United States
Environmental Protection
Agency

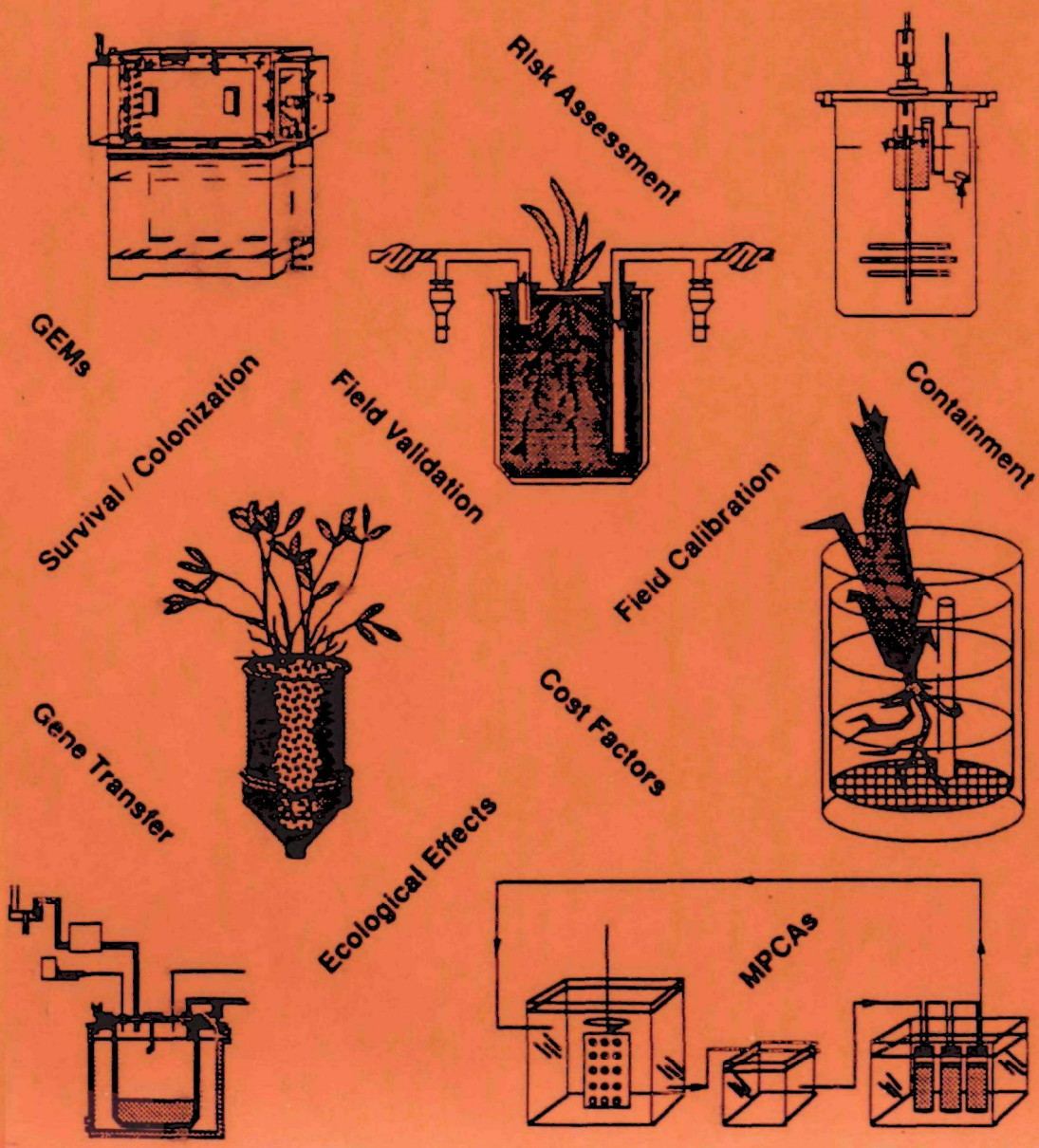
Office of Research and
Development
Washington, DC 20460

EPA/600/R-92/066
May 1992

EPA

Workshop

Application of Microcosms for Assessing the Risk of Microbial Biotechnology Products



