Designation: E1197 – 12 (Reapproved 2021)

Standard Guide for Conducting a Terrestrial Soil-Core Microcosm Test[1]

This standard is issued under the fixed designation E1197; 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 defines the requirements and procedures for using soil-core microcosms to test the environmental fate, ecological effects, and environmental transport of chemicals that may enter terrestrial ecosystems. The approach and the materials suggested for use in the microcosm test are also described.

1.2 This guide details a procedure designed to supply site-specific or possibly regional information on the probable chemical fate and ecological effects in a soil system resulting from the release or spillage of chemicals into the environment in either liquid or solid form.

1.3 Experience has shown that microcosms are most helpful in the assessment process after preliminary knowledge about the chemical properties and biological activity have been obtained. Data generated from the test can then be used to compare the potential terrestrial environmental hazards of a chemical.

1.4 *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.*

1.5 *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:*[2]

D422 Test Method for Particle-Size Analysis of Soils (Withdrawn 2016)[3]

D511 Test Methods for Calcium and Magnesium In Water

D515 Test Method for Phosphorus In Water (Withdrawn 1997)[3]

D1426 Test Methods for Ammonia Nitrogen In Water

D2167 Test Method for Density and Unit Weight of Soil in Place by the Rubber Balloon Method

D2216 Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass

D2488 Practice for Description and Identification of Soils (Visual-Manual Procedures)

D3867 Test Methods for Nitrite-Nitrate in Water

2.2 *U.S. Environmental Protection Agency:*

Environmental Effects Test Guidelines, EPA 560/6-82-002, 1982[4]

Chemical Fate Test Guideline, EPA 560/6-82-003, 1982[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *soil-core terrestrial microcosm*—an intact soil-core containing the natural assemblages of biota surrounded by the boundary material. The system includes all equipment, facilities, and instrumentation necessary to maintain, monitor, and control the environment.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *terrestrial microcosm or micro-ecosystem*— a physical model of an interacting community of autotrophs, omnivores, herbivores, carnivores and decomposers within an intact soil profile. The forcing functions, for example, light intensity and duration, water quality and watering regime, temperature, and toxicant dose for the test system, are under the investigator's control. This test system is distinguished from test tube and single-species toxicity tests by the presence of a natural

[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 Jan. 1, 2021. Published February 2021. Originally approved in 1987. Last previous edition approved in 2012 as E1197–12. DOI: 10.1520/E1197-12R21.

[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.

[4] Available from the Office of Pesticides and Toxic Substances, Washington, DC. Also available as PB82 – 23992 from National Technical Information Service (NTIS), United States Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161.

[5] Available from Office of Pesticides and Toxic Substances, Washington, DC. Also available as PB82 – 233008 from National Technical Information Service (NTIS), United States Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161.

assemblage of organisms. This assemblage creates a higher order of ecological complexity and, thus, provides the capacity to evaluate chemical effects on component interactions and ecological processes. Certain features of this test system, however, set limits on the types of questions that can be addressed. Those limitations are related to scale and sampling, which in turn constrain both (*a*) the type of ecosystems and species assemblages on which one can gain information, and (*b*) the longevity of the test system.

3.2.2 *physical, chemical, and biological conditions of test system*—determined by the type of ecosystem from which the test system was extracted and by either the natural vegetation in the ecosystem or the crops selected for planting. Vegetation and crop selection are constrained and determined by the size (width and depth) of the soil core extracted.

3.2.3 *boundaries*—the boundaries of the test system are determined by the size of the soil-core and the space needed for vegetative growth.

3.2.4 *light*—light for the test system can be supplied by artificial means in either a growth chamber or a greenhouse, or it can be the natural photoperiod occurring in a greenhouse. If the test is performed in a growth chamber, the daily photoperiod should be equal to or greater than the average monthly incident radiation (quantity and duration) for the month in which the test is being simulated. During extremely short natural photoperiods, which might not allow for flowering or seed-set, photoperiod should be artificially lengthened to induce those responses. The spectral quality of visible light supplied during testing should simulate that of sunlight (for example, include commercially available visible full-spectrum lamps).

