# Oligochaete (microdrile) worms in the environmental risk assessment of pesticides in the European Union

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**Abstract**

This paper reviews the use of oligochaete worms for the environmental risk assessment (ERA) of pesticides based on the legal requirements of the European Union (EU) . Such an ERA is a required part of the registration process of each active ingredient before it can be marketed either in Europe or in many other countries. Within environmental risk assessment, three compartments (water, sediment and soil) have to be studied separately. Their important ecological role in soils and sediments make oligochaete worms an appropriate test species for the assessment of pesticides. In soil, as earthworms (not covered here), Enchytraeidae can be used in laboratory tests, semi-field and field studies. Guidelines for laboratory tests, focussing on the mortality and reproduction of these worms, have been published by OECD, ISO and ASTM. A soil bioaccumulation test with enchytraeids is currently validated through an international ring test. In addition, enchytraeids can be used in Terrestrial Model Ecosystems. In sediments different oligochaete families are used, mainly the Tubificidae and Lumbriculidae. In particular the widespread species *Lumbriculus variegatus* is already an accepted part of test strategies in North America, and is increasingly used in acute mortality and chronic toxicity and bioaccumulation tests in the laboratory. After successful validation in international ring tests publication of these test methods as OECD guidelines is expected in the near future. Finally, species of both families are regularly studied in aquatic micro-and mesocosms. To summarise, the use of oligochaete microdrile worms in ecotoxicology is likely to increase.

## Introduction

This contribution provides an overview of the principles of environmental risk assessment (ERA) of pesticides in the European Union (EU), and focuses on the use of oligochaete worms in this framework. Before pesticides are allowed to be marketed in the European Union, they must be registered in a standardised process ("registration") according to Council Directive 91/414 ("Directive concerning the placing of plant protection products on the market" (EU, 1991)) and its accompanying documents. Such an ERA is an obligatory part of the registration process of each active ingredient before it can be marketed in Europe (currently 26 countries) and in other countries that refer to decisions of the EU. This legal procedure aims to ensure a safe and efficient use of these chemicals.

Pesticides can be defined as chemicals designed to protect crops which are intentionally used in the field or greenhouses. They are used as formulations, i.e. a mixture of the active substance(s) (= a.s.) and solvents. Pesticides are classified according to their use (e.g. insecticides, herbicides or fungicides), their chemical class (e.g. organo- phosphates or pyrethroids) or their application (e.g. as a water soluble formulation or granulate etc.). The use of pesticides is defined by the applied rate: often between 0.1 and 3.0 kg a.s. $ha^{-1}$ and the frequency of application (about 1-10 times per season).

In the following, the principles of the Environmental Risk Assessment (ERA) are described briefly (Leeuwen & Hermens, 2001). The term environmental risk assessment can be defined as simply a systematic means of developing a scientific basis for regulatory decision making (Barnthouse et al. 1992). The concept

of ERA was developed in the USA during the 1970s (Fava et al., 1987) for anthropogenic stress factors with potential impacts on the environment. In the 1980s, the risks of chemicals were prospectively, i.e. before being marketed, assessed (EPA, 1992). Shortly afterwards this concept was also adapted by European authorities for standardising the registration of pesticides. The ERA process can be divided in four steps (Fig. 1).

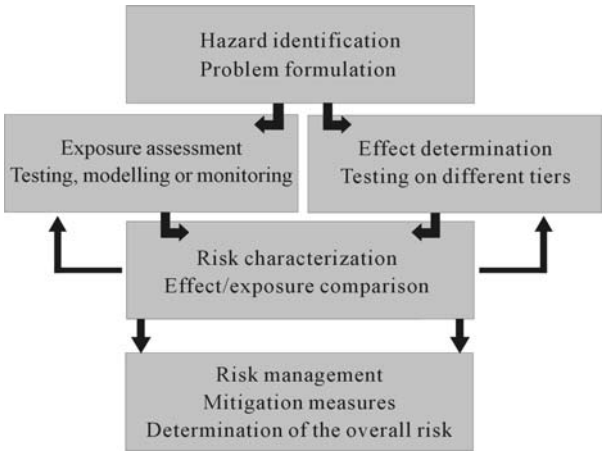


*Figure 1.* Overview on the main steps of ERA

The aim of the hazard identification step is to define whether (and if yes then where) the pesticide may occur in the environment. In the exposure assessment the Predicted Environmental Concentration (=PEC) is determined (in mg a.s. $kg^{-1}$ soil dw). During the effect assessment, the toxic concentration (=TC) is measured in standardised toxicity tests (also in mg a.s. $kg^{-1}$ soil dw). Finally, the potential risk of the pesticide is characterised by calculating the Toxic-Exposure-Ratio (=TER): TC/PEC. A risk is assumed if TER is$\leqslant$1. In such cases the result can be refined by using test methods more relevant for the field (e.g. semi-field tests like Terrestrial Model Ecosystems (Knacker et al., 2004)) or by re-modelling exposure by using actual values from the area where the test substance will be used. Using these new TC/PEC data the risk is assessed again (refined ERA). The process of refining the ERA has to be repeated until it is clear whether there is concern or not. In case of concern, measures to decrease the risk are necessary. If risks cannot be avoided by safety measures the pesticide can be banned (Fig. 2).

