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Bioaccumulation and elimination of ^{14}C-lindane by *Enchytraeus albidus* in artificial (OECD) and a natural soil

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Abstract

Bioaccumulation and elimination of ^{14}C-lindane in *Enchytraeus albidus* was studied in artificial OECD soil and a silty loam from an agricultural field in Central West Portugal. Results showed that enchytraeids were able to bioaccumulate the chemical with a kinetic pattern similar to that of earthworms: fast uptake within a few days and a biphasic elimination pattern. A 10 day period to study uptake was sufficient, but a few more days were probably necessary for elimination. Bioaccumulation was influenced by soil type. The authors suggest that higher organic matter (OM) content and also the higher content on sand particles in the OECD soil may have led to a faster elimination: hydrophobic chemicals tend to adsorb to OM being in this way less bioavailable and therefore less bioaccumulated having bioaccumulation factor value around 6 while in natural soil is 10; the sand could act as abrasive particles (helpers) in the elimination process leading to an elimination of 90% of the chemical in two days while in natural soil 67% was eliminated in the same period of time.

Keywords: Bioaccumulation pattern; Toxicokinetics; *Enchytraeus albidus*; Lindane; Different soils

1. Introduction

Bioaccumulation constitutes an ecological risk and should be considered in the overall risk assessment of chemicals. Toxicokinetic studies are of major importance in soil ecotoxicology, since many effects can hardly be recognized, even in chronic toxicity tests. Lipophilic agricultural chemicals, due to their low water solubility and affinity for soil associated organic matter (OM), become sorbed preferentially to the OM in soil. The organisms associated with the soil or sediment are exposed and may bioaccumulate such materials. Since the environmental hazard of a chemical does not depend directly on its concentration in the environment, it is essential to understand the pathways and the mechanisms through which a chemical enters the organism, and also to evaluate the bioaccumulation potential of the chemical (Peijnenburg et al., 1997). A possible way to evaluate the bioavailability of pollutants in soil is to measure both the concentration inside the organism (Van Straalen, 1996) and the elimination rate from it.

The aim of this study was to evaluate the toxicokinetic behaviour of the test chemical lindane, on *Enchytraeus albidus*, using two different soil types. Lindane was a commonly used pesticide and is still known to be used despite current restrictions. Its hydrophobicity ($\log k_{ow} = 3.85$) and persistency in the environment makes it a suitable test substance for bioaccumulation

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studies, because a certain period of time is needed to see effects.

Soil properties have a major influence on chemical bioavailability making it difficult to predict the toxicity associated with the soil properties. Often, well defined test soils are used (in bioassays) to allow for a standardization between laboratories or a better comparison of test results obtained with different species (Smit and Van Gestel, 1998). Understanding the way soil characteristics interact with chemicals and how it influences toxicity to animals, becomes more important when we extrapolate data to real environments. In this study two soil types are used: OECD artificial soil (OECD guideline no. 207, 1984) and a natural alluvian soil from the Mondego Low Valley. Chronic toxicity tests were performed in both soils (Amorim et al., 1999) showing that these worms were not reproducing at the natural soil pH (4,7) but adults survival was not affected; bioaccumulation testing could occur maintaining the purpose of soil type comparison.

Another purpose of this study, and the reason why we have chosen an enchytraeid species as the test organism, is related to the need of developing a bioaccumulation test for organisms living in the soil layer rather than the humus (as earthworms like *Eisenia fetida* do). Furthermore, enchytraeids are gaining importance in soil ecotoxicology with a reproduction test already validated and about to become an ISO guideline (Römbke and Moser, 1999). Thus, this study may act as a first step in the development of such a bioaccumulation guideline.

2. Materials and methods

2.1. Test chemical

Lindane, 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane, is a pesticide. The unlabeled lindane was obtained from Merk with purity >99.5%. It was solved in acetone and mixed with radiolabeled lindane (^{14}C-γ-HCH) from Internationale Isotope (München, purity >95%). The specific activity of the mixture was 0.019 MBq mg^{-1}. The solution was prepared to achieve a nominal concentration of 20 μg g^{-1} of soil dry weight.

