



Designation: E1963 – 22

## Standard Guide for Conducting Terrestrial Plant Toxicity Tests<sup>1</sup>

This standard is issued under the fixed designation E1963; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

### 1. Scope

1.1 This guide covers practices for conducting plant toxicity tests using terrestrial plant species to determine effects of test substances on plant growth and development. Specific test procedures are presented in accompanying annexes.

1.2 Terrestrial plants are vital components of ecological landscapes. The populations and communities of plants influence the distribution and abundance of wildlife. Obviously, plants are the central focus of agriculture, forestry, and rangelands. Toxicity tests conducted under the guidelines and annexes presented herein can provide critical information regarding the effects of chemicals on the establishment and maintenance of terrestrial plant communities.

1.3 Toxic substances that prevent or reduce seed germination can have immediate and large impacts to crops. In natural systems, many desired species may be sensitive, while other species are tolerant. Such selective pressure can result in changes in species diversity, population dynamics, and community structure that may be considered undesirable. Similarly, toxic substances may impair the growth and development of seedlings resulting in decreased plant populations, decreased competitive abilities, reduced reproductive capacity, and lowered crop yield. For the purposes of this guide, test substances include pesticides, industrial chemicals, sludges, metals or metalloids, and hazardous wastes that could be added to soil. It also includes environmental samples that may have had any of these test substances incorporated into soil.

1.4 Terrestrial plants range from annuals, capable of completing a life-cycle in as little as a few weeks, to long-lived perennials that grow and reproduce for several hundreds of years. Procedures to evaluate chemical effects on plants range from short-term measures of physiological responses (for example, chlorophyll fluorescence) to field studies of trees over several years. Research and development of standardized plant tests have emphasized three categories of tests: (1) short-term, physiological endpoints (that is, biomarkers); (2) short-term

tests conducted during the early stages of plant growth with several endpoints related to survival, growth, and development; and (3) life-cycle toxicity tests that emphasize reproductive success.

1.5 This guide is arranged by sections as follows:

| Section | Title                                      |
|---------|--------------------------------------------|
| 1       | Scope                                      |
| 2       | Referenced Documents                       |
| 3       | Terminology                                |
| 4       | Summary of Phytotoxicity Tests             |
| 5       | Significance and Use                       |
| 6       | Apparatus                                  |
| 7       | Test Material                              |
| 8       | Hazards                                    |
| 9       | Test Organisms                             |
| 10      | Sample Handling and Storage                |
| 11      | Calibration and Standardization            |
| 12      | Test Conditions                            |
| 13      | Interference and Limitations               |
| 14      | Quality Assurance and Quality Control      |
| 15      | Calculations and Interpretation of Results |
| 16      | Precision and Bias                         |

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 8.

1.8 *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:<sup>2</sup>

D1193 Specification for Reagent Water

D4447 Guide for Disposal of Laboratory Chemicals and Samples

<sup>1</sup> 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 published in 1998. Last previous edition published 2014 as E1963–09(2014). DOI: 10.1520/E1963-22.

<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

**D4547** Guide for Sampling Waste and Soils for Volatile Organic Compounds

**D5633** Practice for Sampling with a Scoop

**E943** Terminology Relating to Biological Effects and Environmental Fate

**E1598** Practice for Conducting Early Seedling Growth Tests (Withdrawn 2003)<sup>3</sup>

**E1733** Guide for Use of Lighting in Laboratory Testing

2.2 *Code of Federal Regulations Standard:*

**CFR 49**<sup>4</sup>

2.3 *Other useful references have described phytotoxicity test procedures***(1-11)**.<sup>5</sup>

### 3. Terminology

3.1 *General Terminology*—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 ought to be designed to satisfy the specified condition, unless the purpose of the test requires a different design. “Must” is only used in connection with factors that directly relate to the acceptability of the test (see Section 14). “Should” is used to state that the specified condition is recommended and ought to be met if possible. Although violation of one “should” is rarely a serious matter, 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.”