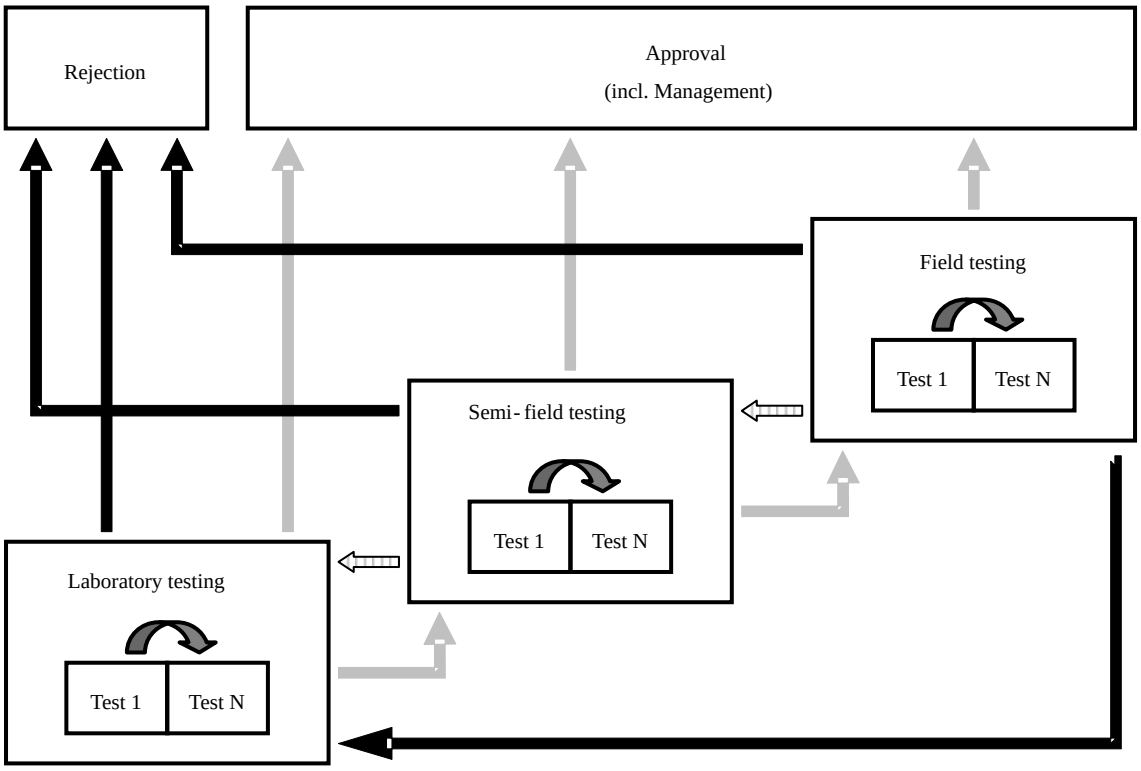


*Figure 2.* Schematic description of testing and risk assessment on different levels (black arrows: rejection based on laboratory, semi-field or field results; grey arrows: approval based on laboratory, semi-field or field results; striped arrows: feedback steps. N = number of tests

In detail, the PEC for a pesticide is determined by using complex models based on the physico-chemical properties of the substance, how and when they reach the environment (i.e. use pattern, application rate etc.) and, to a certain extent, environmental variables. For example, chemicals with high log $P_{ow}$ values can accumulate in the fat tissues of organisms and along the food chain. This may result in "secondary" poisoning of species on higher trophic levels. The determination of the PNEC is usually performed by measuring the effects of a chemical on individual species (e.g. the mortality, growth or reproduction) in laboratory tests, semi-field or field tests. Using "safety" or "assessment factors" each effect concentration determined in tests will be extrapolated to the concentration expected to have no effect in the field (Leeuwen & Hermens, 2001).

The ERA has to be performed separately for each environmental compartment: surface water, ground water, soil, sediment and air. In this paper, only the compartments soil and sediment will be considered. Organisms to be tested in the ERA of pesticides for the soil compartment include micro-organisms, plants, collembolans, beetles and oligochaetes, while in the sediment compartment mainly insects and oligochaetes can be tested. Such a battery of tests is necessary since there is no one species which is "most sensitive" to all chemicals (Cairns, 1986). All tests used for the ERA have to be standardised and validated, mainly by the Organisation for Economic Co-operation and Development (OECD) or the International Standardization Organisation (ISO).

While an evaluation of test methods or assessment of these test methods, e.g. using criteria like those proposed by Giesy & Hoke (1989) or Römbke et al. (1996), is not intended here, this article presents an inventory of standardised and validated microdrile oligochaete tests that are currently (or will in the near future be) used for the environmental risk assessment of pesticides in the European Union.

**The role of Oligochaeta in the ERA of pesticides**

Lumbricid earthworms are most frequently used in soil tests which have often been highlighted in literature (Petersen & Luxton, 1982; Edwards & Bohlen, 1997; Lavelle et al., 1997). However, this contribution focuses onmicrodrile worms (enchytraeids in soil and lumbriculids or tubificids in sediments). Why have these often inconspicuous oligochaetes been chosen for the ERA process?

