



Designation: E2172 – 22

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

This standard is issued under the fixed designation E2172; 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 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) reference 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:

Scope	1
Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Interferences	6
Apparatus	7
Safety Precautions	8
Soil	9
Test Organism	10
Procedure	11

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.

Current edition approved Aug. 1, 2022. Published September 2022. Originally approved in 2002. Last previous edition approved in 2014 as E2172–01(2014). DOI: 10.1520/E2172-22.

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

Analytical Methodology	12
Acceptability of Test	13
Calculation of Results	14
Report	15
Annexes	
	A1. <i>Caenorhabditis elegans</i>
	A2. Artificial Soil Composition

References

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.