



Standard Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates¹

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1. Scope*

1.1 This guide covers procedures for measuring the bioaccumulation of sediment-associated contaminants by infaunal invertebrates. Marine, estuarine, and freshwater sediments are a major sink for chemicals that sorb preferentially to particles, such as organic compounds with high octanol-water-partitioning coefficients (K_{ow}) (for example, polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT)) and many heavy metals. The accumulation of chemicals into whole or bedded sediments (that is, consolidated rather than suspended sediments) reduces their direct bioavailability to pelagic organisms but increases the exposure of benthic organisms. Feeding of pelagic organisms on benthic prey can reintroduce sediment-associated contaminants into pelagic food webs. The bioaccumulation of sediment-associated contaminants by sediment-dwelling organisms can therefore result in ecological impacts on benthic and pelagic communities and human health from the consumption of contaminated shellfish or pelagic fish.

1.2 Methods of measuring bioaccumulation by infaunal organisms from marine, estuarine, and freshwater sediments will be discussed. The procedures are designed to generate quantitative estimates of steady-state tissue residues because data from bioaccumulation tests are often used in ecological or human health risk assessments. Eighty percent of steady-state is used as the general criterion. Because the results from a single or few species are often extrapolated to other species, the procedures are designed to maximize exposure to sediment-associated contaminants so that residues in untested species are not underestimated systematically. A 28-day exposure with sediment-ingesting invertebrates and no supplemental food is recommended as the standard single sampling procedure. Procedures for long-term and kinetic tests are provided for use when 80 % of steady-state will not be obtained within 28 days or when more precise estimates of steady-state tissue residues

are required. The procedures are adaptable to shorter exposures and different feeding types. Exposures shorter than 28 days may be used to identify which compounds are bioavailable (that is, bioaccumulation potential) or for testing species that do not live for 28 days in the sediment (for example, certain *Chironomus*). Non-sediment-ingestors or species requiring supplementary food may be used if the goal is to determine uptake in these particular species because of their importance in ecological or human health risk assessments. However, the results from such species should not be extrapolated to other species.

1.3 Standard test methods are still under development, and much of this guide is based on techniques used in successful studies and expert opinion rather than experimental comparisons of different techniques. Also, relatively few marine/estuarine (for example, *Nereis* and *Macoma*), freshwater (for example, *Diporeia* and *Lumbriculus variegatus*) species, and primarily neutral organic compounds provide a substantial portion of the basis for the guide. Nonetheless, sufficient progress has been made in conducting experiments and understanding the factors regulating sediment bioavailability to establish general guidelines for sediment bioaccumulation tests.

1.4 This guide is arranged as follows:

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*A Summary of Changes section appears at the end of this standard.



- Annex A6.** Special Purpose Exposure Chambers
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References

1.5 Field-collected sediments may contain toxic materials, including pathogens, and should be treated with caution to minimize exposure to workers. Worker safety must also be considered when using laboratory-dosed sediments containing toxic compounds.

1.6 This guide may involve the use of non-indigenous test species. The accidental establishment of non-indigenous species has resulted in substantial harm to both estuarine and freshwater ecosystems. Adequate precautions must therefore be taken against the accidental release of any non-indigenous test species or associated flora or fauna.

1.7 The values stated in SI units are to be regarded as the standard.

1.8 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 8.

2. Referenced Documents

2.1 ASTM Standards:²

- D 1129** Terminology Relating to Water
D 4387 Guide for Selecting Grab Sampling Devices for Collecting Benthic Macroinvertebrates³
E 729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians
E 943 Terminology Relating to Biological Effects and Environmental Fate
E 1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks
E 1367 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Estuarine and Marine Invertebrates
E 1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates⁴
E 1391 Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing and for Selection of Samplers Used to Collect Benthic Invertebrates
E 1525 Guide for Designing Biological Tests with Sediments
E 1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates
SI10-02 IEEE/ASTM SI 10 American National Standard

² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ Withdrawn.