EPA/600/R-92/066
May 1992

WORKSHOP: APPLICATION OF MICROCOSMS FOR ASSESSING THE RISK OF MICROBIAL BIOTECHNOLOGY PRODUCTS

May 19, 1992
Hunt Valley, Maryland

edited by

C.R. Cripe and P.H. Pritchard
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, Florida 32561

A.M. Stern
U.S. Environmental Protection Agency
Office of Toxic Substances
Health and Environmental Effects Division
Washington, DC 20460

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

Disclaimer

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Abstract

The U.S. Environmental Protection Agency (EPA) develops testing methods to support assessments of the environmental risks associated with the release of microorganisms and microbial pest control agents. Microcosms may be used as one step in the progression of product development from laboratory to field experimentation. The utility of microcosms in this process is, in some measure, dependent on the capacity of the test system to simulate environmental complexity, and consequently, to provide relevant answers to questions of environmental concern that may be raised by the regulatory community. The usefulness of current microcosm systems to evaluate and provide relevant information on a variety of regulatory endpoints pertinent to environmental risk assessment of microbial products was examined by workshop participants who met at Hunt Valley, MD, on January 23-27, 1989. A total of 14 generic and site-specific microcosms, portraying terrestrial and aquatic habitats with varying degrees of ecosystem complexity, was examined. The endpoints of ecological effects and other performance characteristics were compared for each microcosm system. Finally, future directions of microcosm research that appear to be required to fill gaps in the state-of-the-science were recommended.

Contents

Application of Microcosms for Assessing the Risk of Microbial Biotechnology Products

1. Introduction

The EPA, under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), is charged with the regulation of microbial biotechnology products such as genetically engineered microorganisms (GEMs) and microbial pest control agents (MPCAs) that might be released to the environment. EPA's regulatory programs developed to evaluate that risk must be able to analyze data gathered from a variety of experimental approaches ranging from relatively simple laboratory studies to more complex field studies.

Field studies, although providing relevant information concerning a particular site, suffer from many drawbacks: (1) they are subject to disruption by meteorological events, (2) they do not allow easy examination of the influence of individual variables (e.g., temperature, nutrients, soil composition, water content) on the interactions of introduced microorganisms with their environment, and (3) introduction of microorganisms at a field test site for research purposes may, by itself, pose an unacceptable risk.

Evaluations of chemical fate and effects have utilized laboratory test systems, such as microcosms, to provide risk assessment information while avoiding some of the problems of field testing. Some test systems are simple enough to offer the advantages of replication and experimental manipulation while maintaining sufficient complexity to include many important ecosystem processes.

Tests conducted in microcosms may be diagnostic in themselves or a surrogate for small-scale field testing, thus allowing regulatory decisions to be made on laboratory-scale testing and reducing the time and expense of the first stage of field testing.

In assessing the risks of microorganisms, the Office of Toxic Substances (OTS) and the Office of Pesticide Programs (OPP) currently use separate but similar criteria called "Points to Consider" (regulatory endpoints) which outline the categories of information that are useful in risk assessments by the EPA. Although the OPP and OTS lists differ somewhat, overall risk assessments address similar issues. Some experimentally-derived information that would satisfactorily address the points in the lists may be obtained by testing in microcosms. It is, therefore, appropriate to evaluate the usefulness of the quality and quantity of information that microcosm systems can provide relative to these regulatory endpoints. This document summarizes such an evaluation performed by a group of scientists.

2. Workshop Background

The Microcosm Workshop was a joint effort of EPA's Office of Research and Development (ORD) and the Office of Pesticides and Toxic Substances (OPTS). Fourteen microcosm systems judged appropriate for testing the fate and potential ecological effects of introduced microorganisms were selected before the workshop for discussion by the participants. These microcosms were not chosen to represent the entire field of appropriate test systems but, rather, to be representative of systems that had provided useful information for chemical risk assessment or that were specifically designed for testing microorganisms. A brief description of each microcosm was contributed by its developer before the workshop.