1. They play an important ecological role in soils and sediments as indicated by high biomass and high diversity (Didden, 1993; Brinkhurst & Jamieson, 1971). Some species act as "ecosystem engineers" (i.e. by their burrowing activity they considerably modify the habitat), e.g. the enchytraeid *Cognettia sphagnetorum* (Vejd., 1877) in acid soils of Scandinavian coniferous forests (e.g. Abrahamsen 1990).
2. Practical to use: At least some species like T*ubifex tubifex*, *Lumbriculus variegatus* or *Enchytraeus albidus* are easy to breed in the laboratory (e.g. ASTM International, 2000, 2002). In several cases the same (or related) species can be handled on various test levels (e.g. laboratory or semi-field tests; Moser et al., 2004).
3. Several microdrile species have shown medium to high sensitivity towards chemicals (e.g. Jensen et al., 2001; Chapman, 2001).
4. Standardised and validated test methods are available from OECD and ISO. They will be described in detail below.
5. Finally, the importance of Oligochaete worms is well understood by the public, i.e. the services provided by microdrile worms can be explained by referring to earthworms – and these worms have been valued since the times of Charles Darwin.

For these reasons oligochaete worms were the first choice when selecting invertebrate test species for soils, and only second to chironomids for sediments in Europe and North America. The Environmental Risk Assessment for the soil compartment is usually performed by testing earthworms (Lumbricidae) as representatives of all soil invertebrates, using acute and chronic laboratory tests (OECD, 1984; OECD, 2004d) or long-term field tests (ISO, 1998).

Earthworm tests are an obligatory part of any pesticide ERA in the European Union, but they will not be described in detail here. An alternative or addition in the Environmental Risk Assessment for the compartment soil is the testing of enchytraeids. Chronic laboratory tests (OECD Guideline 220, 2004c)

as well as a long-term semi-field test with the natural enchytraeid community (Knacker et al., 2004; Moser et al., 2004) and a bioaccumulation test in the laboratory (Bruns et al., 2001 a, b) is available. Enchytraeid tests are not yet an integral part in the ERA of pesticides but they are consistently used with other chemical groups in soil quality assessment (e.g. for metal-polluted soils in The Netherlands; Römbke et al. 2002).

For the Environmental Risk Assessment of the sediment compartment currently two main tests with the midge *Chironomus riparius* (Chironomidae) or related species from the same genus are used (OECD 2004a, b). However, since these midge larvae actually live at the sediment-water interface, feeding mainly on the surface (and not within) the sediment (e.g. Rasmussen, 1984), these organisms may not be fully representative for evaluating the effects of chemicals on sediment-dwelling organisms. Since benthic oligochaetes (Tubificidae, Lumbriculidae) as "true" sediment infaunal organisms are exposed to sediment contaminants by all uptake routes including ingestion of contaminated sediment (e.g. Rodriguez & Reynoldson, 1999; Chapman 2001) these worms are considered an alternative or an addition to the chironomid tests. Various chronic laboratory toxicity tests (e.g. Reynoldson et al., 1991; ASTM International, 2002; OECD, 2006) as well as bioaccumulation tests (e.g. ASTM International, 2000; Egeler et al., 2006) are available. Some of the different methods are described below.

**Description of soil tests with Enchytraeidae**

Each test is first described in a tabular way, followed by an example how this test could be used. A recent overview on enchytraeid tests is provided by Römbke et al. (2005), while examples of toxicity results are given by Didden & Römbke (2001).

*Acute and chronic laboratory tests with enchytraeids*

The Enchytraeid Reproduction Test (Tab. 1) is the first soil test which was standardised by three standardization organizations: OECD, ISO and ASTM. The OECD guideline focuses on the testing of individual chemicals (in particular pesticides), and ISO covers retrospective testing of samples from contaminated sites. The ASTM guideline has a broad approach, covering not only enchytraeids but also earthworms. Acute (mortality) and chronic (reproduction) parameters are used in this test which has often been used for the testing of pesticides (Römbke, 2003; Amorim et al., 2005a, b). Currently it is identified in several legal documents, usually as an alternative to earthworm testing (EPPO, 2003; VICH, 2005; ISO 2006). The species *Enchytraeus crypticus* Westheide & Graefe 1992 is more popular than *E. albidus* because of its broader ecological range and higher practicability (shorter test duration, higher juvenile numbers; Kuperman et al., 2006).

*Table 1.* Description of the Enchytraeid Reproduction Test (ERT; Römbke & Moser, 2002)

| | |
|---|---|
| Guideline& Reference | ASTM E1676-97 (2004), OECD 220 (2004c); ISO 16387 (2004) |
| Test type | Chronic, sublethal laboratory test |
| Test species | *Enchytraeus albidus* Henle, 1837 (Enchytraeidae) or other species of this genus; in all cases originating from mass culture |
| Test parameter | Mortality (adults), reproduction (number of juveniles) |
| Test duration | Range-Finding-Test: 2 weeks; main test: variable, depending on the species; *E. albidus*: 6 weeks; others species: 4 weeks |
| Test substrate | Artificial soil: quartz sand, kaolin, peat, calcium carbonate and water (OECD, 1984); also field soils possible |
| Application of test ubstance | Mixed into the artificial soil; mixtures of contaminated and control soil also possible |
| Test conditions | 10 adult (= clitellate) worms per test glass vessel (with lid; 0.2-0.25 L volume); temperature: 20 ± 2 °C; permanently no light; moisture: 40-60% of the $WHC_{max}$., extraction of the juveniles using Bengal red; weekly feeding with rolled oats |
| Control | Untreated test substrate (e.g. artificial soil or a reference soil such as the German LUFA 2.2) |
| Validity criteria (control) | Mortality＜20% (adults); number of juveniles per test vessel (at the end of the test)＞25 (*E. albidus*) or＞50 (other species) |
| Test ssessment | NOEC (treatment versus control) or $EC_x$ |
| Reference substance | EC50 (reproduction) of Carbendazim: 1.2 ± 0.8 mg/kg dw |
| Limitations and remarks | Possible modifications depending on the *Enchytraeus* - species and, in the case of soil quality assessment, on the test soil |

The following example describes the effects of a pesticide (the fungicide carbendazim) on an enchytraeid species (*Enchytraeus albidus*) in a laboratory test (for more details see Römbke & Moser 2002). It should be noted that these data are taken from an international ring test, where some of the variability is attributed to the individual experience of the participants. At the same time, the higher sensitivity of the chronic (i.e. reproduction) compared to the acute (i.e.

mortality) endpoint is exemplified in Figures 3 and 4. While in the acute tests an LC50 of ＞10 mg kg$^{-1}$ dw soil was found, the EC50 determined in the chronic test was clearly lower: 2.8-3.7 mg kg$^{-1}$ soil dw.

