Research Articles

Avoidance Test with *Eisenia fetida* as Indicator for the Habitat Function of Soils:

Results of a Laboratory Comparison Test

Kerstin Hund-Rinke[1*], Rudolf Achazi[3], Jörg Römbke[2] and Dietmar Warnecke[3]

[1] Fraunhofer Institute for Molecular Biology and Applied Ecology, D-57377 Schmallenberg, Germany
[2] ECT Oekotoxikologie GmbH, 65439 Flörsheim, Germany; [3] Free University of Berlin, Institute for Biology, D-14195 Berlin, Germany

* Corresponding author (hund-rinke@ime.fraunhofer.de)

DOI: http://dx.doi.org/10.1065/jss2002.11.062

Abstract

Intention, Goal, Scope, Background. The habitat function of soils is often assessed using the reproduction test with *Eisenia fetida*. As this test is rather labour-intensive, an alternative is needed which is less cost-intensive in terms of duration and workload, but gives reasonable results. The avoidance test with *E. fetida* is a suitable screening test meeting these criteria. However, before a novel test system can be generally recommended it has to be ensured that comparable results are acquired from different laboratories on the basis of the respective test guideline.

Objective. The avoidance test with *E. fetida* was performed as laboratory comparison test. The results were compared with those of the earthworm acute and reproduction tests carried out with the same soils.

Methods. The three tests were performed by three laboratories using eight contaminated soils and three control soils. The contaminated soils were mixed with the control soils to obtain different concentrations of the contamination.

Results and Discussion. The results of the avoidance test show that despite the partially considerable standard deviations a 72% agreement in the assessment of soils was reached with a toxicity criterion of >80% avoidance response. The sensitivity is at least that for the reproduction test and considerably surmounts the sensitivity of the acute test.

Recommendation and Outlook. The avoidance test is considered to be a suitable screening test for assessing the habitat function of soils. The whole test design could be improved by reducing the standard deviations among parallel test batches. With regard to standardization it is recommended to use control soils which have the same properties as the soils described in respective guidelines (e.g. ISO 11269-2, OECD 216, 217).

Keywords: Avoidance behaviour; earthworm; *Eisenia fetida*; soil fauna, soil quality; terrestrial ecotoxicity test

Introduction

Eisenia fetida is a frequently used test organism for soil quality assessments. In general, standardized test systems are applied using the measurement parameter 'mortality' (acute test) or 'reproduction' according to the DIN ISO guidelines 11268-1, -2, which had been originally developed for substance testing. Both these tests were validated for soil quality assessments as a combined test in a round robin test financially supported by the Deutsche Bundesstiftung Umwelt (German Federal Foundation for the Environment) with national and international involvement (Warnecke et al. 2002, Hund-Rinke et al. 2002). The disadvantages of the acute test and the reproduction test are a low sensitivity and a long test period (56 d), respectively, coupled with the labour-intensive determination of the number of juveniles. As the test period and the work expense dictate the costs of a given test, it is desirable to obtain the results within a short test period and at a high level of sensitivity. Consequently, combining high sensitivity with a short test period would be recommended for the routine use of a test. This feature is offered by the avoidance test with *Eisenia fetida*. The principle of this test is that earthworms are simultaneously applied to the soil sample to be evaluated and to a control soil. The location of the animals is determined after a short test period of a few days only.

Concerning the performance and suitability of a response test for the assessment of chemicals or (contaminated) soils the following conclusions can be drawn based on the studies of Yeardley et al. (1996), Slimak (1997), Stephenson et al. (1998), and Hund-Rinke and Wiechering (2000):

- Simple test design
- Short test period with a low labour intensity compared to the established test procedures
- High sensitivity concerning the assessment of chemicals or contaminated soils
- Additional information on the established test procedures regarding repellent effects
- Estimable influence of physico-chemical soil properties on the migratory behaviour of the earthworms in soil assessment testing opposed to the uncontaminated reference soils
- Criterion for assessing the habitat function of the test soils: evaluation as 'limited habitat function' at >80% avoidance of the test soil by the organisms.

