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# 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 problems, 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.*

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

D422 Test Method for Particle-Size Analysis of Soils
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D515 Test Method for Phosphorus In Water (Withdrawn 1997)[3]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 Procedure)
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 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

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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.

[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.

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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 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

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

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 (30-35kPa) 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 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

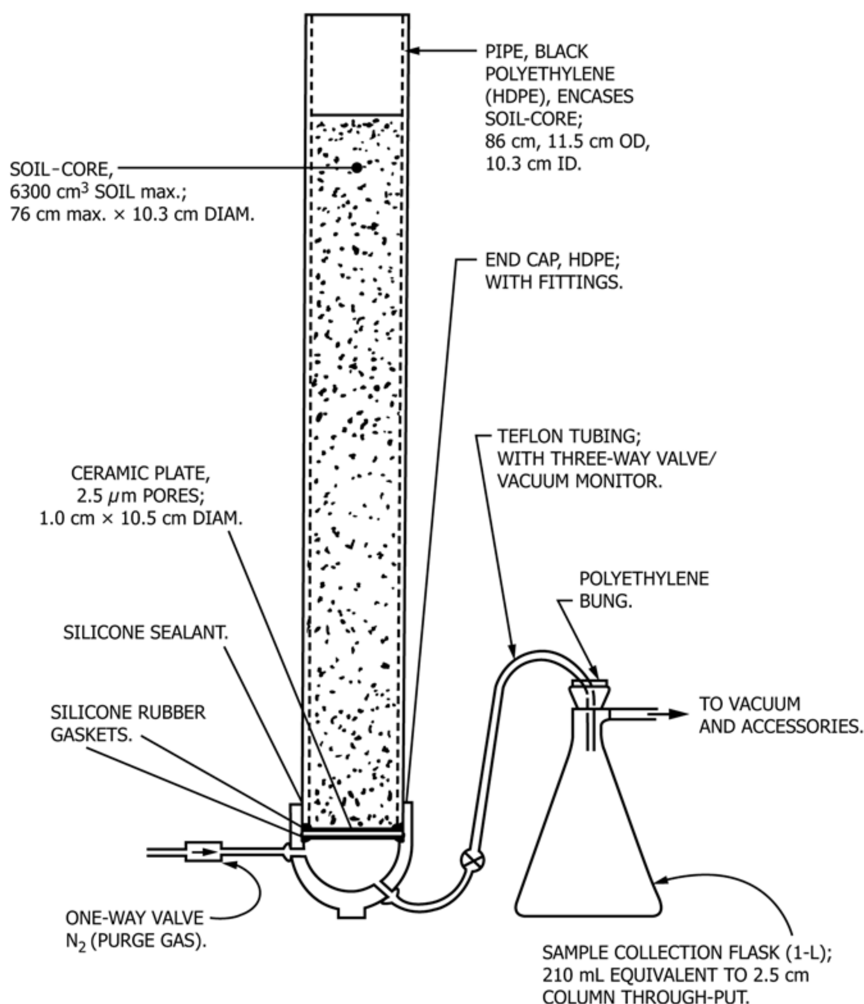


**FIG. 1 Microcosm Structure and Materials (19)**

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 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