⁴ Withdrawn.

for Use of the International System of Units (SI): The Modern Metric System

2.2 Federal Document:

CFR, Title 21, Food and Drugs, Chapter I Food and Drug Administration, Department of Health and Human Services, Part 177, Indirect Food Additives: Polymers⁵

CFR, Title 49, Transportation Chapter 1 Research and Special Programs Administration, Department of Transportation Parts 100–177, Subchapter A—Hazardous Materials Transportation, Oil Transportation and Pipeline Safety, Subchapter B—Oil Transportation and Subchapter C—Hazardous Materials Regulation⁵

3. Terminology

3.1 Definitions:

3.1.1 The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express an absolute requirement, that is, to state that the test needs to be designed to satisfy the specified conditions, unless the purpose of the test requires a different design. “Must” is used only in connection with the factors that relate directly to the acceptability of the test. “Should” is used to state that the specified conditions are recommended and ought to be met in most tests. Although the violation of one “should” is rarely a serious matter, violation of several will often render results questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean “is (are) allowed to,” “can” is used to mean “is (are) able to,” and “might” is used to mean “could possibly.” Thus, the classic distinction between “may” and “can” is preserved, and “might” is never used as a synonym for either “may” or “can.”

3.1.2 For definitions of terms used in this guide, refer to Guide **E 729** and Terminologies **D 1129** and **E 943**. For an explanation of units and symbols, refer to **SI10-02 IEEE/ASTM SI 10**.

3.2 Descriptions of Terms Specific to This Standard:

3.2.1 *alpha*—see *Type I error*.

3.2.2 *apparent steady-state*—see *steady-state*.

3.2.3 *bedded sediment*—see *whole sediment*.

3.2.4 *beta*—see *Type II error*.

3.2.5 *bioaccumulation*—the net accumulation of a substance by an organism as a result of uptake from all environmental sources.

3.2.6 *bioaccumulation factor (BAF)*—the ratio of tissue residue to sediment contaminant concentration at steady-state.

3.2.7 *bioaccumulation potential*—a qualitative assessment of whether a contaminant in a particular sediment is bioavailable.

3.2.8 *bioconcentration*—the net assimilation of a substance by an aquatic organism as a result of uptake directly from aqueous solution.

3.2.9 *bioconcentration factor (BCF)*—the ratio of tissue residue to water contaminant concentration at steady-state.

⁵ Available from Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

3.2.10 *biota-sediment accumulation factor (BSAF)*—the ratio of lipid-normalized tissue residue to organic carbon-normalized sediment contaminant concentration at steady state, with units of g-carbon/g-lipid.

3.2.11 *block*—a group of homogeneous experimental units.

3.2.12 *coefficient of variation (CV)*—a standardized variance term; the standard deviation (SD) divided by the mean and expressed as a percent.

3.2.13 *comparison-wise error*—a Type I error applied to the single comparison of two means. Contrast with *experiment-wise error*.

3.2.14 *compositing*—the combining of separate tissue or sediment samples into a single sample.

3.2.15 *control sediment*—sediment containing no or very low levels of contaminants. Control sediments should ideally contain only unavoidable “global” levels of contaminants. Contrast with *reference sediment*.

3.2.16 *degradation*—metabolic breakdown of the contaminant by a test species.

3.2.17 *depuration*—loss of a substance from an organism as a result of any active (for example, metabolic breakdown) or passive process when the organism is placed into an uncontaminated environment. Contrast with *elimination*.

3.2.18 *dichlorodiphenyltrichloroethane (DDT)*—a common environmental contaminant. Metabolites include dichlorodiphenyldichloroethane (DDD) and dichlorodiphenylethylene (DDE).

3.2.19 *redox potential (Eh)*—a measure of the oxidation state of a sediment.

