Role of Climatic Factors In The Toxicity of Fipronil Toward Earthworms In Two Tropical Soils: Effects of Increased Temperature And Reduced Soil Moisture Content

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Abstract

The aim of this study was to assess the effect of temperature on the toxicity of fipronil toward earthworms (*Eisenia andrei*) in two Brazilian soils (Entisol and Oxisol) with contrasting textures. In the case of Entisol, the influence of the soil moisture content on the toxicity was also investigated. Earthworms were exposed for 56 days to soils spiked with increasing concentrations of fipronil under scenarios with different combinations of temperature (20, 25 and 27 °C) and soil moisture content (60 and 30% of water holding capacity (WHC) for Entisol and 60% WHC for Oxisol). The number of juveniles produced was taken as the endpoint and a risk assessment was performed based on the hazard quotient (HQ). In Entisol, at 60% WHC the fipronil toxicity decreased at 27 °C compared with the other temperatures tested (EC_{50} = 52.58, 48.48 and 110 mg kg⁻¹ for 20, 25 and 27 °C, respectively). In the case of Oxisol at 60% WHC, the fipronil toxicity increased at 27 °C compared with other temperatures (EC_{50} = 277.57, 312.87 and 39.89 mg kg⁻¹ at 20, 25 and 27 °C, respectively). An increase in fipronil toxicity was also observed with a decrease in soil moisture content in Entisol at 27 °C (EC_{50} = 27.95 and 110 mg kg⁻¹ for 30% and 60% WHC, respectively). The risk of fipronil was only significant at 27 °C in Entisol and Oxisol with water contents of 30% and 60% WHC, respectively, revealing that higher temperatures can increase the risk of fipronil toxicity toward earthworms. The results reported herein show that soil properties associated with climatic shifts could enhance the ecotoxicological effects and risk of fipronil for earthworms, depending on the type of soil.

1 Introduction

According to the Intergovernmental Panel on Climate Change (IPCC), increases in global temperatures and the occurrence of extreme weather events, are expected in the coming years. In a global scenario, an increase of around 2 °C in global temperature is expected between the years 2021 - 2040. In Brazil, although the predicted consequences of climate change vary for different regions, if this global warming trend is confirmed, temperatures could rise by around 3.0 – 3.5 °C and reductions of between 10 – 20% in rainfall patterns may occur, which increases the probability of extreme and prolonged drought events (IPCC 2021).

The consequences of climate change, especially those related to temperature and precipitation, are considered an imminent problem due to long-term impacts on terrestrial species. These changes can affect the physical, chemical and biological properties of the soil ecosystem and can also affect the structure and composition of the edaphic community. Disorders in the metabolism of soil organisms can impair their growth, reproduction and behavior, making them susceptible to additional environmental disturbances, such as soil contamination by toxic substances caused by human activities (Noyes et al. 2009). Therefore, it is expected that an increase in temperature and a reduction in rainfall will enhance the risk of toxic substances present in the soil (González-Alcaraz et al. 2015).

Fipronil is a phenylpyrazole pesticide that is efficient in pest control and widely used for seed treatment, with sales of 2,000 tons in 2019 in Brazil (IBAMA 2019). The lack of training for pesticide application

(Waichman et al. 2007) and the accumulation due to repeated inputs may result in greater residues of the active ingredient (a.i.) in the soil, which can impact non-target organisms (Daam et al. 2019). The ecotoxicological effects of fipronil on the survival, reproduction and growth of different soil organisms have been reported in the literature (e.g., San Miguel et al. 2008; Alves et al. 2014; Qin et al. 2014; Qu et al. 2014; Zortéa et al. 2018a; Zortéa et al. 2018b) and reveal that this molecule can cause population imbalances for different species. However, studies on the effects of fipronil used for seed treatment combined with abiotic and climatic factors, in particular toxicity toward earthworms, were not found in the literature.

To address this knowledge gap, in this study the influence of an increase in temperature and reduction in the moisture content of tropical soils on the toxicity of fipronil toward the earthworm species *Eisenia andrei* was evaluated. Bioassays were conducted in two contrasting natural soils (Entisol and Oxisol) with different combinations of temperatures (20, 25 and 27 °C) and soil moisture contents (30 and 60% of water holding capacity (WHC) for Entisol and 60% WHC for Oxisol). The number of juveniles produced by the earthworms was considered as the endpoint. The hypotheses of this study are: 1) the toxicity and risk of fipronil toward *E. andrei* increases at higher temperatures in both tropical test soils; and 2) this toxicity and risk increases in Entisol under drought conditions.