3.2.5 *water*—water for the test system should either be purified, untreated laboratory water, should be precollected, filtered rainwater from the site or region being evaluated, or formulated rainwater (for example, based on rainfall of the region). Chemical characterization of the water, either laboratory or rainwater, is required and must be performed using Test Methods D511, D515, D1426, and D3867.

3.2.6 *soil*—the soil-core used for the microcosm test should be an intact, undisturbed (nonhomogenized) core extracted from a soil type typical of the region or site of interest. The core should be of sufficient depth to allow a full growing season for the natural vegetation or the crops selected, without causing the plants to become significantly rootbound. Disturbances during extraction and preparation should be kept to a minimum. It should be noted that soil characteristics play an important role in how the microcosm responds to a test substance. In addition, within-site soil heterogeneity also influences the microcosm response and contributes to a loss of sensitivity of the test. The approach used in this test system, however, is based on a comparison of responses among and between treatments rather than on the absolute values measured.

3.2.7 *biota*—the biota of the microcosm are characterized by the organisms in the soil at the time of extraction **(1, 2)**[6] and by the natural vegetation or crops introduced as the autotrophic component. The biota may include all heterotrophic and carnivorous invertebrates typically found in the soil and all soil and plant microbes.

4. Significance and Use

4.1 This guide provides a test procedure for evaluating the potential ecological impacts and environmental transport of a chemical in an agricultural (tilled, low-till, or no-till) or natural field soil ecosystem that may be released or spilled into the environment. The suggested test procedures are designed to supply site-specific information for a chemical without having to perform field testing. (See EPA 560⁄6-82-002 and EPA 560⁄6-82-003.)

4.2 This guide is not specifically designed to address fate of chemicals in soils of forested ecosystems. However, with some modifications, it may be adapted for that purpose by the individual investigator.

4.3 Specifically, this guide is used to determine the effect of a chemical on (*1*) growth and reproduction of either natural grassland vegetation or crops, and (*2*) nutrient uptake and cycling within the soil/plant system. Additionally, the soil-core microcosm will provide information on (*1*) potential for bioaccumulation (enrichment) of the chemical into plant tissues, and (*2*) the potential for and rate of transport of the chemical through soil to groundwater.

4.4 The results of this test should be used in conjunction with information on the chemical and biological activity of the test substance to assess the relative environmental hazard and the potential for environmental movement once released.

4.5 The test methods described in this guide are designed specifically for liquid or solid materials. Significant modifications of the exposure system would be necessary to accommodate chemicals that are volatile or that may be released in a gaseous or aerosolized form. For methods that could be adapted for use with volatile or gaseous test substances see Refs **(3, 4, 5, 6).**

4.6 Results of a multi-year soil-core microcosm test have been correlated with data derived from a series of multi-year field plot tests for a limited number of materials. Information on the correlation between microcosm and field results can be found in Refs **(7, 8, 9, 10).**

5. Chemical Characterization of Test Substance and Soil

5.1 *Information Required on Test Substance:*

5.1.1 Minimum information required to properly design and conduct an experiment on a test chemical includes the chemical source, composition, degree of purity, nature and quantity of

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

any impurities present, and certain physiochemical information such as water solubility and vapor pressure at 25 °C (**11**, **12**). Ideally, the structure of the test chemical should also be known, including functional groups, nature and position of substituting groups, and degree of saturation. The octanol-water-partition coefficient, the dissociation constant, the degree of polarity, and the pH of both pure and serial dilutions should also be known. Where mixtures are involved or where a significant impurity (>1 %) occurs, data must be available on as many components as practical. However, the octanol-water-partition coefficient (K_{ow}) stands out as a key value for lipophilic compounds. Soil partition coefficient (K_d) can be determined or estimated, and organic carbon partition coefficient (K_{oc}) can be estimated from log K_{ow} using the organic matter content. Water solubility can be predicted with some degree of accuracy from log K_{ow} if this value is less than seven. In combination with other chemical characteristics, log K_{ow} can also be used to estimate Henry's Law Constant and thus provide a rough estimate of the potential volatility of the test substance from soil solutions.