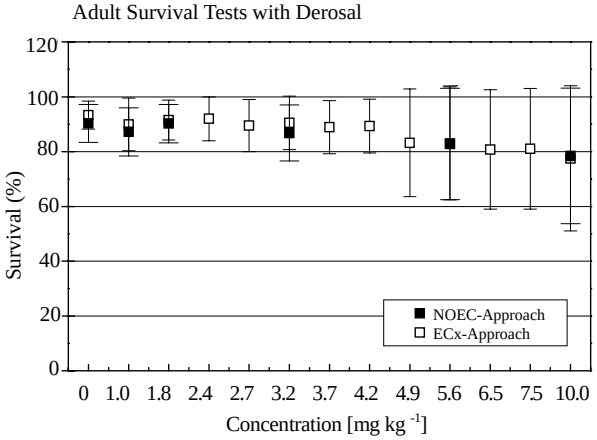


*Figure 3.* Survival of adult individuals of the species *Enchytraeus albidus* in a laboratory test after 6-week exposure to the fungicide derosal (a.i. carbendazim), using two different test designs; data from an international ring test (Römbke & Moser, 2002)

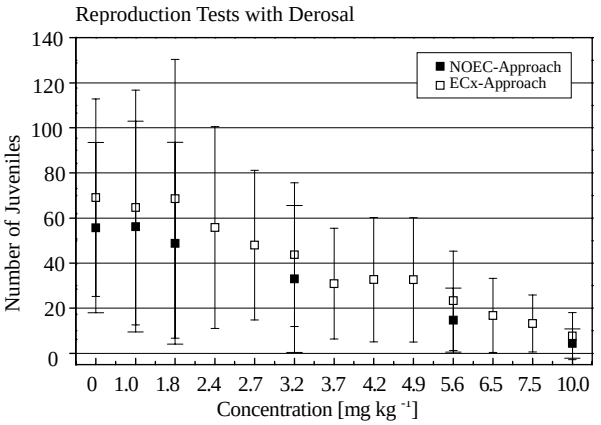


*Figure 4.* Reproduction (i.e. number of juvenile individuals) of the species *Enchytraeus albidus* in a laboratory test after 6-week exposure to the fungicide derosal (a.i. carbendazim), using two different test designs; data from an international ring test (Römbke & Moser, 2002)

An Environmental Risk Assessment for carbendazim in the soil compartment using the data from the enchytraeid reproduction test was performed (Römbke & Moser, 2002). Carbendazim belongs to the chemical class of benzimidazoles and is applied as a fungicide in wheat at application rates of 360 g a.i./ha. The predicted concentration in soil (PECs) is calculated as 0.48 mg/kg soil dw. In a first step the acute mortality (determined as LC50＞3.6 mg kg$^{-1}$ dw) is compared with the PEC (TER=TC/PEC=＞3.6/0.48=7.5. Since this TER ratio is potentially smaller as the trigger value defined by the EU (= 10), concern is identified. Therefore, the ERA has to be repeated using chronic toxicity data (NOEC=0.56 mg kg$^{-1}$ soil dw), meaning that the refined risk assessment ends up in a TER=TC/PEC=1.2 (EC, 2002). Since this TER ratio is smaller than the refined trigger value defined by the EU (=5), the use of carbendazim may cause a risk for the soil environment. After getting such a result, usually further tests (e.g. in the field) are performed in order to verify this result.

*Bioaccumulation test with enchytraeids*

In Table 2, the second standardised test method using enchytraeids is described. This bioaccu- mulation test is currently being validated through an international ring test. It is anticipated that sometime in the next few years this test method will be published as an OECD guideline. The bioaccu- mulation test can be modified to use different enchytraeids as well as earthworm species, but here we describe the performance with *Enchytraeus* species (*E. albidus, E. luxuriosus* or *E. crypticus*).

*Table 2.* Description of the Oligochaete Bioaccumulation Test (part Enchytraeidae; Bruns et al., 2001 a, b)

| Guideline & Reference | Draft Guideline for OECD; in Bruns et al. (2001 b)[1] |
|---|---|
| Test type | Bioaccumulation test (uptake and elimination phase) |
| Test species | *Enchytraeus albidus* (Enchytraeidae), *E. crypticus* or other species of this genus |
| Test parameter | Bioaccumulation factor and uptake and elimination rates |
| Test duration | 21 days each phase |
| Test substrate | Artificial soil: quartz sand, kaolin, peat, calcium carbonate and water (OECD 1984); also field soils possible |
| Test substance | Metals, organometalloids, organic chemicals (usually $^{14}$C-labelled organic substances are used) |
| Application of test substance | Mixed into the artificial or field soil |
| Test conditions | 20 adult (= clitellate) worms per test glass vessel (with lid; 50 mL volume); temperature: 20 ± 2 °C; 16 h : 8 h (light : dark); 100 to 1000 lx; moisture: 40-60 % of the $WHC_{max}$., food added to soil immediately before adding the organisms at start of uptake and elimination phases; no additional feeding |
| Test design | In each phase, (soil and worm) samples are taken at six dates with four replicates each |
| Validity criteria (control) | Worms should burrow into soil; overall mortality during test period≤20% (adults) |
| Test assessment | BAF or BSAF (lipid-normalised) |
| Reference substance | Not specified |
| Limitations and remarks | Depending on the final assessment of the ring test modifications may occur. |