2.2. Soils

Artificial OECD soil and a natural soil were used in this study. Artificial OECD soil was prepared according to the OECD guideline no. 207 (OECD, 1984, earthworm acute toxicity tests) but the pH was adjusted to 4.7 (similar to the natural soil) adding $CaCO_3$. The natural soil, an agricultural alluvial soil from the Low Mondego Valley, was air dried, sieved (2 mm), and stored until use. Soil properties can be seen in Table 1.

Table 1
Summary description of some of the characteristics of the two soil types

	OECD soil[a]	Natural soil
Soil type	Artificial	Clay silt
pH (1 M KCl)	4.7	4.69
Moisture (% water-holding capacity)	40	40
OM content (%)	7.6 (±0.7)	2.96 (±0.9)
Cation exchange capacity (meq/100 g)	9.4 (±0.6)	15.9 (±3.9)
Sand (%)	72.4	9.7
Silt (%)	2.4	63.1
Clay (%)	17.9	24.3

[a] Organization for Economic Cooperation and Development, Paris, France.

The test solution was homogeneously mixed in both soil types. After solvent evaporation (the soil was left for 24 h under ventilation), the soil was dried to 40% water holding capacity and separated into the test vessels (of 7.5 by 4 cm plastic boxes filled with 30 g DW of contaminated soil). Additional soil samples (both OECD and natural soil) were prepared and analysed in order to evaluate the homogeneity of the chemical solution within the soil. In the natural soil, the experiment started immediately after compound addition, whereas in the OECD soil it started after one week.

2.3. Uptake and elimination experiment

2.3.1. Test animals

The enchytraeids came from a laboratory culture maintained under controlled conditions according to Römbke and Moser (1999). Before carrying out the experiment, adult animals of similar size were acclimatised in uncontaminated test substrate (OECD or natural soil) for a period of 24–72 h. After that, each group of five animals was cleaned in water to remove soil particles, gently dried, weighted, placed into the test vessel with no food supply (preliminary tests were carried out and the organisms did not eat the food) and the vessel was covered with a lid. Experiments were carried out at a temperature of 20 ± 2 °C and a photoperiod of 16/8 h (light : dark).

2.3.2. Experimental procedures

A total of 420 animals were used (= 84 replicates) per soil type. The experiment lasted for a period of 20 days, 10 for the uptake phase plus 10 for the elimination phase. Sampling was performed at 12 h, 1, 2, 3, 5, 7 and 10 days after the animals were introduced into the test vessels. At each sampling time six replicate animals were harvested. The animals were removed and cleaned in a

1% acetone solution, gently dried on filter paper, weighted and frozen (−20 °C) for analysis. Soil was weighted and frozen for further analysis.

After the 10 days of uptake the animals from the remaining samples were transferred to clean soil for the elimination phase, which followed the same procedure in terms of sampling scheme and analysis.

2.3.3. Lindane analysis

Lindane was analysed in the animals and soils using a Biological Oxidizer OX 500 (Zinsser Analytic) at 900 °C in the presence of a mixture of O_2 and N_2. The $^{14}CO_2$ (2 min for the animals and 4 min for soil samples) released during the burning period was trapped in a vial containing scintillation liquid (Zinsser Oxysolve 400) and then counted by liquid scintillation counting (Beckman LS 6500) for a period of 20 min.

2.4. Data treatment

Since there was a significant decrease of lindane in soil over time during the uptake phase, the following first order kinetic equation was used to describe the process (Widianarko and Van Straalen, 1996):

$$C(t) = C_0 e^{k_s t} \quad (1)$$

where $C(t)$: concentration in soil at time t (μg lindane g^{-1} soil dry weight), C_0: initial concentration in soil (μg lindane g^{-1} soil dry weight), k_s: rate constant for decrease of the chemical in the medium (day^{-1}).