5.1.2 Several tests may be needed to supply information on environmental mobility and stability. Support information on phytotoxicity, the physicochemical nature of the chemical, its mammalian toxicity, or its ecological effects (for example, species-specific LC_{50}, invertebrate toxicity, biodegradability) not only assist in proper design of the microcosm experiment, but also are useful in assessing the fate and effects of the chemical in a terrestrial microcosm. If the chemical is radioactively labeled, the position and specific element to be labeled should be specified.

5.1.3 It is imperative to have an estimate of the test substance toxicity to mammals as a precaution for occupational safety. In addition, hydrolysis or photolysis rate constants should be known in order to determine necessary handling precautions. When a radiolabeled material is used, normal laboratory techniques for radiation safety provide an ample margin of safety (**13**), except for chemicals in the "very highly toxic" category (rat oral LD_{50} <1 mg/kg). In this case a combination of radiation safety and chemical safety procedures should be followed. For additional information on individual compounds, see Refs (**14**, **15**, **16**, and **17**).

5.1.4 Water solubility, soil sorption and octanol-water partitioning, and vapor pressure largely will control the physical transport and bioavailability of a test chemical in soil. Water-soluble chemicals are likely to move with soil water into the water films surrounding soil particles and root surfaces. Most microbially-mediated biodegradation occurs in the water-containing microsites of soil particles. Plant uptake and bioaccumulation is largely a function of water transfer to roots, active or passive uptake, internal partitioning (hydrophilic and inorganic compounds) and solubility in fatty tissues. In addition, water-soluble chemicals and their transformation products may be leached to groundwater. Water solubility of an organic chemical is a function of the dissociation of ionic compounds and the polarity of nonionic compounds.

5.1.5 Compounds with very high vapor pressures (boiling point <80 °C or vapor pressure >25 mm Hg) are not suitable for testing in the terrestrial soil-core microcosm described in this guide. According to Refs (**6**, **18**), modification of the test system should be useful for handling gaseous or aerosolized chemicals.

5.2 *Information Required on Soil:*

5.2.1 Soil sorption of an organic molecule depends on several properties of the chemical (molecular size, ionic speciation, acid-base properties, polarity, and nature of functional groups) and of the soil (for example, organic matter content, clay content, clay mineralogy and nature, pH, water content, bulk density, cation exchange capacity, and percent base saturation). Highly sorbed chemicals may displace inorganic nutrient ions from exchange sites in the soil and also may be effectively immobilized, depending on soil pH. Thus, chemicals attracted more strongly to soil surfaces than to water may be very immobile in soil. In some cases, this may render the compound relatively resistant to biodegradation. In other cases, however, immobilization of the compound on soil particles may render it susceptible to extracellular enzymatic degradation. Specific information on descriptive data required for soil can be found in 6.2.2.

6. Terrestrial Microcosm Extraction and Maintenance

6.1 *Microcosm and Chamber Design:*

6.1.1 A ≥ 60-cm deep by ≥ 10-cm diameter terrestrial soil-core microcosm is designed to yield pertinent information about a chemical for either a natural grassland ecosystem or an agricultural ecosystem planted with a multiple-species crop (Fig. 1) (**7**, **19**, **8**, **9**, **20**, **21**). The agricultural microcosm is a 10 to 17-cm diameter tube of plastic pipe that is made of ultra-high molecular weight, high-density, and nonplasticized polyethylene and contains an intact soil core (≥ 40 cm) including topsoil. A microcosm for large plants may require an intact totally undisturbed 17-cm diameter by ≥60-cm deep test system. The plastic pipe should be impermeable to water, light-weight, tough, rigid, and highly resistant to acids, bases, and biological degradation. Additionally, one should use plastic pipe that does not release plasticizers or other compounds that may interfere with test results. At the bottom of each pipe containing a soil-core, a controlled-pore ceramic plate should be installed in direct contact with the intact soil-core; this controlled-pore ceramic plate should be installed air-tight, and contained within an appropriate end-cap (**19**) where leachate may flow by gravity for collection into a receiving flask, or transfer into flask accomplished by transfer at intervals using an inert gas (**19**) (Fig. 1 and Fig. 2). The controlled-pore ceramic is included so that a partial-tension (30-35 kPa) may be applied at the bottom of each microcosm to mimic field conditions, thus preventing undue buildup of water within the microcosms that otherwise would change chemical, physical, and biological properties of the microcosm for all except very light-textured soils (for example, sands and loamy sands).