2 Materials And Methods

2.1 TEST SOILS

Samples of two Brazilian soils, Entisol and Oxisol, were collected in the municipalities of Araranguá (29° 00'S, 49° 31'W) and Palmitos (27° 04'S, 53° 09'W), respectively, from the top soil layer (0-20 cm), in areas with no history of contamination. The sampled soils were sieved (2 mm), defaunated applying the procedure described by Alves et al. (2019), air-dried and kept protected from light at room temperature. The soil characteristics are shown in Table 1, where the WHC and pH (1 M KCl) were measured following the ISO 11267 recommendations (ISO 2014), and the cation exchange capacity (CEC), soil organic matter (SOM) and sand, clay and silt contents were determined based on methods described by Tedesco et al. (1995).

Table 1
Mean values ($\pm$ standard deviation; $n = 2$) for physical and chemical characteristics of the tropical soils (Entisol and Oxisol) used in the ecotoxicological tests.

Parameter	Entisol	Oxisol
pH (1M-KCl)	4.2 $\pm$ 0.2	5.5 $\pm$ 0.1
SOM (%)	2.2 $\pm$ 0.1	4.7 $\pm$ 0.1
CEC ($\text{cmol}_c \text{ dm}^{-3}$)	1.4 $\pm$ 0.1	18.3 $\pm$ 2.8
WHC (%)	31.6 $\pm$ 1.1	58.8 $\pm$ 2.2
Sand (%)	93.8 $\pm$ 0.4	28.6 $\pm$ 0.7
Clay (%)	4.1 $\pm$ 0.2	33.0 $\pm$ 0.0
Silt (%)	2.1 $\pm$ 0.1	38.4 $\pm$ 0.7
Soil texture	Sandy	Clay loam
SOM: Soil Organic Matter;		
CEC: Cation Exchange Capacity;		
WHC: Water Holding Capacity.		

2.2 TEST SPECIES

Bioassays were performed using laboratory cultured individuals of the species *E. andrei* (Lumbricidae, Oligochaeta), obtained from Minhobox® Corporation, Minas Gerais State, Brazil. The organisms were maintained in a room with a temperature of 20 ± 2 °C applying a 12 h photoperiod (ISO 2012), in boxes with a moisturized substrate composed of defaunated horse manure (free of contaminants), coconut fiber and sand in the proportion of 2:1:0.3 (w:w:w), respectively. Once a week, the earthworms received cooked oatmeal as food and distilled water was used to adjust the substrate moisture content.

2.3 TEST SUBSTANCE

Ecotoxicological assays were performed using the commercial formulation Shelter®, an insecticide used for chemical seed treatment, which contains 250 g of fipronil L^{-1} as the active ingredient (a.i.). Test soils were spiked to give increasing concentrations (actual) of the a.i. (8.95, 19.48, 38.22, 155.61 and 237.81 mg kg^{-1} for Entisol; 12.99, 27.94, 48.42, 204.67 and 374.29 mg kg^{-1} for Oxisol), which were chosen from range-finding tests (data not shown). A control treatment was also performed using only distilled water.

The spiking was performed via an aqueous solution, with volumes calculated to reach 30 and 60% WHC for Entisol, and only 60% WHC for Oxisol. In the latter case, we could not test lower moisture contents

because the species did not reproduce at 30 and 45% WHC in this soil (data not shown). Soil moisture content and pH were checked at the beginning and the end of each bioassay (Table S1).

The actual concentrations tested in soil samples were estimated applying the modified QuEChERS extraction method without the cleaning step (Gebrehiwot et al. 2019), followed by quantification on an LC-MS (2020, Shimadzu) with electrospray ionization source, quadruple mass analyzer and LabSolution data acquisition system (as described in Hennig et al. (2021)). The limit of detection of the equipment for the soil samples analyzed was 0.01 mg of a.i. per kg of dry soil (mg kg⁻¹).