Thus a one-compartment model (Eqs. (2a) and (2b)) is used to estimate the assimilation rate (a) and elimination rate constant (k_e) simultaneously (Sousa et al., 2000):

for $t \leqslant t_c$:

$$Q(t) = \frac{a}{k_e - k_s}(e^{-k_s t} - e^{-k_e t}) \quad (2a)$$

for $t > t_c$:

$$Q(t) = \frac{a}{k_e - k_s}(e^{-k_s t_c} - e^{-k_e t_c})e^{-k_e(t - t_c)} \quad (2b)$$

where, $Q(t)$: concentration in the organism at time t, (μg g^{-1} animal wet weight), a: assimilation rate (μg g^{-1} animal day^{-1}), k_e: elimination constant (day^{-1}), k_s: rate constant for decrease of the chemical in the medium (day^{-1}), t: time (days), t_c: time at which animals were transferred to clean soil (days).

A biphasic type curve was observed during the elimination phase, thus a double exponential equation was used to improve the model (Belfroid et al., 1994; Egeler et al., 1997).

$$Q(t) = (A^{-k_a t}) + (B^{-k_b t}) \quad (3)$$

where, $Q(t)$: concentration in the organisms at time t (μg g^{-1} animal wet weight), A: size of the compartment with rapid loss of test substance (% of initial concentration), k_a: elimination constant rate of the rapid elimination phase (day^{-1}), B: size of the compartment with slow loss of test substance (% of initial concentration), k_b: elimination constant rate of the slow elimination phase (day^{-1}), t: time (days).

The models were fitted to the raw data (time, concentrations) using the non-linear estimation module of STATISTICA (StatSoft, Inc., 1998) with the quasi-Newton method for calculating least squares.

The bioaccumulation factor (BAF), defined as the ratio between body concentration and soil concentration at steady state (Egeler et al., 1997), was calculated assuming that equilibrium was achieved between days 3 and 10.

3. Results

3.1. Chemical analysis

Chemical analysis of the extra soil samples revealed an homogeneous mixing of the chemical with the soil.

Soil analysis of the natural soil revealed that the lindane average concentration was 18.1 μg g^{-1} soil, at the beginning of the experiment, decreasing to 11.4 μg g^{-1} soil ($k_s = 0.0385$ day^{-1}) in the last days of the uptake period. In the OECD soil, lindane's average concentration in the soil decreased from a value of 8.3–6.3 μg g^{-1} soil, in the same period, with a rate constant for decrease of 0.026 day^{-1} (Fig. 1). This was the result of mineralisation or volatilisation.

3.2. Kinetics

No mortality was observed in either soil. However, a weight reduction in the worms was observed during both phases of the experiment. On natural soil the average initial fresh weight (±SD) was 23.6 ± 2.9 mg ($n = 5$) decreasing, after exposure, to 14.3 ± 3.3 mg ($n = 5$). Worms exposed to the OECD soil exhibited a similar change: weight decreased from an initial average fresh weight of 20.2 ± 2.3 mg ($n = 5$) to 11.3 ± 2.8 mg ($n = 5$).

The curves on Figs. 2 and 3 (solid lines) represent the relationship between the concentration of lindane in the worms and time for both soil types. The uptake of lindane occurred very rapidly in both soils, being detected already on the first sampling time, just 12 h after exposure.

In natural soil (Fig. 2) the concentration in organisms showed a peak after 2 days of exposure, with an average

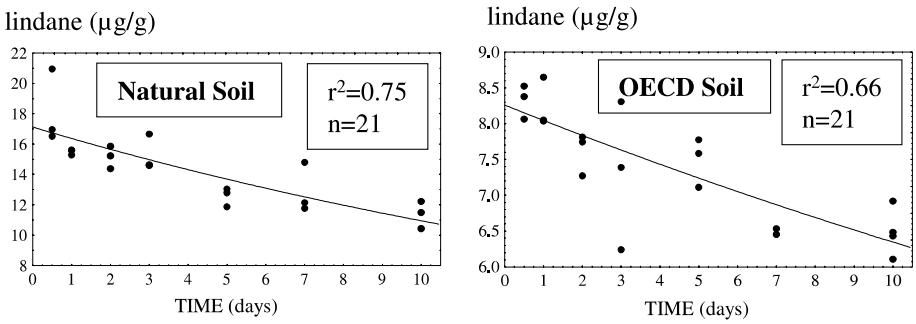

Fig. 1. Lindane concentration in Natural and OECD soils during the 10 days period (uptake period). Data fitted to non-linear regression (see text for further explanation). The decay constant values (k_s) are given in the text.