6.1.2 Six to twelve microcosms and receiving flasks are typically contained within a temperature controlled chamber packed with insulation beads, to reduce drastic changes in temperature profile (**19**, **20**) (Fig. 2). Chamber dimensions are determined by the size required and space availability within the greenhouse. Tops of chambers have aperatures to accommodate each microsm, so that tops of microcosms are exposed

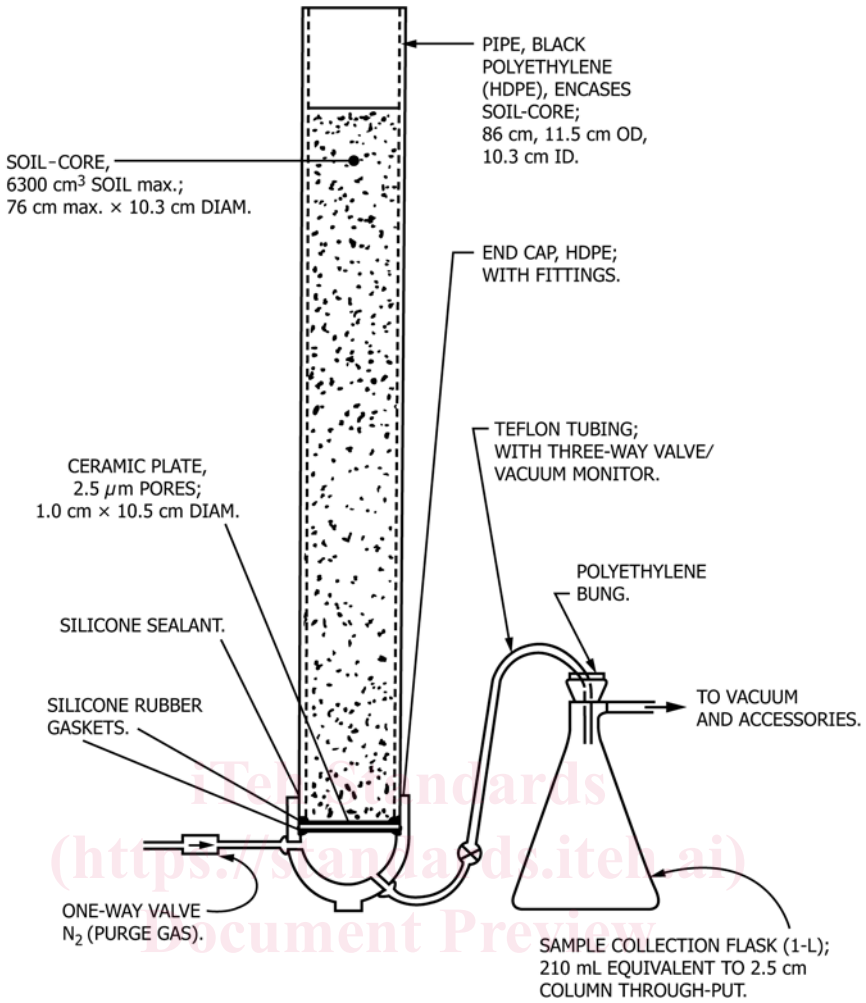

FIG. 1 Microcosm Structure and Materials (19)

to incident light and temperature. Each flask receiving leachate from an indivisual microcosm is housed in darkness within the chamber, at the same controlled temperature as the microsms. Leachates are kept in darkness at the same temperature as the microcosm to simulate field conditions, and avoid undue degradation of chemicals under investigation.

6.2 *Soil Core Extraction:*

6.2.1 Soil cores are extracted from either a natural grassland ecosystem, a typical agricultural soil in the region of interest, or from the ecosystem of interest within the region. The intact system is extracted with a specially designed, steel extraction tube (**7, 19, 8, 9, 20, 21, 22**) (Fig. 3) and a backhoe. The steel extraction tube encases the polyethylene pipe to prevent the tube from warping or splitting, or both, under pressures created during extraction. Once the core is cut by the leading edge of the driving tube, it is forced up to the microcosm tube. For the agricultural microcosm, the plowed topsoil is moved aside and saved. For the natural grassland ecosystem, the vegetation is clipped before the core is extracted. For ecosystem microcosms, existing vegetation may be retained, or removed (especially important when natural vegetation is large); vegetation of interest may then be subsequently planted. The soil-core microcosm is later removed as a single unit (soil and plastic pipe) from the extraction tube and taken to the laboratory. For the agricultural microcosm, the topsoil is backfilled into the upper portion (for example, 20 cm) of the microcosm tube. The extraction procedure as described here does disrupt and compress the soil-core to a certain extent. This should not, however, influence the conclusions drawn from the tests because the evaluation is being performed on the difference between the response of treatments versus controls rather than the absolute response.