A prerequisite for the general recommendation of a test system is that the test guideline ensures the comparability of the results obtained in the different laboratories. Moreover, the sensitivity of a method to recognize a restriction of the habitat function should be known with respect to other test systems.

This paper presents the results of a laboratory comparison test on the avoidance behaviour of *Eisenia fetida,* which was performed in three laboratories using eight different soils. In addition, the obtained results are compared with the results gained in acute and reproduction tests with *E. fetida* using the endpoints mortality, reproduction and biomass change.

1 Material and Methods

1.1 Soils

For all tests, the soils were sieved (contaminated soils: ≤5 mm; control soils: ≤2 mm). The sieved contaminated soils as well as the control soil LUFA 2.2 were supplied by Laboratory 1 to Laboratory 2 and Laboratory 3. The control soils Borstel and Thyrow (see below) were used only by Laboratory 1 and Laboratory 2, respectively. They were sampled and prepared by the respective laboratory. The contaminated soils were mixed with control soils (on dry weight basis) to obtain the different contamination concentrations. The water contents of all soils were below 60% of their maximum water holding capacity and therefore suitable to prepare a homogenous mixture. The mixtures were adjusted to 60% of the maximum water holding capacity (WHC_{max}), and allowed to equilibrate for at least 24 h at room temperature. The maximum water holding capacities of the soils and mixtures were determined by Laboratory 1 except for the set-ups with the control soil Thyrow, for which the values were determined by Laboratory 2.

Table 1: Physico-chemical characterization of the uncontaminated soil samples (control soils)

Parameters	Borstel	Thyrow	LUFA 2.2
Clay [%] (<0.002 mm)	3.6	2.7	8.2
Silt [%] (0.002–0.063 mm]	25.6	14.2	18.5
Sand [%] (0.063–2.0 mm)	70.8	83.1	73.2
Soil type	medium silty sand	slightly silty sand	medium loamy sand
TOC [%] [a]	0.93	0.53	2.2
pH	5.9	7.0	5.8
WHC_{max} [g/100 g dry matter] [b]	27	25	50

[a] TOC: total organic carbon
[b] WHC_{max}: maximum water holding capacity

1.1.1 Control soils

The physico-chemical characteristics of the applied test soils are presented in **Table 1**. All three control soils were sandy soils.

1.1.2 Contaminated soils

The physico-chemical characteristics of the applied test soils are shown in **Table 2**.

Table 2: Physico-chemical characterization of the contaminated soil samples

	Landhausen	Iserlohn	Gaswerk 1:2	PAH-Mix	Sickergraben	Neuohe	IMA SM 66	IMA 927 B
Clay (<0.002 mm) [%]	5.4	6.7	5.4	7.5	6.5	5.4	8.2	28.3
Silt (0.002–0.063 mm) [%]	12.7	10.9	9.8	8.9	0.3	10.8	12.8	36.1
Sand (0.063–2.0 mm) [%]	81.9	82.4	84.8	83.6	93.2	83.8	79.0	35.6
Soil type	slightly loamy sand	slightly loamy sand	slightly loamy sand	slightly loamy sand	slightly clayey sand	slightly loamy sand	loamy sand	slightly clayey loam
pH	7.3	7.5	5.6	7.5	5.0	7.2	7.7	7.8
TOC [%] [a]	8.5	5.9	1.7	3.9	6.2	2.1	0.8	0.4
WHC_{max} [%] [b]	35	24	27	25	51	42	29	41
As [mg/kg]	<15	<15	<15	<15	<15	<15	<15	<15
Cd [mg/kg]	29.6	<13	<13	<13	<13	<13	<13	<13
Cr [mg/kg]	2005	320.3	94.9	195.9	227.1	304.4	61.8	59.3
Cu [mg/kg]	10921	367.8	<34.3	79.1	<34.3	83.1	<34.3	43.8
Ni [mg/kg]	5894	578.5	<33	32.8	<33	50.3	<33	68.7
Pb [mg/kg]	1082	547.5	<24	109.9	38.1	1180	43.0	26.1
Zn [mg/kg]	5065	577.6	22.3	274.9	312.5	54980	106.5	96.8
Mineral oil [mg/kg] [c]	43660	8799	<50	≈ 1000	105.4	<50	170.5	66.9
Σ_{16} PAH [mg/kg]	60	21	2268	2682	2.11	0.42	4.32	0.46
2,4,6-TNT [mg/kg]	–	–	–	–	57048.2	4.05	0.78	8.15
4-A-2,6-DNT [mg/kg]	–	–	–	–	114.1	0.38	0.29	0.28
2-A-4,6-DNT [mg/kg]	–	–	–	–	112.0	0.20	0.39	0.23
1,3,5-TNB [mg/kg]					13.41	0.11	0.02	0.21
1,3-DNB [mg/kg]	–	–	–	–	35.96	<DL [d]	<DL	<DL
2,4-DNT [mg/kg]	–	–	–	–	85.67	<DL	<DL	<DL