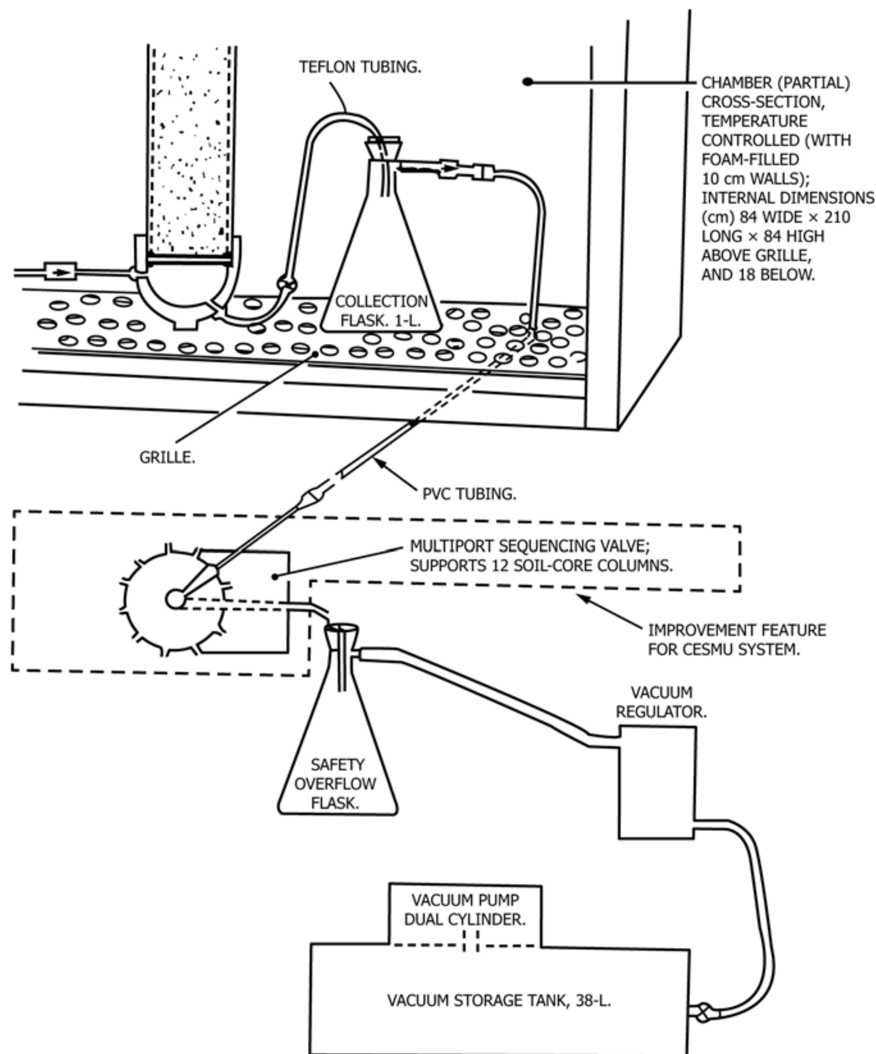


**FIG. 2 Arrangement of Microcosm and Support Apparatus within Temperature Controlled Chamber**

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:*

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

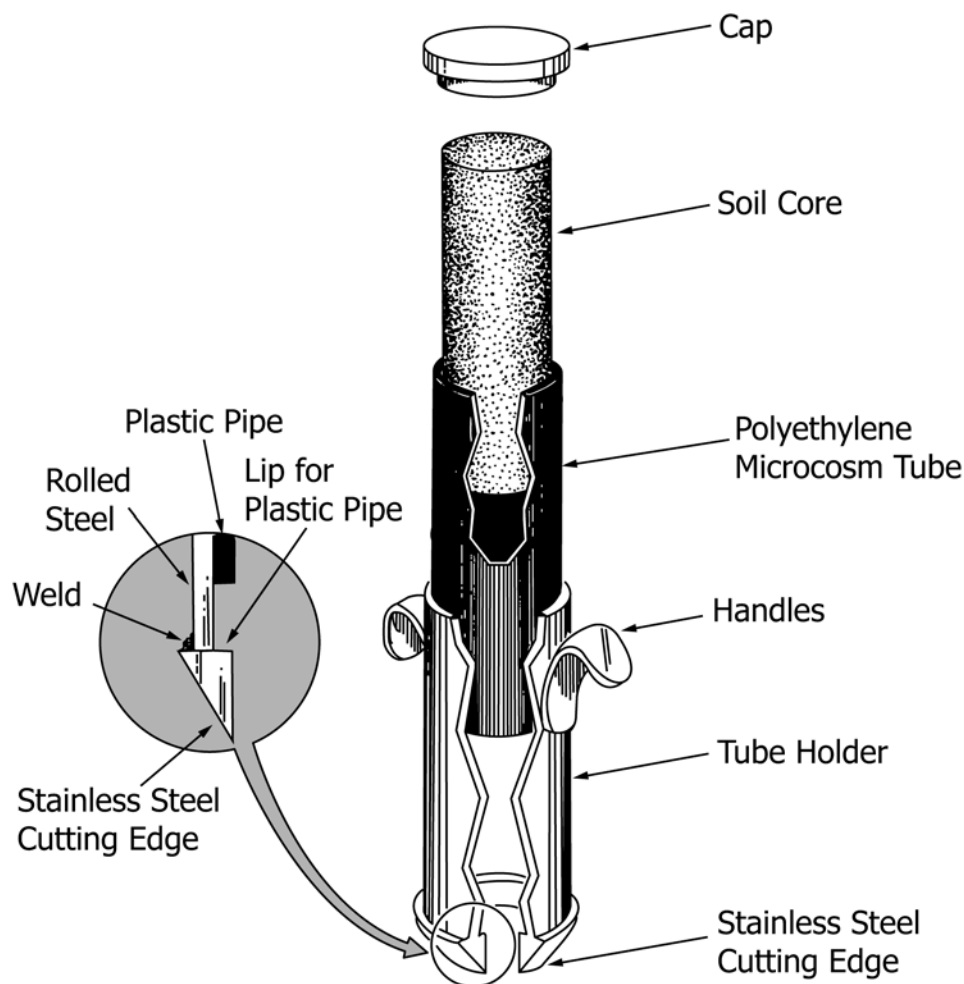


**FIG. 3 Diagram of Microcosm Extraction Tube (8)**

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 ($\Delta$) 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 ($\alpha$) 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 these four values, determine the sample size, or number of replicates for each treatment level. See Refs (**28, 29**) for discussion of power of a test. The three treatments chosen are estimated from the range-finding test data to produce a 20 % to 25 % change in productivity for each subsequent concentration

of the test chemical. Reduce analytical costs associated with the fate studies by using the replicate microcosms in each treatment as replicate pairs. Thus, leachate and plant tissue analyses are conducted on the pooled specimens from paired microcosms. However, pooling of specimens will reduce the power of the test and reduces the effective number of replicates. Productivity data, on the other hand, are analyzed for each individual microcosm. Each cart holds six to twelve microcosms (see Fig. 2). Place the microcosms paired for analyses in different carts to ensure that all microcosms are housed under similar conditions.

7.4.3 Depending on the type of natural vegetation or crop planted, it may be possible to harvest more than once, such as during the middle and at the end of the test. If growth is vigorous, harvest grasses at a pre-arranged height, for example, 2 to 6 cm above soil surface during the middle of the 12-week test period. Multiple harvests permit evaluation of both gross plant yield and plant uptake of the test substance with respect to time **(7, 8, 30).**

7.5 *Exposure Techniques:*

7.5.1 If the primary mode of exposure of the test chemical is anticipated to be by addition of pH-adjusted laboratory water or rainwater containing appropriate concentrations of the test substance, then use the following exposure techniques. In no case shall the total aqueous volume of a single exposure be sufficient to cause leaching any of the microcosms. Test substances that are likely to be released into the environment as a liquid or solid, and which can be mixed with water, are applied as a single exposure sufficient in volume to bring the microcosm to field capacity. The volume of laboratory water or rainwater required for exposure can be determined using an unplanted microcosm of the same soil type. The volume selected should be the same for all microcosms. Carriers other than water are not recommended unless they are likely to be released into the environment in conjunction with the test substance in an effluent stream. If a carrier is necessary, then consider acetone or ethanol. However, avoid the use of carriers unless they are essential to produce a realistic exposure. Also, tests for carrier effects should be required with inclusions of additional microcosms in the experimental design for this purpose.

