Methods for Assessing Sediment Bioaccumulation in Marine/ Estuarine Benthic Organisms

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Introduction

Organic pollutants can be divided into two general classes (see page 1-15). The first consists of compounds with high water solubility and low K_{ow} values, such as acetone, that do not tend to adsorb to particulates. Their major reservoir is the water column, where they are accumulated through bioconcentration. The second suite of organic pollutants are the low water solubility, high K_{ow} compounds, such as DDT, dieldrin, and polychlorinated biphenyls (PCBs), that readily partition to particulates. They accumulate in the sediment where they can be bioaccumulated by benthic organisms. From the benthos, these high K_{ow} compounds can be introduced into higher trophic levels through trophic transfer. Other presentations at this conference are addressing the trophic transport and the effects of these pollutants. This presentation will focus on methods to measure/predict the bioavailability of sediment-associated contaminants to marine/estuarine benthos, and the use of sediment bioaccumulation tests in particular.

When designing any test method, it is critical to assess how are the data are going to be used. Tissue residue data can be used in risk assessments in a number of ways, which are summarized in the figure on page 1-16. The column labeled "Steady-State?" refers to whether estimates of steady-state tissue residues are required to adequately address the particular component of a risk assessment. For the sediment bioaccumulation tests, the need for steady-state data defines how long the test needs to be conducted to assure that steady-state tissue residues have been approached. The column labeled "Max Residue?" refers to whether an upper limit estimate of the tissue residue is required to address the particular risk assessment component. That is, is it necessary to use a duration, species, or test method that tends to maximize uptake?

As suggested in the figure on page 1-16, residues approaching steady-state are required for quantitative ecological or human health risk assessments other than hazard identification and identifying specific uptake routes. Tissue residues substantially less than steady-state residues will underestimate both exposure and effects, and this error will propagate through the risk assessment (e.g., estimate of trophic transport). The need for the more stringent requirement of an upper-limit estimate of tissue residue depends upon the specific question. For example, amphipods metabolize PAHs to a greater extent than most bivalves. If the goal is to assess PAH exposure to amphipods, an amphipod would be a suitable test species. However, if the goal is to extrapolate PAH bioavailability to other species, an upper limit estimate would be more appropriate, and a bivalve species should be used.

Bioaccumulation Methods

There are a suite of methods available to assess or to predict bioaccumulation that will be discussed in various presentations during the conference (see listing on page 1-16). Two approaches provide direct measures of existing conditions: the field approach and the bioaccumulation test. Both approaches involve measuring tissue residues in either field-collected or laboratory-exposed organisms. These direct approaches have high ecological relevance but can be costly, and they have limited ability to predict tissue residues resulting from changes in sediment contamination (e.g., after a clean-up).

Sediment bioaccumulation models can serve as cost-effective "screens" to determine when direct measurements are required and as a method to predict tissue residues when direct measurements are not practical. The two general types of sediment bioaccumulation models are equilibrium-based and kinetic approaches (see Landrum et al., 1992). The equilibrium-based approaches assume steady-state conditions between the organism and the environment, whereas the kinetic approaches describe bioaccumulation as the net effect of rate processes. The two equilibrium models are bioaccumulation factors (BAF = tissue residue/sediment concentration) and the equilibrium partitioning model. The two basic types of

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kinetic approaches are kinetic process models and bioenergetically based toxicokinetic models. Kinetic models can be more accurate than the steady-state models, but they require extensive data. A decision tree has been developed to guide the risk assessor in choosing which tests or models should be used to assess or predict bioaccumulation given the project goals (Boese and Lee, 1992) (see figure on page 1-17).

Equilibrium Partitioning Bioaccumulation Model

The equilibrium partitioning model is based on the theory that neutral organic pollutants partition between the lipid phase in the organism, sediment carbon, and interstitial water until equilibrium is obtained (see page 1-18). Assuming that organic carbon is the only sink for neutral organics in the sediment and that lipids are the only sink in the organism, the model becomes:

Ctss/L = (Cs/TOC) * BSAF

where:

Ctss = tissue concentration at steady-state (µg/g)
L = lipid content (g/g)
Cs = sediment concentration (µg/g)
TOC = total organic carbon in sediment (g/g)
BSAF = biota-sediment accumulation factor (g carbon/g lipid)

The biota-sediment accumulation factor (BSAF) has also been referred to as the "accumulation factor" (AF). BSAFs can be determined empirically for each pollutant from laboratory exposures or field surveys. If partitioning is not a function of lipid or carbon type, the value of the BSAF for a compound should not vary among sediments or species. However, data from bioaccumulation tests indicate that differences in the sediment can affect the BSAF values. Tests were run with spiked sediments using the deposit-feeding clam *Macoma nasuta*, one of the bioassay animals for marine and estuarine systems. Two sediment types were spiked with 13 PCB congeners and hexachlorobenzene (HCB) at concentrations of about 50 parts per billion for each congener or compound. Test results show differences in the BSAFs for the two sediment types (see page 1-18), so the equilibrium model did not completely account for sediment differences. The results also show dramatic differences in BSAF values among congeners. A possible kinetic explanation for these results are that PCB congeners with lower K_{ow} values undergo rapid degradation whereas PCB congeners with higher K_{ow} values have limitations moving across membrane surfaces which can result in low uptake. Nor does the equilibrium partitioning bioaccumulation model totally account for species differences. In a study of a DDT-contaminated site, we found that field-collected *Macoma nasuta* had tissue residues of total-DDT and dieldrin 7 to 9 times higher than filter-feeding bivalves (Lee et al., l994, see Figure 15, page 1-22). It seems, then, that there is about a 2- to 10-fold uncertainty in BSAF values. Even with these uncertainties, BSAFs have utility as a screening tool and in extrapolating among species or sediments. Additionally, this uncertainty can be reduced by extrapolating among similar feeding and sediment types.