[1] Available for download on http://www.umweltbundesamt.org/fpdf-l/2102.pdf

The bioaccumulation factors (BAF) of two organic compounds (lindane, hexachlorobenzene (HCB)) mixed into two test substrates (OECD artificial soil, LUFA St. 2.2 standard field soil) are presented in Table 3 data from Bruns et al., 2001 a). Both substances were clearly accumulated in both soils by *E. albidus* and *E. luxuriosus*. The BAF values of lindane are by a factor of 2-3 higher in the LUFA 2.2 field soil compared to OECD soil, but the BAFs for HCB are not different in the two soils (OECD = 1.5; LUFA=2). The difference between the BAFs is probably caused by the different organic matter contents of the two soils, which is about five times higher in OECD soil than in LUFA soil (OECD: 10% LUFA: 2.2%). With the exception of lindane in OECD soil, the BAFs are consistently higher for the small species *E. luxuriosus* than for the relatively large species *E. albidus*. This difference may be caused by the different volume : surface ratio of the two species, so that small-bodied species can take up higher amounts of the test chemicals from the pore water. This hypothesis is supported by the results from similar earthworm tests: Their BAF values were always lower than those of the enchytraeids (Bruns et al. 2001b).

*Table 3.* Bioaccumulation, and tissue residues at end of elimination of two pesticides (lindane, hexachlorobenzene) in two soils (OECD artificial soil, LUFA 2.2 standard field soil) in two enchytraeid species; BAF: bioaccumulation factor based on concentrations in wet worm and soil; BSAF: biota-soil accumulation factor normalised for concentrations in lipid and organic carbon (OC)

| | Soil type | BAF ± SE | BSAF | Tissue residues at end of elimination |
|---|---|---|---|---|
| | | [kg soil $kg^{-1}$ worm (ww)] | [g OC $g^{-1}$ lipid] | [% of accumulated residues] |
| **Lindane** | | | | |
| *E. albidus* | OECD | 12 ± 0.7 | 9.5 | 50.1 |
| | Lufa 2.2 | 22 ± 0.4 | 7.7 | 8.6 |
| *E.luxuriosus* | OECD | 12 ± 1.1 | 9.4 | 2.6 |
| | Lufa 2.2 | 36 ± 1.1 | 12.7 | 4.1 |
| **HCB** | | | | |
| *E. albidus* | OECD | 14 ± 4.4 | 11 | 14.3 |
| | Lufa 2.2 | 28 ± 0.8 | 9.7 | 10.0 |
| *E. luxuriosus* | OECD | 27 ± 0.9 | 21.2 | 0.02 |
| | Lufa 2.2 | 35 ± 0.7 | 12.1 | 8.4 |

SE: standard error; ww: wet weight

The use of BAF values determined in the uptake phase is well established as part of ERAs. Primarily it addresses the issue of biomagnification at higher trophic levels (e.g. when discussing the effects of DDT on birds of prey (e.g. ospreys, Wiemeyer et al., 1975)). Synopsis of uptake and elimination kinetics gives a more comprehensive picture of the bioaccumulation of a test compound. Additional results from the elimination phase to be used in ERA are the non-eliminated tissue residues (Tab. 3). It can be seen that HCB is eliminated almost completely by both species in both soils at the end of the test. However, 50% of the accumulated lindane is still present in the tissue of *E. albidus* (but not *E. luxuriosus*) at the end of the test. This latter finding may indicate a risk of secondary poisoning for species foraging on *E. albidus* (e.g. carabid beetles, centipedes or gamasid mites). In terms of ERA these results can be interpreted as follows: For lindane a risk of secondary poisoning of predators is likely while HCB seems not to be a problem after short-term exposure of the enchytraeids.

*Further laboratory tests under development*

Already in the 1990s, an avoidance test using enchytraeids was proposed by Achazi et al. (1996). While a comparable test using earthworms is already approved by ISO (2005a), there is still little experience concerning the use of the avoidance behaviour of enchytraeids. However, it seems that pesticides like the fungicides Benomyl and Carbendazim cause effects only in the range known from acute tests (Amorim et al., 2005a). Behavioural tests seem to be a useful complement to existing acute and chronic tests, since within a few hours or days a first evaluation of the toxicity of a substance or a soil sample can be made. However, more tests with different chemicals as well as with different enchytraeid species have to be made before ISO could start to standardise an enchytraeid avoidance test guideline. On the other hand, the use of enchytraeids in aquatic tests did not gain general acceptance (Römbke & Knacker, 1989).

