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Comparison of the effects of zinc nitrate-tetrahydrate and tributyltin-oxide on the reproduction and avoidance behavior of the earthworm *Eisenia andrei* in laboratory tests using nine soils

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ABSTRACT

The aim of this contribution was to evaluate whether the sensitivity of the earthworm avoidance test is comparable to that of the earthworm reproduction test (standard test guidelines for both are available). The objective was to determine if relatively simple short-term tests are useful as an initial screening step for the environmental risk assessment (ERA) of potentially contaminated soils prior to performing long-term, elaborate tests. Therefore, the effects of two model substances, zinc nitrate-tetrahydrate and tributyltin-oxide (TBT-O) on the reproduction and the avoidance behavior of the earthworm *Eisenia andrei* were compared using Organization for Economic Co-operation and Development (OECD) artificial soil as well as eight natural soils, including LUFA St. 2.2 soil, covering a wide range of pH-values, organic matter content and texture. Almost all tests fulfilled the validity criteria defined in the two standard guidelines published by the International Organization for Standardization (ISO), despite the fact that the properties of natural soils differed clearly from those of OECD artificial soil. The median effective concentration (EC50) values estimated for zinc nitrate-tetrahydrate in 48-h avoidance tests and in 56-day reproduction tests were often similar when using the same soil (seven out of nine soils). However, in the case of TBT-O, the outcome was more complex: in two test soils the avoidance EC50 values were by a factor >3 higher than the reproduction EC50 values. In one of the test soils it was the other way around and the remaining soils showed comparable EC50 values in both tests. Summarizing the results obtained here and according to experiences reported in the available literature, the earthworm avoidance test may be recommended as an initial screening tool in the ERA of potentially contaminated soils. However, further research is needed to understand the factors that contribute to the difference in sensitivity between reproduction and avoidance tests.

1. Introduction

To improve the applicability of ecotoxicological tests for the environmental risk assessment (ERA) of potentially contaminated soils, the German Federal Ministry of Education and Research initiated a joint-research project in early 2002 (Project ERNTE No. 03303000) (Römbke et al., 2006a). Despite some national and international efforts (e.g. ISO, 2008b), it is still not clear which tests are most efficient and suitable for the ERA of potentially contaminated soils. However, evidence is increasing that earthworm avoidance tests could be a suitable screening method in lower tiers of the ERA of potentially contaminated sites (e.g. Natal da Luz et al., 2004; Antunes et al., 2008; Ma and Bonten, 2011; Niemeyer, 2012). As part of this project it was studied whether relatively simple short-term tests are useful as an initial screening step in the ERA prior to performing long-term, elaborate tests. In detail, it has to be shown that the sensitivity of the short-term earthworm avoidance test is higher to that of the long-term earthworm reproduction test (Römbke et al., 2006b, 2007). Therefore, in this contribution the effects of two model substances, zinc nitrate-tetrahydrate and tributyltin-oxide (TBT-O) on the reproduction and avoidance behavior of the earthworm *Eisenia andrei* were compared using OECD artificial soil and eight natural soils including LUFA St. 2.2 soil that covers a wide range of pH-values, organic matter content and texture. Based on the results of this comparison and the experience described in the literature, it could be ascertained whether the earthworm avoidance test is recommendable as a screening test for the ERA of potentially contaminated soils.

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Table 1
Physico-chemical characterization of the tested soils (eight natural soils and OECD (Organization for Economic Co-operation and Development) artificial soil).

Soil[a]	WHCmax [mL/kg]	pH ($CaCl_2$)	C_{org} [%]	N [%]	C/N	CEC [cmol+/kg]	Sand [%]	Silt [%]	Clay [%]
OECD	631	6.0	4.7	0.07	67.1	8.9	75.4	16.6	8.04
BRG	601	4.9	2.34	0.29	8.1	14.1	13.6	56.7	29.7
BWZ	307	3.8	1.54	0.05	30.8	3.3	81.3	13.6	5.1
ESo5	468	3.1	5.09	0.18	28.3	5.0	83.8	11.6	4.67
GGI	232	5.5	0.94	0.06	15.7	2.0	80.5	15.7	3.82
HAG	611	5.2	2.64	0.28	9.4	13.2	12.8	62.3	24.9
SBG	653	5.8	3.37	0.33	10.2	11.8	27.0	47.1	25.9
SHA	474	7.4	2.22	0.16	13.9	19.8	7.79	69.7	22.5
SOE	483	6.6	1.63	0.16	10.2	13.8	1.97	83.0	15.0
St 2.2	500	6.1	2.70	0.19	14.2	7.9	76.9	16.3	6.84

[a] Abbreviations and locations of soils: OECD: artificial soil according to OECD (1984); BRG: Breddewarden, Niedersachsen; BWZ: Weitzgrund, Brandenburg; ESo5: Gudow, Schleswig-Holstein; GGI: Raddusch, Brandenburg; HAG: Frankfurt-Harheim, Hessen; SBG: Schmallenberg, Nordrhein-Westfalen; SHA: Schafstädt, Sachsen-Anhalt; SOE: Soest, Nordrhein-Westfalen, St 2.2: LUFA 2.2 standard soil, Speyer, Rheinland-Pfalz; all locations are in Germany.