Sediment Bioaccumulation Test

A laboratory test is often the preferred method to evaluate a specific sediment and to generate BSAFs under controlled conditions. Although these tests had been conducted for over a decade, there was no standardized methodology. Scientists at the U.S. Environmental Protection Agency (EPA) in Newport, Oregon developed a sediment bioaccumulation test for marine and estuarine systems, published it in a guidance manual in 1989, and revised it in 1993 (Lee et al., 1993, see page 1-19). Since then, the EPA scientists have worked with Peter Landrum of NOAA to develop a guide for sediment bioaccumulation tests for marine and freshwater benthic invertebrates (ASTM, 1995). The bioaccumulation test includes six key procedures (see page 1-19): (1) 28-day exposure duration, (2) use of sediment-ingesting organisms, (3) no supplemental food added, (4) independent exposure of species, (5) recommended accuracy of 80 percent of steady-state tissue residues, and (6) use of long-term tests or toxicokinetic approaches for greater than 80 percent accuracy or for slowly accumulated compounds. Information used to support the recommended procedures is discussed below.

The recommendation for testing 28 days was based on a literature review of percentage of steady-state residue levels achieved in 10 days (the period then used for testing dredged materials) and 28 days. Results were available for a variety of compounds such as PCBs, dioxins, furans, PAHs (or PNAs), and metals (see page 1-20). These compounds generally achieved 80 percent of steady-state tissue residues within 28 days. We evaluated the adequacy of the 28-day duration in the experiment exposing *Macoma nasuta* to the sediments spiked with 13 PCBs and HCB (see discussion under Equilibrium Partitioning Bioaccumulation Model). The experiment was run for 120 days. The figure on page 1-20 shows the results for PCB congeners 153 and 209. Though bioaccumulated to different amounts, both PCB congeners approached or exceeded 80 percent of steady-state residue after 28 days, as did the other congeners.

Data from other studies indicate that a period of 28 days can be insufficient for bioaccumulation testing. The United Heckathorn Superfund site in San Francisco Bay is highly contaminated with DDT and dieldrin. A bioaccumulation test was conducted with *Macoma nasuta* for 90 days using sediment from the most contaminated site. Test results for total DDT (DDT, DDE, and DDD) are displayed on page 1-21. At 28 days, the tissue residues only reached about one-third of the steady-state residues. The graphs of the tissue residues for the three most abundant compounds show even worse results (see page 1-21).

These results raise the question of whether DDT and its metabolites are different than PCBs or whether the difference is due to field versus spiked sediments. It also raises the question about the adequacy of the 28-day test. There are practical limitations to consider in setting the length of any laboratory test. A proposal to resolve this problem is to maintain 28 days as the standard duration, but to multiply the 28-day residues by an "adjustment factor" which is the ratio of the steady-state residue to the 28-day residue. Adjustment factors would be developed through long-term lab studies. For the DDT compounds and dieldrin, these adjustment factors ranged from 1.7 to 10.8, with a value of 2.9 for total DDT. For compounds which accumulate rapidly, such as the low molecular weight polycyclic aromatic hydrocarbons (PAHs), the adjustment factor should approach 1.

Several criteria for organism selection for use in marine/estuarine bioaccumulation tests are listed on page 1-22. Of these, the requirement for using sediment-ingesting organisms to maximize uptake of sediment-associated contaminants is critical. The figure on page 1-15 (modified from Landrum, 1989) helps illustrate why. An organism can accumulate sediment-associated contaminants from the interstitial water or from ingested particles. Compounds with higher K_{ow} values will be associated mainly with the particulate phase, so particle ingestion will be the primary uptake route for these chemicals. A series of experiments conducted with hexachlorobenzene, a low solubility compound, showed that at least 70 percent of the uptake in *Macoma nasuta* was from ingested particles. Although it can be argued that if all the phases are in equilibrium the uptake phase does not matter, the 7- to 9-fold higher residues in the sediment-ingesting *Macoma nasuta* compared to filter-feeding bivalves at the United Heckathorn site (see page 1-22) clearly demonstrates the importance of sediment ingestion on bioaccumulation. Based on similar reasoning, supplemental feeding is not recommended as the addition of uncontaminated food could "short-circuit" the solid-phase uptake route and result in an erroneously low evaluation of bioavailability. The marine/estuarine environment contains a number of deposit-feeding animals (various bivalves and polychaetes) that meet the criteria, particularly with respect to providing sufficient biomass for chemical analysis at the end of the test. The 1993 guidance manual and the ASTM guide identify animals suitable for bioaccumulation testing in marine and estuarine systems.

With any laboratory test there is the question of whether it accurately predicts tissue residues in field organisms. At the United Heckathorn Superfund study site, we were able to compare tissue residues in laboratory-exposed *Macoma nasuta* and *Macoma* collected at several of the field sites. As mentioned above, the 28-day exposure underestimated steady-state of DDT and dieldrin, so it was necessary to use the adjustment factors from the 90-day test at station 1 to correct the 28-day residues from the other five sites. After adjustment, ratios of laboratory to field tissue residues for total DDT and dieldrin at each station (see graph on page 1-23) ranged from about 0.5 to 3. At least for this suite of high K_{ow} neutral organics, the standard test appears to predict field residues within 2- to 3-fold.