7.5.2 Several typical exposure modes are suggested for particular types of test substances if either a hypothetical or real (actual) exposure scenario is not available. If the test substance is likely to be a contaminant of irrigation water, apply the test substance daily or weekly in proportionate concentrations, such that the total amount applied equals the desired concentration. If the test substance does not mix with water, apply it as evenly as possible to the top of the unplanted microcosm and mixed into the topsoil prior to planting. If the test substance is normally sprayed on growing plants (for example, pesticide), then mix the desired amount with the volume of solvent or water necessary to wet the soil surface and wet the plants to the point where they begin to drip. Use a chromatography sprayer or nebulizer used to spray plants that are past the seedling stage. Follow the recommendations by the test substance manufacturer for field spraying as closely as possible, but terminate the test (last harvest) at least eight weeks after the plants are sprayed.

7.6 *Waste Disposal:*

7.6.1 Retain all liquid (leachate) and solid (soils and plant tissues) specimens for proper disposal. Clean (acid wash) all specimen collection bottles, collection apparatus, microcosm tubes, and sampling tools thoroughly and analyze for radioactive contamination before they are stored or used on another test system. Dispose of all samples and the remaining, undisturbed portion of the test system in accordance with United States Environmental Protection Agency (USEPA) and Nuclear Regulatory Commission (NRC) regulations, if radiolabeled compounds were used. Treat soil leachate and all other aqueous-sample wastes prior to disposal using one or more of the following techniques: (*a*) filtration, (*b*) activated charcoal filtration, or (*c*) ion exchange.

7.6.2 Soils contaminated with organic residues or radiolabeled compounds, or both, as well as the plastic pipe, sample bottles, glassware, gloves, masks, filters, activated charcoal from aqueous cleanup, and any other potentially contaminated equipment must be either certified as uncontaminated or packaged and disposed of in accordance with existing USEPA and NRC guidelines and regulations.

## 8. Fate and Effects Sampling Procedures

8.1 Sampling procedures have been divided into two basic categories: ecological effects sampling and test-chemical fate sampling. Ecological effects sampling may include productivity measurements, physical appearance of plants, and nutrient loss or uptake measurements. Test-chemical fate sampling may include leachate, soil, and plant analyses.

8.1.1 *Ecological Effects Sampling—Productivity Measurements:*

8.1.1.1 Primary productivity is a commonly measured parameter in terrestrial effects testing. Depending on the plant species, it may be desirable to report total yield or yield by plant part. For example, in the case of grain crops, such as soybeans, oats, and wheat, the total biomass yield can be reported in addition to the grain yield. This will allow total biomass to be compared with grain yields typically reported for local agriculture. In addition, separate grain samples may be useful for later tissue analyses to determine whether the test chemical was enriched in potentially edible plant parts. For other systems, such as natural grassland microcosms, segregation into plant parts may be unnecessary.

8.1.1.2 Productivity should be reported as oven-dry weight. According to Jones and Steyn **(31)** 65°C for 24 h are adequate conditions for drying without unnecessary thermal decomposition of plant material. Evaluate information on the chemical volatility when selecting a drying temperature. It may be desirable in some circumstances to report air-dried productivity or to be able to calculate air-dried yields based on moisture loss after oven-drying. These data could be useful if agricultural crops are the plants used in the microcosm and if it is desirable to compare productivity with yields reported in local agriculture.

8.1.1.3 The number of harvests will depend on the types of plants grown. An agricultural crop, alfalfa/timothy for

example, may require two or more harvests over the course of the testing period (**7, 32**).

8.1.2 *Physical Appearance of Plants*— Throughout the test period, it is desirable to record the physical appearance of plants in the terrestrial microcosm. Monitor symptoms of nutrient deficiency or toxicity, pathogenicity, water stress, or test-chemical-induced toxicity. These observations may be useful in interpreting the specific ecological effects of a test chemical relative to responses in plants elicited by known environmental toxicants or stresses (**33**). Careful observation on physical appearance in controls versus treated microcosms may also aid in determining whether abnormal physical appearance is a result of the test chemical or is a manifestation of microcosm management.

8.1.3 *Nutrient Loss Measurements:*

8.1.3.1 An important ecological effects sampling procedure is to monitor nutrient losses in leachates (**6, 26, 34, 35**). The rationale for such monitoring is explained in detail in Refs (**8, 9**). One of the desirable attributes of the terrestrial microcosm approach to testing chemicals is the relative ease with which soil leachates can be collected. This approach offers the potential to construct nutrient budgets for the model ecosystem (**36, 37**).

8.1.3.2 The final suite of nutrients monitored in leachates probably will depend on the nature of the test chemical (**38, 35, 39, 40**). Consider initially those nutrients during the range-finding test that include calcium, potassium, nitrate-nitrogen, ortho-phosphate, ammonium-nitrogen, and dissolved organic carbon (DOC). Depending on the results of nutrient losses measured during the range-finding test, a set of nutrients can be selected for monitoring during the definitive test.

8.1.3.3 Various methods exist to analyze for nutrients. Standard techniques proven useful include atomic absorption spectrophotometry for Ca and K and analysis using a Technicon Autoanalyzer II for nitrate-nitrogen, ortho-phosphate, DOC, and ammonium-nitrogen. See Method D511, Test Methods D515, D1426, and D3867 and Refs (**41, 42**) for more information. For less rigorous determinations, such as during the range-finding test, ion-specific electrodes may be useful for nitrate- and ammonium-nitrogen detection.

