Subject Area 7.2: Evaluations of the impacts and interactions of chemicals in the environment and their fate in biota

Research Article

Importance of Exposure Route for Behavioural Responses in *Lumbriculus variegatus* Müller (Oligochaeta: Lumbriculida) in Short-Term Exposures to Pb*

Almut Gerhardt[1**,2,3]

[1] LimCo International, An der Aa 5, 49477 Ibbenbueren, Germany <http://www.limco-int.com>
[2] University of Aveiro, Dept. of Biology, Aveiro, Portugal
[3] University of Coimbra, Instituto do Ambiente e Vida, Coimbra, Portugal

** **Correspondence address** (AlmutG@web.de)

DOI: http://dx.doi.org/10.1065/espr2006.12.371

Please cite this paper as: Gerhardt A (2007): Importance of Exposure Route for Behavioural Responses in *Lumbriculus variegatus* Müller (Oligochaeta: Lumbriculida) in Short-Term Exposures to Pb. Env Sci Pollut Res 14 (6) 430–434

Abstract

Goal, Scope and Background. *Lumbriculus variegatus* Müller (Oligochaeta), a common freshwater sediment-dweller, has frequently been used in toxicokinetic studies, although has been less used in ecotoxicity tests.

Methods. For the first time the Multispecies Freshwater Biomonitor® (MFB) was applied in a short-term whole-sediment toxicity test. The MFB automatically and quantitatively recorded the spontaneous locomotory behaviour of *Lumbriculus variegatus* in exposures with two compartments, water and sediment. The study questioned, whether the animals altered their locomotion depending on the compartment which was spiked with lead (Pb).

Results and Discussion. As in the exposures to Pb-contaminated water/clean sediment, the animals exposed to Pb-contaminated sediment/clean water showed higher activities in intermediate Pb-concentrations. This indicates, that spontaneous locomotory activity is affected by Pb-concentrations at sublethal levels regardless of whether the Pb-concentration is found in the water or in the sediment, because these animals use both environmental compartments simultaneously. However, within the same Pb-levels, the animals showed higher locomotory activity in contaminated water compared with contaminated sediment. This indicates a possible tendency to withdraw from ('avoidance') contaminated water into the clean sediment compartment, whereas there was no withdrawal from contaminated sediment into clean water. The latter might be explained by the fact that withdrawal from sediment to water might increase the risk of predation and drift in nature, whereas retracting to sediment might provide shelter.

Conclusions. The study showed that spontaneous locomotory responses of *L. variegatus* to Pb depend on whether the water or sediment is contaminated. The study also concluded that the Multispecies Freshwater Biomonitor® can be applied effectively in sediment toxicity testing.

Recommendations and Perspectives. More emphasis should be given to the interactions of water/sediment in sediment ecotoxicity tests to better simulate field conditions and increase ecological realism in risk assessment, especially as quantitative recording methods exisit.

Keywords: Lead; locomotory activity; Multispecies Freshwater Biomonitor®; whole-sediment test

Introduction

Freshwater Oligochaeta have long been used in aquatic biomonitoring, especially in the classification of the trophic state of lakes and large rivers. Most of the research so far has been conducted to indicate general sediment pollution based on saproby, such as the ratio of *Limnodrilus hoffmeisteri* (a species known to be tolerant to organic pollution) to other tubificid worms, or the ratio of tolerant oligochaetes to chironomids (Gerhardt 2002). In the saprobic system *Lumbriculus variegatus* is regarded as an indicator for a-mesosaprob conditions (Gunkel 1994).

Whole-sediment toxicity tests with true in-benthic species are regarded as ecologically relevant, as they address all routes of exposure, and simultaneously represent a time- and cost- effective approach compared to sediment toxicity testing based on pore-water or different types of extraction procedures. The latter methods might affect and alter the bioavailability of contaminants (Ahlf 1995, Riedhammer and Schwarz-Schulz 2001). Recently several sediment contact tests with different species, such as bacteria, yeast, the fish *Danio rerio* and vascular plants have been proposed (Heise & Ahlf 2005, Hollert et al. 2003, Neumann-Hensel & Melbye 2006, Weber et al. 2006, Feiler et al. 2005). Whole-sediment testing started to be frequently used as additional line of evidence in weight-of-evidence studies. In a recent BMBF-joint project a comparison of whole-sediment tests including bioaccumulation in *Lumbriculus variegates* is under investigation (Feiler et al. 2005).