6.2.2 Detailed chemical and physical properties of the soil in the test systems are to be determined using USDA nomenclature. Information such as pedologic identity, according to the USDA 7th Approximation Soil Classification System, percent organic matter, hydraulic characteristics, cation exchange capacity, bulk density, macro- and micro-nutrient content, organic matter content, mineralogy, exchange capacity, particle-size distribution, hydraulic characteristics, and other important characteristics should be measured before and after the experiment, depending on the relative hazards of the test substance (see Refs (**23, 24**), Test Methods D422, D2216, and D2167, and Practice D2488). The history of the

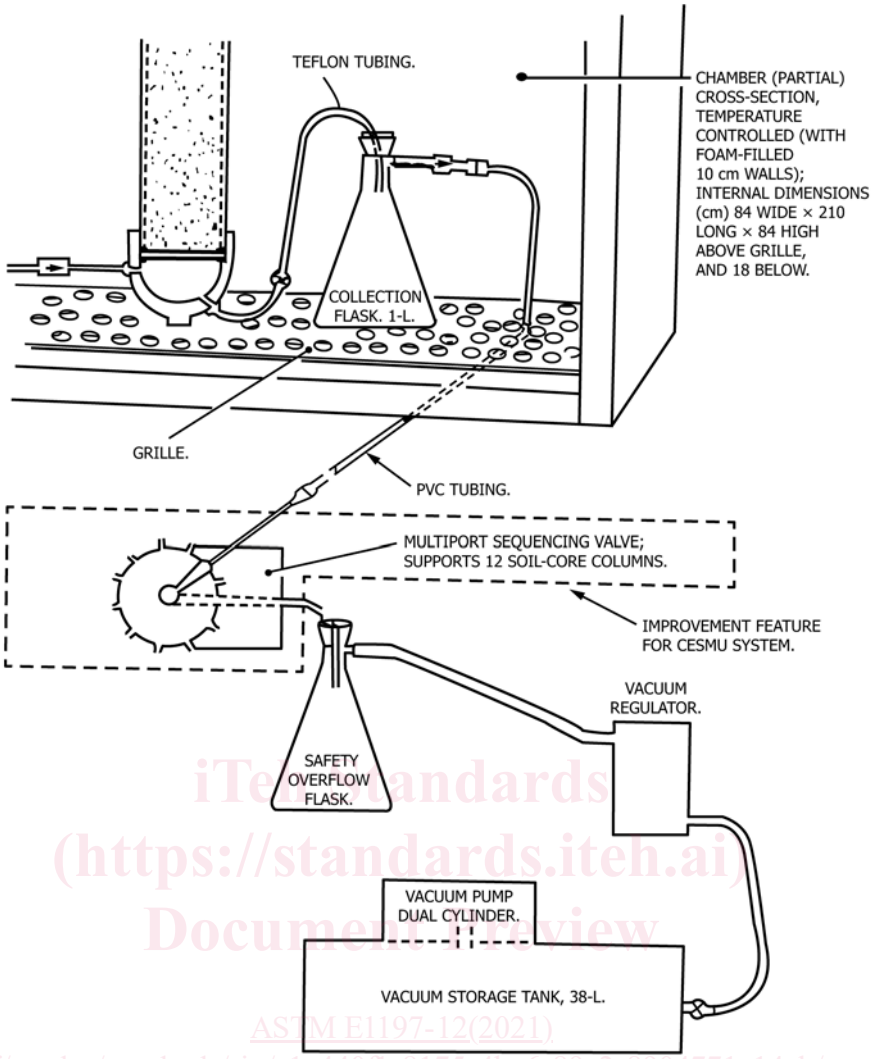

FIG. 2 Arrangement of Microcosm and Support Apparatus within Temperature Controlled Chamber

soil, including previous crops grown, pest control, and other management practices used, should be documented to assist in the interpretation of the results.

