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Oligochaete bioaccumulation tests with organic compounds

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ZUSAMMENFASSUNG:
Die Bioakkumulation organischer Substanzen in Bodenorganismen wird als Indikator für "secondary poisoning" angesehen. Mit dem Ziel eine neu entwickelte Methode zu standardisieren wurde ein internationaler Ringtest vom deutschen Umweltbundesamt (UBA) organisiert. Als Teil dieses Ringtests wurde Toxikokinetik von Hexachlorbenzol in *Eisenia andrei* und *Enchytraeus albidus*, die in OECD Kunsterde bzw. einem mediterranen Freilandboden exponiert waren, untersucht. Die Ergebnisse zeigen, dass die Bioverfügbarkeit und die Akkumulation der Testsubstanz von Bodeneigenschaften wie der Bodenart und dem Gehalt an organischem Material abhängen. Die Ergebnisse zeigen, dass die neue, in Kürze von der OECD standardisierte Testmethode für die Umweltrisikobewertung von organischen Substanzen gut geeignet ist.

SCHLÜSSELWÖRTER:
Bodenbewertung, Regenwürmer, Enchytraeen, Hexachlorbenzol

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SUMMARY:
Bioaccumulation kinetics of organic compounds has been used as an indicator of secondary poisoning. Aiming to standardize the methods to adopt in this type of studies, an international ring test sponsored by the German Federal Environmental Agency was organised in different laboratories. Included in this ring test, the toxicokinetics of Hexachlorobenzene in *Eisenia andrei* and *Enchytraeus albidus*, when exposed to OECD soil and a natural reference Mediterranean soil, was evaluated. Results showed that the bioavailability and the kinetic parameters of the contaminant is closely related to the soil type and the quality and amount of organic matter. The results indicate that this new test method, which will be standardised by the OECD in 2010, is well suitable for higher tiers of Environmental Risk Assessment (ERA) schemes (e.g. in the context of the registration of pesticides, biocides or veterinary pharmaceuticals).

KEYWORDS:
Soil assessment, earthworms, enchytraeids, Hexachlorobenzene

INTRODUCTION

Legal requirements for soil bioaccumulation tests with organics do exist (e.g. in the context of the registration of pesticides), but they are not very specific. However, in the literature some proposals for testing accumulation of organics have been made, usually focussing on the compost worm *Eisenia fetida / andrei* (e.g. Khalil 1990, Belfroid et al. 1994, 1995). The first standard guideline was published in the USA (Annex 3 of ASTM No. 1676, 2004), but it is very brief. Since available data are hardly comparable there is a clear need for a standardized soil bioaccumulation test. Such a test method will be presented in the following (OECD 2010).

DESCRIPTION OF THE OECD TEST METHOD

First of all, the history of the development of the new method is briefly presented:
1993: Inclusion of soil bioaccumulation in the Test Developmental Program of OECD

1996 - 1998: Performance of a literature review on soil bioaccumulation at ECT (Römbke et al. 1998).
1999 - 2001: Sponsoring of the standardisation of the method by UBA (Germany)
2004 - 2007: Sponsoring of a ring test by UBA (Germany), coordinated by ECT
2007: Final ring test workshop in Coimbra (Portugal), guideline submission to OECD
2010: Adoption of the new test!

Three "key issues" were fixed when developing the test: The most suitable test organisms for soil bioaccumulation tests are earthworms (*E. fetida, E. andrei*) and enchytraeids *(Enchytraeus albidus, E. crypticus)* because of their ecological relevance and for reasons of practicability and, partly, experience (Bruns et al. 2001b). Actually, no alternative is available (e.g. isopods are suitable for litter but not soil studies). For the development and validation of the method Hexachlorobenzene (HCB) was selected as model substance due to its persistence and high log Pow. Finally, OECD (1984) artificial soil or standard field soils (e.g. LUFA 2.2) are appropriate test substrates. The test is divided into two phases, an uptake and an elimination phase, which differ in their duration depending on the test organisms: for earthworms it is 21 days each, for enchytraeids 14 days each.