Lumbriculus variegatus (Lumbriculidae), also called blackworm or mudworm, is an ubiquitous sediment dweller, widely spread throughout North America and Europe, preferring shallow habitats at the edges of ponds, lakes, slow flowing habitats or marshes where it feeds on decaying vegetation, microorganisms and sediment (Brinkhurst and Gelder 1991, Penttinen et al. 1996). *Lumbriculus variegatus* inhabits both the sediment and water compartment simultaneously, dwelling with the head first in the sediment and keeping the tail in the water, where respiration and photoreception occurs (Penttinen et al. 1996). In the laboratory no

* **ESS-Submission Editor:** Prof. Dr. Henner Hollert (henner.hollert@bio5.rwth-aachen.de)

sexual reproduction occurs, however asexual autotomy into two or more fragments (morphallaxis) has been observed (Drewes and Brinkhurst 1990). The species is widely used in sediment toxicity testing based on chronic bioaccumulation assays (Ahlf 1995, Dermott and Munawar 1992, Phipps et al. 1993, USEPA 2000, Ingersoll et al. 2003). In particular, long-term bioaccumulation and depuration tests (e.g. Brooke et al. 1996), metabolic rate determination (Penttinen et al. 1996) and toxicokinetic studies (e.g. Schuler et al. 2003) have been performed with freshwater oligochaetes (e.g. Dawson et al. 2003, Ingersoll et al. 2003). However, *Lumbriculus variegatus* may also be suitable for toxicity tests using survival, reproduction, disturbance of normal energy metabolism and behaviour as test parameters (Chapman et al. 1999, Ding et al. 2001, Leppänen et al. 2002, Landrum et al. 2004 a,b). Regeneration behaviour might also be used as a new and sensitive toxicity parameter in *Lumbriculus variegatus*. The use of *Lumbriculus variegatus* could be added to the standard sediment toxicity tests, which up to now have mostly been performed with chironomids (OECD 2002).

Behavioural responses, encompassing the whole-organism level, are non-destructive and sensitive toxicity test parameters (Warner 1967, Lagadic et al. 1994). West and Ankley (1998) recorded avoidance behaviour in *Lumbriculus variegatus* exposed to field-collected sediments with different levels of pollution. Avoidance behaviour is going to be an established toxicity test parameter in earthworm toxicology, as several studies found out that avoidance behaviour is as sensitive as e.g. growth and fecundity(Booth et al. 2005) and offer a quick sensitive screening tool (Schaefer 2003). Moreover, *Lumbriculus variegatus* has been used in behavioural tests that examine rates of feeding and burrowing behaviour, both of which are interdependent in that the rate of burrowing may be a consequence of increased feeding (Landrum et al. 2004a,b). *Lumbriculus variegatus* has proven to be more sensitive to metals than *Tubifex tubifex*, and therefore represents a better choice for assessing sediment toxicity, and in addition *L. variegatus* provides a higher biomass for bioaccumulation studies (Chapman et al. 1999).

Quantitative recording of behavioural responses in the sediment in a fully automated way and on real-time basis has rarely been carried out. The Multispecies Freshwater Biomonitor® (MFB) based on quadropole impedance conversion, a non-optical recording principle (Gerhardt et al. 1994, Gerhardt 1999) is capable of providing this quantitative recording and more recently a number of authors have examined the use of behavioural responses within sediment of various organisms. Gerhardt and Schmidt (2002) applied the MFB with the trichopteran *Hydropsyche* sp., Kirkpatrick et al. 2006a used the marine amphipod *Corophium volutator* under Ammonia-stress.The MFB has been widely used in behavioural studies with several crustaceans (e.g. Gerhardt 1995, Kirkpatrick et al. 2006a, b) and insects (e.g. Gerhardt 1996).