6.3 *Microcosm Vegetation and Harvesting:*

6.3.1 For the natural ecosystem (undisturbed grassland) test system, natural plant cover should be sufficiently diverse to be representative of plant species in the ecosystem of interest. When the agricultural microcosm is used, a mixture of grasses and broad leaves (for example, legumes) should be included. Two or three species of grasses or legumes that are typically grown together as an agricultural crop in the region of interest should be chosen. The species chosen must have compatible growth habits and be able to grow to maturity in the small surface area (for example, 83.3 cm^2 for 10.3–cm diameter to 227 cm^2 for 17– cm diameter) of the microcosm. In some cases, it may be appropriate to select a grain crop normally grown for human consumption to evaluate the uptake of the radiolabeled test substances and their degradation products (**7**).

6.3.2 The seed application rate should duplicate standard farming practice for the region of interest in agricultural microcosms. Seeds should be planted evenly and covered with an appropriate depth of soil. Similarly, the method used to apply the test substance should approximate the way in which the test substance might arrive at the site in question. For example, solid test substances might be mixed with the topsoil before planting, thus mimicking the plowing of an agricultural field before seed is sown. Alternatively, it may be dusted on the surface to simulate dry deposition.

6.3.3 For an agricultural system, harvesting of plant tissues should be consistent with those practices used in a given region. Plants from units are harvested from each microcosm at the end of the test period (**20**, **25**). They are then air dried and then oven dried. In the range-finding test (see 7.3.1) the crop is harvested four weeks after first exposure to the test substance. In the definitive test (see 7.4.1) plants may be harvested one or two times during the 12-week growing period or at the end of the test. The definitive test may need to be extended beyond the 12-week test period to accommodate plant species that take longer to reach the desired maturity (for example, seed production).