3.2.20 *elimination*—a general term for the loss of a substance from an organism that occurs by any active or passive means. The term is applicable in either a contaminated environment (for example, occurring simultaneously with uptake) or a clean environment. Contrast with *depuration*.

3.2.21 *equilibrium partitioning bioaccumulation model*—a bioaccumulation model based on equilibrium partitioning of a neutral organic among organism lipids and sediment carbon.

3.2.22 *experiment-wise error*—a Type I error (alpha) chosen such that the probability of making any Type I error in a series of tests is alpha. Contrast with *comparison-wise error*.

3.2.23 *experimental error*—variation among experimental units given the same treatment.

3.2.24 *experimental unit*—an organism or organisms to which one trial of a single treatment is applied.

3.2.25 *finer*—the silt-clay fraction of a sediment.

3.2.26 *gut purging*—voiding of sediment contained in the gut.

3.2.27 *hydrophobic contaminants*—low-contaminant water solubility with a high K_{ow} and usually a strong tendency to bioaccumulate.

3.2.28 *interstitial water*—water within a wet sediment that surrounds the sediment particles.

3.2.29 *kinetic bioaccumulation model*—any model that uses uptake or elimination rates, or both, to predict tissue residues.

3.2.30 *long-term uptake tests*—bioaccumulation tests with an exposure period greater than 28 days.

3.2.31 *metabolism*—see *degradation*.

3.2.32 *minimum detectable difference*—the smallest (absolute) difference between two means that is distinguishable statistically.

3.2.33 *multiple comparisons*—the statistical comparison of several treatments simultaneously, such as with Analysis of Variance (ANOVA).

3.2.34 *no further degradation*—an approach by which a tissue concentration is deemed acceptable if it is not greater than the tissue concentration at a reference site.

3.2.35 *pairwise comparisons*—the statistical comparison of two treatments. Contrast with *multiple comparisons*.

3.2.36 *power*—the probability of detecting a difference between the treatment and control means when a true difference exists.

3.2.37 *pseudoreplication*—the incorrect assignment of replicates, often due to a biased assignment of replicates.

3.2.38 *reference sediment*—a sediment similar to the test sediment in physical and chemical characteristics and not contaminated by the particular contaminant source under study (for example, dredge material, discharge, and non-point runoff). A reference sediment should ideally contain only background levels of contaminants characteristic of the region. Contrast with *control sediment*.

3.2.39 *replication*—the assignment of a treatment to more than one experimental unit.

3.2.40 *sampling unit*—the fraction of the experimental unit that is to be used to measure the treatment effect.

3.2.41 *standard reference sediment*—a standardized sediment and contaminant used to estimate the variability due to variation in the test organisms.

3.2.42 *steady-state*—a “constant” tissue residue resulting from the balance of the flux of compound into and out of the organism, determined operationally by no statistical difference in three consecutive sampling periods.

3.2.43 *total carbon (TC)*—this value includes organic and inorganic carbon.

3.2.44 *test sediment*—the sediment or dredge material of concern.

3.2.45 *test treatment*—treatment that is compared to the control or reference treatment. It may consist of either a test sediment (compared to a reference or control sediment) or a reference sediment (compared to the control sediment).

3.2.46 *thermodynamic partitioning bioaccumulation model*—see *equilibrium partitioning bioaccumulation model*.

3.2.47 *tissue residues*—the contaminant concentration in the tissues.

3.2.48 *toxicokinetic bioaccumulation model*—a bioaccumulation model based on the feeding and ventilatory fluxes of the organism.

3.2.49 *treatment*—the procedure (type of sediment) whose effect is to be measured.

3.2.50 *Type I error*—the rate at which H_0 is rejected falsely.

3.2.51 *Type II error*—the rate at which H_0 is accepted falsely.

3.2.52 *whole sediment*—consolidated or bedded sediment (that is, not suspended). Also referred to as *bedded sediment*.

3.3 *Symbols* :

H_a —alternate hypothesis.