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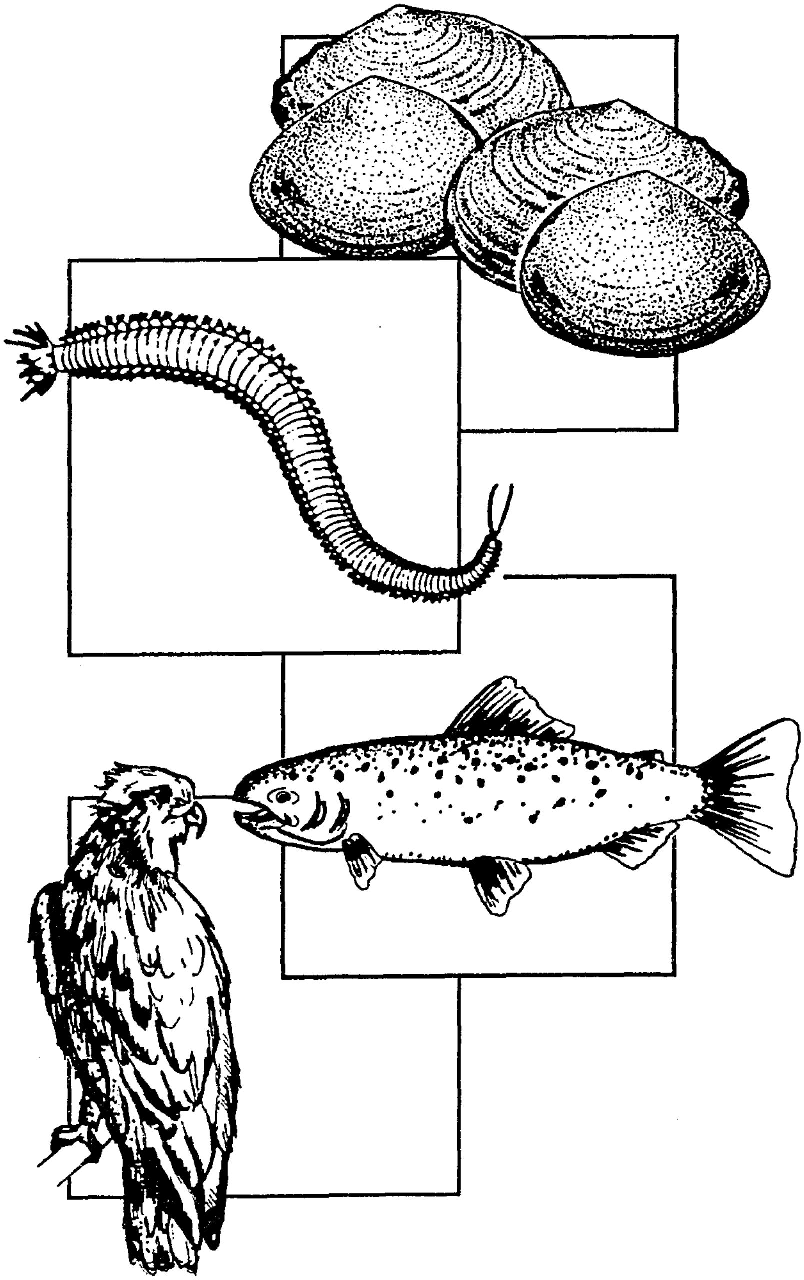

Methods for Assessing Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates

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Over the past 10 years, a variety of methods have been described for evaluating the toxicity of sediment-associated contaminants with freshwater invertebrates (i.e., USEPA, 1994; ASTM, 1997a). However, only a limited number of standard methods are currently available for assessing bioaccumulation of contaminants from field-collected or laboratory-spiked sediments (see page 1-31). Standard guides have recently been published for conducting 28-day bioaccumulation tests with the oligochaete *Lumbriculus variegatus* including determination of bioaccumulation kinetics for different compound classes (USEPA, 1994; ASTM, 1997b). These methods have been applied to a variety of sediments to address issues ranging from site assessments to bioavailability of organic and inorganic contaminants using field-collected and laboratory-spiked samples (Schuytema et al., 1988; Nebeker et al., 1989; Ankley et al., 1991; Call et al., 1991; Carlson et al., 1991; Ankley et al., 1993; Kukkonen and Landrum, 1994; Brunson et al., 1998; see ASTM, 1997b for a listing of these citations). Results of laboratory bioaccumulation studies with *L. variegatus* have been confirmed with comparisons to residues (polychlorinated biphenyls, PCBs; polycyclic aromatic hydrocarbons, PAHs) present from field populations of oligochaetes collected from the same sites as sediments used in the laboratory exposures (Ankley et al., 1992; Brunson et al., 1998). Additional method development is under way to evaluate bioaccumulation kinetics and to provide additional data confirming responses observed in laboratory sediment tests with benthic communities in the field.

