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Toxicity of three pesticides commonly used in Brazil to *Pontoscolex corethrurus* (Müller, 1857) and *Eisenia andrei* (Bouché, 1972)

Andressa Cristhy Buch[a], George Gardner Brown[b,*], Cintia Carla Niva[b], Klaus Dieter Sautter[c], José Paulo Sousa[d]

[a] Federal University of Paraná, Rua dos Funcionários 1540, Setor de Ciências Agrárias, Curitiba, PR CEP 80035-050, Brazil
[b] Embrapa Forestry, Estrada da Ribeira km. 111, C.P. 319, Colombo, PR 83411-000, Brazil
[c] Positivo University, Rua Prof. Pedro Viriato Parigot de Souza 5300, Campo Comprido, Curitiba, PR 81280-330, Brazil
[d] IMAR-CMA, Department of Life Sciences, University of Coimbra, Apartado 3046, 3001-401 Coimbra, Portugal

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ABSTRACT

The indiscriminate and excessive use of pesticides poses serious risks to humans and the environment, including soil biota. Ecotoxicological tests are useful to indicate the extent to which these chemicals are harmful and how and where their effects occur. Some of these tests were standardized by ISO (International Organization for Standartization) using the earthworm species *Eisenia fetida* and *Eisenia andrei*, both native to temperate climates. However, these species may be of lower relevance for soil ecotoxicological studies since they live in the litter and feed on fresh organic matter. The species *Pontoscolex corethrurus*, native to tropical regions, may be an alternative for more relevant ecotoxicological tests as it is an endogeic geophagous species. However, little is known of its sensitivity to pesticides. Therefore, avoidance and mortality tests were performed using *E. andrei* and *P. corethrurus* and three pesticides commonly used in Brazilian agriculture: carbendazim, carbofuran and glyphosate. The tests were conducted in tropical artificial soil (TAS). For carbendazim, the median avoidance concentration (AC_{50}) was 76.1 and 65.8 mg a.i. kg^{-1} and the median lethal concentration (LC_{50}) 19.7 and 15.3 mg a.i. kg^{-1} for *E. andrei* and *P. corethrurus*, respectively. For carbofuran, the AC_{50} was 9.7 and 7.3 mg a.i. kg^{-1} and LC_{50} 13.5 and 9.3 mg a.i. kg^{-1} for *E. andrei* and *P. corethrurus*, respectively. Concentrations applied in the field of these two pesticides have toxic effects on both species. Glyphosate showed no toxic effects for either species even at the highest concentration tested (47 mg a.i. kg^{-1}), although they displayed avoidance behavior at this concentration. The sensitivity of *P. corethrurus* appears to be similar to the standard species for the pesticides evaluated reinforcing the notion that *E. andrei* is a good test species. Nevertheless, further studies should be undertaken using other contaminants to confirm the similar sensitivity of both species and the relevance of *E. andrei* in ecotoxicological tests.

1. Introduction

Brazil is currently the largest consumer of pesticides worldwide and the eighth per cultivated area (Anvisa, 2006; Rebelo et al., 2010). Nevertheless, the side-effects of this high pesticide usage on the soil ecosystem have been little studied in relation to non-target organisms.

Pesticides can affect the soil and its biota by direct contact, or indirectly, by volatilization, leaching and dispersion (Andréa, 2010). The toxicity of a chemical depends on the exposure time, the susceptibility of the organism, concentration, characteristics of the chemical or its applied combinations and environmental factors (Fent, 2004). The toxicity to a test organism of different chemicals may differ between temperate and tropical regions (Laabs et al., 2002; Garcia et al., 2004, 2011; De Silva and van Gestel, 2009b), so that results from the Northern hemisphere may not be directly applicable to tropical ecosystems (Garcia et al., 2011).

