). At day 7, 14, 21, 28, nine earthworms were chosen randomly for each concentration (three for ROS content, three for enzyme activities and MDA content, three for DNA damage). These earthworms were moved from beakers to petri dish with wet filter paper for one night before the test.

### 2.3. Protein content, enzyme activity and MDA content measurements

Earthworms were put in a glass homogenizer and ground up in 50 mM phosphate buffer solution (pH 7.8). The homogenization buffers were centrifuged at 10000 rpm for 15 min. The supernatant was used for analysis of protein, enzyme activities, and MDA content. The entire extraction was performed at 4 °C.

The content of proteins in earthworm enzyme solution was measured by the staining method. The bovine serum albumin was selected to created a standard curve. The absorbance values of samples were measured at 595 nm.

To determine superoxide dismutase (SOD) activity, the inhibition of nitroblue tetrazolium chloride (NBT) was measured by the method of Song et al. (2009) with slight modification. The reaction liquid was prepared in advance including 5 mL deionized water, 30 μL phosphate buffer, 6 mL methionine, 6 mL nitroblue tetrazolium, 6 mL EDTA-$Na_2$, and 6 mL riboflavin. The sample solution (three parallels to per concentration) consisted of 50 μL enzyme solution and 3 mL reaction liquid. The control solution (four parallels) consisted of 50 μL PBS and 3 mL reaction liquid. All treatments were placed in the light for 30 min. At last, absorbance values were recorded at 560 nm.

Catalase (CAT) activities were measured by observing the degradation of $H_2O_2$ absorbance at 250 nm referring to the method of Böhmer et al. (2011). 67 mM CAT-PBS I included 14.612 g Disodium hydrogen phosphate ($Na_2HPO_4{\cdot}12H_2O$), 3.522 g potassium dihydrogen phosphate ($KH_2PO_4$,) and DI water. 160 μL Hydrogen peroxide ($H_2O_2$, 30%) and 100 mL PBS I were combined to obtain CAT-PBS II. The CAT activity was measured by dynamical method for a minute at 250 nm.

Peroxidase (POD) activities were assayed as the method described by Song et al. (2009). The reaction mixture included 50 mL potassium phosphate buffer, 19 mL 30% $H_2O_2$, and 28 mL guaiacol. Then, 3 mL reaction mixture and 20 μL phosphate buffer were poured to the reference cell and the mixture (20 μL enzyme liquid and 3 mL reaction mixture) was poured into the sample cell. Then POD activities were measured by dynamical method for 3 min at 470 nm.

Glutathione S-transferase (GST) activities were measured by 1-chloro-2,4-dinitrobenzene colorimetry (Borah et al., 2016). The control included 2.6 mL phosphate buffer, 20 μL CDNB and 20 μL of GSH. The sample included 20 μL enzyme solution, 2.4 mL phosphate buffer, 20 μL CDNB and 20 μL GSH. The absorbance after 180 s was recorded down at 340 nm.

Malondialdehyde (MDA) content was measured by the way of Simeoli et al. (2015). The reaction mixture (0.2 mL supernatant, 0.2 mL 8.1% SDS, 1.5 mL 20% acetic acid, 1.5 mL TBA and 1 mL of deionized water) was heated in a water bath (90 °C) for 1 h. Then the mixture was centrifuged and absorbance values were recorded at 532 nm.

### 2.4. ROS analysis and DNA damages

In our study, ROS level was measured using the DCFH-DA fluorescence method (Lawler et al., 2003; Han et al., 2014). Three earthworms at each concentration were ground and mixed with potassium phosphate buffer in an ice bath. The mixture was centrifuged for two times, 15 min at 3000 g and 20 min at 2000 g, respectively. Then the precipitate was resuspended with potassium phosphate buffer to achieve and suspension was acquired. All the steps in the experiment were performed at 4 °C. The reaction liquid of the suspension and DCFH-DA solution was heated at 37 °C for 30 min. The mixture was measured at a 488 nm excitation wavelength and at 522 nm for the fluorescence intensity (Shimadzu, RF-5301PC).

The comet assay was used for DNA damage determination. Three earthworms were soaked in 2 mL coelomocyte extract for 2–3 min to expel coelomocytes from the earthworms. After two runs of centrifugation and sediment resuspension, cell suspensions were obtained for SCGE, which was performed following Song et al. (2009). The olive tail moment was measured to assess the level of DNA damage caused by mesotrione to the earthworms.

### 2.5. Statistical analysis

Three replicates were used in the experiment. All data were analyzed by SPSS software (Ver. 20) and Microsoft Office Excel 2010. And the standard deviation (SD) was shown as error bars. In addition, one-way ANOVA was applied for data analysis, and the differences among