Selection of Test Organisms

The choice of a test organism has a major influence on the relevance, success, and interpretation of a test. Various organisms have been suggested for use in studies of chemical bioaccumulation from freshwater sediments (Table 1). The following criteria outlined in Table 1 were used to select *L. variegatus* for bioaccumulation method development by USEPA (1994) and ASTM (1997b): (1) ease of culture and handling, (2) known chemical exposure history, (3) adequate tissue mass for chemical analyses, (4) tolerance to a wide range of sediment physicochemical characteristics, (5) low sensitivity to contaminants associated with sediment, (6) amenability to long-term exposures without feeding, (7) ability to accurately reflect concentrations of contaminants in field-exposed organisms (i.e., exposure is realistic), and (8) data confirming the response of laboratory test organisms with natural benthic populations. Thus far, extensive interlaboratory testing has not been conducted with *L. variegatus*. Other organisms did not meet many of the selection criteria outlined in Table 1, including mollusks (valve closure), midges (short life cycle), mayflies (difficult to culture), amphipods (i.e., *Hyalella azteca*: small tissue mass, too sensitive), cladocerans and fish (not in contact with sediment).

Testing Procedures for *Lumbriculus variegatus*

The 28-day bioaccumulation test with *L. variegatus* described in USEPA (1994) and ASTM (1997b) is conducted with adult oligochaetes at 23°C with a 16L:8D photoperiod at an illuminance of about 500 to 1000 lux. Test chamber size ranges from 4 to 6 L, and the chamber contains 1 to 2 L of sediment and 1 to 4 L of overlying water with five replicates recommended for routine testing. To minimize depletion of sediment contaminants, a ratio of 50:1 total organic carbon in sediment to dry weight of organisms is recommended. A minimum of 1 g (wet weight)/replicate, with up to 5 g/replicate should be tested. Organisms are not fed during a bioaccumulation test (see page 1-36).

Table 1. Selection criteria for sediment bioaccumulation test organisms (EPA, 1994; ASTM, 1997b; Ingersoll et al., 1995). A "+" or "-" rating indicates a positive or negative attribute; "NA" is not applicable; and "?" is unknown.

Criterion	*Lumbriculus variegatus*	Mollusks	Midges	Mayflies	Amphipods	Cladocerans	Fish
Laboratory culture	+	-	+	-	+	+	+
Known chemical exposure	+	-	+	+/-	+	+	+
Adequate tissue mass	+/-	+	-	+	-	-	+
Low sensitivity to contaminants	+	+	-	-	-	-	+/-
Feeding not required during testing	+	+	-	+	-	-	+
Realistic exposure	+	+/-	+	+	+	-	-
Sediment physico-chemical tolerance	+	?	+/-	-	+	NA	NA
Response confirmed with benthic populations	+	?	?	?	+	?	-

If sediments could be toxic to *L. variegatus*, a 4-day toxicity screening test should be conducted before starting a bioaccumulation test (ASTM, 1997b). Endpoints monitored in the toxicity test are survival and behavior. Test organisms should burrow into test sediment because avoidance of test sediment by *L. variegatus* may reduce bioaccumulation. Survival of *L. variegatus* in the toxicity screening test should not be significantly reduced in the test sediment relative to a control sediment. Additional requirements for test acceptability are outlined in USEPA (1994) and ASTM (1997b).

At the end of the bioaccumulation test, live oligochaetes are transferred to a 1-L beaker containing overlying water without sediment for 24 hours to eliminate gut contents (oligochaetes clear more than 90 percent of the gut contents in 24 hours). A correction for the extent of elimination from the body burden may need to be made for compounds with log K_{ow} less than 5. Oligochaetes are not placed in clean sediment to eliminate gut contents because clean sediment can contribute 15 to 20 percent to the dry weight of the oligochaetes, resulting in a dilution of contaminant concentrations on a dry weight basis. Minimum tissue mass required for various analyses at selected lower limits of detection are listed in USEPA (1994) and ASTM (1997b). Depending on study objectives, total lipids can be measured on a subsample of the total tissue mass of each replicate sample. Dry weight of oligochaetes can be determined on a separate subsample from each replicate.

