



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:

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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 Oct. 1, 2014. Published December 2014. Originally approved in 2002. Last previous edition approved in 2008 as E2172–02(2008). DOI: 10.1520/E2172-01R14.

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