6.4 *Microcosm Watering and Leachate Collection:*

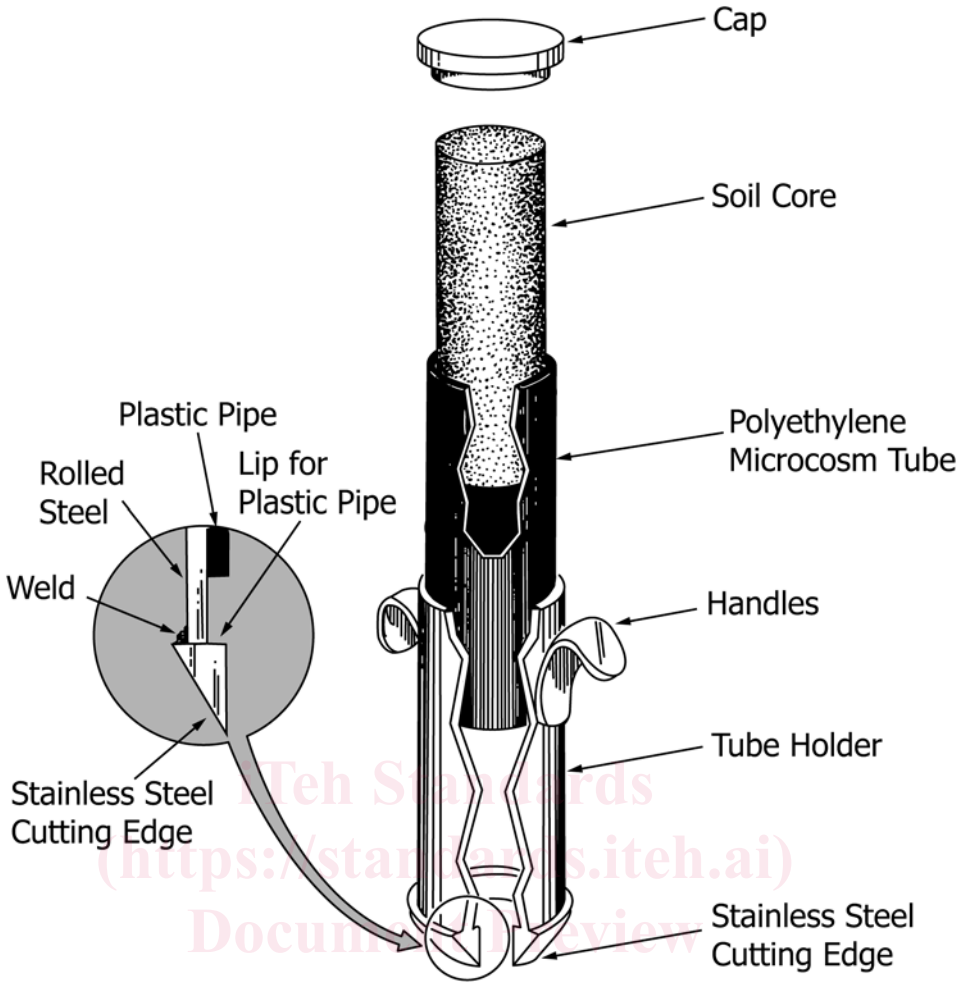

FIG. 3 Diagram of Microcosm Extraction Tube (8)

6.4.1 Microcosms are watered as dictated by a predetermined water regime, usually established on the basis of site history, with either purified laboratory water (for example, distilled, reverse osmosis), or rainwater that has been collected, filtered, and stored in a cooler at 4 °C ; or formulated rainwater (**6, 20, 22**). If comparisons are being made between microcosms and field plots, then parallel watering in both units should be used. Care needs to be taken to deliver sufficient water while preventing overwatering, which can induce fungal disease and stress.

6.4.2 Microcosms are continuously leached by the partial pressure exerted at the controlled-pore ceramic. Natural rainfall amounts should be used to guide selection of the watering regime. Caution should be exercised to prevent overwatering, which may drastically alter the rate of degradation, transformation, translocation and transport of chemicals within the microcosm.

6.4.3 Leachate is collected at regular intervals (for example, every two days) into flasks (previously washed with 0.1 N HCl, rinsed with purified water, and dried). The 500-mL (alternatively 1-L) collection flasks are attached to receiving end-caps (ultra-high molecular weight, high-density, nonplasticized polyethylene) using vinyl tubing or other tubing that is compatible, such as polyvinyl chloride or vinyl tubing (see Fig. 2). Fifteen percent more soil cores are extracted than are required for a combination of both the range finding and definitive tests. When the microcosms are leached before planting, those which do not leach, or leach too quickly, or take longer than two days to produce 100 mL of leachate after the soil has been brought to field capacity are discarded.

6.5 *Greenhouse and Growth Chamber Environments*—Microcosms in chambers are kept in a greenhouse, or within an environmental chamber, where temperature and light can be controlled. Temperatures in environmental chambers and greenhouses are designed to approximate outdoor temperatures that occur during a typical growing season in the region of interest. If the experiment is not conducted in the greenhouse during the normal agricultural growing season, then lights suitable for plant growth, controlled by timing devices, should be used to simulate the photoperiod, intensity, and spectrum for a typical growing season in the area of interest. If the experiment is conducted in the greenhouse during periods when the photoperiod of the natural light is not long enough to induce flowering and seed set, then supplemental lighting will be required.

6.6 *Soil Sampling for Environmental Fate During the Test*—The soil in the microcosm system is not designed to be sampled

during the test. This would alter the leaching and movement of test substance in the system and make that particular microcosm useless for other test results. If it is necessary to take soil samples during the test to determine the rate of movement of the test substance at intermediate time scales, then the number of replicates will have to be increased to account for this sacrificial sample.

7. Test Procedures

7.1 *Test Purpose and Assumptions*—The purpose of the terrestrial soil-core microcosm test is to determine the fate and ecological effects of a test substance, including its transformation products, within a particular natural grassland, agricultural, or other natural ecosystem. The relationship of fate and ecological effects data from treated versus control microcosms is assumed to be very similar to that from treated versus control field plots **(7, 8, 20, 22).** This assumption is supported by the comparisons of microcosms and field results according to Refs **(7, 20, 25, 26).** The fate and effects from the microcosm test should then be related to either the natural or agricultural ecosystems that have the same combination of soil type, vegetation, crop species, and environmental variables used during the microcosm test.