Lead has been chosen as the stressor as it is an ubiquitous toxic and persistent metal, with high affinity to sediment and soil particles, and thus being of special interest for sediment toxicity testing. In addition, particle feeding species are more prone to Pb-stress than fish, and high bioaccumulation rates (> 4000 fold) have been reported in literature (Gerhardt 1993).

This study assesses whether *L. variegatus* shows higher spontaneous locomotory activity in contaminated sediment (escape from toxic sediment into clean water) compared to contaminated water (retracting into clean sediment instead of escaping into contaminated water).

1 Materials and Methods

1.1 Culture of *Lumbriculus variegatus*

Adult worms were kept in three replicates of three separate treatments of sandy sediment (70 g): fine < 1mm, medium 1–2 mm, coarse sand > 2 mm to 6 mm, each with 300 ml of ASTM overlaying water (ASTM 2000) (changed twice per week) and powderized TetraMin as food (5 mg, twice a week) in polyethylene boxes (9.5 x 13.5 x 7 cm). The boxes were aerated by an airstone and closed with a polyethylene lid, they were placed in a climate room, set at 20°C and a photoperiod (30 Watt Neonlights) of 16 h light: 8 h dark with 50% humidity. The sand was pretreated by acid washing using H_2SO_4, (96%) to adjust pH 2 in the wet sediment for a duration of 1 h, and rinsing in tapwater three times, followed by drying (100°C, 24 hrs) and ashing (450°C, 4 hrs). The culture started with 6 adult animals in each treatment and replicate and any asexual reproduction was followed daily over 4 weeks in order to assess the best grain size for optimal growth and health of the test species.

1.2 Short-term exposures to Pb in water or sediment

Pb-acetat-3-hydrat ($(CH_3C00)_2Pb*3H_2O$) was used in the following nominal concentration levels: ASTM (control), 0.1, 1, 5, 10 mg/l dissolved in ASTM water at circumneutral pH, and used for the Pb-water exposures, or spiked to the sand, i.e. added as aqueous solution and stirred for 1 h two days before test start to the sediment and left for evaporation at room temperature. Both experiments (contaminated sediment/clean water and clean sediment/contaminated water) were performed directly after each other. Within each experiment, all Pb- and sediment-type treatments were randomly and sequentially performed.

The adult animals were exposed individually for 24 hrs in test chambers of the MFB, (acryl glass tubes of 7 cm length and 2 cm diameter, filled 50% with sediment (20 g) and 50% with ASTM water): 31 animals were used per sediment treatment (10 per replicate, three replicates per Pb-concentration level) and recorded automatically for four minutes in every ten minutes over 24 hrs. The MFB quantitatively records the changes in impedance, that are caused by an organism moving in the test chamber, which provides an electrical field of high frequency and low voltage alternating current (quadropole impedance technology) (Gerhardt et al. 1994, Gerhardt 1999). The generated electrical signals can be appointed to different types of behaviour, such as locomotion and ventilation, by simultaneous recording and visual observation in a calibration step before the start of the experiments. The spontaneous locomotory activity of *Lumbriculus variegatus* was defined as activity between 0.5 and 2 Hz.

The following treatments were tested: Pb-water/clean sediment for fine, medium, coarse and whole sediment with the above mentioned Pb-concentration, each in 3 replicates. Moreover, Pb-sediment/clean water for fine, medium, coarse and whole sediment with the same Pb-concentrations, each 3 replicates. All Pb-concentrations represented sublethal exposures as determined in parallel experiments, which will be published elsewhere.

1.3 Statistical analyses

Spontaneous locomotory activity was recorded as % time spent on locomotion for each 4 minute trace record and summed up over 24 hrs of exposure. Comparisons among different replicates within a treatment, and among different Pb-concentration levels within one type of sediment were carried out for the exposure in water and sediment with Kruskal Wallis tests followed by Post-hoc Dunn tests. In case of no significant differences among replicates, data were pooled. Pairwise comparisons of the same treatments, e.g. same type of sediment, were carried out for contaminated water versus contaminated sediment with the non-parametric 2-group Sign test (Statistica).