The ISO protocols for ecotoxicological tests using earthworms and contaminated soils recommend *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* Bouché, 1972 native to temperate regions (ISO, 1993, 2007). These epigeic species feed on fresh organic matter on the soil surface and do not ingest soil (Lavelle, 1988), and are recognized as having sub-optimal ecological relevance for assessing exposure effects in natural soils. However, for practical reasons both species are still widely used for these purposes due to their short reproduction time, easy handling in laboratory and the wealth

* Corresponding author. Tel.: +55 41 3675 5707; fax: +55 41 3675 5601.
E-mail addresses: andressabuch@hotmail.com (A.C. Buch), george.brown@embrapa.br, browng@pq.cnpq.br (G.G. Brown), cintiacn@gmail.com (C.C. Niva), ksautter@up.edu.br (K.D. Sautter), jps@zoo.uc.pt (J.P. Sousa).

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of information on their sensitivity to various types of pollutants (Udovic and Lestan, 2010; Gomez-Eyles et al., 2011; Kinney et al., 2012; Wang et al., 2012). Nevertheless, the testing of natural soil or tropical artificial soil (TAS; Garcia et al., 2004) and earthworm species found in tropical areas (particularly geophagous endogeics that are dominant in tropical soils; Lavelle, 1983) in toxicity tests could contribute to a more relevant and reliable risk assessment of chemicals in the tropics (Kuperman et al., 2009).

Pontoscolex corethrurus (Müller, 1857) is a native Brazilian species commonly found in disturbed agricultural and peri-urban soils of tropical regions both in Brazil and across the globe (Brown et al., 2006). It is an endogeic geophagous species that might be a good alternative for more relevant ecotoxicological tests in the tropics, although little is known of the toxicity of pesticides to this earthworm species. Therefore, the present experiment was performed to evaluate the sensitivities of *E. andrei* and *P. corethrurus* to three frequently used pesticides in Brazil – carbendazim, carbofuran and glyphosate.

2. Materials and methods

2.1. Test substrate

The test substrate used was TAS, developed by Garcia et al. (2004) and based on the formulation of the OECD artificial soil (OECD, 1984). In TAS, the organic matter used is powdered coconut fiber, replacing the OECD sphagnum peat (Garcia et al., 2004; De Silva and van Gestel, 2009a). TAS consists of 70% fine sand, 20% kaolinite clay and 10% powdered coconut fiber, with pH adjusted to 6.0 ± 0.5 with calcium carbonate when necessary. The water holding capacity was adjusted to 60% of the TAS water holding capacity (i.e., 54% H_2O on TAS dry matter basis).

2.2. Test species

E. andrei, the species recommended by OECD (1984), ISO (2007) and the Brazilian authorities (IBAMA, 1990; ABNT, 2007), were obtained from vermicompost at the Centro Paranaense de Referência em Agroecologia (CPRA) in Pinhais, Brazil (25°18′47″S; 49°09′28″W), and kept in plastic boxes of 50 L filled with cow manure (from organically raised cows) at the Ecology Laboratory at Embrapa Forestry from March to December 2009. Food (cow manure) was offered weekly. *P. corethrurus* was collected from March to June and September to December 2009 at a rural household garden near Embrapa Forestry in Colombo, Brazil (25°23′30″S; 49°07′30″ W). Prior to the test, the earthworms were kept in plastic boxes of 2 L filled with their natural soil (Cambisol) and fed horse manure (from one organically raised animal) weekly. Previous attempts to keep this species in the laboratory with cow manure were unsuccessful (Buch, personal observation), so horse manure was used instead. Only adults with biomass of 0.7–1.0 g (*P. corethrurus*) and 0.3–0.6 g (*E. andrei*) were used.

