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Effect of temephos on cholinesterase activity in the earthworm *Eisenia fetida* (Oligochaeta, Lumbricidae)

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Abstract

In this study, adult *Eisenia fetida* earthworms were exposed to the sub-lethal concentrations of temephos using the contact filter paper test procedure. Since temephos is an organophosphate pesticide, its effects on earthworms were determined by measuring ChE inhibition—a known biomarker of exposure. The ChE activity was measured after a short time of exposure—1 and 2 h. As expected, the lowest ChE activity (72.70% and 38.03% inhibition) was measured at the highest concentration of temephos (120 ng cm^{-2}) applied. More interestingly, at the 0.12 ng cm^{-2} concentration the ChE activity increased up to 36.28% of activity in the control in all three conducted experiments. Dose-response curves showed an inverted U-shape characteristic for hormesis. This hormetic-like effect could be important for health status of an earthworm.

Keywords: Cholinesterase; Earthworm; Temephos; Hormesis; *Eisenia fetida*; Insecticide

1. Introduction

Mosquitoes take up a significant portion of many species that are subjected to insecticide control around the world. Decisions to use insecticides are based on an evaluation of the risks to the general public from diseases transmitted by mosquitoes or on an evaluation of the nuisance level that communities can tolerate from a mosquito infestation (USEPA, 2005). Mosquito control is based on both larvicidal and adulticidal treatments and account must be taken of adverse impact on non-target organisms and pesticide residues in the aquatic medium. Among larvicides, the only and the most widely used organophosphate is temephos. It is particularly used to control mosquito, midge, and black fly larvae (USEPA, 2000). Temephos is applied most commonly by helicopter directly in the mosquito breeding sites, which mostly includes lakes, ponds, and wetlands. Additionally, it can also be applied, in either liquid or granular form, by backpack sprayers, fixed-wing aircraft, and sprayers on the vehicles. Although it is not expected that temephos have a direct impact on terrestrial animals, treatment by helicopter can be imprecise and some of the larvicide may end up in the soil, especially when large marsh areas or wetlands are treated. Pierce et al. (1989) reported that with an aerial application 50% and 60% of the larvicide was captured by the mangrove vegetation and persisted in the mangrove foliage for 7 days (Pierce et al., 1996). Furthermore, the low water-solubility (0.001 mg L^{-1}) (EXTOXNET, 1996) reported for temephos suggests that it has a high affinity for soils and sediments (soil organic partition coefficient: $\log K_{oc} \approx 5$). The estimated half-life for soils and sediments is 30 days (EXTOXNET, 1996).

The majority of the researches on impact of temephos so far have been based on freshwater and marine aquatic organisms (Jacob et al., 1982; Hanazato et al., 1989; Jumel and Lagadic, 1998; Fourcy et al., 2002), both fish and invertebrates and some birds and bees, but none on the soil invertebrate fauna. The results showed that temephos has a variable toxicity depending on the species tested (USEPA, 1999). Temephos is highly toxic to very highly toxic to

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freshwater and marine/estuarine aquatic invertebrates and moderately to highly toxic to various bird species and according to its high affinity for soils and sediments experiments with soil invertebrates should be conducted as well.

Earthworms can represent a major fraction of the soil invertebrate biomass (>80%) and play an important role in improving structure and fertility of the soil (Edwards and Bohlen, 1996). Therefore, those characteristics along with a reasonably easy maintenance in the laboratory make them a good indicator species for ecotoxicological research. Although their complexity is low compared to vertebrates, they have highly differentiated organs and tissues (Stenersen et al., 1992); they also possess an immune system that is comparable with that of vertebrates (Goven et al., 1998). Earthworms can be exposed to pesticides either through dermal contact or by ingestion. The uptake of organic chemicals in soil- and sediment-dwelling organisms is usually described as a simple hydrophobic partitioning between pore water and the organism's lipids (Jager, 2004). Belfroid et al. (1995) concluded that food intake becomes a relevant additional uptake route for very hydrophobic chemicals (octanol-water partition coefficient: $\log K_{ow} > 5$). As a $\log K_{ow}$ for temephos is 4.9 dermal exposure is relevant uptake route which can be assessed by filter paper contact test. The specific effect of OPs on the activity of cholinesterase (ChE) can be used as an effective biomarker of exposure to insecticides (Booth and O'Halloran, 2001). A number of studies have been conducted using this biomarker effectively to determine OP effects on earthworms. All of these studies were effectuated with diazinon, chlorpyrifos, or azodrin, but none with temephos (Booth et al., 1998; O'Halloran et al., 1999; Venkateswara Rao et al., 2003; Venkateswara Rao and Kavitha, 2004). The endpoints in these studies were various: from mortality, reproduction, neurological functions to behavioral changes.