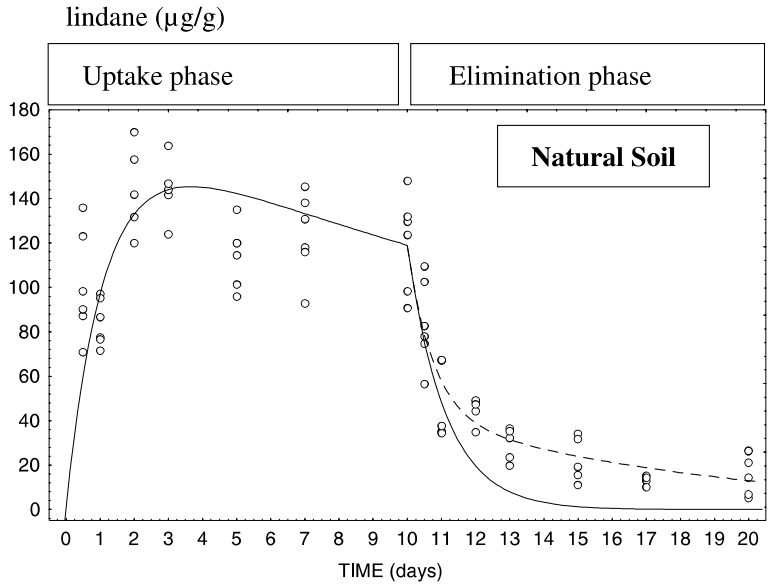

Fig. 2. Kinetic behaviour of [^{14}C]lindane in *E. albidus* during uptake and elimination phases. Animals exposed to contaminated natural soil. Solid line results from fitting individual concentration values from both phases to Eqs. (2a) and (2b). Dashed line results from fitting concentration values from elimination phase to Eq. (3). Data fitted by non-linear regression (see text and Table 2 for further explanation).

value of 144.3 µg lindane mg^{-1} animal, followed by a smooth decrease until the end of the exposure period, whereas the chemical concentration in the animals reached 120.4 µg lindane mg^{-1} animal. Data from the uptake period showed some degree of variation, in particular in the 12 h and 7th day samples which present slightly higher values. During the elimination phase, lindane concentration in the animals started to decrease immediately after they were transferred to clean soil. Within the 10 days animals were able to eliminate almost all lindane present in their bodies, reaching an average value of 16.8 µg lindane g^{-1} animal at the end of the experiment (<15% of the initial value at the beginning of the elimination phase). Estimated kinetic parameters were 150.7 µg lindane g^{-1} animal day^{-1} for the assimilation rate (a) and 0.9 day^{-1} for the elimination rate constant (k_e) (Table 2). The poor fit between the curve and the raw data points on the elimination phase suggested that this process followed a normal biphasic pattern (e.g., fast initial decrease followed by a slower phase). In this case a two-exponential (or two compartment) curve seems to be more appropriate to describe this decreasing process. Fitting individual elimination data to Eq. (3) resulted in the kinetic curve on Fig. 2 (dashed line) and in the estimation of an initial fast decrease rate (k_a) of 1.3 day^{-1} with size compart-