2.4 ESTIMATION OF PEC AND PNEC

The predicted environmental concentrations (PEC) after 56 days were calculated according to the method of the European and Mediterranean Plant Protection Organization (EPPO 2003) using the software ESCAPE (2013). A scenario with a soybean crop and soil densities of 1.5 and 1.0 g cm⁻³ for Entisol and Oxisol, respectively, was considered. Also, the worst-case scenario of the Shelter® application was considered, at a sowing density of 60 kg of seed per ha (EMBRAPA 1988), with the highest recommended pesticide dose for the soybean crop (37.5 g a.i. per 60 kg of seeds, according to the manufacturer's recommendations), and with 5% of interception by plants, during only one planting cycle (Jackson et al. 2005). Dissipation half-life (DT₅₀ values) values of 68 days (Ying & Kookana 2002), 31 days (EFSA 2006) and 28 days (Shuai et al. 2012) were considered for the temperatures of 20, 25 and 27 °C, respectively. The values for the predicted no-effect concentrations (PNEC) were estimated based on the ratio between the EC₁₀ values and an assessment factor of 100 (EC 2003).

2.5 CHRONIC TOXICITY ASSAYS

The toxicity assays were performed according to ISO 11268-2 (ISO 2012), where organisms were exposed to increasing concentrations of fipronil in two types of soil (Entisol and Oxisol) and, in the case of Entisol, two different soil moisture contents (30 and 60% WHC) simulating different scenarios of water availability. For Oxisol, assays were performed only at 60% WHC. All assays were performed at temperatures of 20, 25 and 27 °C, simulating different scenarios of global warming.

Plastic containers (15 cm diameter and 10 cm height) with perforated lids (to allow gas exchange) received around 650 g of wet soil (control or spiked with the a.i. concentrations tested). Ten grams of horse manure moisturized with 20 mL of distilled water were offered as food at the start of the tests. Ten earthworms with known individual weights (250 - 600 mg; Table S2) were inserted in each replicate. Four replicates were performed for each treatment. Once a week, the soil moisture was adjusted with distilled water (weight-based) and horse manure was added as food (consumption-based). After 28 days from the beginning of the test, surviving adult earthworms were removed. During the next 28 days, only the soil and the cocoons and juveniles produced remained in the containers. At the end of the test (56 days), the experimental units were immersed in a water bath (60 ± 5 °C) for one hour and the *E. andrei* juveniles were manually counted.

2.6 DATA ANALYSIS

The homoscedasticity and normality of the data were tested via Bartlett and Kolmogorov-Smirnov tests, respectively. Logarithmic transformations were applied only to the data obtained for Oxisol at 20 °C, to achieve the assumptions. Significant differences ($p < 0.05$) between treatments were tested using ANOVA. Factorial ANOVA was applied to evaluate the interaction of fipronil concentrations, temperature and soil moisture in relation to the fipronil toxicity toward earthworms. A generalized linear model (GLM) was applied to the data to estimate the contribution of the same factors to the production of juveniles. The effective concentrations (ECs), that is, those at which 10% and 50% reductions in the species reproduction were observed (EC_{10} or EC_{50} , respectively), were estimated using non-linear regression models, according to Environment Canada (2007). The statistical analysis was performed using Statistica®, version 13.5.0.17 (TIBCO DATA SCIENCE 2013).

To identify significant differences between the EC values obtained applying the three temperatures tested (20 vs 25 vs 27 °C) for each soil, and as a function of the soil moisture content for the Entisol soil (30 vs 60% WHC), a generalized likelihood ratio test ($p < 0.05$) was used, as described by Natal-da-Luz et al. (2011).

The risk assessment regarding the toxicity of fipronil toward *E. andrei* was carried out using the hazard quotient (HQ) approach, dividing PEC by PNEC, according to the procedure recommended by the European Commission (EC 2003). When $HQ > 1$, the risk is considered to be significant.

3 Results

All validity criteria for chronic ecotoxicological tests with earthworms (ISO 2012) were met (Table S2).

In all exposure scenarios, there was a decrease in the number of juveniles *E. andrei* produced with increasing fipronil concentrations (Fig. 1). In addition, the a.i. toxicity (based on the EC_{50}) toward earthworms was higher in Entisol compared to Oxisol (Table 2).

Table 2

Ecotoxicological parameters (EC_{50} and EC_{10} , with 95% confidence intervals) obtained from chronic toxicity assays with *Eisenia andrei* exposed to increasing concentrations of fipronil in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27 °C. Different lowercase letters indicate significant differences between EC_{50} or EC_{10} at different temperatures for the same soil. Different capital letters indicate significant differences between EC_{50} or EC_{10} at different soil moisture contents for Entisol at the same temperature.