2 Results and Discussion

Lumbriculus variegatus grew tendentially better in fine and medium sediment compared to coarse and whole sediment (**Table 1**).

In general, locomotory behaviour of *Lumbriculus variegatus* showed high variation and decreased towards the end of the experiments (**Tables 2** and **3**), which might be caused by (1) high genetic variation in the animals tested, (2) some animals responding to the stressor and others not. Such a typical dichotomous response has been observed before and might be based on behavioural plasticity (Gerhardt, in press).

The results for the experiment involving clean sediment/Pb-contaminated water were as follows: Whithin each sediment type a comparison of spontaneous locomotory activity in the different Pb-concentration levels was performed, these showed significant concentration-dependent differences in locomotion for fine ($p = 0.001$) and whole sediment ($p = 0.003$) with higher locomotory activity in intermediate Pb-concentrations. In fine sand, there was a higher activity in the 1 and 5 mg/l Pb treatments ($p < 0.05$). In coarse sand a higher activity compared to other treatments was found in 1 mg Pb/l ($p < 0.05$), and in whole sand of all mixed grain sizes a higher activity was found in 0.1 and 1 mg Pb/l. At 10 mg Pb/l locomotory activity was decreased by half and inactivity doubled in alive animals, compared to other treatments. As true sediment organisms, up to 1/3 of the control animals remained inactive, but were alive, in the control, particularly in the coarse and whole sand.

When the animals were exposed to Pb-contaminated sediment and overlaying clean water, significant concentration-dependent differences in spontaneous locomotory activity were seen in all the treatments except for fine sediment (medium: $p = 0.0001$, coarse: $p = 0.03$, whole sand: $p = 0.001$). As in the exposures to Pb-contaminated water, the animals exposed to Pb-contaminated sediment showed higher activities in intermediate Pb-concentrations. This indicates, that

Table 1: Growth of *Lumbriculus variegatus* in sand of different grain size

Sediment	Number of worms		Growth factor
Sand	at start mean (sd)	after 4 weeks mean (sd)	
Fine (< 1 mm)	6 (0)	19 (4)	3.2
Medium (1–2 mm)	6 (0)	19 (5)	3.2
Coarse (2–6 mm)	6 (0)	15 (4)	2.5
Whole (0–6 mm)	6 (0)	16 (2)	2.7

Table 2: Locomotory activity (mean time spent on locomotion (% of 4 Min records, summed up over 24 hrs) and SD: standard deviation) of *Lumbriculus variegatus* over 24 h (R: 3, N: 10) exposed to different types of sediment and Pb-concentrations in water: Zeros: Animals that did not show any spontaneous locomotory activity in the MFB, but were alive

Sediment	Control	0.1 mg/l	1.0 mg/l	5.0 mg/l	10.0 mg/l
Fine sand					
Mean (SD)	475 (526)	158 (216)	1298 (3363)	1086 (2416)	159 (504)
Zeros	4	10	9	2	18
Medium sand					
Mean (SD)	391 (438)	238 (297)	183 (297)	400 (996)	499 (141)
Zeros	7	3	8	4	7
Coarse sand					
Mean (SD)	303 (360)	282 (294)	988 (192)	206 (333)	125 (201)
Zeros	10	3	15	10	11
Whole sand					
Mean (SD)	144 (207)	549 (1845)	546 (2418)	149 (285)	68 (174)
Zeros	10	8	17	8	20

Table 3: Locomotory activity (as defined in Table 2) of *Lumbriculus variegatus* over 24 h (R: 3, N: 10) exposed to different types of sediment and Pb-concentrations spiked in the sediment: Zeros: Animals that did not show any spontaneous locomotory activity in the MFB, but were alive. Nd: not determined