3.1.1 For definitions of terms used in this guide, refer to Terminology **E943**.

#### 3.2 Definitions:

3.2.1 *control, n*—the treatment group in a toxicity test consisting of reference soil or artificial soil that duplicates all the conditions of the exposure treatments, but contains no test substance. The control is used to determine if there are any statistical differences in endpoints related to the test substance.

3.2.2 *eluate, n*—solution obtained from washing a solid with a solvent to remove adsorbed material.

3.2.3 *hazardous substance, n*—a material that can cause deleterious effects to plants, microbes, or animals. (A hazardous substance does not, in itself, present a risk unless an exposure potential exists.)

3.2.4 *inhibition, n*—a statistically lower value of any endpoint compared to the control values that is related to environmental concentration or application rate.

3.2.5 *leachate, n*—water plus solutes that has percolated through a column of soil or waste.

3.2.6 *test material, n*—any formulation, dilution, etc. of a test substance.

3.2.7 *test substance, n*—a chemical, formulation, eluate, sludge, or other agent or substance that is the target of the investigation in a toxicity test.

3.2.8 *toxicant, n*—an agent or material capable of producing an adverse response (effect) in a biological system, adversely impacting structure or function or producing death.

3.2.9 *toxicity endpoints, n*—measurements of organism response such as death, growth, developmental, or physiological parameters resulting from exposure to toxic substances.

#### 3.3 Definitions of Terms Specific to This Standard:

3.3.1 *chlorotic, adj*—the discoloration of shoots that occurs as chlorophyll is degraded as a result of disease, toxic substances, nutrient deficiencies, or senescence.

3.3.2 *coleoptile, n*—the protective tissues surrounding the growing shoot in a monocotyledonous plant.

3.3.3 *cotyledon, n*—a primary leaf of the embryo in seeds, only one in the monocotyledons, two in dicotyledons. In many of the latter, such as the bean, they emerge above ground and appear as the first leaves.

3.3.4 *cutting, n*—a vegetative segment of a plant, usually a stem that contains several nodes and associated buds, that can be used to regenerate an entire plant.

3.3.5 *dead test plants, n*—those individuals that expired during the test observation period as indicated by severe desiccation, withering, chlorosis, necrosis, or other symptoms that indicate non-viability.

3.3.6 *desiccated, adj*—the plant, or portion of the plant, that is dried in comparison to the control plant.

3.3.7 *development, n*—the series of steps involving cell division and cell differentiation into various tissues and organs.

3.3.8 *dicotyledon, n*—in the classification of plants, those having two seed leaves.

3.3.9 *dormancy, n*—a special condition of arrested growth in which buds, embryos, or entire plants survive at lowered metabolic activity levels. Special environmental cues such as particular temperature regimes or photoperiods are required to activate metabolic processes and resume growth. Seeds that require additional treatment besides adequate moisture and moderate temperature to germinate are said to be dormant. (See *quiescence*.)

3.3.10 *emergence, n*—following germination of a plant, the early growth of a seedling that pushes the epicotyl through the soil surface.

3.3.11 *enhanced growth and yield, n*—when a treated plant exhibits shoot growth, root elongation, lateral root growth, or yield significantly greater than the control values, the plant is “enhanced” or “stimulated.”

3.3.12 *epicotyl, n*—that portion of an embryo or seedling containing the shoot. It is delineated anatomically by the transition zone which separates the epicotyl from the hypocotyl.

<sup>3</sup> The last approved version of this historical standard is referenced on [www.astm.org](http://www.astm.org).

<sup>4</sup> Available from Standardization Documents Order Desk, DODSSP, Bldg. 4, Section D, 700 Robbins Ave., Philadelphia, PA 19111-5098, <http://www.dodssp.daps.mil>.

<sup>5</sup> The boldface numbers in parentheses refer to the list of references at the end of this guide.