7.2 *Evaluation of Test Substance:*

7.2.1 Physicochemical information supplied for the test substance (see 5.1.1) is used to tailor the general range-finding test procedures to the specific substance. Phytotoxicity, or bacteriostatic, action, or both, if known, should be taken into account when designing the exposure concentrations of the range-finding experiment. If the information is available, only one concentration above that known to cause at least 50 % change in plant growth or 50 % change in bacterial growth/respiration will need to be tested. In any case, the lowest treatment level should not be less than 10 times greater than the analytical limits of detectability of the parent compound at the start of the experiment.

7.2.2 The water solubility and soil sorption capacity can be used to determine the appropriate frequency of leachate analyses for the radiolabeled test substance and its transformation products. This same information will also determine the design of the soil sampling procedures for the range-finding test. Chemical structure and any degradation information is used to determine which transformation products for the soil, leachate, and plant tissue will be analyzed.

7.2.3 As stated in 6.3.2, exposure should approximate a reasonable scenario. Additionally, one must account for the water solubility, dissociation constant(s), and soil pH when determining the concentration and when selecting the specific formulation of the chemical to apply. Solubility, however, may be markedly altered by ionization in soil. If the soil pH is such that a more soluble form is likely, adjust accordingly the test substance pH with either sodium hydroxide or hydrochloric acid before adding to the soil in the microcosm. If the pH adjustment to increase solubility is extreme ($4 < pH > 9$), chemical and photolytic degradation may be enhanced when preparing the chemical solutions.

7.3 *Range-finding Test:*

7.3.1 The range-finding test should last a minimum of four weeks from first exposure of the test substance to final harvest. At the start of the test, the microcosms are dosed with a minimum of five concentrations of the test substance. Three replicate microcosms are used for each of the four or five treatment levels and the controls, resulting in a total of 15 or 18 microcosms. Concentrations typically used are 0.1, 1.0, 10, 100, and even 1000 µg/g within the upper 20 cm of topsoil of the microcosm if a realistic scenario is not known. The logarithmic scale for concentration in a range-finding test is suggested by Rand **(27).** The bulk density (g/cm^3) of the dry topsoil is used to calculate the concentrations. Depending on mode of release of the test chemical, select either a single, or a multiple application, based on a reasonable exposure scenario.

7.3.2 When possible, randomly move each chamber, holding one replicate of each of the four or five test concentrations and a control, in the greenhouse each week to avoid location-induced effects. When such rotation is not possible, chambers should include a complete random set(s) of treatments and block effects investigated.

7.3.3 The range-finding tests yield two necessary types of information. These are (*1*) estimates of the bounds of toxicity within which the 50 % response (for example, LC_{50}) lies, and (*2*) initial estimates of variance in response. Given the identification of bounds of toxicity for the range-finding tests, the concentrations for the definitive tests may be refined. Use the variance estimates to determine sample sizes needed in the definitive tests to achieve statistical tests able to detect specified differences (Δ) among concentrations with a specified power ($1-\beta$).

7.4 *Definitive Test Experimental Design:*

7.4.1 The definitive test lasts for 12 or more weeks from first exposure of the test chemical to final harvest. Test results may be influenced by extraneous environmental sources of variation, such as temperature or light gradients within a greenhouse. These sources of variation may be accounted for by randomly repositioning the chambers, or by using randomized block, latin-square, or other more complex experimental designs. If such extraneous sources of variability in test results are not taken into account, results may be biased, thus jeopardizing the outcome of the experiment. The types of statistical analyses to be performed are decided at this point and are dictated largely by the experimental and treatment designs. The experimental design determines the method of randomization of the treatments to account for extraneous sources of variability in the experiment environments. The treatment design determines the number of treatments and the arrangement of treatments with respect to one another.

7.4.2 At the start of the test, the microcosms are dosed with three concentrations of the test substance. Determine the number of microcosms to be dosed by the desired power of the statistical tests. Power is influenced by the variance of the response (estimated from range-finding tests), the size of the difference to be detected among the treatments, and the alpha (α) level. The desired power, alpha level, and detectable difference are specified by the researcher, and the variance estimates are obtained from the range-finding tests. Based on