Research Needs

Areas requiring further study to advance the science related to sediment bioaccumulation assessment are summarized on page 1-23. They include: (1) interlaboratory round-robin testing; (2) field validation of the bioaccumulation test, particularly for PAHs and metals; (3) identification of local test species, especially for subtropical, subarctic, and oligohaline habitats; (4) "standardization" of lipid methods for derivation of BSAFs; (5) evaluation of effects of sediment storage and spiking on bioavailability; (6) refinement of the experimental design, including criteria for controls and references; and (7) evaluation of kinetic and physiological-based alternatives to the 28-day bioaccumulation test. Of these, perhaps the most troubling question is whether there is slower uptake or a lower bioavailability with field-contaminated sediments compared to spiked sediments, as suggested by the slow uptake rates for DDT compared to the rates from the spiked PCBs. Enhanced bioavailability of spiked sediment could potentially result in erroneously high BSAFs and toxicity.

The above research needs address how to conduct tests and their accuracy and precision. The overriding question, however, is: "What is the ecological significance of tissue residues?" This needs to be addressed at several scales. At the scale of individual benthos, the question is, "What are the effects of the accumulated toxicants on growth, fecundity, and survival?" Use of critical body residues, as are being developed for neutral narcotics, is a promising approach. However, in nearly all cases, the goal of marine/estuarine ecological risk management is to protect a resource or higher levels of biological organization (e.g., "ecological integrity"). To achieve this goal, we will need to develop the insights and methods to translate toxic effects on individuals into effects on populations and communities. Addressing these higher levels of biological organization will require assessments at larger spatial scales than the classical "end-of-the-pipe" evaluations, and will often require evaluation of contaminated sediment effects in the context of multiple stressors. This will be a critical challenge for the future.

References

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Boese, B., and H. Lee II. 1992. *Synthesis of methods to predict bioaccumulation of sediment pollutants.* ERLN N232. U.S. Environmental Protection Agency, Newport, OR. 87 pp.

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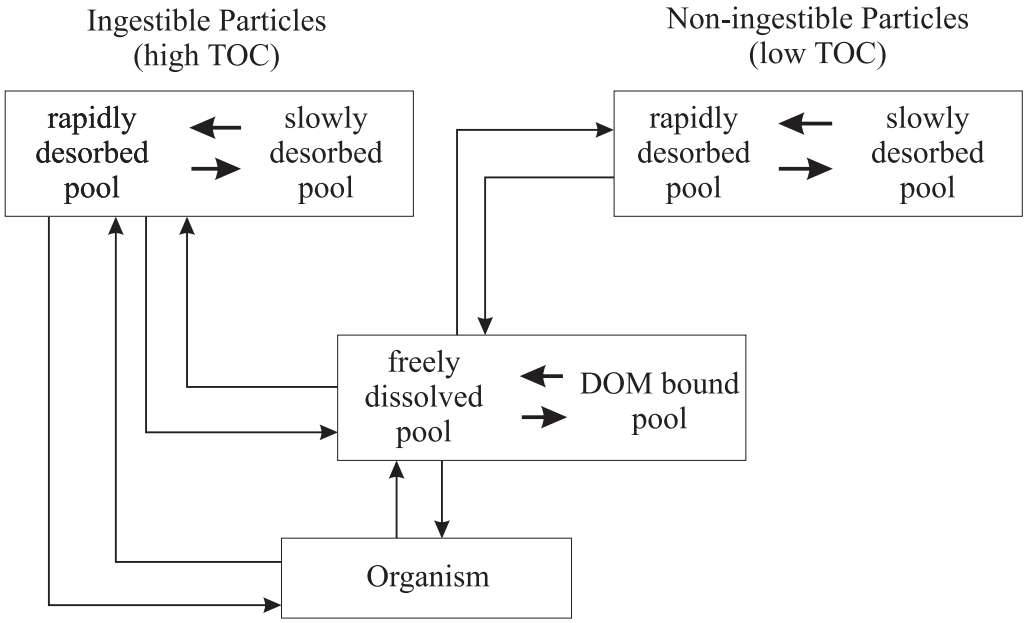
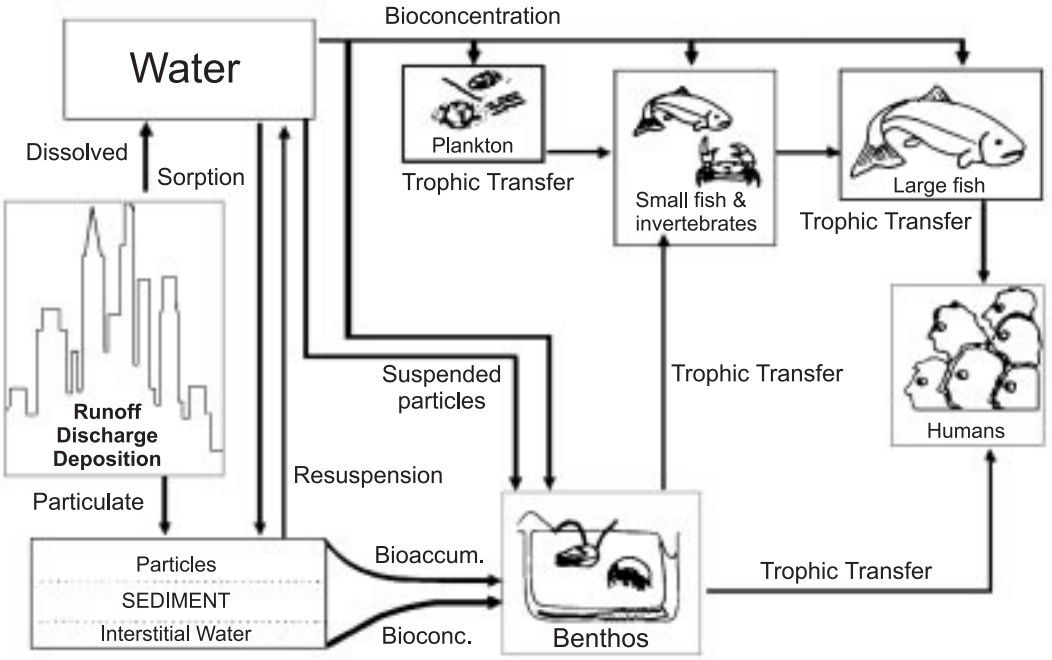
BIOAVAILABILITY OF SEDIMENT-ASSOCIATED POLLUTANTS
AQUEOUS AND PARTICULATE POOLS