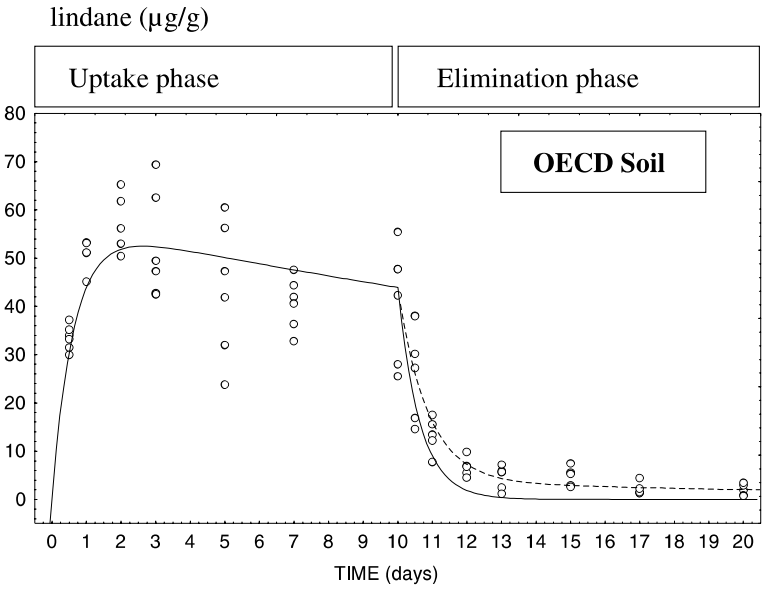

Fig. 3. [^{14}C]lindane kinetics in *E. albidus* derived from Eq. (3) during uptake and elimination phases. Animals exposed to contaminated OECD soil. Solid line results from fitting concentration values from both phases to Eqs. (2a) and (2b). Dashed line results from fitting concentration values from elimination phase to Eq. (3). Data fitted by non-linear regression (see text and Table 2 for further explanation).

Table 2
Toxicokinetic parameters, a (µg g^{-1} animal day^{-1}) and k_e (day^{-1}), estimated when using Eqs. (2a) and (2b); A (%), k_a (day^{-1}), B (%) and k_b (day^{-1}) are relative to the double exponential equation used for the elimination period (Eq. (3))

Soil type	a	k_e	r^2	BAF	
Eqs. (2a) and (2b) (uptake plus elimination)					
Natural	150.65	0.91	0.75	9.6	
OECD	88.85	1.58	0.85	6.4	
	A	k_a	B	k_b	r^2
Eq. (3) (elimination)					
Natural	80.98 (66.8%)	1.35	40.21 (33.2%)	0.11	0.88
OECD	37.83 (90.4%)	1.16	4.01 (9.6%)	0.05	0.85

The BAF is also calculated, assuming that equilibrium was achieved between days 3 and 10.

ment A of 66.8% (during the first two days), followed by a slow elimination rate (k_b) of 0.1 day^{-1} with size compartment B of 33.2% (Table 2).

In the OECD soil (Fig. 3), the kinetics of the compound followed the same pattern as in the natural soil, both on uptake and elimination phases. A similar peak was observed after 2 days of exposure (57.4 µg lindane g^{-1} animal) followed by a decrease to 41.1 µg lindane g^{-1} animal at the end of the uptake period. This slow decrease of lindane in the worms, caused by elimination mechanisms, presented a similar amplitude as in the natural soil. The difference in absolute values between both soils at the end of the exposure period is likely to be related to differences in initial concentration of the chemical in the soil. During the elimination phase animals were able to eliminate almost all of the lindane (>95%) present at the start of this period, with the concentration in the animals reaching a value of 1.9 µg lindane g^{-1} animal at the end of the experiment. Due to the much lower concentration levels found in exposed worms, estimated kinetic parameters were quite different from those values found for the natural soil; the assimilation rate (a) was 88.9 µg lindane g^{-1} animal and the estimated elimination rate constant was 1.6 day^{-1} (Table 2). Fitting elimination data to the double-exponential equation (Eq. (3)) resulted in the curve presented in Fig. 3 (dashed line) and the kinetic parameters presented in Table 2: elimination of almost all chemical substance,

compartment A (90,4%), during the fast elimination phase ($k_a = 1.16\ \text{day}^{-1}$).

4. Discussion

4.1. Kinetics—comparison between soil types

The use of a one compartment model allowed the simultaneous estimation of the uptake and elimination rates in both soil types. This was particularly true during the uptake period where this model proved to give the best fit. However, due to the observed biphasic shape of the elimination curve, the use of a double-exponential equation gave a better fit when applied to the elimination data.

Due to the fact that both experiments started with different initial chemical concentration, it is difficult to make a direct comparison of the obtained results, especially of the uptake phase. Higher uptake rate in natural soil may be related both to the higher initial concentration, and to the OM content of the soil. The chemical availability and bioaccumulation is inversely correlated with the amount of OM (or organic carbon) in a soil or sediment (Belfroid and Sijm, 1998). Thus, it is likely that even if the initial chemical concentrations were similar in both soils, the uptake and therefore the internal concentration, in animals exposed to the natural soil would be higher compared to animals exposed in OECD soil. BAFs found in similar bioaccumulation experiments with *E. albidus* with lindane and hexachlorobenzene (Bruns et al., 2001), were also lower in the OECD soil compared to a natural soil (Lufa 2.2), being 12 and 22 respectively (14 and 28 with HCB). In the present study the BAF values were for OECD soil 6 and for natural soil 10, comparable to those considering the different test design.