[a] WHC_{max}: maximum water holding capacity
[b] TOC: total organic carbon
[c] Mineral oil hydrocarbons
[d] DL: detection limit

1.2 Test design

1.2.1 Avoidance test

According to the reproduction test vessels with a surface of about 200 cm^2 were used. The volumes of the vessels used by the three laboratories were 1,0–1,7 l. The containers were filled with soil up to a height of about 5–6 cm (about 1000 g soil, dry weight). To avoid lateral effects of light, non-transparent vessels or vessels wrapped with aluminum foil were used. At the beginning of the test, the vessels were divided into two equal sections by a vertically introduced plexiglass divider. One half of the vessel was filled with test soil (Section A) and the other half with control soil (Section B). Then the separator was removed, and ten adult worms of the species *Eisenia fetida* (weight: 300–600 mg) were placed on the separating line of each test vessel. To prevent the worms from escaping from the vessels, these were covered with gauze permeable to light and air. The vessels were incubated at 20 ± 2°C at a day/night-rhythm of 16/8 hours for 48 h. The light intensity was 700–800 lux. At the end of the test period the control and test soils were separated by inserting the plexiglass divider. The number of worms was determined for both sections of the vessels. Worms divided due to the introduction of the plexiglass divider were counted as 0.5 independent of the length of the remaining body. Mortality was observed in some setups with contaminated soils. This kind of effect goes beyond the scope of an avoidance test. To consider this effect in the assessment, dead worms were classified as escaped animals. The tests were generally run in 5 replicates.

Soils were considered to be toxic (habitat function reduced) if >80% of the worms stayed in the control soil (Hund-Rinke and Wiechering 2000). Furthermore, differences between control and test soil were determined by statistical analyses (t-, u-test). In this case, a soil was considered to be toxic if a statistically significant difference ($p < 0.05$) was detected.

1.2.2 Acute and reproduction Tests with Earthworms

The tests were performed following standardized guidelines (ISO 11268-1 and ISO 11268-2). Instead of the artificial soil, the test soils were used. In the reproduction test with earthworms the soil was supplemented with the required amount of cattle manure.

Soils are considered to be toxic if the mortality rate is >20% in the acute test (Dott et al. 1995), a reduction of >50% in the number of offspring is obtained in the reproduction test compared to a control soil (LUFA 2.2) (Dott et al. 2001), and if a 20% reduction of the biomass after 28 d compared to the beginning of the test is determined (Warnecke et al. 2002).

2 Results and Discussion

2.1 Avoidance test

Fig. 1 presents the results obtained with the four soils Landhausen, Iserlohn, Gaswerk 1:2 and PAH-Mix. The three institutions used the same control soil (LUFA 2.2). With the exception of the soil PAH-Mix which was avoided by the worms at both tested concentrations and in all three laboratories, a dose-response curve is recognizable for all test batches.