Idealized Pollutant Pathways in Marine Ecosystems



-- = High water solubility, low Kow, rapidly metabolized
-- = Low water solubility, high Kow, slowly metabolized

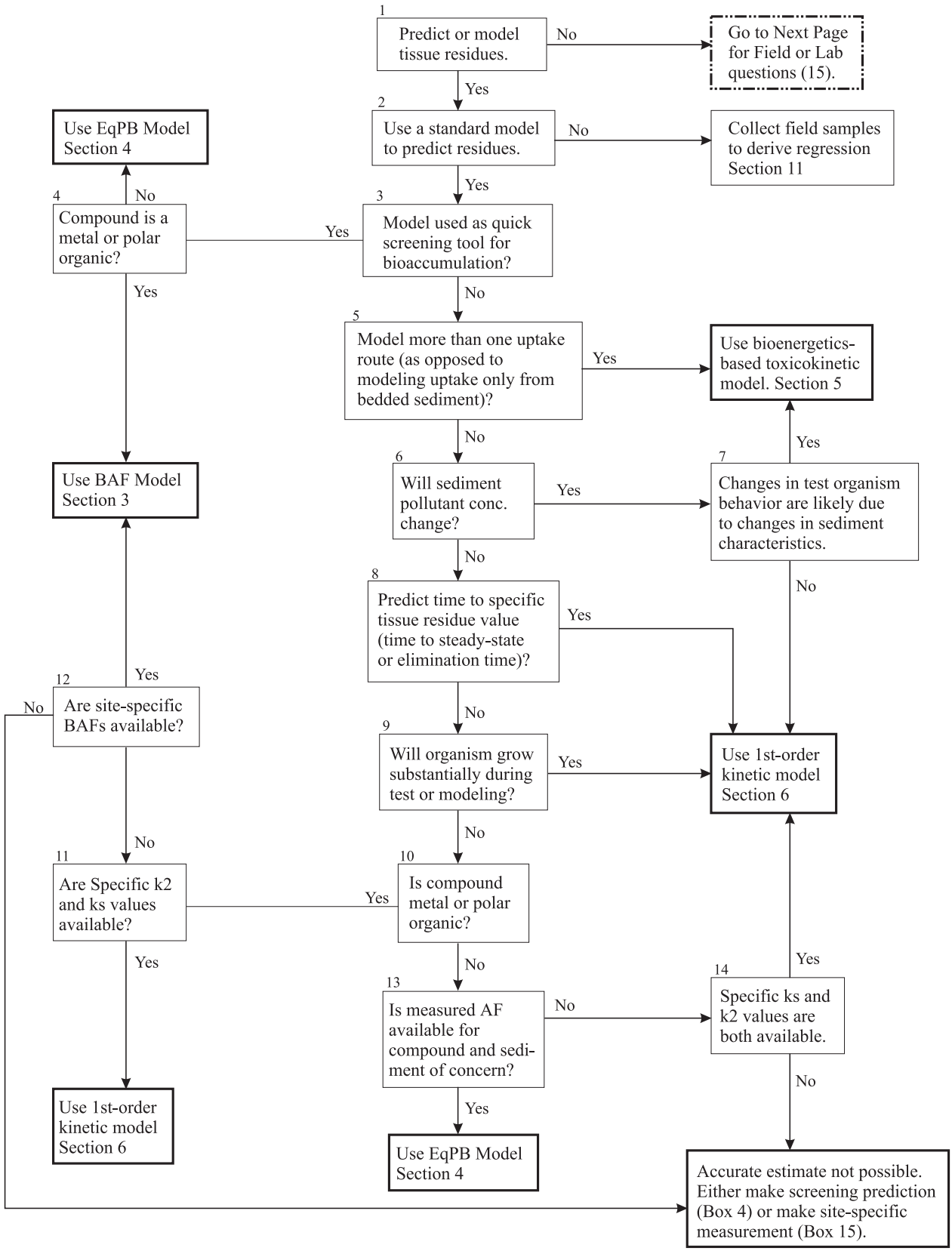
USE OF TISSUE RESIDUE DATA IN
RISK ASSESSMENTS

	Steady-State?	"Max" Residue?
I. HAZARD IDENTIFICATION		
● Identify bioavailable compounds	No	No
● Quantitative measure of bioavailability	No	No
II. EXPOSURE ASSESSMENT		
● Quantify exposure to assessment endpoint species (e.g., edible clam)	Yes	No
● Test species is an indicator (measurement endpoint) for exposure to other species	Yes	Yes
● Test species is prey for higher trophic levels	Yes	Yes
III. ECOLOGICAL EFFECTS ASSESSMENT		
● Tissue residue effects on benthos	Yes	Yes/No
● Derive "Tissue Residue Criteria"	Yes	Yes
IV. HUMAN HEALTH EFFECTS ASSESSMENT	Yes	No
V. RESEARCH		
● Evaluate Sediment Quality Criteria and bioaccumulation models	Yes	Yes
● Determine importance of uptake routes	No	Yes/No

