

***Enchytraeus albidus* (Oligochaeta) Exposed to Several Toxicants: Effects on Survival, Reproduction and Avoidance Behaviour**

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(Received 2 February 2010; accepted 8 February 2010)

Abstract—In this study, the effect of pesticides with different modes of action is assessed in the standard test species *Enchytraeus albidus*. These are important inhabitants of a wide variety of soil types and suitable test organisms for environmental risk assessment through the determination of effects on survival and reproduction. The main goals of this study were to test the toxicity of a range of pesticides to *E. albidus* in a natural soil and to assess if avoidance can be used to predict survival and reproduction effects. Moreover, it was proposed to investigate if the organisms' response to different toxics can be grouped into the respective chemical classes. Pesticides selected were the: (1) herbicides: phenmedipham and atrazine; (2) fungicides: carbendazim and pentachlorophenol; (3) insecticides: dimethoate and lindane. All tested pesticides caused effects in the survival and reproduction of *E. albidus*. The compounds that showed a higher toxicity were carbendazim, dimethoate and atrazine. Reproduction was usually the most sensitive endpoint and avoidance the least. The effect concentrations were not chemical class dependent.

Keywords: pesticides, toxicity, soil, enchytraeid worms

INTRODUCTION

Enchytraeids (Oligochaeta) are dominant members of the soil biocenosis in many temperate biotopes and are distributed in soils worldwide. These organisms are indirectly involved in regulating the degradation of organic matter, as well as improving the pore structure of the soil (Didden, 1993; Amorim *et al.*, 2005). The fact that they inhabit the upper layers of the soil (being specially exposed to pesticides) makes of them suitable test-species for environmental risk assessment of pesticides. Furthermore, the use of enchytraeids to test the effects of pesticides is of ecological relevance since they can be negatively affected by this group of contaminants either by a direct impact of the chemicals dissolved in pore water or adsorbed to organic particles, or via a reduction in food supply—directly by fungicides or indirectly by herbicides (Didden and Römbke, 2001).

There are currently standardized tests available to evaluate various toxicity parameters for enchytraeids, such as survival and reproduction (ISO, 2003; OECD, 2004). Avoidance behaviour can be also evaluated following the

earthworms ISO (International Organization for Standardization) guideline (ISO, 2007).

It has been suggested (Natal-da-Luz *et al.*, 2004, 2010) that the avoidance test with earthworms could be used as a first tier testing, due to its rapidness (48 h), followed by survival and reproduction tests. To test whether avoidance can predict responses on the survival and reproduction, 6 compounds were chosen. It is unknown what chemical composition causes avoidance in enchytraeids. Thus, compounds were selected so that they could represent different modes-of-action and high tonnage used pesticides. Therefore, the following two compounds of three classes of pesticides are selected: phenmedipham and atrazine as herbicides; carbendazim and pentachlorophenol as fungicides; dimethoate and lindane as insecticides.

Because of the extensive use (past or present) of these pesticides in agriculture and human activities, they are found in many European soils making it essential to know their effects on soil organisms. The main goals of this study were to test the toxicity of a range of pesticides to *Enchytraeus albidus* in a natural soil and to assess if avoidance can be used to predict survival and reproduction effects. Moreover, it was proposed to investigate if the organisms' response to different toxics can be grouped into the respective chemical classes (herbicides, fungicides and insecticides).

MATERIALS AND METHODS

Test species

The test species used was the Oligochaete *E. albidus*, Henle (1837). Organisms were maintained in laboratory cultures, being bred in moist soil (50% OECD soil, 50% natural garden soil) at 17°C with a photoperiod of 16:8 h light:dark and fed twice a week with finely ground and autoclaved rolled oats (Cimarron, Portugal). Details of the culturing process are given in Römcke and Moser (2002).

Test soil

All exposures were performed in the natural standard soil LUFA 2.2 (Løkke and van Gestel, 1998). This soil type is commercially available. The properties of this soil are summarised as follows: pH = 5.5, OM = 3.9%, texture = 6% clay; 17% silt; 77% sand.