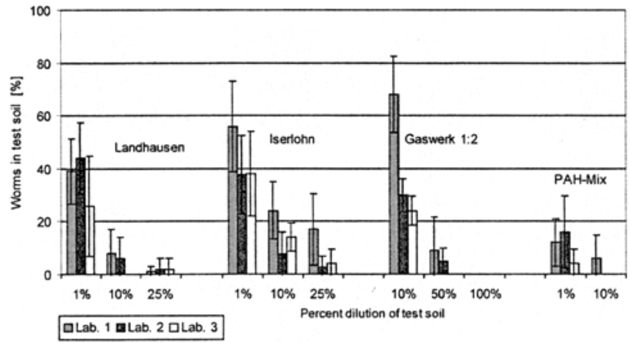

Fig. 1: Percentage of *Eisenia fetida* (mean ± standard deviation) in the batches with the soils. Landhausen, Iserlohn, Gaswerk 1:2 and PAH-Mix; (control soil: LUFA 2.2); the soils were tested independently in three different laboratories

The number of worms in the test soil decreases with increasing fractions of test soil in the test batch. If the threshold of 20% is applied as the toxicity criterion, with one exception all soils or soil mixtures would be assessed equally by all three laboratories. Only the batch containing 10% of Iserlohn soil would still be evaluated nontoxic by one laboratory, while it would be assessed as toxic by the other two laboratories.

Fig. 2 illustrates the results obtained with two contaminated soils (Sickergraben, Neuohe) and with two remediated soils (IMA SM 66, IMA 927 B). The three participants used different control soils for these test batches. The 1% batch of Sickergraben soil tested in Laboratory 2 showed a clearly lower avoidance response than in Laboratory 1 and Laboratory 3. For the batch with 50% IMA 927 B, however, the avoidance response observed in Laboratory 2 was clearly stronger and reached that of the 100% batch. Based on these opposing tendencies the properties of the control soil presumingly did not considerably affect the worm distribution.

In the 100% batch of the soil IMA SM 66, there was a high number of worms in the control soil of Laboratory 3 at the end of the test. The result also does not seem to be caused by *E. fetida* being especially attracted to this soil. The evaluation is based on the fact that in the other tests (e.g. with the test soils Neuohe and IMA 927 B) such an attraction was not found.

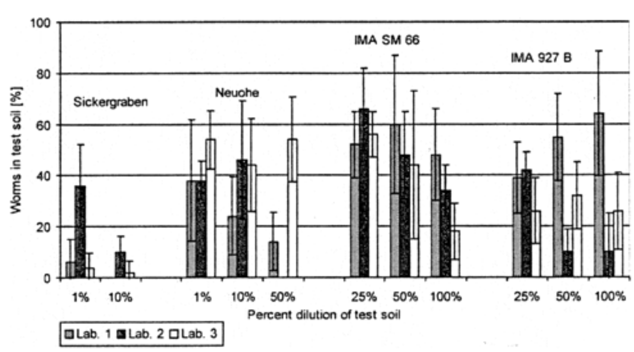

Fig. 2: Percentage of *Eisenia fetida* (mean ± standard deviation) in the batches with the soils. Sickergraben, Neuohe, IMA SM 66 and IMA 927 B; (control soil: Lab. 1: Borstel; Lab. 2: Thyrow; Lab. 3: LUFA 2.2); the soils were tested independently in three different laboratories

Table 3: Assessment of the soils based on '>80% avoidance response' (20% limit value) and 'statistical significance compared to the control' (+: habitat function limited, basis <20% worms in the test soil, corresponding to >80% avoidance response, or statistical significance; –: habitat function not limited)