Despite the above mentioned difference between soils, it is possible to draw some comparisons between it. The kinetic pattern of lindane on animals exposed to the different soil types was similar. A fast uptake was observed in the initial stages of exposure, followed by a rapid decrease in concentration immediately after the beginning of the elimination period, assuming a biphasic elimination behaviour.

This two-step elimination has been observed in experiments with *Eisenia andrei* exposed to different organic chemicals (Belfroid et al., 1994), in tubificid worms (Egeler et al., 1997) and more recently in the enchytraeid species *E. albidus* and *E. luxuriosus* exposed to lindane and HCB (Bruns et al., 2001). This pattern suggests that for these species (including *E. albidus* in this study) exposed to soil and sediment, a second compartment exists. Several explanations for this behaviour have been suggested (Belfroid et al., 1994). In particular, one of the hypotheses relates to the adsorption/desorption of the chemical to the gut wall, which could act as an important uptake/elimination route when animals are exposed to contaminated soil or sediment. The desorption of the chemical could be related to the passage of abrasive sand particles through the gut and by the repartitioning between gut wall and the clean soil particles passing by. Moreover, this could determine the duration of the fast elimination phase, with higher lipophilic compounds causing longer initial phases.

Initial rapid elimination in both soils lasts only for 2 days. This agrees with the fact that lindane has a lower $\log k_{ow}$ when compared with the compounds studied by Belfroid et al. (1994), which observed an increase of the duration of the initial phase (up to 10 days) with an increase of $\log k_{ow}$. The abrasive effect of sand particles passing through the gut may explain the differences found in terms of the percentage of the compound eliminated in the initial phase on both soils. In OECD soil (with 72% of sand) 90.4% of the compound was eliminated in the first phase, while in natural soil (with approximately 10% of sand) only 66.8% of the compound was eliminated in the first 2 days.

OM content can also exert a strong influence on the elimination rate of a given chemical. Contrary to the common accepted assumption that the elimination is only conditioned by the elimination rate constant, and the amount of chemical present in the animal body, the study of Belfroid and Sijm (1998) showed that the elimination of hydrophobic compounds was faster, and the initial elimination phase lasted longer, in soils with higher OM content. This relation is stronger for compounds with high $\log k_{ow}$ (>5). The explanation suggested by these authors is related to the higher adsorptive capacity of soils with higher OM content, not only in the vicinity of the organism's body but also on the gut. This suggests that with the higher adsorptive capacity of a soil with high OM content the fast initial elimination phase will last longer. Only after the saturation of this fraction, the elimination will be controlled by passive diffusion through the water phase, causing a shift from the fast to the slow elimination phase. When comparing the elimination pattern on both soils, we can observe that the animals were able to eliminate more than 95% of the lindane in OECD soil (85% in natural soil) although, it was not clear that the fast elimination phase took longer in this soil type.

4.2. The suitability of enchytraeids as test organisms for bioaccumulation studies

This study shows that *E. albidus* is a suitable test organism for bioaccumulation studies. Moreover, this organism accumulates and eliminates lindane in a manner similar to that observed in earthworms. Although a decrease of lindane concentration in the soil over time led to a decrease in the concentration in the animals during the uptake period, we strongly believe

that for stable compounds (or in a stable situation), a steady state could be achieved, allowing the calculation of BAFs (e.g., BAF, BSAF). As seen by Bruns et al. (2001), who performed the test in an apparatus that minimised the leaking of radioactivity and allowed the determination of a mass balance at the end of the tests, it is possible to avoid the chemical decrease. It is clear that the study of more chemicals would be very important.