Test substances and spiking

The substances tested were the following pesticides from different classes: a) herbicides: phenmedipham (Betosip, Stähler Agrochemie, 157 g L⁻¹ of active ingredient (a.i.)) and atrazine (Sigma-Aldrich, 97.4% a.i.); b) fungicides: carbendazim (Sigma-Aldrich, 97% a.i.) and pentachlorophenol (Sigma-Aldrich, 98% a.i.); c) insecticides: dimethoate [Sigma-Aldrich (Riedel-de Haën), 99.8% a.i.] and lindane (γ -HCH, Sigma-Aldrich, 97% a.i.).

The chemical compounds dimethoate and phenmedipham were spiked into

Table 1. Effect concentrations for survival and reproduction of *Enchytraeus albidus* exposed to phenmedipham, atrazine, carbendazim, pentachlorophenol, dimethoate and lindane. Results of the avoidance behaviour from literature are also presented.

	Herbicides			Fungicides		Insecticides	
	Phenmedipham	Atrazine	Carbendazim	Pentachlorophenol	Dimethoate	Lindane	
Survival							
LC ₅₀ (95%-CL)	50 (n.d.)	12 (n.d.)	1 (0-2)	38 (n.d.)	7 (n.d.)	47 (6-95)	
NOEC	32.0	1.0	<0.1	3.2	0.32	<5.6	
LOEC	100.0	3.0	<0.1	10.0	1.0	5.6	
R.M.	Probit	Probit	Probit	Probit	Probit	Probit	
Reproduction							
EC ₁₀ (95%-CL)	14 (13-16)	0.1 (n.d.-1)	n.d.	0.1 (0-1)	0.1 (0-1)	9 (4-14)	
EC ₂₀ (95%-CL)	19 (17-20)	0.2 (n.d.-1)	n.d.	0.4 (0-2)	0.2 (0-1)	14 (8-19)	
EC ₅₀ (95%-CL)	28 (27-30)	2 (0-7)	0.1 (n.d.)	4 (0-15)	1 (1-2)	33 (25-43)	
NOEC	10.0	1.0	<0.1	<0.3	1.0	18.0	
LOEC	32.0	3.0	<0.1	<0.3	3.2	32.0	
R.M.	Weibull	Weibull	Logit-2P	Weibull	Weibull	Logit-2P	
Avoidance							
EC ₅₀	7 ^a	38 ^a	0.1 ^a	703 ^a	58 ^a	173 ^a	
	51 ^b						

*All values are in mg of active ingredient per kg of dry weight LUFA 2.2 soil. LC₅₀ = 50% Lethal Concentration; EC₁₀ = 10% Effect Concentration; EC₂₀ = 20% Effect Concentration; EC₅₀ = 50% Effect Concentration; 95%-CL = 95% Confidence Limits; NOEC = No-Observed-Effect Concentration; LOEC = Lowest Observed Effect Concentration; R.M. = Regression Model used; n.d. = not determined.

^aAmorim *et al.*, 2008; ^bAmorim *et al.*, 2005.

the pre-moistened soil as aqueous solutions, each test concentration into the whole batch of soil. In the case of chemicals non water soluble (atrazine, pentachlorophenol, carbendazim and lindane) acetone was used as a solvent being this solution homogeneously mixed with the soil. Solvent was left to evaporate overnight and then deionised water was added to moisten the soil to approximately 50% of the water holding capacity (WHC). In this case, a solvent control was added—control soil spiked with the same amount of acetone. After homogeneous mixing, the soil was introduced into the test vessels. All pesticides were tested in at least 5 concentrations given as active ingredient per kg of soil dry weight. The ranges of concentrations were between 0.1 and 100 mg kg⁻¹.

Experimental procedure

The assays to determine the survival and reproduction of *E. albidus* were performed according to the standardized guidelines for enchytraeids reproduction testing (ISO, 2003). Avoidance bioassays were performed under identical conditions and as described by Amorim (Amorim *et al.*, 2008).

Statistical analysis

Effect concentrations (EC_x), no-observed-effect concentrations (NOECs) and lowest-observed-effect concentrations (LOECs) were calculated using ToxRat statistical software (ToxRat, 2003). For the calculation of the NOEC, data were checked for normality (Shapiro-Wilk's test) and variance homogeneity (Levene's test). Depending on the results, either the Welch *t*-test for inhomogeneous variances with Bonferroni adjustment or the Williams multiple sequential *t*-test for homogeneous variances were used. Different regression models were used to calculate EC values as indicated in Table 1. For comparisons between control and solvent control, Student's *t*-test was performed.

