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Standard Guide for Conducting Terrestrial Plant Toxicity Tests[1]

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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:

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 and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

D4547 Guide for Sampling Waste and Soils for Volatile Organic Compounds

D5633 Practice for Sampling with a Scoop

E1598 Practice for Conducting Early Seedling Growth Tests (Withdrawn 2003)[3]

E1733 Guide for Use of Lighting in Laboratory Testing

[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] 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] The last approved version of this historical standard is referenced on www.astm.org.

2.2 *Code of Federal Regulations Standard:*
CFR 49 [4]

2.3 *Other useful references have described phytotoxicity test procedures* **(1-11)** .[5]

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."

3.2 *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.

3.3.13 *fruits, n*—the reproductive tissues derived from the ovary in the case of epigenous flowers or the ovary and accessory tissues in the case of hypigenous flowers.

3.3.14 *germination, n*—the physiological events associated with re-initiation of embryo growth and mobilization of reserve nutrients in seeds. The emergence of the seedling radicle from the seed coat defines the end of germination and the beginning of early seedling growth.

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

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

3.3.15 *growth, n*—a change in size or mass measured by length, height, volume, or mass.

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

3.3.17 *inhibited plant growth and yield, n*— plant growth, root length and lateral root growth, or yield are "inhibited" when their measurements are significantly less than the control values.

3.3.18 *lateral roots, n*—roots growing off the primary roots, also referred to as secondary roots.

3.3.19 *monocotyledon, n— in the classification of plants*, those having a single seed leaf.

3.3.20 *mottled, adj*—marked with lesions, spots or streaks of different colors. This includes the discoloration of leaf margins.

3.3.21 *phytotoxicity, n*—a lethal or sub-lethal response of plants to a toxicant.

3.3.22 *quiescence, n*—a condition in buds, embryos, or entire plants characterized by lowered metabolic rates and limited or no growth. Seeds that germinate when supplied with adequate moisture and moderate temperature are said to be quiescient. (See *dormancy*.)

3.3.23 *radicle, n*—the emerging root of an embryo during germination.

3.3.24 *seed, n*—the propagule of a plant derived from an ovule. It consists of an embryo, a protective covering (seed coat), and may have storage tissue (endosperm).

3.3.25 *shoot, n*—the above-ground portion of a plant consisting of stems, leaves, as well as any reproductive parts that may be attached.

3.3.26 *surviving plants, n*—test plants that are alive at the time observations are recorded.

3.3.27 *viable, adj*—plants capable of resuming metabolic functions and growth are considered "viable." Buds, embryos, or entire plants may be dormant or quiescient and therefore exhibit no growth during the period of observation. Distinguishing dead plants from viable plants with certainty is difficult without special training and sophisticated measures of metabolic function.

3.3.28 *withering, v*—becoming limp or desiccated, deprived of moisture; often the result of root damage.

4. Summary of Phytotoxicity Tests

4.1 The terrestrial phytotoxicity tests covered under this guide apply to a range of test conditions and test species that can be adapted to meet project-specific objectives. Test organisms are maintained either as seeds or as cuttings until a particular test is to be conducted. A prescribed number of individual plants are introduced into test treatments that include a negative control, a series of positive controls, and one or more test-substance treatment concentrations. The treatment concentrations may be known or unknown; nominal or measured, depending on the nature of the investigation. In the case where the test substance is evaluated as an additive to soil, a range of concentrations is recommended. In tests of environmental samples that already contain a putative phytotoxic substance, the tests may be conducted with either the test soil as collected from the field, or as diluted with a suitable reference soil. Another variant of the tests allows for amendments, or spikes, of selected toxic substances to be added to environmental samples. Finally, in the case of the root elongation assay, eluates, effluents, or other aqueous derivatives of a soil sample are tested.

4.2 Plants are exposed to the test substances in the form described in the specific variations of the tests for a discrete period of time that ranges from 96 h to several months. For short tests, no nutrient additions or amendments are needed or recommended as the amendments may interact with the toxicant and alter the toxicity response. For tests lasting more than two weeks, nutrient additives may be warranted, depending on the test objectives, in order to maximize the potential for plant growth and development. Thinning, culling, or replacing individual plants must not be done once exposure of plants to a test substance has begun as such actions invalidate the test through the introduction of bias or variable test duration among test organisms. At intermediate times, and at the conclusion of the exposure period, tallies of survival and measures of shoot growth and development are made.

4.3 For phytotoxicity tests, 100 to 200 µmol m^{-2} s^{-1} of visible light (or photosynthetically active radiation, 400 to 700 ηm) has been found to be a broadly applicable fluence rate. In some cases, different light levels or spectral ranges (for example, solar ultraviolet) may be required. Guide E1733.

4.4 Measured endpoints and other observational data are used to calculate response levels in terms of ECxx or ICxx (where xx refers to a specified percentage response), or categorical descriptions of phytotoxic effects (for example, proportion of plants exhibiting abnormal development or other symptomatic indices that might be scored in qualitative terms) relative to controls. These are interpreted to characterize phytotoxic effects attributed to test substances.

5. Significance and Use

5.1 Terrestrial phytotoxicity tests are useful in assessing the effects of environmental samples or specific chemicals as a part of an ecological risk assessment (**3-6, 12, 13**).

5.2 Though inferences regarding higher-order ecological effects (population, community, or landscape) may be made from the results, these tests evaluate responses of individuals of one or more plant species to the test substance.

5.3 This guide is applicable for: (*a*) establishing phytotoxicity of organic and inorganic substances; (*b*) determining the phytotoxicity of environmental samples; (*c*) determining the phytotoxicity of sludges and hazardous wastes, (*d*) assessing the impact of discharge of toxicants to land, and (*e*) assessing the effectiveness of remediation efforts.

6. Apparatus

6.1 *Facilities*—The preparation of the test, test soil medium, storage of soil and seeds, and all stages of a test procedure must