Soil [a]		Assessment: Limit value 20%			Assessment: Statistics		
		Lab. 1	Lab. 2	Lab. 3	Lab. 1	Lab. 2	Lab. 3
Landhausen							
	1%	–	–	–	+	–	+
	10%	+	+	+	+	+	+
	25%	+	+	+	+	+	+
Iserlohn							
	1%	–	–	–	–	–	–
	10%	–	+	+	+	+	+
	25%	+	+	+	+	+	+
Gaswerk 1:2							
	10%	–	–	–	(+) [b]	+	+
	50%	+	+	+	+	+	+
	100%	+	+	+	+	+	+
PAH-Mix							
	1%	+	+	+	+	+	+
	10%	+	+	+	+	+	+
Sickergraben							
	1%	+	–	+	+	+	+
	10%	+	+	+	+	+	+
Neuohe							
	1%	–	–	–	–	+	–
	10%	–	–	–	+	–	–
	50%	+	+	–	+	+	–
IMA SM 66							
	25%	–	–	–	–	(+) [b]	–
	50%	–	–	–	–	–	–
	100%	–	–	+	–	+	+
IMA 927 B							
	25%	–	–	–	–	+	+
	50%	–	+	–	–	+	+
	100%	–	+	–	–	+	+
% complete agreement of the three laboratories			72			60	

[a] Percentage of the test soil in the test batch
[b] (+): Statistically significant, but due to an *increased* number of worms in the test soil

In part the results led to considerable standard deviations. This is apparently not attributed to the specific test soils, control soils or institutions, since a correlation between one of these factors and the variability between replicates is not observed. Despite the partially high standard deviations, by considering the mean values, 72% of the test batches showed complete agreement in the assessment of the habitat function using the criterion >80% avoidance of the test soil (**Table 3**). If the assessment was based on a statistically significant difference between the control batch and the test soil batch (Table 3), further samples would be classified as having a limited habitat function. Complete agreement between the three laboratories are reached in 60%. Based on a statistical evaluation the batch with 10% Iserlohn soil of Laboratory 1 or the 1% Sickergraben soil of Laboratory 2 would also be assessed as having a limited habitat function, which seems to be reasonable when looking at the results of the other two laboratories. On the other hand, there are statistically significant differences which are difficult to accept. For example, the habitat function would be assessed as 'limited', though no dose-response relationship exists (1% Neuohe/Laboratory 2; IMA 927 B/Laboratory 3). Furthermore, laboratory 2 investigated set-ups containing the same soil in both sections of the vessels (data not shown). In three of eight set-ups statistically significant differences in the distribution of the worms were detected. However, in no set-up the threshold value of 80% was exceeded. Statistically significant effects do not necessarily correspond to environmentally relevant effects (Paine 2002); study design, n, variances, tests and transformations as well as the level of statistical significance influence the results. Considering the results obtained with the presented test design and following the aim of minimizing potential effects by different physico-chemical properties of the test soil and the control soil the introduction of a fixed threshold value seems to be a useful tool for the assessment of the habitat function of soils. To strengthen the results a maximum coefficient of variation could be defined as a validity criterion.

Table 4: Assessment of the habitat function of soils based on various earthworm tests (habitat function limited: +; habitat function intact: –; signs in parentheses: with the performance by several laboratories, percent agreement in the assessment less than 100, but between 56 and 78)

Soil [a]	Avoidance	Reproduction	Mortality	Biomass
Landhausen				
1%	–			
10%	+			
12,5%		+	–	–
25%	+	+	(–)	(+)
50%		+	(+)	+
100%		+	+	+
Iserlohn				
1%	–			
10%	(+)			
12,5%		(–)	–	–
25%	+	+	(–)	(+)
50%		+	(–)	(+)
100%		+	+	+
Gaswerk 1:2				
10%	–			
12,5%		–	–	–
50%	+	(–)	–	–
100%	+	+	(+)	+
PAH–Mix				
1%	+			
10%	+			
100%		+	+	+
Sickergraben				
1%	+			
10%	+			
100%		+	+	+
Neuohe				
1%	–			
10%	–			
50%	(+)			
100%		+	–	–
IMA SM 66				
25%	–			
50%	–			
100%	(–)	–	–	–
IMA 927 B				
25%	–			
50%	(–)			
100%	(–)	+	–	–

[a]: Percentage of the test soil in the test batch

2.2 Comparison of test systems

Table 4 shows the assessment of the habitat function of the eight test soils on the basis of various test parameters in the different earthworm tests. The strict criterion >80% avoidance response is used for the comparison. The results of the mortality and reproduction tests for the soils Landhausen, Iserlohn and Gaswerk 1:2 originated from round robin tests (Warnecke et al. 2002). In this case, the majority opinion decided on the assessment of the habitat function. For the other five soils the reproduction test was only performed by laboratory 2. The test parameter mortality, which represents the greatest damage that an organism can show, expectedly proved to be the least sensitive one.

