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Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia Fetida* and the Enchytraeid Potworm *Enchytraeus albidus*[1]

This standard is issued under the fixed designation E 1676; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This guide covers procedures for obtaining laboratory data to evaluate the adverse effects of contaminants (for example, chemicals or biomolecules) associated with soil to earthworms (Family Lumbricidae) and potworms (Family Enchytraeidae) from soil toxicity or bioaccumulation tests. The methods are designed to assess lethal or sublethal toxic effects on earthworms or bioaccumulation of contaminants in short-term tests (7 to 28 days) or on potworms in short to long-term tests (14 to 42 days) in terrestrial systems. Soils to be tested may be *(1)* reference soils or potentially toxic site soils; *(2)* artificial, reference, or site soils spiked with compounds; *(3)* site soils diluted with reference soils; or *(4)* site or reference soils diluted with artificial soil. Test procedures are described for the species *Eisenia fetida* (see Annex A1) and for the species *Enchytraeus albidus* (see Annex A4). Methods described in this guide may also be useful for conducting soil toxicity tests with other lumbricid and enchytraeid terrestrial species, although modifications may be necessary.

1.2 Modification of these procedures might be justified by special needs. The results of tests conducted using atypical procedures may not be comparable to results using this guide. Comparison of results obtained using modified and unmodified versions of these procedures might provide useful information concerning new concepts and procedures for conducting soil toxicity and bioaccumulation tests with terrestrial worms.

1.3 The results from field-collected soils used in toxicity tests to determine a spatial or temporal distribution of soil toxicity may be reported in terms of the biological effects on survival or sublethal endpoints (see Section 14). These procedures can be used with appropriate modifications to conduct soil toxicity tests when factors such as temperature, pH, and soil characteristics (for example, particle size, organic matter content, and clay content) are of interest or when there is a need to test such materials as sewage sludge and oils. These methods might also be useful for conducting bioaccumulation tests.

1.4 The results of toxicity tests with *(1)* materials (for example, chemicals or waste mixtures) added experimentally to artificial soil, reference soils, or site soils, *(2)* site soils diluted with reference soils, and *(3)* site or reference soils diluted with artificial soil, so as to create a series of concentrations, may be reported in terms of an LC50 (median lethal concentration) and sometimes an EC50 (median effect concentration). Test results may be reported in terms of NOEC (no observed effect concentration), LOEC (lowest observed effect concentration) or as an ECx (concentration where x % reduction of a biological effect occurs. Bioaccumulation test results are reported as the magnitude of contaminant concentration above either the Day 0 tissue baseline analysis or the Day 28 tissues from the negative control or reference soil (that is, 2×, 5×, 10×) (see A3.9).

1.5 This guide is arranged as follows:

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fate and is the direct responsibility of Subcommittee E47.02 on Terrestrial Assessment and Toxicology.

An ASTM guide is defined as a series of options or instructions that do not recommend a specific course of action.

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

1.7 *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.* While some safety considerations are included in this guide, it is beyond the scope of this standard to encompass all safety requirements necessary to conduct soil toxicity tests. Specific precautionary statements are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 653 Terminology Relating to Soil, Rock, and Contained Fluid

D 4447 Guide for the Disposal of Laboratory Chemicals and Samples

E 380 Practice for Use of the International System of Units (SI) (the Modernized Metric System)

E 943 Terminology Relating to Biological Effects and Environmental Fate

E 1023 Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses

E 1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates

E 1688 Guide for Determination of the Bioaccumulation of Sediment-Associated Contaminants by Benthic Invertebrates

E 1706 Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates

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 must be designed to satisfy the specified condition, 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 (see Section 13). “Should” is used to state that the specified condition is recommended and ought to be met if possible. Although a violation of one “should” is rarely a serious matter, the violation of several will often render the 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 Terminology E 943 and Guide E 1023. For an explanation of units and symbols, refer to Practice E 380.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *artificial soil*—a synthetic soil, prepared with a specific formulation, designed to simulate a natural soil (see Annex A2). Artificial soil may be used as a diluent medium to prepare concentrations of site or reference soil and may be used as a negative control medium.