Participants worked in both plenary sessions and small subgroup sessions to generate information about the potential uses and limits of the selected microcosms with respect to the assessment of the survival and ecological effects of introduced microorganisms as well as their potential for transferring genetic material to indigenous microorganisms. Workshop participants also identified areas in microcosm technology where further research was required to expand microcosm applicability or to increase confidence in data outputs. This information was supplemented by the results of questionnaires distributed to microcosm developers which requested more details about their test systems after the workshop had concluded.

3. Workshop Objectives

The overall workshop objectives were to determine the current state-of-the-art in microcosm design and to ascertain the extent to which microcosms could be applied to biotechnology risk assessment. Specific goals were to:

1. Identify the most appropriate of the currently available microcosms to evaluate the fate and effect parameters of microorganisms released to the environment.
2. Provide sufficient information to allow assessment of advantages and disadvantages of each microcosm with respect to:

Table 1. Summary of test systems examined by workshop.

Name of Microcosm	Habitat	Developer(s)	Page
Benthic-Pelagic	Marine	Perez	9
Compartmentalized Lake	Freshwater	Kroer	19
Mixed Flask Culture	Freshwater	Shannon	29
Pond	Freshwater	Giddings	37
Sediment Core	Marine	Pritchard/Clark	45
Standard Aquatic	Freshwater	Taub	55
Stream	Freshwater	Bott	65
Waste Treatment	Wastewater	Gealt	75
Root System	Terrestrial	Klein	87
Soil Core	Terrestrial	Fredrickson	97
Soil in a Jar	Terrestrial	Stotzky	109
Terrestrial Chamber	Terrestrial	Gillett	119
Terrestrial System	Terrestrial	Seidler/Armstrong	129
Versacore	Terrestrial	Holben/Jansson	137

a. Potential for, and confidence in, the extrapolation of laboratory data to field predictions with regard to critical fate and effect endpoints.

b. Cost and expertise required to construct and operate the microcosms.

c. Potential for the development of possible modifications to expand microcosm utility.

3. Identify gaps in current knowledge regarding microcosm development and application for biotechnology risk assessment.

4. Microcosm Descriptions and Questionnaire Design

Selection of an appropriate microcosm design to assess the potential environmental risk of a microorganism requires knowledge of microcosms that have demonstrated value in other types of risk assessment activities (e.g., chemical effects, fate, transport). A questionnaire was completed by developers of each of the 14 microcosms listed in Table 1 to provide specific information about their potential use in assessing the risk of microbial biotechnology products. Collating this information produces a useful, structured, comparison of these systems relative to risk assessment needs and to each other.

The questionnaire examines general characteristics of each test system: a description of the physical design and size, lighting, temperature control, purpose for which microcosm was originally designed, habitat represented as well as trophic levels and method of establishing communities, sampling of environmental media, provisions for air or water exchange/circulation, equilibrium period prior to use, lifespan of test system, and environmental parameters routinely monitored. These ancillary details may find important application in simulation or assessment modeling.

Questions concerning containment focus on whether current designs are adequate for working with genetically engineered microorganisms or if specific modifications would improve containment. A section on protocols details the development of standard operating procedures for microcosm construction, operation or output analysis. Modifications (other than those related to containment) that would improve a test system's use for risk assessment are solicited. Sampling strategies (repetitive, destructive, etc.) are examined, along with information on test system cost.

A section on applicability for evaluating ecological parameters describes techniques that have been used to monitor five types of ecological effects factors in the test system: community structure, trophic interactions, energy flow, biogeochemical cycling, or other effects. Results of field calibration tests (comparison of the responses of ecological parameters in microcosms with the field in the absence of stress agents) for each of these five factors was also solicited, as was information on problems encountered with making these comparisons.

A final questionnaire section addresses field verification studies; these are tests with genetically engineered organisms or surrogate organisms to compare survival, colonization, and microbial/gene mobility observed in microcosms with those observed in the field.

Microcosm questionnaire responses are grouped according to aquatic or terrestrial application (Appendix B and C, respectively). At the end of each summary is listed additional information such as the name, address, and telephone number of the microcosm developer or contact person, pertinent publications, protocols, other documents relating to the microcosm, data that have been derived from its use, and, if available, a diagram of the test system.

