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Assessment of the toxicity of ethyl-parathion to earthworms (*Aporrectodea caliginosa*) using behavioural, physiological and biochemical markers

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ABSTRACT

The aim of this study was to determine the influence of soil properties on ethyl-parathion toxicity to earthworms (*Aporrectodea caliginosa*) using different markers (mortality, cholinesterase (ChE) activity, body mass change and burrowing behaviour). In a first experiment, the toxicity of five different concentrations of ethyl-parathion was studied in four different soils, three from Mexico and one from France, which were previously seen to have different adsorption partition coefficient (Kd) values for ethyl-parathion. In a second experiment, which focused on the sublethal effects of ethyl-parathion, the markers were compared in the French soil only. In all four soils, high mortality was observed at 70 mg active ingredient kg^{-1} of dry soil, significant body mass loss at 7 mg kg^{-1} and significantly decreased ChE activity at 0.7 mg kg^{-1} (the normal application rate). Mortality was highest in one of the Mexican soils, an Andosol characterised by the lowest pH and highest Kd. Nevertheless, almost no differences were observed between body mass change and cholinesterase activity values among the four soils suggesting that ethyl-parathion toxicity was not greatly affected by Kd values or soil characteristics. The second experiment showed that (i) body mass change was the least sensitive toxicity marker whereas (ii) ChE activity and some characteristics of the burrow systems (burrow length and the number of branches) were sensitive parameters showing responses at very low ethyl-parathion (0.07 mg kg^{-1}) concentrations. Even if the burrowing behaviour of earthworms is not so easy to measure, it appears preferable to ChE activity, which is only a biomarker of exposure and cannot be used to predict environmental effects on the soil ecosystem or functioning.

1. Introduction

All pesticides have been assessed according to sets of standardised protocols to detect potential negative effects on model organisms. Earthworms are a terrestrial model organism, on which ecotoxicity tests have to be carried out since these animals play a key role in soils. Indeed, earthworms, as ecosystem engineers (Jones et al., 1994), have a great influence on many physical (transfer properties), chemical (biogeochemical cycles) and biological (interactions

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with other components of the soil ecosystem) processes that take place in the soil (McCoy et al., 1994; Görres et al., 2001).

Organophosphates are long-established insecticides with high acute toxicity and low specificity and are still massively used worldwide (Garcia de la Parra et al., 2006). These pesticides inhibit acetylcholinesterases, leading to an accumulation of acetylcholine, which interferes with the function of the nervous system. Until recently the organophosphate ethyl-parathion was used to control mites, aphids, moths and other arthropods on a wide variety of plant cultures (orchards, vines and arable land). In 2001, the European Commission (EEC, 2001) banned this insecticide due to risks for operators and non-target organisms such as bees and birds. In the same document, it was concluded that usual application rates would not cause risk to earthworms (14-d LC50 = 65 mg kg^{-1} soil and NOEC = 32 mg a.i. kg^{-1} soil). However, van Gestel et al. (1992), using *Eisenia andrei* and an artificial soil, reported that ethyl-parathion had detrimental effects on growth and fertility at concentrations as low as 10 and 18 mg a.i. kg^{-1} soil, respectively, even if the LC50 was greater than 180 mg a.i. kg^{-1} soil.

In the 1990s, it was recommended that artificial soils be used for ecotoxicity tests (Reinecke, 1992). The main expected advantage was the ability to compare results from different laboratories. However, ten years later, in the synthesis from the 3rd International Workshop on Earthworm Ecotoxicology, van Gestel and Weeks (2004) recommended using natural soils because results obtained with artificial soils "cannot be translated directly to field soils". This does not mean, however, that soil has no effect on pollutant bioavailability (Lanno et al., 2004) and implies that the soil used for the ecotoxicity tests must be thoroughly characterised (van Gestel and Weeks, 2004). Indeed, in a review of the main characteristics of soils that could influence pesticide bioavailability, van Gestel (1992) referred to previous studies by Ma (1983) and Lofs-Holmin (1982) who observed that lindane and benomyl toxicity to earthworms was affected by soil texture and organic matter content, respectively.

Therefore, in the present study, aimed at determining the lethal and sublethal effects of ethyl-parathion on the earthworm *Aporrectodea caliginosa*, we decided to investigate the detrimental effects of ethyl-parathion in four different soils. The sorption of ethyl-parathion in these four soils was characterised in previous papers (Olvera-Velona et al., 2008; Saffih-Hdadi et al., 2002) and showed that its fate was quite different depending on the soil characteristics. The toxic effects of ethyl-parathion on exposed earthworms were assessed by measuring mortality, as well as physiological (body mass change), biochemical (cholinesterase (ChE) activity) and behavioural (burrowing) markers. These markers were used in this study as "indirect biological measures of bioavailability" (sensu Lanno et al., 2004). We were then able to (i) determine whether soil type influences marker response under our experimental conditions and (ii) compare the qualities and drawbacks of these 4 markers for estimating pollutant toxicity to earthworms.

2. Materials and methods

2.1. Soils and earthworms

Three Mexican soils were selected on the basis on their relative prevalence at the regional scale (Morelos state in central Mexico), texture and organic matter content (Table 1). According to the FAO soil classification (WRB, 1998), the Mexican soils were classified as Andosol (Huitzilac site) and Vertisols (Ayala and Yautepec sites). In Morelos state, Andosols constitute 12% of the land and are predominant in the northern temperate region of the state. Developed on slopes of the volcanic sierras, these soils, which were naturally covered by forests, have been recently converted for agriculture (INEGI, 2004). Vertisols are found in 21% of the total surface area and are mainly distributed in the temperate-sub tropical area in the south of Morelos state. Historically, these soils have been used for agriculture due to their fertility (INEGI, 2004). A fourth soil was sampled in Caumont (SE of France), near Avignon, and is a Calcosol typical of soils currently found in this area.

The three Mexican soils were sampled from the surface layers (0–20 cm) of cultivated plots. The French soil was collected from the surface layer (0–20 cm) of a natural meadow. The samples were air-dried and sieved at 2 mm. Soil properties are summarised in Table 1. The Andosol was acid whereas the others were neutral (Vertisol 2) to moderately basic (Vertisol 1 and Calcosol). According to texture, the soils were classified as sand (Andosol), sandy clay (Vertisol 1) clay (Vertisol 2) and clay loam (Calcosol). The soil organic carbon content was much higher in the Andosol than in the Vertisols

Table 1 – Main characteristics of the four soils used for determining the toxicity of ethyl-parathion for *Aporrectodea caliginosa*

Location Soil type and name	Ayala Vertisol 1	Yautepec Vertisol 2	Huitzilac Andosol	Caumont Calcosol
pH_{water}	8.0	7.1	5.6	8.3
Sand (g kg^{-1})	49.4	41.8	55	19.3
Silt (g kg^{-1})	10.8	22.4	27.2	46.5
Clay (g kg^{-1})	39.8	35.8	17.8	34.2
Organic carbon (g kg^{-1})	10.8	17.8	54.3	15.5
Total N (g kg^{-1})	0.80	1.44	3.67	1.36
C/N	13.5	12.4	14.8	11.4
C.E.C. ($cmol_c$ kg^{-1})	44.6	23.9	21.9	14.0
Kd (l kg^{-1}) for parathion	38.6	68.6	74.9	24.4