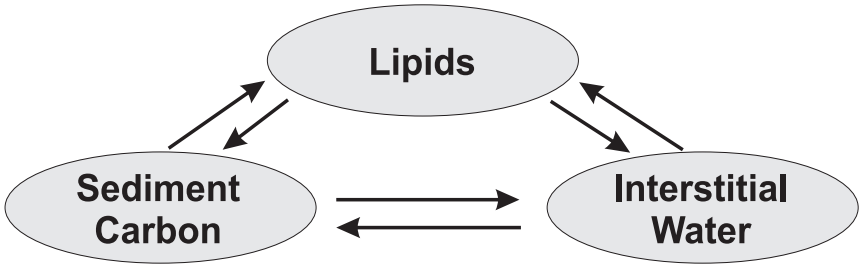
PREDICTING BIOACCUMULATION OF
SEDIMENT POLLUTANTS BY BENTHIC ORGANISMS

- FIELD APPROACH
- BIOACCUMULATION TEST
- STEADY-STATE MODELS
  - BIOACCUMULATION FACTORS (BAFs)
  - EQUILIBRIUM PARTITIONING (BSAFs)
- KINETIC MODELS
  - COMPARTMENT-BASED MODELS (1ST-ORDER KINETIC MODEL)
  - PHYSIOLOGICAL- & ENERGETIC-BASED MODELS

SUMMARY OF QUESTIONNAIRE CHOICES. NUMBERS ABOVE DECISION BOXES REFER TO QUESTIONNAIRE CHOICES.



EQUILIBRIUM PARTITIONING BIOACCUMULATION MODEL



$$Ctss / L = (Cs / TOC) * AF$$

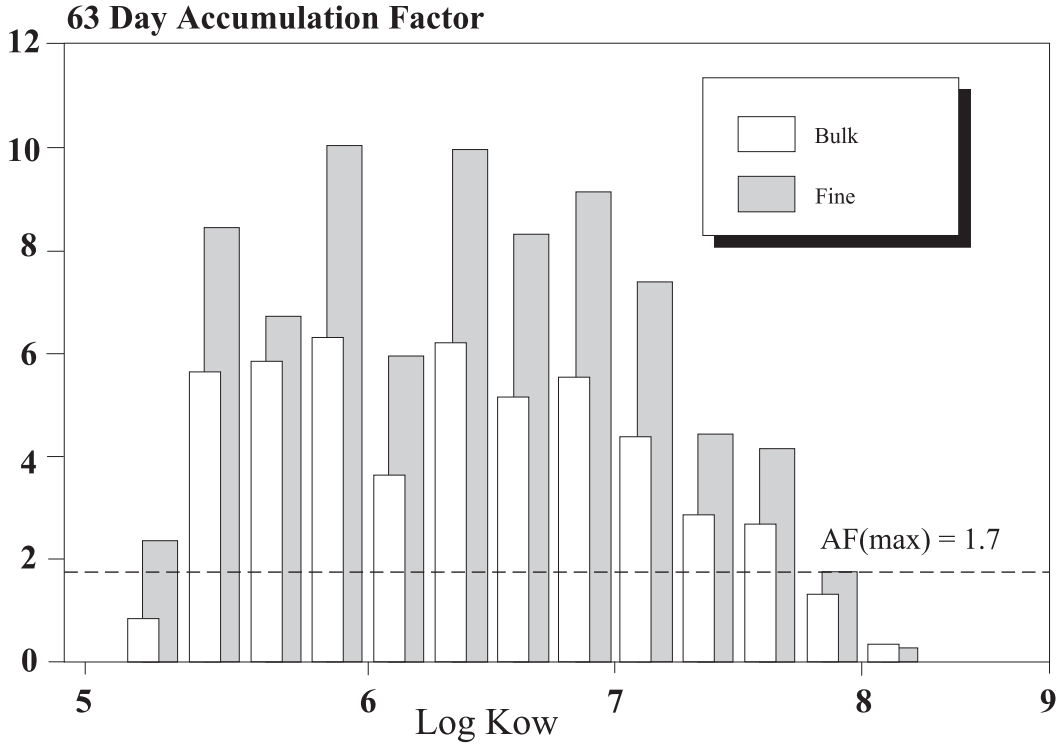
$$AF = (Ctss / L) / (CS / TOC)$$

Where:

Ctss = Tissue conc. at steady-state (ug/g)
L = Lipid content (g/g)
TOC = Total organic carbon in sediment (g/g)
Cs = Sediment conc. (ug/g)
AF = Accumulation Factor (g carbon/g lipid)

1) Tissue residues cannot exceed the concentration set by partitioning (AF < = 2)
2) AFs do not vary among species, sediments, or compounds

PCB Congener AFs in Fine and Bulk Sediments



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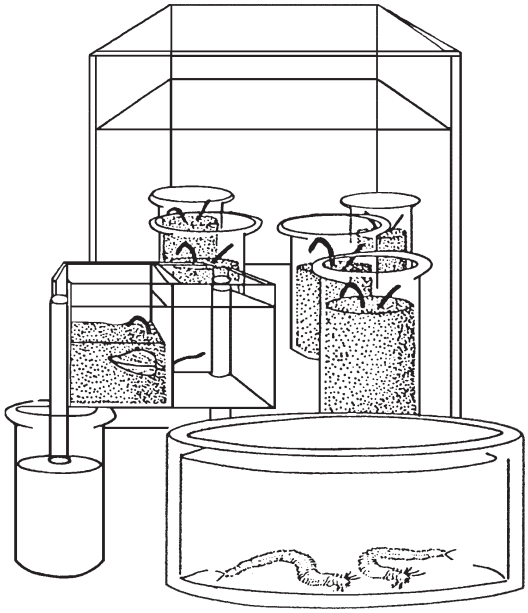
EPA