Soil and moisture content	Ecotoxicological parameter	Fipronil concentration (mg kg ⁻¹)		
		Temperature		
		20 °C	25 °C	27 °C
Entisol 30% WHC	EC_{50}	58.71aA	17.19bA	27.95bB
	Limits (95%)	(32.89 – 84.52)	(10.97 – 23.41)	(18.56 – 37.33)
	EC_{10}	12.40aA	6.07aA	2.32bA
	Limits (95%)	(-) ^a	(3.15 – 8.98)	(0.44 – 4.19)
Entisol 60% WHC	EC_{50}	52.58bA	48.48bA	110.00aA
	Limits (95%)	(24.22 – 80.93)	(7.91 – 89.04)	(66.60 – 153.39)
	EC_{10}	3.67aB	0.44aB	5.44aA
	Limits (95%)	(-) ^a	(-) ^a	(-) ^a
Oxisol 60% WHC	EC_{50}	277.57a	312.87a	39.89b
	Limits (95%)	(82.94 – 472.20)	(126.55 – 499.19)	(15.30 – 64.47)
	EC_{10}	7.72a	4.97a	1.33a
	Limits (95%)	(-) ^a	(-) ^a	(-) ^a
^a Confidence limit (95%) could not be estimated.				

In Entisol, applying the drier condition (30% WHC), fipronil toxicity was higher at 25 and 27°C compared to 20°C. Also, in the same soil, at the standard moisture content (60% WHC), the fipronil toxicity was lower at 27 °C compared to 20 and 25 °C (Table 2). In Oxisol, the fipronil toxicity (based on the EC_{50}) was significantly higher at 27 °C compared to 20 and 25 °C.

The soil moisture content affected the reproduction of earthworms in Entisol, revealing a poor reproductive performance in control replicates at 30% WHC in comparison with 60% WHC, regardless of

the temperature (Fig. 1; Table S2). Also, fipronil toxicity (based on the EC₅₀) was higher under the drier condition (30% WHC) at 27°C, while at 20 and 25 °C the soil moisture did not significantly influence the effect (Table 2).

The factorial ANOVA results indicated a significant interaction between fipronil concentration, temperature and soil moisture content with regard to the production of juveniles in Entisol, while in Oxisol the reproduction was influenced by the concentration of fipronil and the temperature in isolation (Table S3).

According to the GLM model (Table 3), for both types of soil, the effects of fipronil concentration, soil moisture content and temperature can play different influences on the number of juveniles produced. According to the coefficients of the generated models, the effect of the fipronil concentration on earthworm reproduction in Entisol is twice the effect observed in Oxisol, while for the temperature the contrary trend was observed (with a stronger effect in Oxisol). Regarding the influence of soil moisture content in Entisol, a higher number of juveniles was produced at the higher value (60% WHC).

Table 3

Generalized linear model (GLM) for the effects of fipronil concentration, temperature and soil moisture content on *Eisenia andrei* reproduction in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27°C.

Soil	Model	R ²
Entisol	Juveniles = 86.7844700 - (0.37665615 × Fipronil concentrations) - (2.5578166 × Temperature) + (1.26135746 × Moisture)	0.68
Oxisol	Juveniles = 181.727511 - (0.12642862 × Fipronil concentrations) - (4.5245980 × Temperature)	0.49

Based on the HQ approach (Table 4), fipronil represented a risk to earthworms in Entisol (30% WHC) and Oxisol (60% WHC) only at 27 °C, revealing that an increase in temperature could increase the environmental risk associated with fipronil. However, in Entisol with 60% WHC, the HQ results indicated that fipronil represents a risk at 20 and 25 °C, with a lower risk at 27 °C, in agreement with the a.i. toxicity behavior observed for Entisol at 60% WHC, as noted previously (Table 2; Fig. 1).

Table 4

Hazard quotient (HQ) and predicted no-effect concentrations (PNEC) calculated for the exposure of *Eisenia andrei* to the time-weight average predicted environmental concentrations of fipronil 56 days after seed planting ($PEC_{TWA-56d}$) in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 and 27 °C.

Soil and moisture content	20 °C			25 °C			27 °C		
	PEC_{TWA} 56d	PNEC	HQ	PEC_{TWA} 56d	PNEC	HQ	PEC_{TWA} 56d	PNEC	HQ
Entisol 30% WHC	0.04	0.12	0.33	0.03	0.06	0.50	0.03	0.02	1.50
Entisol 60% WHC	0.04	0.04	1.00	0.03	0.03	1.00	0.03	0.05	0.60
Oxisol 60% WHC	0.05	0.08	0.62	0.04	0.05	0.80	0.04	0.01	4.00
HQ values higher than 1 (in bold) indicate significant/unacceptable risk;									
PNEC: $EC_{10}/100$.									