Sediment	Control	0.1 mg/l	1.0 mg/l	5.0 mg/l	10.0 mg/l
Fine sand					
Mean (SD)	102 (246)	436 (1335)	nd	397 (168)	83 (216)
Zeros	16	11		16	17
Medium sand					
Mean (SD)	99 (288)	48 (231)	122 (291)	226 (438)	262 (390)
Zeros	17	20	12	7	7
Coarse sand					
Mean (SD)	132 (288)	203 (957)	623 (1605)	142 (288)	209 (333)
Zeros	13	19	4	16	11
Whole sand					
Mean (SD)	154 (423)	831 (2622)	75 (207)	nd	239 (393)
Zeros	21	3	9		11

spontaneous locomotory activity is dependent on Pb-concentrations at sublethal levels, regardless of whether this concentration is found in the water or the sediment, because usually these animals use both compartments simultaneously.

Comparing spontaneous locomotory activity of the worms in each sediment type considering water versus sediment exposures revealed significant differences for fine ($p = 0.0001$), medium ($p = 0.0001$) and coarse ($p = 0.046$) sand, however not for whole sand, showing that the animals were generally more active when exposed to Pb-contaminated water than when exposed tp Pb-contaminated sediment. This might indicate a possible tendency to withdraw from ('avoidance') contaminated water into the clean sediment compartment, whereas there was no withdrawal from contaminated sediment into clean water. Hence, the shelter of sediment even if contaminated with sublethal Pb-concentrations might be the preferred option compared to increased risk of predation and/or drift in the situation of withdrawing from contaminated sediment into clean water. Both increased or decreased locomotory activity due to toxicant exposure has been observed in the literature e.g. whereas previous studies reported decreased burrowing rates with increased contaminant concentration, supporting the assumption of narcotic effects, increased fluoranthene concentration in the sediment led to increased reworking rate, i.e. feeding at depth and depositing faeces at the surface, of *L. variegatus* after 28 d of exposure (Landrum et al. 2002).

3 Conclusions

The study showed that spontaneous locomotory responses of *L. variegatus* to Pb depend on whether the water or sediment compartment is contaminated. The study also concluded that the Multispecies Freshwater Biomonitor® can be applied effectively in sediment toxicity testing. Quantitatively recorded behavioural parameters in whole sediment toxicity testing might represent a fast and sensitive tool in sediment quality assessment.

Acknowledgements. The study was financed by POCTI/BSE/48131/2002 (FCT, Portugal) and by LimCo International (Germany). Practical help during the experiments was gratefully provided by Anke Pagels. The test species has kindly been provided for starting up our culture by Jussi Kukkonen and Matti Leppänen (Univ. of Joenssuu, Finland). Sharon Stewart and Anita Kirkpatrick (University of Belfast, UK) are kindly acknowledged for linguistic assistance.

References

Ahlf W (1995): Biotests an Sedimenten. In: Steinberg CEW, Calmano W, Klapper H, Wilken, RD (Hrsg), Handbuch Angewandte Limnologie, ecomed Verlag, Landsberg, Lech, V-3.6.1, 44 pp

ASTM (2000): Standard test methods for measuring the toxicity of sediment associated contaminants with freshwater invertebrates. E170695b. Water and Environmental Technology Vol 11.05. American Society for Testing and Materials. West Conshohocken, PA

Booth LH, Heppelthwaite VJ, O'Halloran KO (2005): Effects-based Assay in the earthworm *Aporrectodea caliginosa*. J Soils Sediments 5 (2) 87–94

Brinkhurst RO, Gelder SR (1991): Annelida: Oligochaeta and Branchiobdellida. In: Thorp TH, Covich AP (eds), Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York

Brooke LT, Ankley GT, Call DJ, Cook PM (1996): Gut content weight and clearance rate for three species of freshwater invertebrates. Environ Toxicol Chem 15 (2) 223–228

Chapman K, Benton MJ, Brinkhurst RO, Scheuermann PR (1999): Use of the aquatic oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex* for assessing the toxicity of copper and cadmium in a spiked-artificial-sediment Toxicity test. Environ Toxicol 14, 271–278

Dawson TD, Lott KG, Leonard EN, Mount DR (2003): Time course of metal loss in *Lumbriculus variegatus* following sediment exposure. Short Comm. Environ Toxicol 22 (4) 886–889