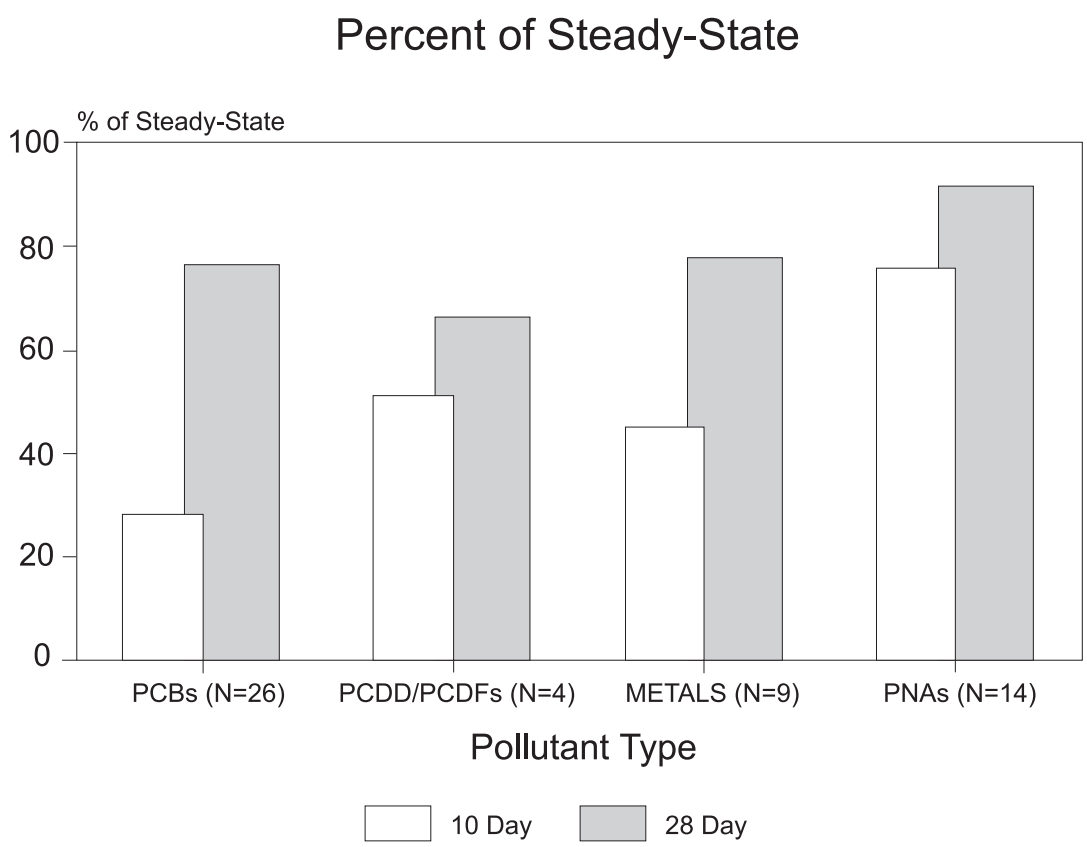
Guidance Manual

Bedded Sediment Bioaccumulation Tests

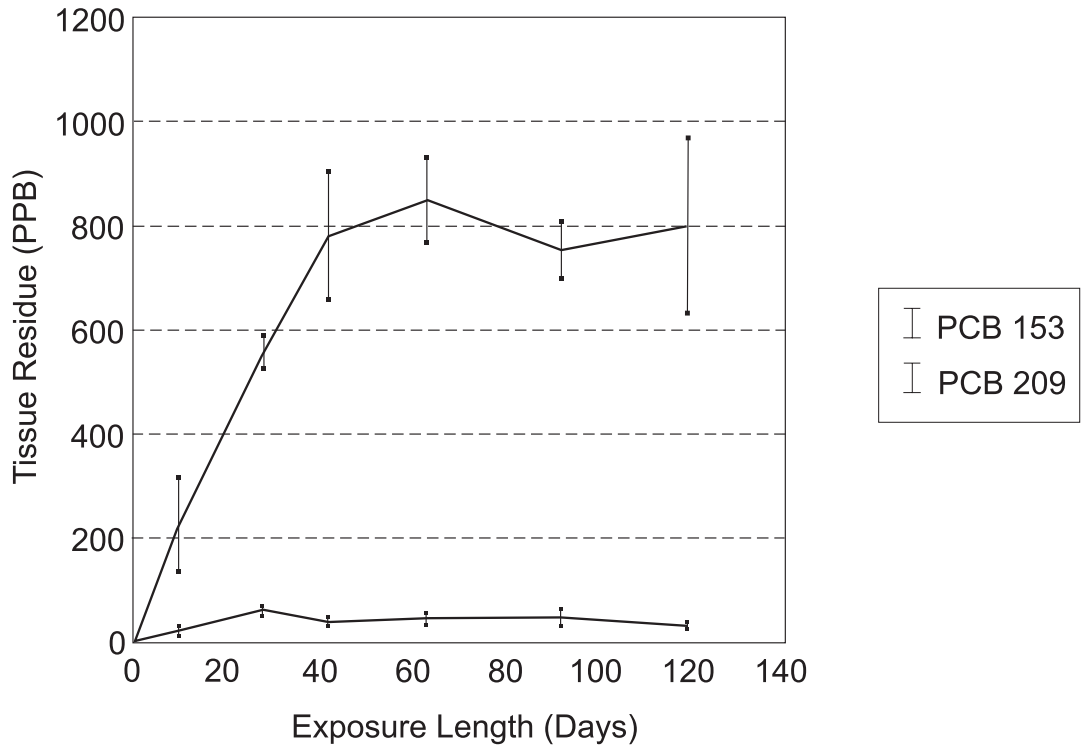


SEDIMENT BIOACCUMULATION TEST
KEY PROCEDURES

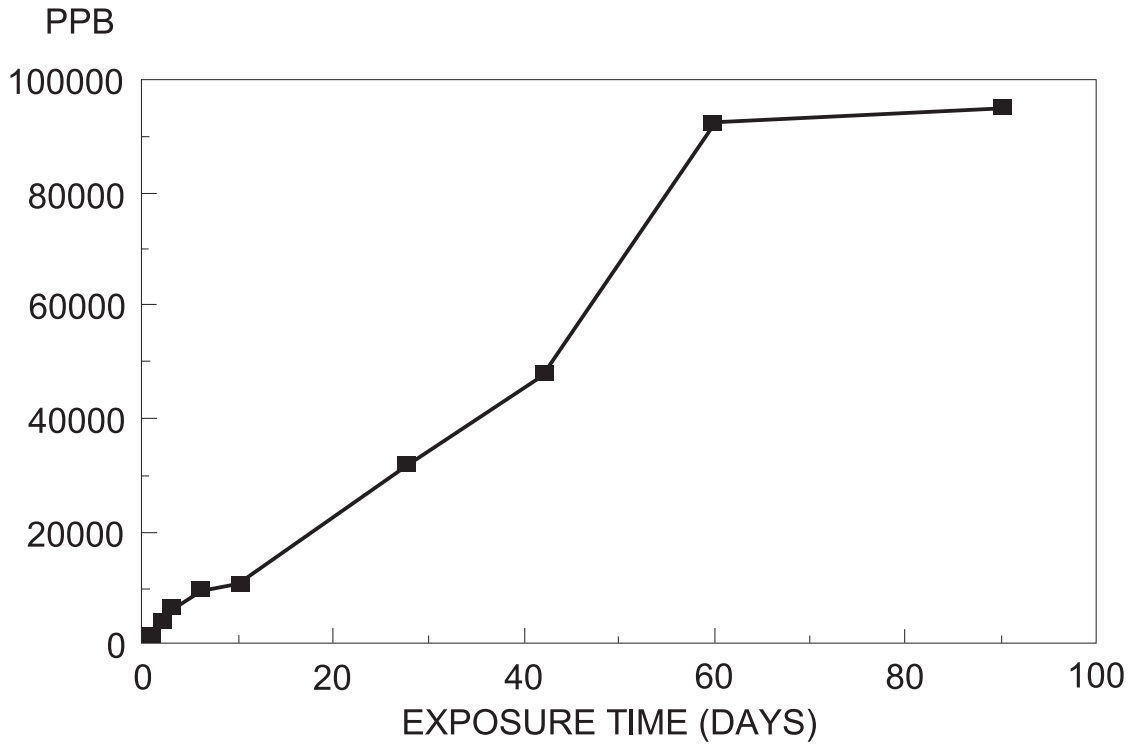
1. 28-DAY EXPOSURE DURATION.
2. SEDIMENT-INGESTING ORGANISM REQUIRED.
3. NO SUPPLEMENTAL FOOD USED.
