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Do recommended doses of glyphosate-based herbicides affect soil invertebrates? Field and laboratory screening tests to risk assessment

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HIGHLIGHTS

- Recommended doses of glyphosate herbicides were applied to desiccate oat.
- Bait lamina (field) and laboratory tests were run to screening the ecotoxicity.
- Earthworms and isopods not avoided soil and oat straw from field in multispecies test.
- Reproduction of collembolans was not impaired but they avoided one product.
- Reduction on soil fauna feeding activity was observed to the red label product.

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ABSTRACT

Despite glyphosate-based herbicides are widely used in agriculture, forestry and gardens, little is known about its effects on non-target organisms. The present work evaluated the ecotoxicity of four formulated products (Roundup® Original, Trop®, Zapp® Qi 620 and Crucial®) on soil invertebrates. Screening ecotoxicity tests were carried out with soil and oat straw collected in a field experiment, besides laboratory-spiked soils. Screening tests included avoidance behaviour of earthworms (*Eisenia andrei*), collembolans (*Folsomia candida*) and isopods (*Porcellio dilatatus*) in single and multispecies tests; reproduction of collembolans (*F. candida*), and bait lamina in field. Non-avoidance behaviour was observed in standard tests (earthworms) in soil, neither in multispecies tests (earthworm + isopods) using oat straw, while for collembolans it occurred for the product Zapp® Qi 620 even at the recommended dose. Reproduction of *F. candida* was not impaired even at high doses in laboratory-spiked soils. Feeding activity on bait lamina test was impaired in treatment corresponding to the red label product, Crucial®. Results showed the relevance of bait lamina test on screening the impact of herbicides in the field. The findings highlight the importance of considering different formulations for the same active ingredient in risk assessment of pesticides.

1. Introduction

Nowadays, agricultural intensification is heavily dependent on herbicides for weed control (Allegrini et al., 2015). Considered the major agrochemical consumer's country (Carneiro, 2015), the main reasons for increasing consumption of herbicides in Brazil are agriculture frontiers' expansion and increasing on land use for no-till practice (Tavella et al., 2011).

As the most popular herbicide used worldwide, the acid equivalent glyphosate [(N-phosphonomethyl) glycine] will probably remain as the world's most used agrochemical during the next years (USEPA, 2012) and thus the interest in quantifying its impacts on the environment and human health are needed (Benbrook, 2016). It is a non-selective systemic broad-spectrum inhibitor of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) which is not present in human or animal cells. EPSPs

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inhibition causes the interruption of three aromatic amino acids (phenylalanine, tyrosine and tryptophan) vital to plant synthesis (Giesy et al., 2000; Lane et al., 2012). Although the foliar application, a great amount of glyphosate can reach the soil (Lane et al., 2012) causing adsorption by minerals and organic phase (Maqueda et al., 2017) leading the residue to soil invertebrates.

Despite glyphosate-based herbicides are widely used in agriculture, forestry and gardens, surprisingly little is known about their effects on non-target organisms (Zaller et al., 2014). The existent studies on glyphosate ecotoxicity and its metabolites for soil fauna are still non-conclusive (Domínguez et al., 2016). While Santos et al. (2012) found no effects for collembolans and earthworms in glyphosate recommended doses applied in Mediterranean soil, Casabé et al. (2007) related damage to soil fauna and ecosystem services in areas with glyphosate application in soybean plantation in Argentina. Kanashiro (2015) found chronic effects for earthworms just in high doses, otherwise another study involving one of the main metabolites of glyphosate, aminomethylphosphonic acid (AMPA) pointed out that earthworms from parents exposed to contaminated soil can have size and growth restricted and may affect ecosystem function carried out by them in agricultural or forestry soil (Domínguez et al., 2016).

Other uncertainties concerns to the herbicides formulation. Commercially formulated as potassium, isopropyl amine, monoammonium, diammonium and trimesium salts, these formulations may include inert substances known as surfactants which, in some cases, can have higher toxicity when compared to the acid equivalent itself (Giesy et al., 2000; Aguiar et al., 2016).

The aim of this work was to evaluate four commercial glyphosate herbicides, applied in recommended doses in field and higher doses in laboratory, presenting a screening of its ecotoxicity and expected effects on soil invertebrates.

2. Material and methods

2.1. Field experiment

Four commercial glyphosate herbicides were used in this study:

- Roundup®Original: 360.00 g L^{-1} acid equivalent (a.e.) glyphosate, isopropylamine salt – 480.00 g L^{-1}, soluble (liquid) concentrate - SL;
- Trop®: 354.61 g L^{-1} a.e. glyphosate, isopropylamine salt – 480.00 g L^{-1}, SL;
- Zapp® QI 620: 500.00 g L^{-1} a.e. glyphosate, potassium salt 620.00 g L^{-1}, SL;
- Crucial®: 540.00 g L^{-1} a.e. glyphosate, isopropylamine salt 400.8 g L^{-1} and potassium salt 297,75 g L^{-1}, SL.

