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Standard Guide for Conducting Laboratory Soil Toxicity Tests with the Nematode *Caenorhabditis elegans*[1]

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1. Scope

1.1 This guide covers procedures for obtaining laboratory data to evaluate the adverse effects of chemicals associated with soil to nematodes from soil toxicity tests. This standard is based on a modification to Guide E1676. The methods are designed to assess lethal or sublethal toxic effects on nematodes in short-term tests in terrestrial systems. Soils to be tested may be (*1*) references soils or potentially toxic soil sites; (*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 *Caenorhabditis elegans* (see Annex A1). Methods described in this guide may also be useful for conducting soil toxicity tests with other terrestrial species, although modifications may be necessary.

1.2 *Summary of Previous Studies*—Initial soil toxicity testing using the free-living, bacterivorous soil nematode *Caenorhabditis elegans* was developed by Donkin and Dusenbery (**1**).[2] Following the development of an effective method of recovery of *C. elegans* from test soils, the organism was used to identify factors that affect the toxicity of zinc, cadmium, copper, and lead (**2**). Freeman et al. further refined the nematode bioassay by decreasing the quantity of soil and spiking solution volumes, determining test acceptability criteria, and developing control charts to assess worm health using copper as a reference toxicant (**3**). More recently, the toxicological effects of nitrate and chloride metallic salts in two natural soils were compared (**4**). LC50 values for *C. elegans* exposed for 24-h to nitrate salts of cadmium, copper, zinc, lead and nickel in an artificial soil (see Annex A2) were found to be similar to LC50 values for the earthworm, *Eisenia fetida* (**5**). Increasing the exposure time to 48-h resulted in much lower LC50 values (**6**). However, longer exposure times necessitate the addition of food and lead to lower recovery percentages in soils high in organic matter. A modification of the recovery method has also been used with a transgenic strain of *C. elegans* used as a soil biomonitoring tool to assess sub-lethal effects of metal exposures in soil (**7**). A variety of sub-lethal endpoints have been developed using *C. elegans* in aquatic media and may prove useful for assessing soil exposures (**8**).

1.3 Modification of these procedures might be justified by special needs. The results of tests conducted using typical 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 tests with terrestrial worms.

1.4 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. 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. These methods might also be useful for conducting bioaccumulation tests.

1.5 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).

1.6 This guide is arranged as follows:

[1] This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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

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, health, and environmental 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.

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D4447 Guide for Disposal of Laboratory Chemicals and Samples

E943 Terminology Relating to Biological Effects and Environmental Fate

E1295 Guide for Conducting Three-Brood, Renewal Toxicity Tests with *Ceriodaphnia dubia*

E1676 Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation Tests with the Lumbricid Earthworm *Eisenia Fetida* and the Enchytraeid Potworm *Enchytraeus albidus*

E1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater 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 E943.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *artificial soil, n*—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, n*—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 *concentration, n*—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.

3.2.4 *diluent soil, n*—the artificial or reference soil used to dilute site soils.

3.2.5 *hydration water, n*—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 3.2.18); however, depending on the nature of the test being implemented, site surface water or ground water may also be utilized for hydration.

3.2.6 *negative control soil, n*—artificial or field collected soil to be used for evaluating the acceptability of a test.

3.2.7 *reference soil, n*—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.

3.2.8 *sampling station, n*—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.

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

3.2.10 *sediment, n*—particulate materials that usually lie below water. Formulated particulate material that is intended to lie below water in a test.

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

3.2.12 *site soil, n*—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 contaminants.

3.2.13 *soil, n*—solid particles produced by the physical and chemical disintegration of rocks, which may or may not contain organic material.

[3] 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.

3.2.14 *spiking, v*—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 carder, the soil is mixed thoroughly to distribute the test material evenly throughout the soil.

3.2.15 *test chamber, n*—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.

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

3.2.17 *test soil, n*—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.

3.2.18 *test water, n*—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 should be deionized 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 *Toxicity of Test Soils is 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 for 24 h exposures to the terrestrial nematodes *C. elegans*.

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, nematodes have a number of characteristics that make them appropriate organisms for use in the assessment of potentially hazardous soils. Bacterial-feeding nematodes such as *C. elegans* feed on soil microbes and contribute to the breakdown of organic matter. They are also of extreme importance in the cycling and degradation of key nutrients in soil ecosystems (**9**). Soil nematodes also serve as a source of prey and nutrients for fauna and microflora such as soil nematophagous fungi (**10**). A major change in the abundance of soil invertebrates such as nematodes, 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 Results from soil tests might be an important consideration when assessing the hazards of materials to terrestrial organisms.

5.3 The soil test might be used to determine the temporal or spatial distribution of soil toxicity. Test methods can be used to detect horizontal and vertical gradients in toxicity.

5.4 Results of soil tests could be used to compare the sensitivities of different species.

5.5 An understanding of the effect of these parameters on toxicity may be gained by varying soil characteristics such as pH, clay content, and organic material.

5.6 Results of soil tests may be useful in helping to predict the effects likely to occur with terrestrial organisms in field situations.

5.6.1 Field surveys can be designed to provide either a qualitative or quantitative evaluation of biological effects within a site or among sites.

5.6.2 Soil surveys evaluating biological effects are usually part of more comprehensive analyses of biological, chemical, geological, and hydrographic conditions. Statistical correlation can be improved and costs reduced if subsamples of soil for laboratory tests, geochemical analyses, and community structure are taken simultaneously from the same grab of the same site.

5.7 Soil toxicity tests can be an important tool for making decisions regarding the extent of remedial action necessary for contaminated terrestrial sites.

6. Interferences

6.1 Limitations to the methods described in this guide might arise and thereby influence soil test results and complicate data interpretation. The following factors should be considered when testing soils:

6.1.1 The alteration of field samples in preparation for laboratory testing (for example, transport, screening, or mixing).

6.1.1.1 Maintaining the integrity of soils during their removal, transport, and testing in the laboratory is extremely difficult. The soil environment is composed of a myriad of microenvironments, redox gradients, and other interacting physicochemical and biological processes. Many of these characteristics influence soil toxicity and the availability of compounds to organisms, microbial degradation, and chemical sorption. Any disruption of this environment complicates interpretations of treatment effects, causative factors, and in situ comparisons.

6.1.1.2 Soils tested at temperatures other than those from the field in which they are collected might affect chemical