4. SPECIES EXPOSED INDEPENDENTLY.
5. 80% OF STEADY-STATE TISSUE RESIDUES RECOMMENDED ACCURACY.
6. LONG-TERM TESTS OR TOXICOKINETIC APPROACHES USED FOR >80% ACCURACY OR SLOWLY ACCUMULATED COMPOUNDS.



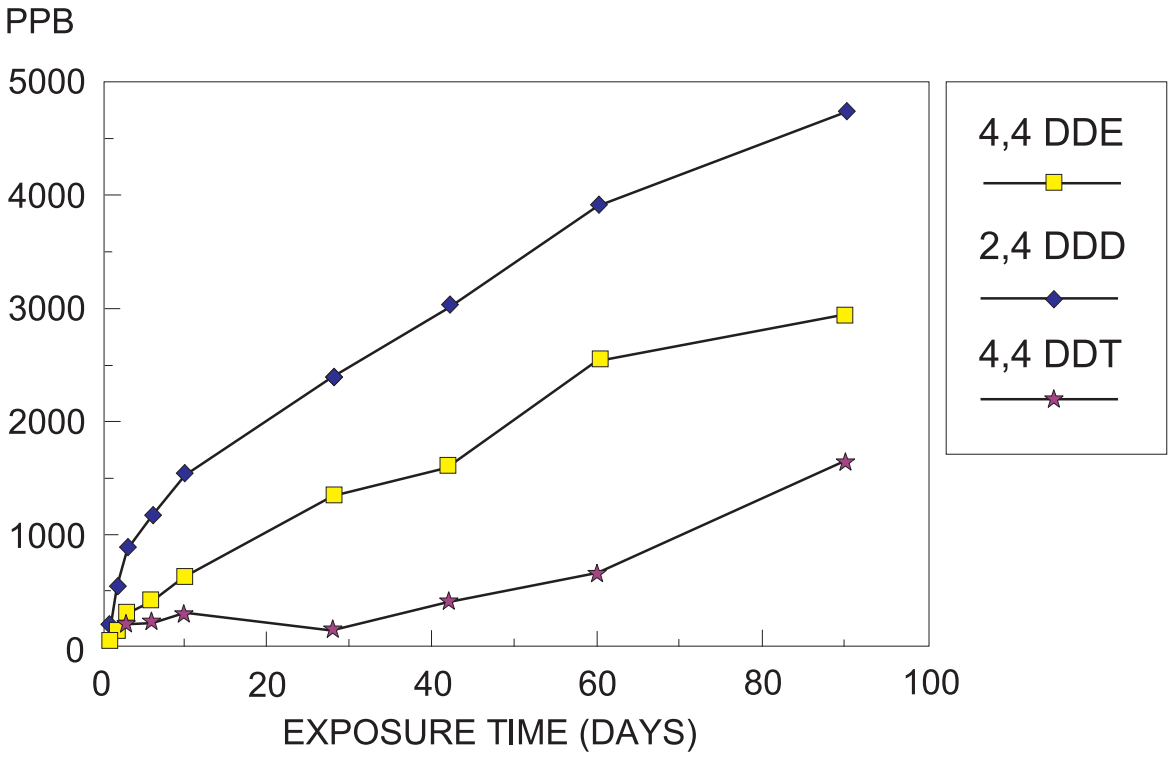
UPTAKE OF PCB CONGENERS 153 VS 209 BY *MACOMA NASUTA*