These herbicides were applied in field to burndown black oat in recommended dose of 720 g L^{-1} a.e. for each product, evaluated at the Experimental Farm of Federal University of Santa Catarina (UFSC), in Curitibanos, SC, Brazil, in three plots per treatment, in a randomized block design. Application using a pressurized sprayer with CO_2 was used with a 2.0 m long bar containing four sprays tips AVISO 11002 interspaced 50 cm from each other at a distance of 50 cm of the cultivation, kept at a work pressure of 2.8 kgf cm^{-2} and speed of 3.6 km $^{h-1}$ providing an application volume of 200 L ha^{-1}. Three plots without application of herbicides were used as control. Soil was classified as haplic cambisol (2.2% organic matter, clay texture) and climate is classified as Cfb, with mean temperatures reaching less than 22 °C in the warmer month and annual precipitation around 1.400 mm.

2.2. Collection and preparation of the samples for ecotoxicity tests

Eleven days after the field application, soil samples were collected at surface layer (20 cm), sieved (5 mm mesh) and immediately used in ecotoxicity tests. The moisture was adjusted to 50% of the water holding capacity and pH measured at the beginning and at the end of each test.

Oat straw samples were collected at each treatment, transported to the lab and cut in 2 cm size fragments using a paper-cutter. This process was adopted in order to facilitate the use of oat straw in avoidance tests, standardizing the volume of straw in each side of test's recipients.

2.3. Preparation of contaminated soil in laboratory

Additional tests were carried out with lab -contaminated soil with higher concentrations than that expected to be found in field. For this purpose, extra quantities of control soil were collected, sieved (5 mm mesh) and stored at the lab. Soil samples were treated using the formulated products at nominal concentrations of:

- Avoidance tests with *Eisenia andrei* Bouché, 1972: Roundup® Original (89.29 mg a.e. Kg^{-1}); Trop® (89.29 mg a.e. Kg^{-1}); Zapp® Qi 620 (64.29 mg a.e. Kg^{-1}); Crucial® (59.52 mg a.e. Kg^{-1});
- Reproduction test with *Folsomia candida* Willem, 1902: Roundup® Original (6; 12; 24 e 48 mg a.e. Kg^{-1}); Trop® (6; 12; 24 e 48 mg a.e. Kg^{-1}); Zapp® Qi 620 (7.75; 15.5; 31 e 62 mg a.e. Kg^{-1}); Crucial® (8.73; 17.45; 34 e 69.8 mg a.e. Kg^{-1}).

These tests started in the same day of product's addition.

2.4. Test organisms

Earthworms of the species *E. andrei*, collembolans of the species *F. candida* and isopods of the species *Porcellio dilatatus* Brandt, 1833 were obtained from laboratory cultures, maintained under photoperiod of 12 h light: 12 h dark and temperature of 20 ± 1 °C. Earthworms and collembolans were cultured according to ISO guidelines 17512-1 (ISO, 2008) and 11267 (ISO, 2014), respectively. Isopods were cultured following recommendations from the literature (Garcia, 2004; Niemeyer et al., 2009), because no standardized ISO guideline is currently available. However, isopods are considered good candidates for future standardization because of their easily manipulation and role in soil ecosystems (Van Gestel, 2012).

Earthworms were cultured in plastic boxes of 11 L of capacity, in a mixture of cattle dung free of antibiotics and coconut dust (1:1, v:v), moistened with distilled water, fed weekly with cooked oat. Collembolans were cultured in culture vessels lined with a mixture of plaster of Paris and activated charcoal in a ratio of 10:1, fed twice a week with granulated dry yeast *Saccharomyces cerevisiae*. Isopods were cultured in culture vessels lined with worm humus, weekly moistened with sprays of distilled water, fed with slices of potatoes and fish food.

Synchronized organisms were used in the tests: *E. andrei* (2–12 months old; 250–600 mg weight and clitellated), *F. candida* (adults in avoidance tests; 10–12 days old in reproduction tests), and *P. dilatatus* (adult isopods with synchronized weight of 35 ± 10 mg).

2.5. Avoidance behaviour tests

Avoidance tests were carried out following ISO guidelines: ISO 17512-1 (ISO, 2008) for earthworms and ISO 17512-2 (ISO, 2011) for collembolans.