4 Discussion

The production of juveniles was not affected by an increase in temperature in this study, based on the control replicates (Fig. 1). However, regardless of the exposure scenario, there was a reduction in *E. andrei* reproduction with an increase in the fipronil concentration (Fig. 1). This can be explained by the bioactivity of the molecule in soil invertebrates, which involves the loss of neuronal signaling control and disturbance in the central nervous system (Cole et al. 1993).

Various effects of fipronil on earthworms have been reported in the literature. It has been found to affect their growth (Qin et al. 2014), reproduction (Alves et al. 2013; Zortéa et al. 2018a) and survival (Qu et al. 2014). Therefore, these studies reinforce our findings, despite being conducted under standard conditions of soil, temperature and moisture, indicating that fipronil may have harmful effects on the earthworm population in the soil.

The results of this study confirm our first working hypothesis, since an increase in temperature enhanced the fipronil toxicity toward earthworms in Entisol at 30% WHC and in Oxisol at 60% WHC (Table 2). These effects can be explained by interaction between the effects of higher temperature and a.i. concentration (Table S3). Temperature is the main factor that models the reproduction of earthworms, mainly in Oxisol (Table 3). A higher temperature may increase the activity of earthworms, inducing greater contact with and uptake of chemicals in the soil (Belfroid et al. 1994). In addition, an increase in temperature can promote the desorption of chemicals from the solid matrix of the soil, increasing their bioavailability and

the degree of contact with and uptake by soil organisms (Navarro et al. 1992; Belfroid et al. 1994). Simultaneously, high temperatures reduce the activity of detoxification enzymes, interfering with the recuperation of organisms exposed to contamination (Hackenberger et al. 2018). These effects have also been reported for imidacloprid (Bandeira et al. 2020), carbaryl (Lima et al. 2015), carbofuran and chlorpyrifos (De Silva et al. 2009) in studies on earthworms, where the toxicity of the pesticide increased with temperature, corroborating the results of this study.

In the tests on Entisol with 60% WHC, however, the first working hypothesis was not confirmed, since fipronil toxicity was lowest at the highest temperature applied (Table 2; Fig. 1). A possible explanation for this may be related to a loss of the a.i. through its degradation at higher temperatures in association with the greater moisture content in this soil. Some authors have reported that the sorption (Freundlich coefficient - Kf) and persistence (half-life values) of fipronil are lower in sandy soils compared to fine-textured soils (Doran et al. 2006; Spomer et al. 2009; Mandal and Singh 2013). These factors may enhance the bioavailability of fipronil in the soil pores of Entisol, where it is more susceptible to the degradation process, catalyzed by high temperatures and water moisture content (Scorza Junior and Franco 2013). Increasing the temperature can increase the activity of decomposer microorganisms as well as catalytic substances that act in the degradation of xenobiotics (Navarro et al. 1992). Higher water contents in soil with lower WHC, such as Entisol, can also accelerate the fipronil degradation process due to the breaking of the molecule by hydrolysis processes (Tingle 2003). The results reported herein are consistent with those observed by Hennig (2021), who found that fipronil toxicity toward collembolans in Entisol was lower at 27 °C compared to 25 and 20 °C. However, the cited authors, as in this study, did not measure fipronil degradation for the respective contamination scenarios and climatic factors, therefore, we are not able to verify this assumption.

The soil moisture content interacted with the fipronil concentration (Table S3), which influences the fipronil toxicity in Entisol and also affects the reproduction performance of earthworms in this soil, regardless of the temperature (Table 3; Fig. 1). A reduction in the soil moisture content can reduce the extent of water films around soil particles, known as hygroscopic water (Coleman et al. 2004). This can affect the survival, growth and reproduction of earthworms in the soil (Singh et al. 2019), since these organisms are soft-bodied, with highly permeable and sensitive skin (Peijnenburg et al. 2012). In this study, for Entisol at 27 °C, drier conditions revealed significantly higher a.i. toxicity in comparison with the standard soil moisture (based on the EC₅₀; Table 2). Effects of increased toxicity toward earthworms with reduced soil moisture content have been reported for fluoranthene (Long et al. 2009), carbaryl (Lima et al. 2011) and propiconazole (Hackenberger et al. 2018). According to Lima et al. (2011), dehydration due to drought conditions can enhance concentrations of a.i. in the bodies of earthworms, increasing the toxicity of pollutants. Moreover, as observed from our data (Table 3), when high temperatures are combined with drought conditions the toxicity of chemicals toward poikilothermic organisms, such as earthworms, may be enhanced due to the greater uptake rates at higher temperatures (Donker et al. 1998; Šustr and Pižl 2010; González-Alcaraz and Van Gestel 2016).