SUM DDT UPTAKE



TISSUE UPTAKE



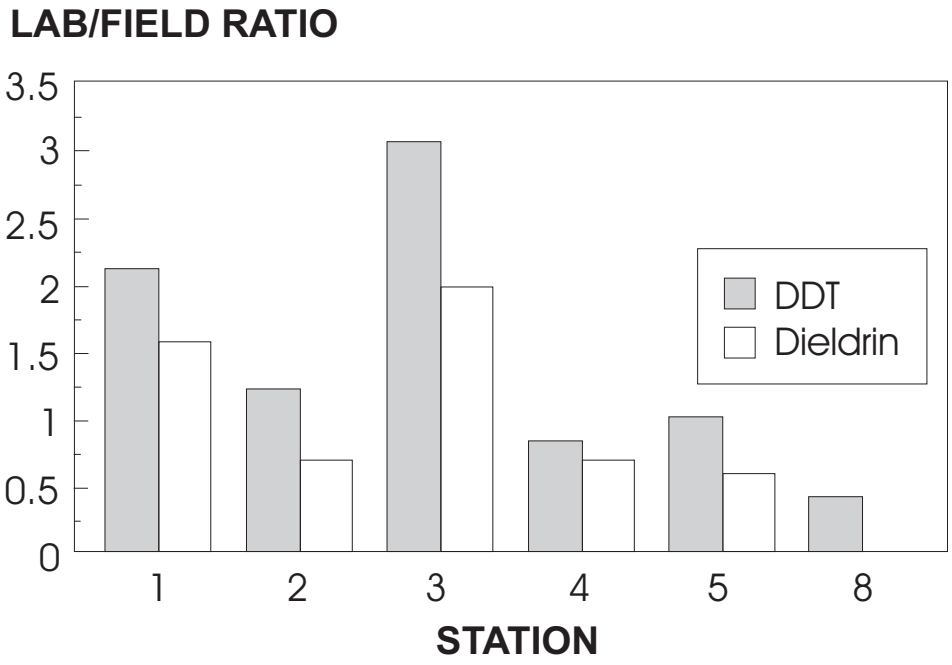
CRITERIA FOR ORGANISM SELECTION

1. Sediment ingester
2. Infaunal (preferably non-tubicolous)
3. Hardy
4. Easily collected or cultured
5. Sufficient biomass for chemical analysis
6. High bioaccumulation potential
7. Feeding behavior understood
8. Suitable for mechanistic/kinetic studies

BSAFs IN FIELD-COLLECTED *MACOMA NASUTA* VERSUS
FILTER-FEEDING BIVALVES IN RICHMOND HARBOR

	Macoma	Filter-Feeding Bivalves	Difference
ΣDDT	0.75	0.10	7.5X
DIELDRIN	1.13	0.13	8.7X

LAB/FIELD TISSUE RESIDUE RATIOS



SEDIMENT BIOACCUMULATION
RESEARCH NEEDS

- INTERLABORATORY ROUND-ROBIN
- FIELD VALIDATION (PAHs, METALS)
- LOCAL TEST SPECIES, ESP. FOR SUBTROPICAL, SUBARCTIC, AND OLIGOHALINE HABITATS
- "STANDARDIZATION" OF LIPID METHODS FOR BSAFs
- EFFECTS OF SEDIMENT STORAGE AND SPIKING ON BIOAVAILABILTY
- REFINEMENT OF EXPERIMENT DESIGN, INCLUDING CRITERIA FOR CONTROLS AND REFERENCES
- EVALUATION OF KINETIC & PHYSIOLOGICAL-BASED ALTERNATIVES TO 28-DAY TEST

WHAT IS ECOLOGICAL SIGNIFICANCE?

- PREDICT TISSUE RESIDUE EFFECTS
- INTEGRATE TISSUE RESIDUE EFFECTS INTO ECOLOGICAL RISK ASSESSMENTS
  - ECOLOGICALLY RELEVANT SPATIAL SCALES
  - MULTIPLE STRESSORS - MULTIPLE ENDPOINTS
  - COMPARATIVE RISK TO OTHER TRADITIONAL & NON-TRADITIONAL STRESSORS

Next