Dermott R, Munawar M (1992): A simple and sensitive assay for evaluation of sediment toxicity using *Lumbriculus variegatus* (Muller). Hydrobiologia 235/236, 407–414

Ding J, Drewes, CD, Hsu WH (2001): Behavioural effects of ivermectin in a freshwater oligochaete, *Lumbriculus variegatus*. Environ Toxicol Chem 20 (7) 1584–1590

Drewes CD, Brinkhurst RO (1990): Giant fibers and rapid escape reflexes in newly hatched aquatic oligochaetes, *Lumbriculus variegatus* (Family Lumbriculida). Invertebrate Reproduction and Development 17, 91–95

Feiler U, Ahlf W, Höss S, Hollert H, Neumann-Hensel H, Meller M, Weber J, Heininger P (2005): The SeKT Joint Research Project: definition of Reference Conditions, Control Sediments and Toxicity Thresholds for Limnic Sediment Contac Tests. Env Sci Pollut Res 12 (5) 257–258

Heise S, Ahlf W (2005): A New Microbial Contact Assay for Marine Sediments. J Soils Sediments 5 (1) 9–15

Hollert H, Keiter S, König N, Rudolf M, Ulrich M, Braunbeck T (2003): A New Sediment Contac Assay to Assess Particle-bound Pollutants using Zebrafish (*Danio rerio*) Embryos. J Soils Sediments 3 (3) 197–207

Gerhardt A (1993): Impact of heavy metals on stream invertebrates with special emphasis on acid conditions. Water, Air and Soil Pollution 66, 289–314

Gerhardt A (1995): Monitoring behavioural responses to and effects of metals in *Gammarus pulex* (Crustacea) with impedance conversion. Env Sci Pollut Res 2 (1) 15–23

Gerhardt A (1996): Behavioural early warning responses to polluted surface water: Performance of *G. pulex L.* (Crustacea) and *H. angustipennis* Curtis (Insecta) to a complex industrial effluent. Env Sci Pollut Res 3 (2) 63–70

Gerhardt A (1999): Recent trends in online biomonitoring for water quality control. In: Gerhardt A (ed), Biomonitoring of Polluted Water. Reviews on Actual Topics. Environmental Research Forum 9, 95–118, TTP Switzerland, 301 pp

Gerhardt A (2002): Indicator species in biomonitoring. In: Encyclopedia of Life Support systems (ed: UNESCO), EOLSS Publishers, Oxford, UK <http://www.eolss.net>

Gerhardt A (in press): Aquatic behavioural ecotoxicology – Prospects and limitations. Journal of Human and Ecotoxicological Risk Assessment

Gerhardt A, Clostermann M, Fridlund B, Svensson E (1994): Monitoring of behavioral patterns of aquatic organisms with an impedance conversion technique. Environ Int 20 (2) 209–219

Gerhardt A, Schmidt S (2002): The Multispecies Freshwater Biomonitor as tool for sediment biotests and biomonitoring. J Soils Sediments 2 (2) 67–70

Gunkel G (1994): Bioindikation in aquatischen Ökosystemen. Gustav Fischer Verlag, Jena, Stuttgart, 530 pp

Ingersoll CG, Brunson EL, Wang N, Dwyer FJ, Ankley GT, Mount DR, Huckins JN, Petty J, Landrum PF (2003). Uptake and depuration of nonionic organic contaminants from sediment by the oligochaete, *Lumbriculus variegatus*. Environ Toxicol Chem 22 (3) 872–885

Kirkpatrick AJ, Gerhardt A, Dick JTA, McKenna M, Laming P, Berges JA (2006a): Use of the Multispecies Freshwater Biomonitor to assess behavioural changes in *Corophium volutator* (Pallas, 1766) (Crustacea: Amphipoda) in response to toxicant exposure in sediment. Ecotoxicology, Environmental Safety 64 (3) 298–303