2. Material and methods

2.1. Test soils and soil properties

Nine uncontaminated natural soils (including LUFA St. 2.2 standard soil) and OECD artificial soil, covering a wide range of soil properties (pH, texture, organic carbon content, WHC, and C/N ratio), were selected (Römbke et al., 2006a). Soils were air-dried, sieved (5 mm mesh size) and stored in 25 L plastic buckets at room temperature for no longer than three months prior to use. Their main properties were determined using ISO standards as follows: maximum water holding capacity (WHC) (ISO, 1998b), pH (ISO, 2005), carbon (ISO, 1995a), nitrogen (ISO, 1995b), cation exchange capacity (ISO, 1994) and soil texture (ISO, 1998a) (Table 1).

2.2. Test chemicals

Zinc nitrate-tetrahydrate ($Zn(NO_3)2 \times 4H_2O$) with a molar mass of 261.44 g/mol was obtained from Merck (Darmstadt, Germany; CAS–19154-63-3). Bis(tri-*n*-butyltin)oxide ($C_{24}H_{54}OSn_2$) with a molar mass of 596.07 g/mol was obtained from Crompton (Bergkamen, Germany; CAS-No. 56-35-9). Aqueous solutions of the test substances were mixed into the soils. Each treatment (=concentration) was set up separately. The concentrations of the two compounds were not analytically determined.

2.3. Test organisms and test performance

The earthworms used, from the species *Eisenia andrei* (Lumbricidae), were adult, had a fresh weight between 300 and 600 mg and were at least two months but not more than one year old (those used in the reproduction test came from a synchronized culture). The worms selected were acclimatized in the respective soil under test conditions for at least 24 h before starting the test. The soil ESo5 was not tested since in an earthworm reproduction pretest, the validity criterion for the number of juveniles (at least 30 per test vessel) was not fulfilled (Römbke et al., 2006b). This was probably due to its very low pH of 3.1 (the preferred pH-range of *E. andrei* is about 5–7; Jänsch et al., 2005). The test performance is summarized in Table 2. In the avoidance test, individuals found between the sections on the separating line, were counted according to the direction in which they were moving, i.e. considered in the section where the anterior part of the body was. Dead earthworms were classified as escaped animals. For both substances, five concentrations were selected according to literature data (Wilke et al., 2004) or own range-finding tests (Table 3).

2.4. Statistics

Statistical analyses of data from reproduction tests were performed using the program ToxRat, Version 2.09 (ToxRat®, 2003). The reproduction median effective concentration (EC50) values and respective 95%-confidence limits were estimated through a linear regression via probit analysis. In the avoidance tests, the EC50 values and their 95%-confidence limits were determined using probit analysis with a maximum log-likelihood method and optimization algorithm by Davidon–Fletcher–Powell (variable metric method). These calculations were based on the mean reproduction (number of juveniles) and avoidance of the four and five replicates, respectively. Statistical software PriProbit Version 1.63 was used.

Table 2
Comparative presentation of the test performance.

	Reproduction test	Avoidance test
Guideline	ISO 11268-2	ISO 17512-1
Principle	Exposure of adult worms to the test substance and determination of the number of juveniles per vessel produced during the duration of the test	Worms are introduced on the transverse line between two soil batches: a control and a test soil; determination of their number in the two halves of the vessel after the test duration
Species	10 adult *Eisenia andrei* (300–600 mg/worm) per vessel	
Duration	56 d	48 (or 24) h
Endpoint	No. of juveniles (after 56 d)	Avoidance behavior (48 h)
Extraction	Water bath (60 °C)	Hand-sorting
Design	5 concentrations with 4 replicates	5 concentrations with 5 replicates
Soil moisture	40–60% WHCmax*	
Temperature	18–22 °C	
Light	16 h light (400–800 Lx), 8 h dark	
Feeding	5 g cattle dung/vessel per week	None
Vessels	Bellaplast No. 590, Polarcup, Alf, Germany), 11 × 15.5 × 6 cm	
Soil amount	500 g dry weight soil	250 + 250 g dry weight soil
Validity criteria (control)	Reproduction > 30 juveniles per vessel; CV (reproduction) ≤ 30%; mortality < 10%;	Mortality < 10%; Distribution in both sections with the same soil (=dual tests): 50 ± 10%

* These parameters were measured according to ISO guidelines.