3.2.2 *batch*—the total amount of test soil prepared for each concentration in a test. A batch is any hydrated test soil ready for separation into replicates.

3.2.3 *bioaccumulation*—the net accumulation of a substance by an organism as a result of uptake from all environmental sources. (See Guide E 1688.)

3.2.4 *bioaccumulation factor (BAF)*—the ratio of tissue residue to sediment contaminant concentration at steady-state. (See Guide E 1688.)

3.2.5 *bioaccumulation potential*—a qualitative assessment of whether a contaminant in a particular sediment is bioavailable. (See Guide E 1688.)

3.2.6 *bioconcentration*—the net assimilation of a substance by an organism as a result of uptake directly from aqueous solution. (See Guide E 1688.)

3.2.7 *bioconcentration factor (BCF)*—the ratio of tissue residue to water contaminant concentration as steady-state. (See Guide E 1688.)

3.2.8 *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. (See Guide E 1688.)

3.2.9 *clitellum*—the fleshy “ring” or “saddle” of glandular tissue found on certain mid-body segments of oligochaete (Lumbricidae and Enchytraeidae) worms. It is the most visible feature of an adult earthworm or potworm and secretes the cocoon into which eggs and sperm are deposited.

3.2.10 *concentration*—the ratio of the weight of test materials to the weight of soil (artificial, reference, or site), usually expressed on a dry weight basis as percent or milligram/kilogram.

3.2.11 *depuration*—loss of a substance from an organism as a result of any active (for example, metabolic breakdown) or passive process.

3.2.12 *diluent soil*—the artificial or reference soil used to dilute site soils.

3.2.13 *enchytraeid*—potworm members of the Family Enchytraeidae of the Class Oligochaeta of the Phylum Annelida.

3.2.14 *hydration water*—water used to hydrate test soils to create an environment with a moisture level suitable for the species being tested. The water used for hydration is often test water (see 3.2.27); however, depending on the nature of the test being implemented, site surface water or groundwater may also be utilized for hydration.

3.2.15 *lumbricid*—earthworm members of the Family Lubricidae of the Class Oligochaeta of the Phylum Annelida.

3.2.16 *negative control soil*—artificial or reference soil to be used for evaluating the acceptability of a test.

[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.

3.2.17 *reference soil*—a field-collected soil that has physicochemical and biological properties as similar as possible to the site soil but does not contain the potentially toxic compounds of the site soil. It is used to describe matrix effects on the test in question. It may be used as a diluent medium to prepare concentrations of site soil and may be used as a negative control medium.

3.2.18 *sampling station*—a specific location, within a site or sampling unit, depending on the field study design, at which soil is collected for chemical, physical, and biological evaluation.

3.2.19 *sampling unit*—an area of land within a site distinguished by habitat and topography.

3.2.20 *site*—a delineated tract of land that is being considered as a study area, usually from the standpoint of its being potentially affected by xenobiotics.

3.2.21 *site soil*—a soil collected from the field to be evaluated for potential toxicity. A site soil may be a naturally occurring soil or one that has been influenced by xenobiotics.

3.2.22 *soil*—sediments or other unconsolidated accumulations of solid particles produced by the physical and chemical disintegration of rocks, and that may or may not contain organic material. (See Terminology D 653.)

3.2.23 *spiking*—the experimental addition of a test material to an artificial, site, or reference soil, such that the toxicity of the material added can be determined. After the test material is added, which may involve a solvent carrier, the soil is mixed thoroughly to distribute the test material evenly throughout the soil.

3.2.24 *test chamber*—an enclosed space or compartment in which environmental parameters such as temperature and lighting are controlled (for example, incubator or modified room). Test containers are placed in the test chamber for biological evaluation.