8.1.3.4 A standard procedure, described below, has proven to be useful in handling leachates. As soon as soil water (that is, leachate) samples are collected, the sample volume is recorded and the pH determined using a glass electrode. Samples are centrifuged at low speed (5000 r/min) to remove large particles and the remaining liquid is passed through a 0.45-µm filter. Divide the specimen into two aliquots prior to storage in the dark at 4°C. Prepare and store similarly blanks consisting of distilled water and reference standards in instrument calibration quantities.

8.1.4 *Test-Chemical Fate Sampling:*

8.1.4.1 The fate of the test chemical (see 2.2) will be determined by methods appropriate to the test, including sensitivity factors adequate to verify exposure and distinguish between parent material, transformation products, and naturally occurring materials present in the test system. Usually this test will involve use of a radiolabeled parent compound and subsequent analysis of microcosm components for radioactivity and chemical identity. Methods appropriate to the latter may be adequate for quantification of fate, but usually cannot reveal bound residues in soil or plants and frequently are inadequate for cost-effectively tracing movement and transformation. To the extent that the fate in soil and plants is well enough understood from other experiments and depending on the degree to which the microcosm test is being used to verify fate and exposure hypotheses, analytical requirements may be reduced (**43, 44, 45, 46, 47**). If sampling of soils is planned during the experiment, then increase the number of replicates accordingly.

8.1.4.2 *Radiolabeling the Parent Compound*— Label the parent compound with $^{14}C$ either in an appropriate aromatic, cyclic carbon group, or in a linear chain (**13, 48**). Other labels, including stable isotopes such as $^{15}N$, may be more useful and informative. In order for the microcosm test to permit an analysis of the fate of the parent compound, or its metabolites, or both, consider the known or hypothesized metabolic pathways for test substances. Hence, the location and form of label is an integral part of the total test design. The laboratory conducting the test is not required to have the capability for radiolabeling, since this is routinely handled by specialty chemical firms. Sufficient radioactivity must be present in order to detect at least 1 % of the initial parent compound in a typical sample of leachate, soil, or plant tissue.

8.1.4.3 *Compartment Analysis for Labeled Compounds*— Analyze several compartments of the terrestrial microcosm for radioactivity. The components include samples of soil leachate, plant tissue, including roots and shoots, and soil from different depths. Select different soil depths used for radiochemical analyses based on information on soil sorption of the compound of interest. Experience indicates that these depths should be relatively close to the soil surface (1 to 2 cm) for radiolabeled chemicals that are strongly sorbed to soils. If any isotope appears in the leachate, the depth selection should be lower in the soil profile. Homogenize and extract specimens with solvents appropriate for the parent compound. Additional extraction steps may be necessary. These include acidification and extraction with nonpolar solvents, soxhlet extractions with polar or non-polar solvents, or both, alkaline or acid hydrolysis with or without heat, detergent extractions, and protease digestion. Oxidize and analyze as $^{14}CO_2$, according to Ref (**44**), the $^{14}C$ in the soil or plant samples that cannot be extracted or dissolved as described by Cole (**46**). The extracts and the oxidized or dissolved samples should be counted by $^{14}C$ liquid scintillation (**46, 47**).

8.1.4.4 At the termination of the range-finding test, collect soil samples from the top, middle, and bottom of the 60-cm soil cores. If the labeled compounds or their metabolites are not detected by liquid scintillation in the deeper soil samples, then take soil samples at the end of the definitive test closer to the top of the soil column. For definitive tests, subsamples of sections of the soil-core can be analyzed to determine fate parameters. This may be accomplished by radioanalyses, described above, or when radiolabeling is not employed by appropriate extraction and analytical determination.

8.1.5 *Identification of Degradation Products*—Liquid scintillation should identify the presence of $^{14}C$-labeled compounds in sample extracts, but the identification and quantification of the parent compound or its degradation products require gas-liquid chromatography (GLC), and thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or other appropriate analytical methods (see 2.2). Thin-layer chromatography autoradiographs using no-screen X-ray film for chromatographed fractions found to be radioactive by liquid scintillation counting (**46, 47**) is cost-effective. Whenever possible, verify the identity of the parent compound and probable degradation products in fractions found to be radioactive by liquid scintillation counting (**46, 47**) by gas-liquid chromatography methods. Also, verify the concentration of the parent compound and degradation products by an alternative chromatographic methods system (for example, HPLC or GLC) with known standards.

## 9. Data Analysis

9.1 *Ecological Effects Analysis*—Various statistical methods are recommended for analyzing data and assessing ecological effects. Regression, correlation, and covariance analyses, as well as analysis of variance (ANOVA) procedures described in the following section, may be appropriate methods. Use of these or other techniques is dictated by the objectives of the experiments and by the original experimental and treatment designs. A number of statistical references describe the common methods of statistical analysis (**49, 50, 51**). Unless otherwise specified, the level of significance for all tests is set at the 5 % level ($\alpha = 0.05$) and the power of the test ($1-\beta$) is set at 0.90 or 0.95. The results of all statistical tests performed must be fully documented. In addition, graphs and tables of all raw data should be appended to the final report describing the chemical effects on the system.

9.1.1 *Test Substance Versus Carrier Chemical Effects*—The soil-core microcosm can be exposed to test materials with or without the use of carrier chemicals. When carrier chemicals are used, their effects on the test system must be evaluated separately and in a manner identical to that recommended for the test chemical. The resulting data should be analyzed such that the influence of the carrier on the effects of the test substance can be accounted for.