The concentration in worms at a given time point of the uptake phase is calculated as:
$Ca(t) = (K_s/K_e)*(1-e-K_e*t)$
where Ks is the Uptake rate constant and Ke the elimination rate constant. The bioaccumulation factor at a given time point of the uptake phase is given as:
$BAF(t) = BAF*(1-e-K_e*t)$
Accordingly, the concentration in the worms at a given time point of the elimination phase is calculated as:
$Ca(t) = Ca*e -ke*t$

The test is performed as follows:

Temperature:	18 - 22 °C
Light conditions:	16 : 8 (light : dark) at 100 - 1000 lx
Moisture:	35 – 55% of dry wt.
Feeding:	As in culture; once in each test phase

Details of the earthworm test:
Soil amount / replicate – 50g dw
Nº of organisms / replicate – 1 adult
Validity criterion – mortality ≤ 10%
Details of the enchytraeid test:
Soil amount / replicate – 20g dw
Nº of organisms / replicate – 20 adults
Validity criterion – mortality ≤ 20%

In the following table, the main properties of OECD Artificial Soil and a field soil from the Mediterranean are presented.

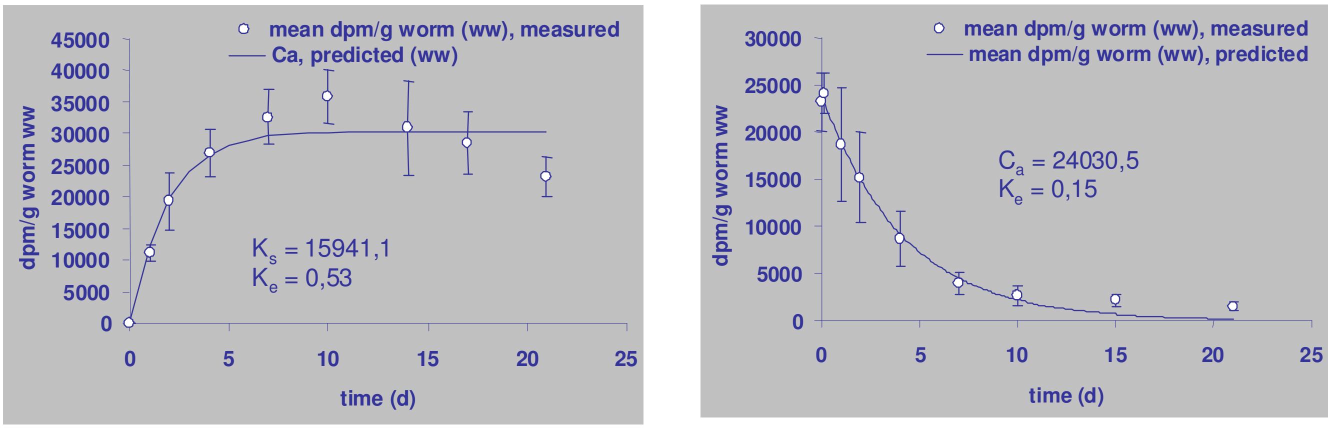
Parameter	Artificial Soil	Mediterranean soils Luv
Source	OECD 207	Chelinho, S. 2006
pH-value	6.0 ± 0.5	4.35
Org. C.	5.6 %	1.2 %
Sand	70 %	68 %

EXAMPLES OF TEST RESULTS

Uptake and elimination of HCB by earthworms and enchytraeids kept in OECD artificial soil and in a Mediterranean field soil (only earthworms) are presented in Figures 1, 2 and 3. In the case of earthworms, bioaccumulation factors (BAFs) were higher than in the natural Mediterranean soil (LUV). However, the kinetics of the HCB followed the same pattern in all soils in both uptake and elimination phases. The uptake of HCB occurred very rapidly, being detected since the first sampling date (after 24h of the beginning of the test).