Because bioaccumulation tests are often used in ecological or human health risk assessments, the procedures are designed to generate estimates of steady-state tissue residues. Eighty percent of steady state is used as the general goal for a test (ASTM, 1997b). An option when conducting a bioaccumulation test is to perform a kinetic study to estimate steady-state concentrations instead of conducting a 28-day bioaccumulation test (e.g., sample on Days 1, 3, 7, 14, 28). A kinetic test can be used when 80 percent of steady state will not be obtained within 28 days or when more precise estimates of steady-state tissue residues are required (see page 1-37).

Case Studies

Methods for conducting bioaccumulation tests with *L. variegatus* have varied slightly over the years; however, test conditions (e.g., test length, exposure systems) have been consistent enough for evaluation of the robustness of the guidance outlined in USEPA (1994) and ASTM (1997b). In a study with sediments from the lower Fox River in Green Bay, Wisconsin, Ankley et al. (1992) compared the bioaccumulation of PCBs by *L. variegatus* exposed in the laboratory to PCB residues in collections of oligochaetes from the field. Good agreement was observed between PCB concentrations in the laboratory and field organisms, particularly for those congeners with K_{ow} values <7 (see Figure 1). This indicates that for super-hydrophobic chemicals, laboratory exposures longer than 28 days may be required to reach equilibrium.

Good agreement was also observed in bioaccumulation between *L. variegatus* exposed in the laboratory for 28 days and field-collected oligochaetes from sediments collected from the upper Mississippi River (Brunson et al., 1998). About 90 percent of the corresponding concentrations of PAHs were within a factor of 3 between the laboratory-exposed and field-collected oligochaetes (see Figure 1). Concentrations that differed by more than a factor of 3 included

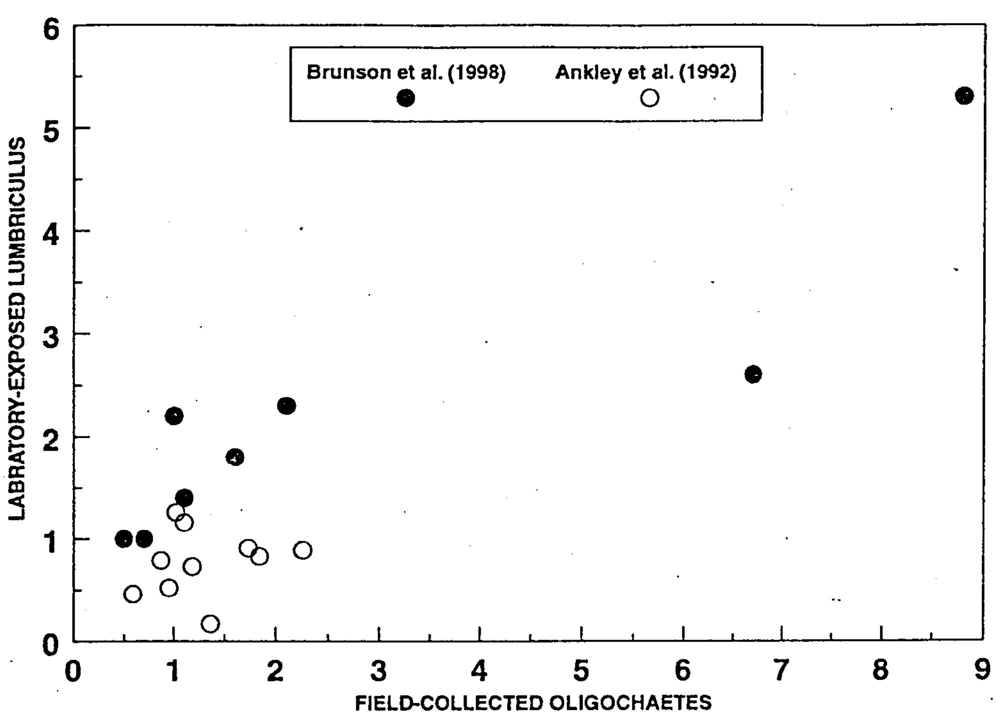

Figure 1. Biota-sediment accumulation factors (BSAFs) for laboratory-exposed *Lumbriculus variegatus* and field-collected oligochaetes for PAHs (Brunson et al., 1998) and PCB homologs (Ankley et al., 1992).

naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, 2,6-dimethylnaphthalene, 1,6,7-trimethylnaphthalene, phenanthrene, 1-methylphenanthrene, and benz(a)anthracene. Tissue concentrations of naphthalenes were generally higher in field-collected oligochaetes relative to laboratory-exposed oligochaetes (naphthalenes are low molecular weight (LMW) PAHs with log K_{ow} values less than 4.5). Compounds with similar concentrations in both the laboratory-exposed and field-collected oligochaetes included a similar number of high molecular weight (HMW) and LMW PAHs. These compounds included biphenyl, fluorene, 1-methylphenanthrene, pyrene, fluoranthene, chrysene, and benzo(e)pyrene. Most of these compounds are intermediate in molecular weight and log K_{ow} (except for benzo(e)pyrene, which has the highest molecular weight and log K_{ow} compared to these other compounds). Compounds with concentrations typically higher in the laboratory-exposed oligochaetes compared to field-collected oligochaetes were primarily HMW PAHs. These compounds included phenanthrene, benz(a)anthracene, benzo(b,k)fluoranthene, and perylene (with log K_{ow} greater than 4.5).