9.1.2 *Productivity of Natural or Planted Vegetation*—The sum total of both air-dried and oven-dried biomass expressed in grams per square meter, $g/m^2$ collected during and at the end of the definitive test, should be evaluated initially by comparing histograms. The histograms should display the calculated means and the 95 % confidence intervals for controls and all concentrations. This method of comparison allows early visual evaluation of the effects of the chemical by exposure level. Variance estimates may indicate whether logarithmic or some other transformation of the data may be necessary for graphic display and analysis. Analysis of variance (ANOVA) calculations (**49, 51**) should be carried out first to test for position effects within the carts and within the environmental area where the test was performed. Position effects may be accounted for in the design of the experiment (for example, by blocking), and any effect of position can then be accounted for in subsequent analyses. If these tests prove to be significant at the 5 % level ($\alpha = 0.05$), then the effects of position will need to be accounted for in the remainder of the statistical analyses. Pair-wise comparisons of variables that are measured only once during the 12-week experiment may be necessary (**50**).

9.1.3 *Statistical Methods:*

9.1.3.1 Randomly assign all experimental microcosms to an experimental treatment level. This may be accomplished by using a completely randomized, randomized block, Latin-square, or other appropriate experimental design. As stated earlier, if an appropriate experimental design is not used, the results and analyses may be biased. ANOVA procedures should be performed on biomass data to determine whether or not an ecological effect resulted from the parent compound, the transformation products or the carrier compound, or a combination thereof, if used. If the ANOVA is significant, then orthogonal comparisons or a multiple-range comparison such as Duncan's Multi-Range Test (**50**) should be performed to determine which of the treatment means were different from the others. The undosed controls are considered to be one of the treatment levels. Again, the 5 % level ($\alpha = 0.05$) should be considered as the level of significance for all tests, and the power should be 0.90 or 0.95 or calculated and reported. All values, whether significant or not, must be reported for each statistical test being performed. A factorial ANOVA test should be conducted where more than a single factor or treatment is incorporated into the original experimental design.

9.1.3.2 Regression analysis should subsequently be performed on the productivity results. Outlier data, defined as an obvious data recording or reporting error, should be excluded; however, these data and the fact that they have been excluded must be reported. If a substantial number of data points have been declared as outliers, deficiencies in quality control may necessitate repeating the test. Once outlying values have been detected and removed from further statistical evaluations, use regression models to estimate $EC_{50}$ for the test substance or the concentration that reduced productivity by 50 % of mean for controls. Ordinary linear least-squares regression analysis may be performed to define the response of relevant production parameters as functions of dose. If it appears that productivity is nonlinear with respect to dose, it may be necessary to transform the data or fit either a quadratic or cubic least-squares regression model to the data for this type of response. Utilization of computer software packages such as Statistical Analysis System (SAS) or Biomedical Computer Program (BMDP) may prove useful.

9.1.4 *Physical Appearance of Plants*— Report changes in the physical appearance of plants in terrestrial microcosms for all test units. Effects of a chemical or its transformation products on plant appearance should be analyzed statistically only when a pattern of effects is evident. Clearly recognizable patterns of injury may be ranked in terms of severity. A nonparametric test, such as the Kruskal-Wallis test, may be used to test for differences in plant injury.

9.1.5 *Nutrient Losses:*

9.1.5.1 The cumulative nutrient loss of each soil-core microcosm should be calculated for each nutrient. First, the nutrient loss concentration from each collection date should be

multiplied by the total volume leached from that microcosm for that collection date. Second, that product should be added to the previous sum of total loss. Plots of the collection date versus means (±SE) of the cumulative nutrient losses for each treatment level should be graphed as a function of days after seeding for the agricultural microcosm or days after exposure for the natural grassland microcosm. Zero loss should be the starting point; if there was no leachate for any microcosm during a particular collection period, that datum should be recorded as zero so that all data are accounted for.

9.1.5.2 A one-way ANOVA should be performed on the total cumulative nutrient loss data at the end of the experiment, to evaluate the effect of treatment levels. A multiple comparison procedure, such as Duncan's **(50),** may be used to determine which specific treatment means were different from each other.

9.1.5.3 A regression/correlation analysis comparing nutrient losses with productivity should be performed to determine the relationship between these two independently measured variables. The test can be performed, however, only if the productivity and cumulative leachate loss measures are matched unit for unit. This test should be performed for each nutrient analyzed.

9.2 *Chemical Fate Analysis:*

9.2.1 At the end of test, the budget or distribution of the parent compound and transformation products is calculated for each concentration. This entails determining the amount of the parent compound that was added and the subsequent distribution of the radioactivity and transformation products through chemical analysis in each of the primary compartments: soil, plant tissue, and air of the test system as well as in the soil leachate. Fate analysis should result in distribution values for above-ground plant tissues, plant roots, each soil depth, and loss to soil leachate. Gaseous loss can be estimated. This is followed by performing statistical analyses for each concentration on any differences in distribution of the compound throughout the test system. Multicompartmental modeling and multivariable analyses, such as multivariate analysis of variance, may also prove useful in assessing the fate of parent compounds and transformation products.

9.2.2 *Radioactivity Budgets*—The calculation of a complete budget of all radioactivity must be submitted with the results of this test. Details for all calculations described in 9.2.3 – 9.4.2 can be found in Ref **(13).** The budget must show the percent of the compound that was tagged and the location of the tag. The label may be $^{14}C$, stable $^{15}$ N, or another suitable label and should be located in a portion of the molecule expected to persist or have biological activity, or both.