It is acknowledged that any microcosm selected for a risk assessment application may incorporate specific features (such as size, containment, or ecological endpoints) from one or more of the 14 systems examined here or elsewhere, to address questions unique to the microorganism being tested.

5. Research Needs

5.1 Introduction

In addition to surveying and describing useful test systems, workshop participants expressed a general confidence in the use of microcosms to assist in assessing risks of

microorganisms and microbial biotechnology products, but they also generated substantial lists of related research efforts required to maximize the utility of microcosms for this purpose. These suggestions have been incorporated into narratives of microcosm research topics.

5.2 Conducting Comparison Studies

More studies of field calibration (baseline studies of various ecological parameters that are observed in a microcosm in the absence of a stress agent relative to those observed in the field) and field validation (comparison of stress-response relationships among ecological endpoints in a microcosm and in the field in the presence of a specific stressor) are needed to improve confidence in the ability to extrapolate microcosm-derived data on microbial fate, effects, and/or gene transfer to field data. Seasonal information from microcosm studies of site-to-site comparisons between geographical areas and among habitats is also required to examine the effects of spatial and temporal variability. Effects of successional changes in microcosms and extrapolation of those data to natural systems also should be examined.

Comparisons between different types of microcosms utilizing a variety of endpoints should substantially improve the selection and design features of test systems used for specific risk assessments. Finally, interlaboratory comparisons of the same test systems were suggested to assess lab-to-lab variability.

5.3 Evaluating Increased Test System Diversity and Interactions

It is necessary to expand the scope of microcosm research to include the study of higher trophic levels, greater species diversity, and community-level ecological processes and interactions. Although careful consideration must be given to cost effectiveness, such expansions would appreciably increase the utility of microcosms and the relevance of the data obtained from their use.

5.4 Developing Mathematical Models and Appropriate Statistical Methodologies

A greater emphasis on the development of field-validated mathematical models to enhance the ability to extrapolate fate and effects data obtained with microcosms to a field site is required. Development and application of appropriate quantitative methods to measure the effects of potential perturbation of ecosystems with respect to specific variables as they vary in both laboratory test systems and in the field is also necessary to achieve a sufficient level of confidence in the use of microcosms and models.

The lack of appropriate aquatic transport microcosms suggested a special need for hydrodynamic modeling, as it relates to microbial transport; chemical and particle movement models are probably not adequate for this purpose. Effects of factors such as microbe size, shape, and physical surface characteristics on physical transport should be examined.

5.5 Developing Test Organisms/Markers

Model test organisms (i.e., bacteria, fungi, viruses) with appropriate markers for assessing fate and effects must be identified and developed. Methods of detection, must be improved, and the spectrum expanded and tested for applicability to different types of microcosms and field tests. Markers should not pose an ecological (or health) risk or affect microcosm structure or function.

There is also a need to develop techniques to measure the movement and expression of genetic material introduced into a microcosm.

5.6 Identifying New and Relevant Endpoints

Additional development of structural and functional endpoints, especially those requiring non-destructive sampling techniques, is needed. The scope of the endpoints should allow testing for ecological effects that include investigating the increased susceptibility of a system to secondary disturbances (e.g., invasion, chemical stress, physical stress) when the microbial agent is introduced simultaneously with, or subsequent to, the introduction of a secondary stress agent.

5.7 Basic Microbial Ecology

Limitations in the understanding of microbial ecology remain one of the most serious hindrances to microbial risk assessment. For example, a variety of factors that control microbial production and biomass (e.g., substrate and predator control) may be known, but the extent of their influence, and the effects introduced in a system as a consequence of the interactions taking place among its components, are not known.

5.8 Microcosm Design and Testing Considerations

The mode and magnitude of introduction of a microbial agent may affect fate, ecological effects, or transfer of novel genetic material and, thus, should be considered typical variables in microcosm testing.

It is not clear which or how many environmental variables (e.g., temperature, light, water content) should be measured and controlled for microcosm tests, although this will probably depend on the specific application. The degree of environmental control necessary for field comparisons also needs to be determined.