2.3. Pesticides and their concentrations

Carbendazim is a highly persistent, systemic carbamate fungicide used to control diseases in fruit, vegetable and grain production and for seed treatment. At present, it is registered for four crops in Brazil and the 10th most widely used pesticide in Brazil (Rebelo et al., 2010) as well as the reference substance for ecotoxicological tests established by the OECD (1984). Carbofuran is a carbamate systemic insecticide and nematicide used in several crops, and has recently been used for earthworm control in rice fields in Southern Brazil, although it is not registered for this crop (Gassen, 2006; Bartz et al., 2009). Farmers in the State of Rio Grande do Sul have complained that earthworms (*Eukerria* spp.) were causing lodging of rice plants, reducing harvests, so they have been applying carbofuran, a known vermicide (Barrion and Litsinger, 1996; De Silva et al., 2010) to try and reduce earthworm populations. Glyphosate is a systemic herbicide recommended for control of monocot and dicot weeds, and is registered for 26 different crops. It is the most widely used active ingredient in Brazil, accounting for 75% of total herbicide consumption nationwide (Rebelo et al., 2010). According to the Brazilian Federal Law No. 7802/89 (Brazil, 1989), carbofuran is extremely toxic (Class I), while glyphosate and carbendazim are moderately toxic (Class III) (Rebelo et al., 2010).

The nominal concentrations of carbendazim – Derosal® 500 SC ($500\,g\,L^{-1}$ a.i.) – used for the avoidance test were 0, 1, 3.2, 10, 31.6, 100, 316 and 1000 mg a.i. kg^{-1} TAS, and for the mortality test 0, 1, 3.16, 10, 31.6 and 100 mg a.i. kg^{-1} TAS, based on application rates used for soybean.

The nominal concentrations of carbofuran – Furadan® 350 SC ($350\,g\,L^{-1}$ a.i.) – used for the avoidance tests were 0, 0.5, 1, 2.5, 5, 10, 20 and 40 mg a.i. kg^{-1} TAS, based on application rates used for cotton, rice and soybean production. For the mortality test, concentrations of 0, 2.5, 5, 10, 16 and 32 mg a.i. kg^{-1} TAS were used.

The nominal concentrations of glyphosate – Pica-Pau® 480 SC ($480\,g\,L^{-1}$ a.i.) – used for both the avoidance and mortality tests were 0, 7, 14, 21, 30 and 47 mg a.i. kg^{-1} TAS, based on application rates used in citrus, cotton, rice and maize production.

Separate soil batches were spiked independently with individual pesticides to prepare the required nominal concentrations, mixed thoroughly, and then weighed out for the ecotoxicological tests with each species.

2.4. Ecotoxicological tests

2.4.1. Avoidance test

This experiment was carried out according to ISO 17512-1 (ISO, 2007). Acclimatization of the animals was performed in TAS for 24 h in plastic boxes of 2 L. Separate tests were performed for each pesticide using each earthworm species. Each pesticide concentration had five replicates. Transparent plastic boxes (26.2 cm × 17.7 cm × 8.5 cm) were filled with TAS up to 4–5 cm height (ca. 500 g dry weight). Half of the box was filled with untreated TAS (control) and the other half with the treated TAS (pesticide-contaminated) with a cardboard divider between them. The cardboard separator was removed and ten *E. andrei* or six *P. corethrurus* were placed on the separating line in each test box. The number of individuals was lower for the latter species due to their larger biomass and body size (*E. andrei* measures 4–5 cm, while *P. corethrurus*, 8–10 cm). The boxes were closed and kept in the dark at 20 ± 4 °C. After 48 h the cardboard divider was reintroduced between the treated and untreated soils in the box and the number of worms on each side counted. The individuals found at the border between the two soil treatments in each box were assigned to the treatment where the anterior part of the body was located.

2.4.2. Acute test

This experiment was carried out according to ISO 11268-1 (ISO, 1993). Acclimatization of the animals was performed in TAS for 24 h in plastic boxes of 2 L. Separate tests were performed for each pesticide using each earthworm species and five replicates for each concentration. Ten *E. andrei* or six *P. corethrurus* were weighed and placed in 500 mL glass jars filled with ca. 500 g dry weight control and contaminated TAS. Each jar received 20 g defaunated (three 3-d freezing cycles) manure (from cows for *E. andrei* and from a horse for *P. corethrurus*), dried and sieved (4 mm) at the beginning of the experiment and on the seventh day, on the surface of the TAS. The jars were closed and kept in the dark at 20 ± 4 °C.