Differences between tissue concentrations in the laboratory-exposed and field-collected oligochaetes may be the result of differential exposure, including the following factors: (1) LMW PAHs may be lost during the sampling of sediments from the field; (2) spatial heterogeneity of contaminants in the field may have resulted in differential accumulation; (3) the route of exposure for oligochaetes in the field is through sediment, food, and overlying water, while the primary route of exposure to oligochaetes in the laboratory is sediment; and (4) species-specific differences in exposure exist between *L. variegatus* and the native oligochaetes.

Concentrations of DDT reached 90 percent of steady state by Day 14 of a 56-day test with *L. variegatus* exposed to field-collected sediments (unpublished data). However, LMW PAHs (i.e., acenaphthylene, fluorene, phenanthrene) generally peaked by Day 3 and tended to decline to Day 56. Concentrations of HMW PAHs (i.e., benzo (b)fluoranthene, benzo(e)pyrene, indeno(1,2,3-c,d)pyrene) typically either peaked by Day 28 or continued to increase during the 56-day exposure. Bioaccumulation of contaminants by indigenous oligochaetes that were recovered on Day 28 from the same chamber with introduced *L. variegatus* were also evaluated. Peak concentrations of select PAHs and DDT were similar in the indigenous oligochaetes and in *L. variegatus* exposed in the same chamber (unpublished data). Bioaccumulation of metals from sediments has also been evaluated using *L. variegatus*. Ankley et al. (1991) reported elevated concentrations of Cd and Ni in worms after 10-day exposures to field-collected sediments where the metal (Cd + Ni):acid-volatile sulfide ratio exceeded 1, but not in samples where the ratio was <1. Ankley et al. (1994) also found that worms did not bioaccumulate metals from three sediments containing elevated concentrations of Cd, Ni, Zn, Cu and Pb, when there was sufficient acid-volatile sulfide to complex metals.

Biota-Sediment Accumulation Factors

Biota-sediment accumulation factors (BSAFs) were calculated for *L. variegatus* by dividing the lipid-normalized tissue concentrations by the organic carbon-normalized sediment concentrations (Table 2; Brunson et al., 1998). For laboratory-exposed oligochaetes, mean BSAFs ranged from 1.1 for benz(a)anthracene to 5.3 for naphthalene. For field-collected oligochaetes, mean BSAFs ranged from 0.5 for benz(a)anthracene to 8.8 for naphthalene. For individual samples, BSAFs for naphthalene ranged from 1.6 to 10.1 in laboratory-exposed oligochaetes and

Table 2. Mean biota-sediment accumulation factors (range in parentheses) reported by Lee (1992) and by Brunson et al. (1998). NR is not reported.

Compound	Lee (1992)	Brunson et al. (1998) Lab-exposed oligochaetes	Brunson et al. (1998) Field-collected oligochaetes
Naphthalene	NR	5.3 (1.6-10.1)	8.8 (2.5-26.6)
2-methyl naphthalene	NR	2.6 (0.9-5.1)	6.7 (2.2-12.2)
Pyrene	0.4 (0.18-0.5)	2.3 (0.8-3.9)	2.2 (0.7-5.6)
Fluoranthene	NR	1.8 (0.9-3.9)	1.6 (0.6-4.9)
Chrysene	NR	1.5 (0.7-2.4)	1.1 (0.3-2.0)
Benz(a)anthracene	0.4 (0.2-0.6)	1.1 (0.4-2.5)	0.5 (0.4-0.7)
Benzo(b,k)fluoranthene	0.4 (0.2-1.0)	NR	NR
Perylene	NR	2.24 (0.5-4.7)	1.02 (0.3-1.9)

2.5 to 26.6 in field-collected oligochaetes. The BSAFs for pyrene, benz(a)anthracene, and benzo(b,k)fluoranthene were typically greater that BSAFs reported for marine organisms in Lee (1992) for these compounds (Table 2). BSAFs were also calculated using PCB homolog data reported in Ankley et al. (1992) for laboratory-exposed *L. variegatus* and field-collected oligochaetes (Figure 1). BSAFs were similar between laboratory-exposed and field-collected oligochaetes in both Ankley et al. (1992) and Brunson et al. (1998); however, BSAFs reported in Brunson et al. (1998) were typically greater (0.5 to 8.8) than BSAFs from Ankley et al. (1992; 0.17 to 2.26; Figure 1).