3.2.25 *test container*—the experimental unit; the smallest physical entity to which treatments can be assigned independently.

3.2.26 *test soil*—a soil prepared to receive a test organism. Site or reference soil mixed with artificial soil or reference soil mixed with site soil in known concentrations for evaluation are test soils. Artificial, site, or reference soils spiked with test materials such as chemicals, oils, or manufacturing products are test soils. Once a site, reference, or artificial soil is hydrated, even though it is not mixed with artificial or reference soil or spiked with a material, it may be called a test soil.

3.2.27 *test water*—water used to prepare stock solutions, rinse test organisms, rinse glassware, and apparatus or for any other purpose associated with the test procedures or culture of the test organism. Test water must be deionized or distilled water or better, such as reagent-grade water produced by a system of reverse osmosis, carbon, and ion-exchange cartridges.

4. Summary of Guide

4.1 The toxicity of test soils or the bioavailability of contaminants are assessed during the continuous exposure of terrestrial organisms. Soils tested may be the following: (*1*) soils collected from potentially contaminated sites, (*2*) soils collected from reference sites, (*3*) artificial soil (see Annex A2) spiked with compounds, (*4*) site soil spiked with compounds, (*5*) reference soil spiked with compounds, (*6*) site soil diluted with artificial soil, (*7*) site soil diluted with reference soil, or (*8*) reference soil diluted with artificial soil. A negative control of artificial or reference soil is used for the following: (*1*) to yield a measure of the acceptability of the test; (*2*) to provide evidence of the health and relative quality of the test organisms; (*3*) to determine the suitability of test conditions, food, and handling procedures; and (*4*) to provide a basis for interpreting data obtained from the test soils. Specified data are obtained to determine the toxic effects on survival or sublethal endpoints for 7 to 28-day exposures or containment bioaccumulation for 28-day exposures to terrestrial lumbricids and the toxic effects on survival or sublethal endpoints for 4 to 42-day exposures to enchytraeids.

4.2 *Summary of Changes*—This current version of the standard is a revision of the E 1676-97 version. Changes made since 1997 involve toxicity testing procedures for the Enchytraied potworm, *Enchytraeus albidus*. There has been an additional annex added (Annex A4) and the main document has been modified to include this species.

5. Significance and Use

5.1 Soil toxicity tests provide information concerning the toxicity and bioavailability of chemicals associated with soils to terrestrial organisms. As important members of the soil fauna, lumbricid earthworms and enchytraeid potworms have a number of characteristics that make them appropriate organisms for use in the assessment of potentially hazardous soils. Earthworms may ingest large quantities of soil, have a close relationship with other soil biomasses (for example, invertebrates, roots, humus, litter, and microorganisms), constitute up to 92 % of the invertebrate biomass of soil, and are important in recycling nutrients **(1, 2)**.[3] Enchytraeids contribute up to 5.2 % of soil respiration, constitute the second-highest biomass in many soils (the highest in acid soils in which earthworms are lacking) and effect considerably nutrient cycling and community metabolism **(94-96)**. Earthworms and potworms accumulate and are affected by a variety of organic and inorganic compounds **(2-7, 97-100)**. In addition, earthworms and potworms are important in terrestrial food webs, constituting a food source for a very wide variety of organisms, including birds, mammals, reptiles, amphibians, fish, insects, nematodes, and centipedes **(8, 9, 94)**. A major change in the abundance of soil invertebrates such as lumbricids or enchytraeids, either as a food source or as organisms functioning properly in trophic energy transfer and nutrient cycling, could have serious adverse ecological effects on the entire terrestrial system.

5.2 A number of species of lumbricids and enchytraeid worms have been used in field and laboratory investigations in the United States and Europe. Although the sensitivity of various lumbricid species to specific chemicals may vary, from their study of four species of earthworms (including *E. fetida*) exposed to ten organic compounds representing six classes of

[3] The boldface numbers in parentheses refer to the list of references at the end of this standard.