RESULTS AND DISCUSSION

All the tests fulfilled the validity criteria as described by the ISO and OECD guidelines (ISO, 2003; OECD, 2004). No significant changes occurred in soil pH due to chemical spiking or test duration. For atrazine and pentachlorophenol, no significant differences were found between the two controls and all further analysis was performed versus a pool of the two controls. Significant differences occurred between control and solvent control for carbendazim and lindane ($p < 0.05$), with the solvent causing an increase in the reproductive rate hence in these cases all further analysis was performed versus the solvent control.

The effect concentrations for survival and reproduction were assessed and are shown in Table 1. Results on the avoidance behaviour, previously published (Amorim *et al.*, 2008), are also shown in Table 1 for comparison purposes.

Regarding the herbicides, atrazine showed to have a higher toxicity to *E. albidus* (EC₅₀ = 2 mg kg⁻¹; LC₅₀ = 12 mg kg⁻¹) than phenmedipham (EC₅₀ = 28 mg kg⁻¹; LC₅₀ = 50 mg kg⁻¹). Regarding the studied fungicides, carbendazim was more toxic (EC₅₀ = 0.1 mg kg⁻¹; LC₅₀ = 1 mg kg⁻¹) than pentachlorophenol (EC₅₀

= 4 mg kg⁻¹; LC₅₀ = 38 mg kg⁻¹). For both fungicides the sensitivity was decreasing in the following order: reproduction > avoidance > survival. In the case of the tested insecticides, dimethoate showed a higher toxicity with an EC₅₀ for reproduction of 1 mg kg⁻¹ and a mortality LC₅₀ of 7 mg kg⁻¹ when compared to the ECs of lindane: EC₅₀ = 33 mg kg⁻¹ and LC₅₀ = 47 mg kg⁻¹. For both insecticides, the least sensitive endpoint was the avoidance behaviour.

The rank order of chemical toxicity (on reproduction and survival) to this enchytraeid species was, from the most toxic: carbendazim > dimethoate > atrazine > pentachlorophenol > phenmedipham > lindane (Table 1). For avoidance behaviour, the rank order of toxicity was: carbendazim > phenmedipham > atrazine > dimethoate > lindane > pentachlorophenol (Table 1). The toxicity was not related to the chemical class, i.e., it was not possible to define one class of pesticides as the most toxic to *E. albidus*.

Overall, avoidance behaviour was less sensitive and more variable, being either within the EC ranges of reproduction or survival. There was a tendency to have higher EC values in the following rank order: reproduction < survival < avoidance. Nevertheless, there was no consistency on the degrees of magnitude that characterize the differences, nor a constant rank order. The present results confirm the previous knowledge that survival and reproduction do not always have a correlation, and that there seem to be no clear relation to avoidance. Moreover, the different results obtained for avoidance with phenmedipham (Table 1) shows the high variability of this endpoint. Difference in EC values may also be indicative of the different sensitivity of different batches of organisms.

Exposure to pentachlorophenol, dimethoate and lindane caused a mortality approximately 20, 10 and 4 times lower than the avoidance EC₅₀ value (Table 1). Therefore, this means that the organisms cannot avoid the soil where these compounds are present in concentrations that already cause severe effects on their reproduction. In the case of lindane and dimethoate, there are evidences that such a difference in sensitivity between avoidance and survival might be explained by the effect of these compounds at the nervous system of the organisms, impairing their capacity of escaping from the contaminated soil. Lindane is known as a GABA-gated chloride channel antagonist that can thus over-stimulate the central nervous system. As to dimethoate, it inhibits acetylcholinesterase activity, which can lead to neuromuscular paralysis due to the excessive acetylcholine accumulation.

Reproduction was the most sensitive endpoint in the majority of pesticides tested, which is in accordance with the results by Frampton and co-authors (2006) where they concluded that chronic reproduction was preferred in comparison to acute lethality to provide the most protective risk assessment.

The present study highlights the ecological relevance of avoidance, i.e., if an organism is not present in a field that may not only be due to mortality but also due to avoidance. Additionally, the present study also showed that avoidance is of little use as a rapid screening for hazard assessment when using enchytraeids, as it does not predict survival and reproduction effects.

Acknowledgments—This study was sponsored by Fundação para a Ciência e Tecnologia, Portugal, through a PhD grant to Sara Novais (SFRH/BD/36253/2007).

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