A theoretical value of 1.7 for BSAFs has been estimated based on partitioning of nonionic organic compounds between sediment carbon and tissue lipids (ASTM, 1997b). A BSAF of less than 1.7 indicates less partitioning into lipids than predicted, and a value greater than 1.7 indicates more uptake than can be explained by partitioning theory alone (Lee, 1992). The majority of the BSAFs in Figure 1 and Table 2 were within a range of about 0.5 to 2.6, suggesting the theoretical BSAF value of 1.7 could be used to predict these mean BSAFs with a fair amount of certainty. However, mean BSAFs for naphthalene (8.8) and 2-methyl naphthalene (6.7) in the field-collected oligochaetes were elevated relative to a theoretical BSAF of 1.7 (Table 2), with BSAFs for individual samples as high as 10.1 for laboratory-exposed oligochaetes and 26.6 for field-collected oligochaetes. The higher BSAFs in the field-collected oligochaetes may be the result of (1) exposure to contaminants in the overlying water; (2) spatial differences in sediment contamination (i.e., sediments were not sampled from a depth representative of the habitat of the oligochaetes); or (3) taxon-specific differences in exposure. BSAFs substantially different from the theoretical value of 1.7 may also result from the system not being at equilibrium (i.e., depletion or release of contaminants in pore water).

In summary, procedures for evaluating the bioaccumulation of contaminants associated with freshwater sediment using the oligochaete *L. variegatus* have been well described. Results of laboratory studies using these procedures are generally similar to the bioaccumulation of contaminants exhibited by oligochaetes in the field. Ongoing research includes further evaluations of bioaccumulation kinetics and field validation of laboratory bioaccumulation methods, use of formulated sediments and sediment spiking, and standardization of micro-lipid analytical methods.

References

Ankley G.T., G.L. Phipps, E.N. Leonard, D.A. Benoit, V.R. Mattson, P.A. Kosian, A.M. Cotter, J.R. Dierkes, D.J. Hansen, and J.D. Mahony. 1991. Acid-volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediment. *Environ. Toxicol. Chem.* 10:1299-1307.

Ankley, G.T., P.M. Cook, A.R. Carlson, D.J. Call, J.A. Swenson, H.F. Corcoran, and R.A. Hoke. 1992. Bioaccumulation of PCBs from sediments by oligochaetes and fishes: Comparison of laboratory and field studies. *Can. J. Fish. Aquat. Sci.* 49:2080-2085.

Ankley, G.T., E.N. Leonard, and V.R. Mattson. 1994. Prediction of bioaccumulation of metals from contaminated sediments by the oligochaete *Lumbriculus variegatus*. *Water Res.* 28:1071-1076.

ASTM. 1997a. Standard test methods for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. E1706-95b. In *ASTM annual book of standards*, Vol. 11.05, American Society for Testing and Materials, Philadelphia, PA, pp. 1138-1220.

ASTM. 1997b. Standard guide for determination of bioaccumulation of sediment-associated contaminants by benthic invertebrates. E1688-97a. In *ASTM annual book of standards*, Vol. 11.05, American Society for Testing and Materials, Philadelphia, PA, pp. 1072-1121.

Brunson, E.L., T.J. Canfield, F.J. Dwyer, N.E. Kemble, and C.G. Ingersoll. 1998. An evaluation of bioaccumulation with sediments from the upper Mississippi River using field-collected oligochaetes and laboratory-exposed *Lumbriculus variegatus*. *Arch. Environ. Contam. Toxicol.* In press.

Ingersoll, C.G., G.T. Ankley, D.A. Benoit, G.A. Burton, F.J. Dwyer, I.E. Greer, T.J. Norberg-King, and P.V. Winger. 1995. Toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates: A review of methods and applications. *Environ. Toxicol. Chem.* 14: 1885-1894.

Lee, H. II. 1992. Models, muddles and mud. In *Sediment toxicity assessment*, ed. G.A. Burton, Lewis Publishers, pp. 267-293. Chelsea, Michigan.

USEPA. 1994. *Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates*. EPA 600/R-94/024. U.S. Environmental Protection Agency, Duluth, MN.

USEPA/USDOI. 1997. *An assessment of sediments from the upper Mississippi River*. Final report. EPA 823-R-97-005. U.S. Environmental Protection Agency, Office of Water, Washington, DC.