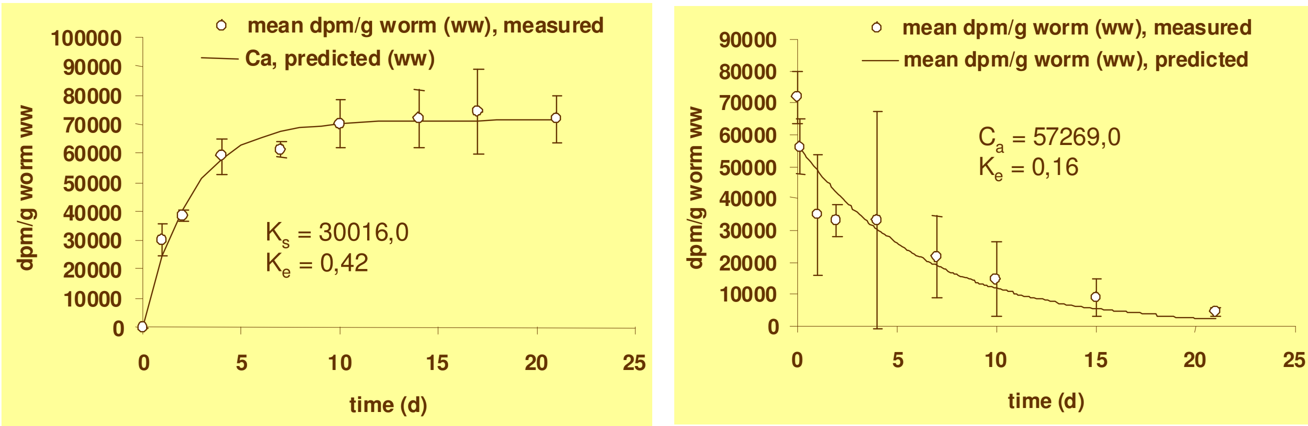
In the case of earthworms, steady state was reached after about ten days of exposure, while in the case of enchytraeids this happened already after about four days. The same difference was found in the elimination phase: after 4 to 5 days the enchytraeids were able to eliminate on average 96% of the accumulated HCB, while the earthworms needed 5 to 10 days for a mean elimination of 94%. Using all results (n = 14) of the ring test, *E. albidus* tended to accumulate more HCB on average than *E. andrei/fetida* (BAFs: 9.5 – 30.0 versus 1.0 – 12.6).

Figure 1: Uptake and elimination of HCB in the earthworm *E. andrei* in OECD artificial soil



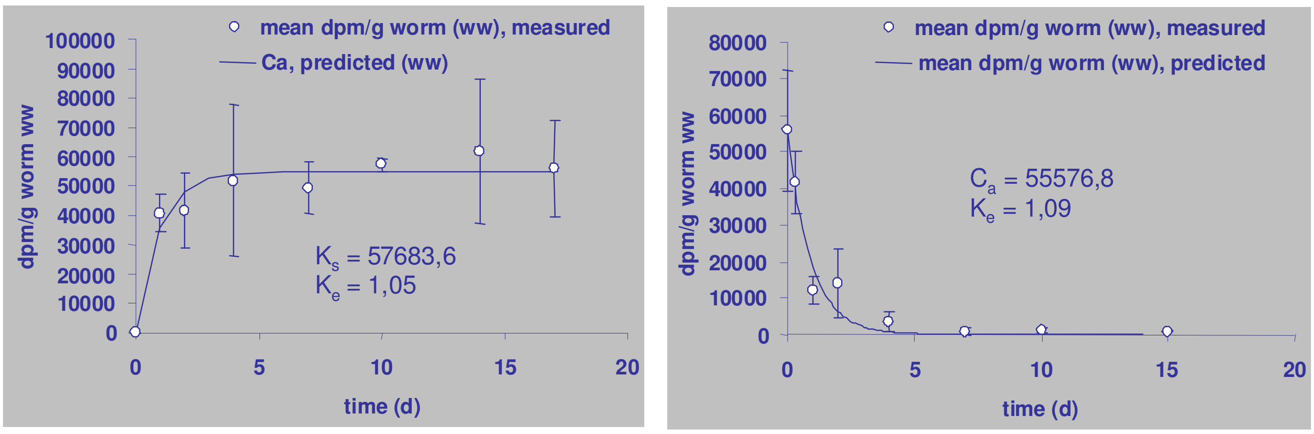
Bioaccumulation factor (BAF): 6.86 (5.9 – 7.8)
Non-eliminated residues (NER): 6.6%

Figure 2: Uptake and elimination of HCB in the earthworm *E. andrei* in LUV field soil



Bioaccumulation factor (BAF): 12.6 (11.9 – 13.3)
Non-eliminated residues (NER): 6.0%

Figure 3: Uptake and elimination of HCB in the enchytraeid *E. albidus* in OECD artificial soil



Bioaccumulation factor (BAF): 9.5 (8.5 – 10.5)
Non-eliminated residues (NER): 1.5%