In this study, earthworms from *Eisenia fetida* species were exposed to field-relevant concentrations of temephos using the contact filter paper test procedure. Field-relevant concentrations are usually sub-lethal and often sub-effective; nevertheless earthworms adversely affected by exposure to them become less able to perform their beneficial and essential functions in the soil ecosystem (Slimak, 1997).

2. Materials and methods

2.1. Earthworms

The adult earthworms *E. fetida* (Savigny, 1826) (Oligochaeta, Lumbricidae) were obtained from the culture maintained in our laboratory with cow dung as a substrate and food. They were removed from culture, rinsed with tap water, and stored in Petri dishes on damp filter paper for 48 h (in the dark at 20 ± 1 °C) to void gut contents. The earthworms used in this assay were all adults with well-developed clitellae (0.22 ± 0.07 g—after voiding gut content). Due to the fact earthworms are hermaphrodite; no sexual differences were taken into account.

2.2. Chemicals

All reagents, except temephos, used in the study were of analytical grade. Abate® 4-E (temephos 44%), the commercial name of temephos (*O*,*O*′-[thiodi-4,1-phenylene]bis[*O*,*O*-dimethyl] phosphorothioate), was acquired from the Institute of Public Health of Osijek-Baranja County and is the same product that is used use for the larvicidal treatments by helicopter in the surrounding area.

2.3. Filter paper contact tests

All four experiments were conducted by the paper contact toxicity method (OECD, 1984). The sides and bottom of a flat-bottom glass vials 4.4 cm in length and 5 cm in diameter were lined with the filter paper without overlapping. The test chemical was suspended in water. The control was distilled water for all experiments. The filter paper was left to dry before 1 mL of distilled water was added to each vial to moisten the filter paper. One earthworm per vial was added, and 10 replicates of each concentration were prepared in all experiments. After addition of earthworms, each vial was closed with a cap with a small ventilation hole and placed in the dark. In the first-preliminary experiment, earthworms were exposed to four different concentrations of temephos (120, 12, 1.2, and 0.12 ng cm^{-2}) for a period of 72 h. The concentrations were chosen and calculated according to the recommended application rates of 0.5–1.0 fl.oz./A. for the emulsifiable concentrate (USEPA, 1999). The time was recorded as the first symptom of toxicity (morphological changes) or mortality occurred. The morphological changes observed and set as an endpoint were excessive coelomic fluid secretion, constriction, and swelling of segments. According to the first observed morphological changes in the first-preliminary experiment (after 3 h), the length of exposure for the following experiments was set to 2 h for the second and fourth and 1 h for the third experiment. It was based both on the assumption that the molecular changes had occurred before morphological (observable) changes (already after 1/3 of the time of exposure, e.g., 1 h) and other laboratory experiments that had been conducted and which showed that 1 h exposure to the highest used concentration (120 ng cm^{-2}) causes molecular changes. Furthermore, the data from the first experiment were used for calculating EC_{50}s and LC_{50}s. Four different concentrations of temephos (120, 12, 1.2, and 0.12 ng cm^{-2}) in the second experiment and seven different concentrations of temephos (120, 12, 5, 1.2, 0.5, 0.12, and 0.05 ng cm^{-2}) in the third experiment were applied, 1 mL volume, on the whole surface of the filter paper. The fourth experiment was conducted after the results from the second and third experiment had been obtained in order to confirm the achieved hormetic effect. Accordingly, eight concentrations of temephos (2.5, 1.24, 0.5, 0.25, 0.12, 0.1, 0.05, and 0.025 ng cm^{-2}), in concentration range in which hormetic effect was previously assumed, were chosen and applied in the same manner as in the above described experiments.

2.4. Sample preparation and cholinesterase activity (biochemical assay)

Cholinesterase activity was measured in earthworms from second, third and fourth experiments. Whole earthworms, with addition of cold TRIS buffer (pH 7.4) (1:5), were homogenized on ice with Ultra-Turrax T18 homogenizer. The homogenates were then centrifuged for 30 min at $9000 \times g$ and 4 °C to yield the post-mitochondrial fraction (supernatant: S9). Aliquots of the supernatant were frozen at −80 °C until use. ChE activity was determined according to the method of Ellman et al. (1961), using Shimadzu UV-1601 spectrophotometer. Samples were analyzed for total protein content using the Bradford method (Bradford, 1976) with bovine serum albumin as standard.

Kinetic measurements were performed with acetylcholine iodide (AChI) as the substrate. Reactions were performed in a cuvette containing: (a) 1 mL of 100 mM phosphate buffer (pH 7.4) + 0.33 mM DTNB (5,5′-dithio-(2-nitrobenzoic acid)), (b) 100 μL of tissue extracts (S9), and