**Sediment tests with Lumbriculidae and Tubificidae**

*Chronic laboratory test with lumbriculids*

In Table 4, a recently standardised toxicity test with *Lumbriculus variegatus* is presented. The aim of this method is to determine effects of a test substance on the reproduction and biomass of test organisms. Spiked artificial sediment which is very similar to

artificial soil (see Chapter 3), is used as test substrate. The method is based on the chironomid tests as described by OECD (2004c) but also takes into account experiences reported in U.S. guidelines (mainly ASTM International, 2002), which primarily focus on the bioassessment of contaminated field sediments. The suitability of the new test was demonstrated in an international ring test (Egeler et al. 2005).

*Table 4.* Description of the *Lumbriculus variegatus* sediment toxicity test

| Guideline & Reference | OECD Draft Guideline: Sediment-water *Lumbriculus* Toxicity Test Using Spiked Sediment; OECD (2006) |
|---|---|
| Test type | Chronic, sublethal laboratory test |
| Test species | *Lumbriculus variegatus* (Müller) |
| Test parameter | Reproduction, biomass |
| Test duration | Four weeks |
| Test substrate | Artificial sediment: quartz sand, kaolin, peat, calcium carbonate and water (OECD 1984); addition of *Urtica* powder or *Urtica* powder/cellulose (0.4-0.5% on dry sediment) before application of test item; no additional feeding; overlying water according to OECD (1992) |
| Application of test substance | Spiked into the artificial sediment |
| Test conditions | 10 adult worms per test vessel (glass with lid; 0.2-0.25 L volume); temperature: 20±2℃; 16 light : 8 dark; 100-1000 lx |
| Test design | At least 5 concentrations with four replicates each plus an untreated control (e.g. artificial sediment) with 4-6 replicates: 24-26 vessels in total |
| Validity criteria (control) | Number of worms increase by at least 1.8 pH 6-9, oxygen at least 60% of air saturation |
| Test assessment | NOEC or ECx (treatment versus control) |
| Reference substance | EC50 (reproduction) of pentachlorophenol: 20 mg $kg^{-1}$ dry sediment |
| Limitations and remarks | Survival and reproduction not separable due to asexual reproduction mode (fragmentation). |

The effects of exposure to pentachlorophenol (PCP) on *L. variegatus* are shown as derived from the report of an international ring test of the method (Egeler et al. 2005). Figure 5 shows the concentration-dependent total number of worms after 28-day exposure to PCP, while Figure 6 contains the concentration-response curve for the same parameter. These data show a steep concentration-response relationship, with small 95%-confidence intervals. The results demonstrate the applicability of the test method for assessing effects of the sediment-spiked test compound with *L. variegatus*. In contrast to the example provided in Chapter 3.1 it is not possible to perform an environmental risk assessment with these data since PCP was chosen as a model chemical which does not have practical relevance any more since it was banned in the European Union already many years ago.

### *Bioaccumulation test*

The last example of a microdrile ecotoxicological test, the Sediment Bioaccumulation Test, is presented in Table 5. Like the *Lumbriculus* toxicity test, it has recently been standardised on the basis of an international ring test (Egeler et al. 2006). In fact, both tests share many properties: For example, in both cases the same test substrate (artificial sediment) is used which is spiked with the test chemical. However, in the bioaccumulation test not only *L. variagatus* is recommended but also the tubificid sludge worm.

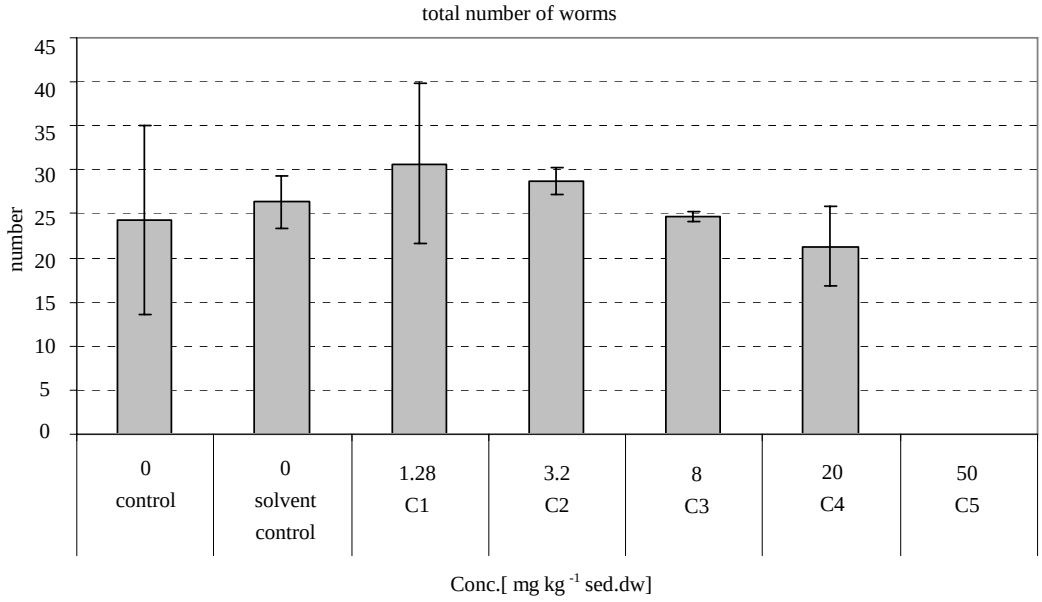


*Figure 5.* Total number of worms (*Lumbriculus variegatus*) per replicate (treatment mean values and SD; n = 3) after 28 days of exposure to PCP-spiked artificial sediment; data from an international ring test (Egeler et al., 2005)