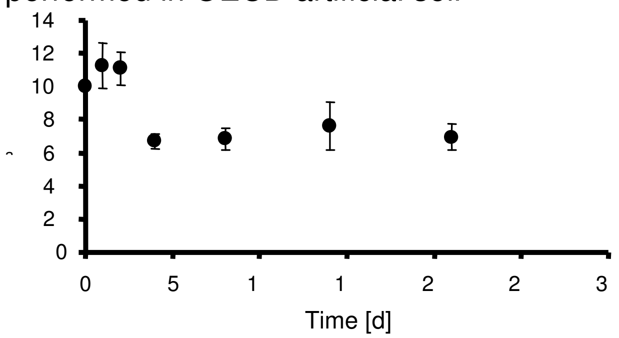
DISCUSSION

Generally speaking, the uptake and the elimination of HCB is much faster in *Enchytraeus albidus* then in *Eisenia andrei.* On average, *E. albidus* tends to bioaccumulate more HCB then *Eisenia andrei/fetida* (internal concentrations and BAF at steady state are higher). In parallel, the non-extractable residues (NERs) at the end of the elimination phase are higher in earthworms (6 – 7% in different soils) than in enchytraeids (about 1.5% in artificial soil). The uptake rate constant (K_s) seems to be strongly influenced by the organic matter content of the soil (higher OM content, lower K_s value). These results point out the importance of considering these kinetic parameters measured in natural soils in order to improve the Environmental Risk Assessment (ERA) of such chemicals.

Finally, an example using the insecticide Lindane is presented which shows the importance of testing different soils, various species (two enchytraeids and two earthworms) and both uptake and elimination phase (Bruns et al. 2001a): Data listed in the following table prove the higher accumulation of small enchytraeid species living in pore water compared to soil-dwelling earthworms. In addition, as also found in the ring test, BAFs are higher in a field soil with low C-content in comparison to OECD artificial soil.

	Lindane BAF	
Species	**OECD soil**	**Lufa 2.2 soil**
E. luxuriosus	12	36
E. albidus	12	22
E. fetida	1.3	3.9
L. rubellus	2.6	3.8

Fig. 4: Elimination of Lindane in a test with the earthworm *Lumbricus rubellus* performed in OECD artificial soil



After a quick elimination of about 50% of the accumulated insecticide, the process stops and no further elimination happens. Obviously, such high NERs indicate much more hazard for earthworm-eating organisms such as mammals or birds as in the case of chemicals like HCB which are quickly eliminated. The new guideline is intended to identify chemicals like Lindane indicating risk of secondary poisoning.

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REFERENCES:

ASTM International (2004): Standard guide for conducting laboratory soil toxicity or bioaccumulation tests with the lumbricid earthworm *Eisenia fetida* and the enchytraeid potworm *Enchytraeus albidus*. ASTM International, E1676-04: 26 pp.

Belfroid, A., Meiling, J., Sijm, D., Hermens, J.; Seinen, W. & Van Gestel, K. (1994): Uptake of hydrophobic halogenated aromatic hydrocarbons from food by earthworms (*Eisenia andrei*). Arch. Environ. Contam. Toxicol. 27: 260-265.

Belfroid, A., Meiling, J., Drenth, H., Hermens, J., Seinen, W. & Van Gestel, K. 1995): Dietary uptake of superlipophilic compounds by earthworms (*E. andrei*). Ecotox. Environ. Safety 31: 185-191.

Bruns, E., Egeler, Ph., Moser, Th., Römbke, J., Scheffczyk, A. & Spörlein, P. (2001a): Standardisierung und Validierung eines Bioakkumulationstests mit terrestrischen Oligochaeten. ECT-Bericht für UBA F & E 298 64 416, 126 + 32 S.

Bruns, E., Egeler, Ph., Römbke, J. Scheffczyk, A., Spörlein, P. (2001b). Bioaccumulation of lindane and hexachlorobenzene by the oligochaetes *Enchytraeus luxuriosus* and *Enchytraeus albidus* (Annelida). Hydrobiologia 463: 185-196.

Chelinho, S. (2006). Selection of Mediterranean reference soils for ecotoxicological testing. 106p. Thesis (Master of Science), University of Coimbra.

Khalil, A. (1990): Aufnahme und Metabolismus von ^{14}C-Hexachlorbenzol und ^{14}C-Pentachlornitrobenzol in Regenwürmern. Diss. Uni. München, 137 S.

OECD (1984): Guideline for the testing of chemicals no. 207; "Earthworm, acute toxicity tests". Organisation for Economic Co-Operation and Development, Paris.

OECD (2010): Guideline for the testing of chemicals (draft); "Bioaccumulation in terrestrial oligochaetes". Organisation for Economic Co-Operation and Development, Paris.

Römbke, J., Egeler, P. & Füll, C. (1998): Literaturstudie über Bioakkumulationstests mit Oligochaeten im terrestrischen Medium. UBA-Texte 28/98, 84 S.