9.2.3 *Total Radioactivity Added*—The total radioactivity added per test unit is based on the decay rate of the radioactive tag, the total amount of radioactive tag added to the compound when initially formulated, the length of time between formulation and test unit exposure (radioactive decay), and the particular exposure level of the test unit.

9.2.4 *Total Radioactivity Removed*—The total radioactivity removed from the microcosm is determined by the concentration of the radiolabel in (*a*) the soil-leachate concentration times the volume of soil leachate lost per collection date, (*b*) the calculated gas phase losses of the compound, and (*c*) the type of radiolabel and the rate of radioactive decay of that label over the length of the experiment.

9.2.5 *Total Radioactivity Remaining*— The total radioactivity remaining in the microcosm is determined by analyzing the radiolabel in each of the primary compartments: (*a*) above-ground plant tissues, (*b*) plant roots (cleaned of soil particles), and (*c*) the distribution of the label through the different soil depths. The soil depths to be analyzed will be determined by a range-finding evaluation as suggested in 8.2.3.

9.3 *Fate of Parent Compound and Transformation Products:*

9.3.1 The percent distribution of the test substance for each concentration should be calculated for (*a*) above-ground plant tissues, (*b*) below-ground plant tissues, (*c*) each depth through the soil profile, (*d*) losses to soil leachate, and (*e*) calculated total vaporization or gaseous losses. In addition, an analysis of the time to reach steady-state loss of the chemical compound in the soil leachate and the time to initiate compound leaching from each concentration should be calculated. For those exposure levels where the time to reach steady-state loss in the soil leachate is greater than the length of the experimental period (12 weeks), an extrapolation of a regression model may be used to estimate the time necessary to reach steady-state **(50).** This should be done cautiously and with the assumption that the shape of the response beyond the experimental period is the same as that estimated for the experimental period.

9.3.2 The calculations for the final distribution of the test material and transformation products are based on the measured radioactivity in that compartment on a per gram basis times the total weight or volume of material in that compartment, expressed on a dry-weight basis, where appropriate. All calculations are subsequently corrected for the radioactive decay that occurred since the beginning of the test. The quantities of compound are expressed as a percent of the original compound added to the test system. An ANOVA test should be performed on the calculated mean arc-sine transform for percentages remaining for each of the four main compartments to discern any differences among exposure concentrations. It may be necessary to transform the percentage data prior to ANOVA in order to satisfy the assumptions of the analysis. In addition, regression analyses should be performed comparing above-ground productivity versus concentration of parent compound and transformation products in above-ground plant tissues, and cumulative nutrient losses for each exposure concentration versus parent compound and transformation products in the soil leachate.

9.4 *Bioconcentration and Enrichment:*

9.4.1 Calculate the concentration of the radioactivity in the above-ground plant tissues and in the top 15 cm of soil on a concentration per unit dry-weight basis. The ratio of the plant tissue concentration to soil concentration is then defined as the bioconcentration factor. Side-by-side histograms displaying the ratios should then be compared for statistical differences.

9.4.2 Enrichment ratios should be calculated for the higher concentration by dividing the activity expressed on a dry-weight basis in a higher concentration by the lowest concentration in which a significant or detectable concentration could

be analytically determined. The enrichment ratio is then regressed against exposure concentration to determine the linearity of the uptake of the chemical as measured through the radiolabeled portion of the chemical. If it appears that an ordinary least-squares regression analysis is not the appropriate function defining the enrichment of the chemical, then an attempt to fit a quadratic or cubic equation should be made. The above guidelines for calculating bioconcentration and enrichment assumes that no wet analytical chemistry has been performed to separate the parent compounds from the transformation products in each compartment. If this has been done, then individual bioconcentration factors and enrichment ratios should be calculated as outlined above.

## REFERENCES


**(1)** Parkinson, D., Gray, T. R. G., and Williams, S. T., "Methods for Studying the Ecology of Soil Micro-organisms," *IBP-Handbook No. 19*, Blackwell Scientific Publications, Oxford, England, 1971.

**(2)** Phillipson, J., "Methods of Study in Quantitative Soil Ecology, Population, Production and Energy Flow," *IBP-Handbook No. 18*, Blackwell Scientific Publications, Oxford, England, 1971 .

**(3)** Nash, R. G., and Beall, M. L., Jr., "A Microagroecosystem to Monitor the Environmental Fate of Pesticides," *Terrestrial Microcosms and Environmental Chemistry*, National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC, 1977, pp. 86–94.

**(4)** Witt, J. M., and Gillett, J. W., eds., *Terrestrial Microcosms and Environmental Chemistry*, National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC, 1977.

**(5)** Gillett, J. W., and Witt, J. M., eds., "Terrestrial Microcosms," *Symposium on Terrestrial Microcosms and Environmental Chemistry*, National Science Foundation, Division of Problem-Focused Research Applications, Washington, DC, 1977.

**(6)** Van Voris, P., O'Neill, R. V., Emanuel, W. R., and Shugart, H. H., Jr., "Functional Complexity and Ecosystem Stability," *Ecology*, Vol 61, 1980, pp. 1352–1360.

**(7)** Van Voris, P., Tolle, D. A., Arthur, M. F., Chesson, J., and Zwick, T. C., "Development and Validation of Terrestrial Microcosm Test System for Assessing Ecological Effects of Utility Wastes," *Final Project Report RP1224-5, EPRI Publication No. EA-3672*, Electric Power Research Institute, Palo Alto, CA, 1984 .

**(8)** Van Voris, P., Tolle, D. A., Arthur, M. F., and Chesson, J., "Terrestrial Microcosms: Validation, Applications, and Cost-Benefit Analysis," *Multi-species Toxicity Testing*, Pergamon Press, New York, NY, 1985a, pp. 117 –142.