The effects of measures to contain microbial biotechnology products may reduce the capability of a microcosm to simulate a real ecosystem. Likewise, containment of a field test site may alter normal community structure or functions; this potential should be considered when comparing results from a microcosm with those in a field test.

5.9 Final Considerations

The successful use of microcosms and models for risk assessment will depend on definitive articulation of the objectives of a particular application. For example, attention must be given to study objectives (e.g., screening vs. a more definitive assessment) and to the degree of detail required (e.g., the required levels of confidence and ability to extrapolate to the field) to meet these objectives. Such decisions affect the practicality of expanding the scope of microcosm research and the further development of mathematical models and microcosms to accommodate this expansion.

6. Summary

Fourteen microcosm designs, using a variety of terrestrial and aquatic habitats, are described. Most systems were originally designed to assess the fate/effects of xenobiotic compounds. Only a few were actually developed with microbial biotechnology risk assessment in mind, but all should provide some useful information in evaluating microbial products.

Initially, the workshop focused on the suitability of each microcosm to assess persistence, ecological effects, or exchange of novel genetic material. However, it became apparent that confidence in these assessments must be tempered by gaps in our knowledge of microbial ecology. A variety of relevant research topics was compiled by each subgroup to address the information necessary for risk assessment testing of microbial biotechnology and for interpretation of test results.

The 14 microcosms described in Appendices B and C provide a basis for the selection of microcosm designs appropriate for specific applications. These systems should be viewed as tools for the generation of some of the information necessary for microbial biotechnology risk assessment. Various aspects of a selected system (e.g., trophic levels, structural or functional endpoints, physical habitat) may have to be modified to answer a specific question. Information provided by such microcosms will only be as applicable for extrapolation to the natural environment as the ecological processes included in the test systems. Thus, field calibration and field verification remain two of the most critical components of microcosm development and testing.

Appendices

Appendix A
Workshop Participants

Dr. Dick Anderson
U.S. Environmental Protection Agency
Environmental Research Laboratory
6201 Congdon Blvd.
Duluth, MN 55804
(FTS) 780-5616

Dr. Thomas Bott
Stroud Water Research Center
Division of Environmental Research
Academy of Natural Sciences
R.D. #1 Box 512
Avondale, PA 19311
(215) 268-2153

Dr. James Clark
Environmental Toxicology Division
Exxon Biomedical Sciences, Inc.
Mettlers Road, CN 2350
East Millstone, NJ 08875-2350
(908) 873-6039

Dr. Rick Coffin
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, FL 32561
(FTS) 228-9255

Mr. Rick Cripe
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, FL 32561
(FTS) 228-9340

Dr. Bob Frederick (RD-682)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460
(FTS) 382-5989

Dr. James Fredrickson
Battelle Pacific NW Laboratories
Po.O. Box 999
Richland, WA 99352
(509) 375-3908

Dr. Michael Gealt
Dept. of Bioscience & Biotechnology
Drexel University
32nd and Chestnut Streets
Philadelphia, PA 19104
(215) 885-5092

Dr. Jeffrey Giddings
Springborn Bionomics, Inc.
790 Main Street
Wareham, MA 02571
(508) 295-2550

Dr. James Gillett
16 Fernow Hall
ICET Cornell University
Ithaca, NY 14853-3001
(607) 255-2163

Dr. Mike Heitkamp
Monsanto Company
800 Lindberg Ave.
St. Louis, MO 63167
(314) 694-3296

Dr. William Holben
Michigan State University
East Lansing, MI 48824
(517) 355-9282

Dr. Donald Klein
Colorado State University
Dept. of Microbiology
Fort Collins, CO 80523
(303) 491-6947

Mr. Niels Kroer
National Environmental Research Institute
Department of Marine Ecology and Microbiology
Frederiksborgvej 399
P.O. Box 358
DK-4000 Roskilde, DENMARK
(45 46 30 13 88)

Dr. Wayne Landis
Institute of Environmental Toxicology
and Chemistry
Huxley College
Western Washington University
Bellingham, WA 98225
(206) 647-6109