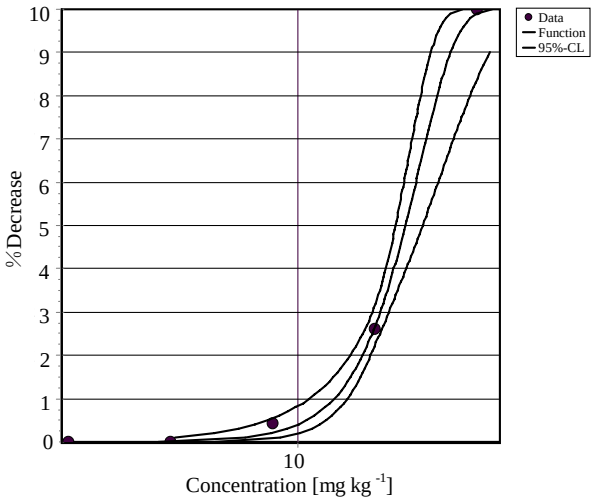


*Figure 6.* Total number of worms (*Lumbriculus variegatus*) per replicate after 28 days of exposure to PCP: concentration-response curve (Weibull function) with 95%-confidence limits (CL); data from an international ring test (Egeler et al., 2005)

*Tubifex tubifex*. This recommendation is based on experiences from earlier studies performed in the 1990s (Reynoldson et al., 1991, Egeler et al., 1997). Despite the fact that it has so far not been validated in a ring test, the species *Branchiura sowerbyi* can also be used in this test. This recommendation is based on experiences from literature (e.g. Marchese & Brinkhurst, 1996; Roghair et al. 1996). Since this species is often found in tropical regions, its inclusion extends the applicability of this guideline considerably. It can be expected that the inclusion of non-temperate species into OECD guidelines will become a general issue in the near future.

In Figures 7 and 8 two typical results from this test are presented, using the highly persistent chemical hexachlorbenzene (HCB) and *L. variegatus* as examples. Figure 7 shows the accumulation kinetics of HCB in the worms during the exposure or uptake phase. The data points in the plots represent the mean accumulation factor (AF) as the ratio of the concentration in the worms to that in the sediment of a given replicate on each sampling date. Radioactivity increased rapidly in the worms during the initial part of the uptake phase. After approximately two weeks, AF reached a plateau. The curves in Figures 7 and 8 were fitted to these data using a 1 - compartment model and nonlinear regression analysis. The uptake phase was terminated by transferring the remaining worms to vessels containing clean water and sediment for the elimination phase (Fig. 8). The accumulated HCB was lost from the worms nearly completely. The BAF as well as non-eliminated residues persisting in the worms after 10 days can be used for evaluating the bioaccumulation behaviour of the test chemicals in risk assessment schemes (e.g. Franke et al. 1994).

*Table 5.* Description of the sediment bioaccumulation test

| | |
|---|---|
| Guideline & Reference | Draft Guideline for OECD; in Egeler et al. (2006) |
| Test type | Bioaccumulation test |
| Test species | *Lumbriculus variegatus* (Müller) or *Tubifex tubifex* (Müller) |
| Test parameter | Concentration of the test substance in worm tissue and test substrate |
| Test duration | Radioanalysis in water, sediment & worms: e.g. on days 0, 1, 3, 7, 14, 21, 28 (uptake phase); e.g. on days 0.5, 1, 3, 5, 7, 10 (elimination phase) |
| Test substrate | Artificial sediment: quartz sand, kaolin, peat, calcium carbonate and water (OECD 1984); addition of *Urtica* powder or *Urtica* powder/cellulose (0.4-0.5% on dry sediment) before application of test substance; no additional feeding; overlying water according to OECD (1992) |
| Application of test substance | Spiked into the artificial sediment |
| Test conditions | 10 adult worms per test vessel (e.g. 0.1 L volume); temperature: 20 ± 2 °C; 16 light : 8 dark; 100-1000 lx |
| Control | Untreated test substrate (e.g. artificial sediment) |
| Validity criteria (control) | mortality in control replicates ⩽ 20%; worms should burrow in the sediment |
| Test assessment | Bioaccumulation factor (BAF), uptake rate constant elimination rate constant(s), non−eliminated residues after 10−d elimination |
| Reference substance | None identified |
| Limitations and remarks | Test compounds: $^{14}$C- or $^{3}$H- labelled with a concentration in definitive test: approx. 20000 dpm/g dry sediment |

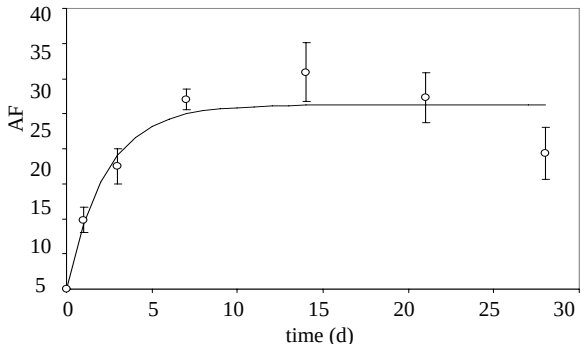


*Figure 7.* Uptake kinetics of $^{14}$C-HCB in *L. variegatus*; AF: bioaccumulation factor as ratio of dpm/kg worm dry weight and dpm/kg sediment dry weight (mean values, n = 3; error bars are SD); data from an international ring test (Egeler et al., 2006)