**(9)** Van Voris, P., Tolle, D. A., and Arthur, M. F., "Experimental Terrestrial Soil-Core Microcosm Test Protocol. A Method for Measuring the Potential Ecological Effects, Fate, and Transport of Chemicals in Terrestrial Ecosystems," *EPA/600/ 3-85/047*, PNL-5450, Corvallis Environmental Research Laboratory, Corvallis, OR, 1985b.

**(10)** Tolle, D. A., Arthur, M. F., and Van Voris, P., "Microcosm/Field Comparison of Trace Element Uptake in Crops Grown in Fly Ash-Amended Soil," *Science of the Total Environment*, Vol 31, 1983, pp. 243–261.

**(11)** Laskowski, D. A., Goring, C. A. I., McCall, P. J., and Swann, R. L., "Terrestrial Environment," *Environmental Risk Analysis for Chemicals*, Van Nostrand Reinhold Company, New York, NY, 1982, pp. 198–240.

**(12)** Swann, R. L., Laskowski, D. A., McCall, P. J., Vander Kuy, K., and Dishburger, H. J., "A Rapid Method for the Estimation of the Environmental Parameters Octanol/Water Partition Coefficient, Soil Sorption Constant, Water to Air Ratio, and Water Solubility," *Residue Reviews*, Vol 85, 1983, pp. 17–28.

**(13)** Wang, C. H., and Willis, D. L., *Radiotracer Methodology in Biology, Environmental, and Physical Sciences*, Prentice-Hall, Inc., Englewood Cliffs, NJ, 1975.

**(14)** Walters, D. B., ed., *Safe Handling of Chemical Carcinogens, Mutagens, Teratogens and Highly Toxic Substances*, Vols I and II, Ann Arbor Science Publishers Inc., Ann Arbor, MI, 1980.

**(15)** Keith, L. H., and Walters, D. B., ed., *Compendium of Safety Data Sheets for Research and Industrial Chemicals*, Vols I, II, & III, VCH Publishers, Inc., Deerfield Beach, FL, 1985.

**(16)** Sittig, M., *Handbook of Toxic and Hazardous Chemicals and Carcinogens, 2nd Edition*, Noyes Publications, Park Ridge, NJ, 1985.

**(17)** Windholz, W., ed., *Merck Index*, 10th Edition, Merck and Co., Rahway, NJ, 1980.

**(18)** Van Voris, P., O'Neill, R. V., Emanuel, W. R., and Shugart, H. H., Jr., "Functional Complexity and Ecosystem Stability: An Experimental Approach," *ORNL/TM-6199*, Oak Ridge National Laboratory, Oak Ridge, TN, 1978.

**(19)** Checkai, R.T., Wentsel, R.S., and Phillips, C.T., "Controlled Environment Soil-Core Microcosm Unit for Investigating Fate, Migration, and Transformation of Chemicals in Soils", *Journal of Soil Contamination*, Vol 2, 1993, pp. 229-243.

**(20)** Van Voris, P., Arthur, M. F., and Tolle, D. A., "Evaluation of Terrestrial Microcosms for Assessing Ecological Effects of Utility Wastes," *EPRI No. EA-2364*, Electric Power Research Institute, Palo Alto, CA, 1982.

**(21)** Zwick, T. C., Arthur, M. F., Tolle, D. A., and Van Voris, P., "A Unique Laboratory Method for Evaluating Agro-Ecosystem Effects of An Industrial Waste Product," *Plant Soil*, Vol 77, 1984, pp. 395–399.

**(22)** Van Voris, P., Arthur, M. F., Tolle, D. A., Morris, J. P., and Larson, M., "Use of Microcosms for Monitoring Nutrient Cycling Processes in Agroecosystems," *Nutrient Cycling in Agricultural Ecosystems*, University of Georgia College Agricultural Experimental Station Special Publication 23, Tifton, GA, 1983, pp. 171–182.

**(23)** Black, C. A., ed., *Methods of Soil Analysis: I. Physical and Mineralogical Properties*, American Society of Agronomy, Madison, WI, 1965.

**(24)** Black, C. A., ed., *Methods of Soil Analysis: II. Chemical and Microbiological Properties*, 2nd Edition, American Society of Agronomy, Madison, WI, 1982.

**(25)** Tolle, D. A., Arthur, M. F., Van Voris, P., Feder, P. I., Matthews, M. C., Chesson, J., Larson, M. E., and Zwick, T. C., "Evaluation of Terrestrial Microcosms for Environmental Studies of Utility Wastes," *Third Annual Progress Report on RP1224-5 to Electric Power Research Institute*, Palo Alto, CA, 1982.

**(26)** Jackson, D. R., Selvidge, W. J., and Ausmus, B. S., "Behavior of Heavy Metals in Forest Microcosms: II. Effects on Nutrient Cycling Processes," *Water, Air and Soil Pollution*, Vol 10, 1978, pp. 13–18.

**(27)** Rand, G. M., "Detection: Bioassay," *Introduction to Environmental Toxicology* , Elsevier North Holland, Inc., New York, NY, 1980, pp. 390–403.

**(28)** Neter, J., and Wasserman, W., *Applied Linear Statistical Models*, Irwin, Homewood, IL, 1974.

**(29)** Kastenbaum, M. A., and Hoel, D. G., "Sample Size Requirements: One-way Analysis of Variance," *Biometrika*, Vol 57, 1970, pp. 421–430.

**(30)** Elseewi, A. A., Straughan, I. R., and Page, A. L., "Sequential Cropping of Fly Ash-Amended Soils: Effects on Soil Chemical Properties and Yield and Elemental Composition of Plants," *Science of the Total Environment*, Vol 15, 1980, pp. 247–259.