The PEC values (Table 4) indicated that, in the highest exposure scenario, residues of fipronil at levels of 0.03 to 0.05 mg kg⁻¹ may be present in the soil, even after 56 days of application. Some studies indicate that these levels may be realistic since concentrations of 0.003 to 0.15 mg kg⁻¹ have been found in a peanut field (Li et al. 2015), 0.01 - 0.15 mg kg⁻¹ in a cotton field (Chopra et al. 2011) and 0.01 to 2.06 mg kg⁻¹ in other agricultural fields (Ying and Kookana 2006).

Even though the EC₁₀ values for fipronil toxicity toward earthworms (0.44 - 12.40 mg kg⁻¹; Table 2) were higher than the estimated PEC and the concentrations reported in the literature in agricultural fields, the HQ approach indicated a risk associated with fipronil, in agreement with the toxicological effects (Table 2). In the case of Entisol and Oxisol with moisture contents of 30% and 60% WHC, respectively, the risk observed only at 27 °C may be due to additive effects of fipronil concentration and high temperature, as previously reported. However, the risk identified for Oxisol 60% WHC was around three times higher compared to Entisol 30% WHC, probably due to the pedogenetic characteristics of this soil. In this case, in addition to the effects of the contaminant, the high clay content (Table 1) could hamper the colonization of earthworms (Chelinho et al. 2011). In Entisol 60% WHC, the highest temperature tested could lead to a loss of the a.i. through catalyzation of the degradation processes, while at 20 and 25 °C, the bioavailability of the a.i. to soil organisms is likely to be higher, representing a risk to earthworms in these scenarios. It should be noted that soil moisture content was not considered in the calculation of PEC using ESCAPE and thus the PEC values for Entisol are the same for the two moisture regimes studied. In addition, since the degradation of the molecules was not assessed in this study, the reported data on increased/reduced risk should be interpreted with caution.

The HQ approach revealed a potential environmental risk for earthworms from the application of fipronil to natural tropical soils within a scenario of climate change and indicated that soil type can modulate the influence of temperature on fipronil toxicity toward earthworms. Abiotic factors related to soil type and climate, which directly influence the dynamics and bioavailability of pollutants to soil organisms, therefore need to be considered in risk assessments.

5 Conclusions

The results reported herein demonstrate that: 1) higher temperatures increase the toxicity of fipronil toward earthworms in Oxisol and under drier conditions, in Entisol, while in wetter Entisol the fipronil toxicity was lower; 2) the reduction in soil moisture content increased the toxicity of fipronil toward earthworms only at the highest temperature; and 3) fipronil posed a risk to earthworms in Oxisol and drier Entisol only at the highest temperature, while in wetter Entisol a risk was not observed at the highest temperature, but was detected at the lower temperatures tested. Our findings reinforce the need to include abiotic and climatic factors in risk and ecotoxicological assessments of pesticides, such as different soil types and different temperature and moisture scenarios.

Statements & Declarations

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Competing interests: The authors declare that they have no conflict of interest.

Authors' contributions: TBH.: Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft. PRLA.: Conceptualization, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing - review & editing. FOB: Investigation, Data curation, Formal analysis. LCC: Investigation, Data curation, Formal analysis. JSD: Investigation. MATS: Supervision and Data curation. DB: Resources, Supervision, Writing - review & editing.

Ethics approval and consent to participate: All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (Brazilian regimentation for the scientific use of animals - Law no. 11.794). Consent to participate and publication is not applicable.

Availability of data and material: The data that support the findings of this study are available and should be requested by e-mail.

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Figures

Figure 1

Dose-effect response curves representing the effects of fipronil on the reproduction of *Eisenia andrei* exposed for 56 days to increased concentrations (actual) of fipronil in Entisol (30 and 60% WHC) and Oxisol (60% WHC) at 20, 25 or 27 °C.

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