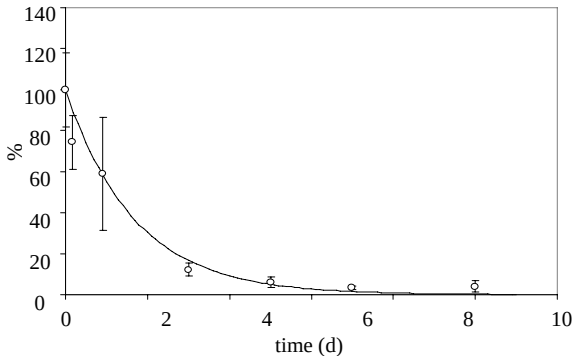


*Figure 8.* Elimination kinetics of $^{14}$C-HCB in *L. variegatus*; %: per cent of dpm/g worm w.w. at end of uptake phase (mean values, n = 3; error bars are SD); data from an international ring test (Egeler et al., 2006)

## Discussion of the use of microdriles in the environmental risk assessment of pesticides

### *Soil*

The "ideal" microdrile test species has not yet been identifed: The species *E. albidus* – the one most often used so far-is sensitive towards soil properties which limits its use when testing field soils. The smaller species *E. crypticus* is easier to handle (e.g. the mean number of juveniles is higher, more or less independent from the soil properties), but its origin is unknown. Fortunately, the test guideline allows the use of different species. More generally speaking, the limited knowledge about the ecological requirements of many enchytraeid species is problematic, since potentially the effect of properties of the soil and the effect of toxic chemicals in soils cannot be distinguished in such cases. In addition, no standard test species was found so far for acid soils (e.g. Canadian or Scandinavian boreal forests).

Finally, no higher-tier test (i.e. a field tests and an evaluation scheme) with enchytraeids has been standardised so far, but it was proposed to include enchytraeids in Terrestrial Model Ecosystems (TMEs; Knacker et al., 2004). This semi-field method consist of intact soil cores (Fig. 9) which are kept under controlled conditions, e.g. in a greenhouse (Fig. 9). Pesticides can be applied on the soil surface before or directly on the soil core after their extraction. Usually, after a test duration of 16 weeks samples are taken using soil corer in order to determine the abundance and species composition of the enchytraeids. Based on experiences with the fungicide carbendazim it could be shown that enchytraeids are well suitable for the risk assessment of pesticides (Moser et al., 2005).

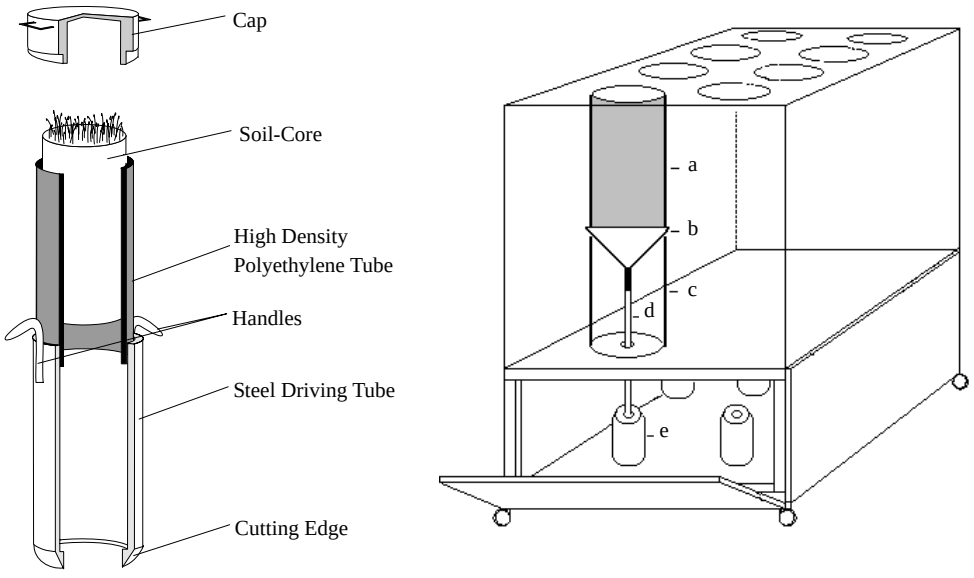


*Figure 9.* Left: Soil corer for the extraction of Terrestrial Model Ecosystems. Right: Diagram of apparatus for keeping TMEs

The main problem of the applicability of enchytraeids in soil ecotoxicology is that clear legal requirements do not exist., at least for the risk assessment of pesticides. In other areas, like the assessment of contaminated land, such requirements have been formulated recently (e.g. ISO 2005b and 2006).

*Sediment*

Aquatic oligochaete tests are not yet formally implemented in the ERA of pesticides. However, they are increasingly requested for testing other chemical groups, e.g. industrial chemicals (Riedhammer & Schwarz- Schulz 2001, EC 2003), where an exposure of benthic organisms to contaminated sediments is to be represented. Oligochaetes are also used as a component in aquatic outdoor mesocosms as described e.g. by Warren et al. (1998) and Verdonschot & Ter Braak (2004). The acceptance of such higher-tier tests is growing, so that they are becoming a regular part of the registration process of pesticides. Experiences, as well as guidance information, in particular guidance on test design (see for example the outcome of the HARAP workshop: Campbell et al. 1999) and multivariate evaluation tools (Maltby et al., 2005), are more developed for aquatic mesocosms than for soil model ecosystems. Especially for compounds which tend to associate to sediments, the presence and evaluation of oligochaetes in aquatic mesocosms can provide valuable information, as these organisms are an integral part of benthic communities.

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