**(31)** Jones, J. B., and Steyn, W. J. A., "Sampling, Handling, and Analyzing Plant Tissue Samples," *Soil Testing and Plant Analysis*, Soil Science Society of America, Inc., Madison, WI, 1973 , pp. 249–270.

**(32)** Malanchuk, J. L., Mueller, C. A., and Pomerantz, S. M., "Microcosm Evaluation of the Agricultural Potential of Fly-Ash-Amended Soils," *Microcosms in Ecological Research*, CONFERENCE-781101, U.S. Department of Energy, Technical Information Center, 1980, pp. 1034–1049.

**(33)** Daubenmire, R., "A Canopy Cover Method of Vegetation Analysis," *Northwest Science*, Vol 33, 1959, pp. 43–64.

**(34)** O'Neill, R. V., Ausmus, B. S., Jackson, D. R., Van Hook, R. I., Van Voris, P., Washburne, C., and Watson, A. P., "Monitoring Terrestrial Ecosystems by Analysis of Nutrient Export," *Water, Air and Soil Pollution*, Vol 8, 1977, pp. 271–277.

**(35)** Jackson, D. R., Ausmus, B. S., and Levin, M., "Effects of Arsenic on Nutrient Dynamics of Grassland Microcosms and Field Plots," *Water, Air and Soil Pollution*, Vol 11, 1979, pp. 3–12.

**(36)** Schindler, J. E., Waide, J. B., Waldron, M. C., Hains, J. J., Schreiner, S. P., Freeman, M. L., Beng, S. L., Pettigrew, D. R., Schissel, L. A., and Clark, P. J., "A Microcosm Approach to the Study of Biogeochemical Systems: I. Theoretical Rationale," *Microcosms in Ecological Research*, CONFERENCE-781101, U.S. Department of Energy, Technical Information Center, 1980, pp. 192–203.

**(37)** Gile, J. D., and Gillett, J. W., "Fate of Selected Fungicides in a Terrestrial Laboratory Ecosystem," *Journal of Agricultural Food Chemistry*, Vol 27, 1979, pp. 1159–1164.

**(38)** Ausmus, B. S., Dodson, G. F., and Jackson, D. R., "Behavior of Heavy Metals in Forest Microcosms: III. Effects on Litter-Soil Carbon Metabolism," *Water, Air and Soil Pollution*, Vol 10, 1978, pp. 19–29.

**(39)** Harris, W. F., Ausmus, B. S., Eddlemon, G. K., Giddings, J. M., Jackson, D. R., Luxmore, R. J., O'Neill, E. G., O'Neill, R. V., Ross-Todd, M., and Van Voris, P., "Microcosms as Potential Screening Tools for Evaluating Transport and Effects of Toxic Substances," *ORNL/ EPA-4, EPA-600/3-80-042*, Publication No. 1506, Oak Ridge National Laboratory, Environmental Sciences Division, Oak Ridge, TN, 1980.

**(40)** Ausmus, B. S., Kimbrough, S., Jackson, D. R., and Lindberg, S., "The Behavior of Hexachlorobenzene in Pine Forest Microcosms: Transport and Effects on Soil Processes," *Environmental Pollution*, Vol 20, 1979, pp. 103–111.

**(41)** Association of Official Analytical Chemists (AOAC), *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12th Edition, Washington, DC, 1975.

**(42)** Environmental Protection Agency, "Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples," Health Effects Research Laboratory, Research Triangle Park, NC, 1979.

**(43)** Harvey, J., Jr., "A Simple Method of Evaluating Soil Breakdown of $^{14}C$ Pesticides under Field Conditions," *Residue Review*, Vol 85, 1983, pp. 149–158.

**(44)** Lichtenstein, E. P., Schulz, K. R., and Fuhremann, T. W., "Movement and Fate of Dyfonate in Soils under Leaching and Nonleaching Conditions," *Journal of Agricultural Food Chemistry*, Vol 20, 1972, pp. 831–838.

**(45)** Lichtenstein, E. P., Fuhremann, T. W., Schulz, K. R., and Liang, T. T., "Effects of Field Application Methods on the Persistence and Metabolism of Phorate in Soils and its Translocation into Crops," *Journal of Economic Entomology*, Vol 66, 1973, pp. 863–866.

**(46)** Cole, L. K., Sanborn, J. R., and Metcalf, R. L., "Inhibition of Corn Growth by Aldrin and the Insecticide's Fate in the Soil, Air, Crop, and Wildlife of a Terrestrial Model Ecosystem," *Environmental Entomology*, Vol 5, 1976, pp. 583–589.

**(47)** Metcalf, R. L., Kapoor, I. P., Lu, P., Schuth, C. K., and Sherman, P., "Model Ecosystem Studies of the Environmental Fate of Six Organochlorine Pesticides," *Environmental Health Perspectives*, Vol 4, 1973, pp. 35–44.

**(48)** Lichtenstein, E. P., Fuhremann, T. W., and Schulz, K. R., "Translocation and Metabolism of ($^{14}C$) Phorate as Affected by Percolating Water in a Model Soil-Plant Ecosystem," *Journal of Agricultural Food Chemistry*, Vol 22, 1974, pp. 991–996.

**(49)** Sokal, R. R., and Rohlf, F. J., *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd Edition, W. H. Freeman and Company, San Francisco, CA, 1981.

**(50)** Snedecor, G. W., and Cochran, W. G., *Statistical Methods*, 7th Edition, Iowa State University Press, Ames, IA 1980 .

**(51)** Steel, R. D. G., and Torrie, B. H., *Principles and Procedures of Statistics* , 2nd Edition, McGraw-Hill Book Co., New York, NY, 1980.



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