If in the avoidance test and reproduction test the batches are compared to each other by using dilution series, the avoidance response is shown to be at least as sensitive as the reproduction rate. The test period of 48 h in comparison to 56 d, however, is clearly shorter. An exception is the remediated soil IMA 927 B. Based on the reproduction test, the 100% test batch was assessed as having restricted habitat function, while according to the avoidance test such an effect was not indicated. However, it should be considered that the reproduction test was performed only in Laboratory 2. Unlike the other two laboratories, this participant assessed the soil to be toxic in the avoidance test too, with the result that the 100% batch produced only 67% concurrence. Therefore, despite the high effort in the homogenization of the soil, it is feasible that laboratory 2 had been supplied with a more toxic soil batch.

A high sensitivity of the avoidance test could also be determined in earlier studies (Hund-Rinke and Wiechering 2000). On the contrary, Schaefer (2001) determined a lower sensitivity than that for the reproduction test with regard to crude oil. A >80% avoidance response was detected in only one out of three samples which led to a reduction of >50% in the number of offspring. The study employed the test system of Stephenson et al. (1998) in which the worms migrate from a central chamber to peripheral chambers. The peripheral soil-filled chambers are separated from each other by plates with a few holes. The difficulty for the worms to migrate from one chamber to another may be the reason for them to avoid only stronger contaminated soils and perhaps tolerate less contaminated ones. However, using the same chambers, Stephenson et al. (1998) concluded that the avoidance test is predictive of the results of the traditional acute and chronic tests when assessing a condensate-contaminated soil. It seems that the sensitivity of the behavioural test depends strongly on the type of contamination as well as on the test design.

The test parameter reproduction responds to toxic substances which can be taken up by an organism via the water path or via food. When applying the avoidance response parameter on *Eisenia fetida*, however, only contaminants perceived via the chemoreceptors can be detected. Based on the soils included in the study, this seems to be the case for mineral oil hydrocarbons, polyaromatic hydrocarbons, TNT and zinc, as these contaminants were present as main contaminants in single soils. The existing results do not indicate conclusively whether a reaction to further heavy metals occurs, since these were present in high concentrations only in the soil Landhausen which, however, was likewise heavily contaminated with mineral oil hydrocarbons and zinc. It is known from other experiments that *E. fetida* reacts to KCl (5000 ppm), NH_4Cl (150 ppm) as well as to manganese and a mixed contamination consisting of zinc, manganese, iron and copper (Yeardley et al. 1996). However, no concentration data are reported for the heavy metals. Furthermore, studies with the earthworm *L. terrestris* demonstrated that this organism shows avoidance behaviour towards numerous pesticides (Slimak 1997).

Based on the results presented here, the sensitivity of the parameter biomass change (biomass reduction) seems to be intermediate between the endpoints mortality and reproduction. Nevertheless, in some studies an inverse relationship between reproduction and body weight change was obtained. In other studies the animals in the treated test batches gained more weight than those in the control batches (Kula 1998).

3 Recommendations and Outlook

The avoidance test with *Eisenia fetida* has numerous advantages (short test period, comparatively lower work expense, sensitivity at least equal to that of the reproduction test). Furthermore, the applied methodological procedure leads to comparable results by different laboratories. Consequently, it is considered to be a suitable screening test for assessing the habitat function of soils. The use of a fixed limit value is recommended for the assessment in order to obtain reasonable results and to minimize potential effects by control soils with physico-chemical properties that differ from those of the test soil. To strengthen the results a maximum coefficient of variation as validity criterion could be defined. A prerequisite for this would be the reduction of the standard deviations among parallel test batches, which would also improve the whole test design. Starting points might be changing the test period (e.g. 24 or 72 h), increasing the number of replicates or varying the water content. With regard to standardization it is recommended to use soils with the same properties as a suitable control soil like in the plant test (ISO 11269-2) and the soil microflora test (OECD 216, 217): a TOC content of 1.5% at the highest, a sand content of 50–75%, less than 20% in the fine particle fraction and a pH value of 5–7.5.