The experimental design used here is appropriate to perform studies in artificial and natural soils. The period of 10 days seemed sufficient for the uptake period of lindane (and to reach an equilibrium), but the same period of time is clearly insufficient for a complete elimination of this chemical; further studies in this subject should be made. However, there is a possibility of having a false steady state; experiments with *Lumbricus rubellus* performed by Füll and Nagel (1994), showed that for lindane in equilibrium of uptake and elimination seemed to be achieved within 15 days, but after 22 days there was a further increase of the BAF and no steady state was reached within 43 days.

Also, since both uptake and elimination patterns are strongly dependent on the soil type and chemical tested, more studies are needed for method improvement and standardisation. Nevertheless, this study can represent the first step towards the development of a guideline using enchytraeids as test organisms in bioaccumulation studies.

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References

Amorim, M.J., Sousa, J.P., Nogueira, A.J.A., Soares, A.M.V.M., 1999. Comparison of chronic toxicity of lindane (γ-HCH) to *Enchytraeus albidus* in two soil types: the influence of soil pH. Pedobiologia 43, 635–640.

Belfroid, A., Sijm, D., 1998. Influence of soil organic matter content on elimination rates of hydrophobic compounds in the earthworm: possible causes and consequences. Chemosphere 37 1221–1234.

Belfroid, A., Sikkenk, M., Seinen, W., Van Gestel, C.A.M., Hermens, J., 1994. The toxicokinetic behavior of chlorobenzenes in earthworm (Eisenia andrei): experiments in soil. Environ. Toxicol. Chem. 13, 93–99.

Bruns, E., Egeler, P., Römbke, J., Scheffczyk, A., Spoerlein, P., 2001. Bioaccumulation of lindane and hexachlorobenzene by the oligochaetes *Enchytraeus luxuriosus* and *Enchytraeus albidus* (Enchytraeidae, Oligochaeta, Annelida). Hydrobiologia 463, 185–196.

Egeler, P., Römbke, J., Meller, M., Knacker, Th., Franke, C., Studinger, G., Nagel, R., 1997. Bioaccumulation of lindane and hexachlorobenzene by tubificid sludgeworms (Oligochaeta) under standardised laboratory conditions. Chemosphere 35, 835–852.

Füll, C., Nagel, R., 1994. Bioaccumulation of lindane (γ-HCH) and Dichlorprop (2,4-DP) by Earthworms (*Lumbricus rubellus*). Poster, Third European Conference on Ecotoxicology, Zürich 1994, p. 4.03.

Organization for Economic Cooperation and Development, 1984. Earthworm acute toxicity tests. OECD Guideline 207. Paris, France.

Peijnenburg, W.J.G.M., Posthuma, L., Eijsackers, H., Allen, H.E., 1997. Implementation of bioavailability for policy and environmental management purposes. In: Herrchen, M., Debus, R., Pramanik-Srehlow, R. (Eds.), Bioavailability as a Key Property in Terrestrial Ecotoxicity Assessment and Evaluation. Fraunhofer IRB Verlag, Stuttgart, Germany, pp. 35–54.

Römbke, J., Moser, T., 1999. Organisation and Performance of an International Ringtest for the Validation of the Enchytraeid Reproduction Test. Final Report. German Federal Environmental Agency, Flörsheim am Main, Germany.

Smit, C.E., Van Gestel, C.A.M., 1998. Effects of soil type, percolation, and ageing on bioaccumulation and toxicity of zinc for the springtail *Folsomia candida*. Environ. Toxicol. Chem. 17, 1132–1141.

Sousa, J.P., Loureiro, S., Pieper, S., Frost, M., Kratz, W., Nogueira, A.J.A., Soares, A.M.V.M., 2000. Soil and plant diet exposure routes and toxicokinetics of lindane in a terrestrial isopod. Environ. Toxicol. Chem. 19, 2557–2563.

StatSoft, Inc., 1998. STATISTICA for Windows [version 5.1]. Tulsa, USA.

Van Straalen, N.M., 1996. Critical body concentrations: their use in bioindication. In: Van Straalen, N.M., Krivolutsky, D.A. (Eds.), Bioindicator Systems for Soil Pollution. Kluwer Academic Publishers, The Netherlands, pp. 5–16.

Widianarko, B., Van Straalen, N., 1996. Toxicokinetics-based survival analysis in bioassays using nonpersistent chemicals. Environ. Toxicol. Chem. 15, 402–406.