Kirkpatrick AJ, Gerhardt A, Dick JTA, Laming P, Berges JA (2006b): Suitability of *Crangonyx pseudogracilis*(Crustacea: Amphipoda) as an Early Warning Indicator in the Multispecies Freshwater Biomonitor. Env Sci Pollut Res 13 (4) 242–250

Lagadic L, Caquet T, Ramade F (1994): The role of biomarkers in environmental assessment. 5. Invertebrate populations and communities. Ecotoxicology 3, 193–208

Landrum PF, Gedeon ML, Burton GA, Greenberg MS, Rowland CD (2002): Biological responses of *Lumbriculus variegatus* exposed to fluoranthene spiked sediment. Arch Environ Contam Toxicol 42, 292–302

Landrum PF, Leppanen M, Robinson SD, Gossiaux DC, Burton GA, Greenberg MS, Kukkonen JVK, Eadie BJ, Lansing MB (2004a): Effect of 3,4,3',4'-tetrachlorobiphenyl on the reworking behavior of Lumbriculus variegatus exposed to contaminated sediment. Environ Toxicol Chem 23 (1) 178–186

Landrum PF, Leppanen M, Robinson SD, Gossiaux DC, Burton GA, Greenberg M, Kukkonen JVK, Eadie BJ, Lansing MB (2004b): Comparing behavioral and chronic endpoints to evaluate the response of Lumbriculus variegatus to 3,4,3',4',-terachlorobiphenyl sediment exposures. Environ Toxicol Chem 23 (1) 187–194

Leppänen M, Greenberg MSG, Burton Jr A (2002): Assessing the use of *Lumbriculus variegatus* (Oligochaeta) in sediment toxicity testing. 12th SETAC-Europe Annual Meeting, Vienna, 12–16 May 2002, Abstract no. 49–13, p 194

Neumann-Hensel H, Melbye K (2006): Optimisation of the Solid-Contact Test with *Arthrobacter globiformis*. J Soils Sediments 6 (4) 201–207

OECD (2002): Proposal for an OECD guideline for testing of chemicals No. 218: Sediment-water chironomid toxicity test using siked sediment. OECD, Paris, France

Penttinen OP, Kukkonen J, Pellinen J (1996): Preliminary study to compare body residues and sublethal energetic responses in benthic invertebrates exposed to sediment-bound 2,4,5-trichlorophenol. Environ Toxicol Chem 15 (2) 160–166

Phipps GL, Ankley GT, Benoit DA (1993): Use of the aquatic oligochaeta *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. Environ Toxicol Chem 12, 269–279

Riedhammer C, Scharz-Schulz B (2001): The newly proposed EU Risk Assessment Concept for the sediment compartment. J Soils Sediments 1 (2) 105–110

Schaefer M (2003): Behavioural Endpoints in Earthworm Ecotoxicology. J Soils Sediments 3 (2) 79–84

Schuler LJ, Wheeler M, Bailer AJ, Lydy MJ (2003): Toxicokinetics of sediment-sorbed benzo(a)pyrene and Hexachlorobiphenyl using the freshwater invertebrates *Hyalella azteca*, *Chironomus tentans* and *Lumbriculus variegatus*. Environm Toxicol Chem 22 (2) 439–449

US EPA (U.S. Environmental Protection Agency) (2000): Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. 2nd Edition, EPA 600/R-99/064. Office of Health and Environmental Assessment, Washington, DC, USA, March 2000

Warner PH (1967): Bioassays for microchemical environmental contaminants with special reference to water supplies. Bull WHO 36, 181–207

Weber J, Kreutzmann J, Plantikow, Pfitzner S, Claus E, Manz W, Heininger P (2006): A Novel Particle Contact Assay with the Yeast *Sacharomyces cerevisiae* for Ecotoxicological Assessment of Freshwater Sediments. J Soils Sediments 6 (2) 84–91

West CW, Ankley GT (1998): A laboratory assay to assess avoidance of contaminated sediments by the freshwater oligochaete *Lumbriculus variegatus*. Arch Environ Contam Toxicol 35, 20–24

Received: September 20th, 2006
Accepted: December 14th, 2006
OnlineFirst: December 15th, 2006