Acknowledgement. The authors wish to thank Monica Armorim, Rosi Auste, Grazyna Chroszcz, Gabi Mascialino, Nicole Nielow, Ricarda Noeker, Rainer Peters and Patrick Sittinger for their capable technical assistance and the 'Deutsche Bundesstiftung Umwelt' (German Federal Foundation for the Environment) for financial support.

References

Dott W et al. (1995): Bioassays for Soils. DECHEMA. ISBN 3-926-59-52-5

Dott W, Achazi R. Eisenträger A, Hund-Rinke K, Kördel W, Neumann-Hensel H, Pfeifer F, Römbke J, Wiesner J, Wilke BM (2001): Biologische Testverfahren für Böden [Biological test procedures for soils]. DECHEMA. ISBN 3-89746-026-2

Hund-Rinke K, Wiechering H (2000): Earthworm avoidance test for soil assessment: An alternative for acute and reproduction tests. JSS – J Soils & Sediments 1, 15–20

Hund-Rinke K, Kördel W, Hennecke D, Wilke B-M, Winkel B, Achazi R, Warnecke D, Heiden St (2002): Bioassays for the ecotoxicological and genotoxicological assessment of contaminated soils – Results of a round robin test. Part II: Assessment of the habitat function of soils – Tests with soil microflora and fauna. JSS – J Soils & Sediments 2, 83–90

ISO 11268-1 (1993): Soil quality; effects of pollutants on earthworms (*Eisenia fetida*) – Part 1: determination of acute toxicity using artificial soil substrate

ISO 11268-2 (1998): Soil quality – Effects of pollutants on earthworms (*Eisenia fetida*) – Part 2: Determination of effects on reproduction

Kula C (1998): Endpoints in laboratory testing with earthworms. Experience with regard to regulatory decisions for plant protection products. In Sheppard St, Bembridge J, Holmsturp M, Posthuma L (Eds.): Advances in earthworm ecotoxicology. SETAC Press, pp 3–14

Paine MD (2002): Statistical significance in environmental effects monitoring (eem) programs. Setac Globe 3, No 1, 23–24

Schaefer M (2001): Earthworms in crude oil-contaminated soils: toxicity tests and effects on crude oil degradation. Contaminated Soil, Sediment & Water, 6. August 2001, 35–37

Slimak KM (1997): Avoidance response as a sublethal effect of pesticides on *Lumbricus terrestris* (Oligochaeta). Soil Biol Biochem 29, 719–715

Stephenson GL, Kaushik A, Kaushik NK, Solomon KR, Steele T, Scroggins RP (1998): Use of an avoidance-response test to assess the toxicity of contaminated soils to earthworms. In: Sheppard S, Bembridge J, Holmstrup M, Posthuma L. (Eds.) Advances in Earthworm Ecotoxicology., Setac Press, Pensacola, pp 67–81

Warnecke D, Chroszcz G, Schäfer R, Achazi R (2002): Bodenfauna-Tests. In: Hund-Rinke K, Kördel W, Heiden St, Erb R, (Eds): Ökotoxikologische Testbatterien – Ergebnisse eines DBU-geförderten Ringtests [Ecotoxicological test batteries – Results of a round robin test, financially supported by DBU]. Erich Schmidt Verlag, Berlin

Yeardley RB, Lazorchak JM, Gast LC (1996): The potential of an earthworm avoidance test for evaluation of hazardous waste sites. Environ Toxicol Chem 15, 1532–1537

Received: May 7th, 2002
Accepted: November 22nd, 2002
OnlineFirst: November 25th, 2002