Dr. Mark Luckenbach
Virginia Institute of Marine Science
College of William and Mary
Gloucester Point, VA 23062
(804) 642-7000

Dr. Robert Miller
Loyola University
Department of Biochemistry and Biophysics
2160 South First Avenue
Maywood, IL 60153
(312) 531-3360

Dr. Vincent J. Nabholz (TS-796)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460
(FTS) 382-4271

Dr. P.H. Pritchard
U.S. Environmental Protection Agency
Environmental Research Laboratory
Gulf Breeze, FL 32561
(FTS) 228-9260

Dr. Gary Sayler
University of Tennessee
Microbiology/Ecology Department
10515 Research Drive
Suite 200
Knoxville, TN 37996
(615) 974-5219

Dr. Mark Segal (TS-796)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460
(FTS) 382-3389

Dr. Ray Seidler
U.S. Environmental Protection Agency
Environmental Research Laboratory
200 S.W. 35th Street
Corvallis, OR 97333
(FTS) 420-4661

Dr. Lyle Shannon
University of Minnesota
Biology Department
Duluth, MN 55812
(218) 726-8000

Dr. Francis Sharples
Oak Ridge NAS Laboratory
P.O. Box Y
Oak Ridge, TN 38731
(615) 576-0524
(615) 691-0452

Dr. Frank Stay
U.S. Environmental Protection agency
Environmental Research Laboratory
6201 Congdon Blvd.
Duluth, MN 55804
(FTS) 780-5542

Dr. Art Stern (TS-796)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460
(FTS) 382-4281

Dr. Guenther Stotzky
New York University
Department of Biology
1009 Main Washington Square
New York, NY 10003
(212) 998-8266

Dr. Glenn W. Suter II
Environmental Sciences Division
Oak Ridge National Lab
Oak Ridge, TN 37831
(615) 574-7306

Dr. Frieda Taub
School of Fisheries, HF-15
University of Washington
Seattle, WA 98195
(206) 685-2115

Dr. In-Soon You (TS-796)
U.S. Environmental Protection Agency
401 "M" Street, S.W.
Washington, DC 20460
(FTS) 382-4237

Appendix B
Aquatic Microcosms

BENTHIC-PELAGIC MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: K. PEREZ

1. Briefly describe the physical design, including microcosm vessel material. If possible, include a labeled diagram.

Each microcosm consists of a glass or fiberglass tank containing a pelagic phase (150 liters of hand-bucketed seawater) and a coupled benthic phase (relatively undisturbed 169 cm^2 x 20 cm deep benthic box core). The two phases are linked by an air-driven displacement pump continuously exchanging seawater from the water column to the benthic box core. The water turbulence of the pelagic phase is controlled by a rotating, reversible stirring paddle.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	*neuston, plankton, benthos*
Primary producers (specify)	Yes_x_ No___	phytoplankton
Invertebrates (specify)	Yes_x_ No___	amphipods, bivalves, polychaetes & hydroids
Vertebrates (specify)	Yes_x_ No___	intertidal fish larvae (low frequency)
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Whole sampling of environment, i.e., no reconstruction. Whole assemblages of organisms are determined by the size of water column (volume) and benthic core (cross-sectional diameter and vertical depth); surface microlayer communities develop after the microcosm is established in the laboratory.

4. If environmental media are used, how is the environment sampled?

Sediment is cored; water is hand-dipped.

5. What habitats are represented?

   a. Typically:

Sediment habitat: benthic organisms, aerobic and anaerobic sediment zones; water column: pelagic fauna.

   b. What factor(s) limit the habitats that could be represented?

Size limits the incorporation of an intertidal zone and large top carnivores.

GENERAL CHARACTERISTICS (CONTINUED)

6. Microcosm size:

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
a. Typically:	Depth = 100	150	170-500
b. What factor(s) limit these size characteristics?	Ratio of the sediment surface area to water column volume of the natural system being simulated		
c. How much space is required per microcosm unit?	Approximately 1 m^3		

7. For what purpose was the microcosm originally designed?

To estimate the fate and ecological effects of chemicals in natural aquatic environments.

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

Seawater containing living organisms and other material is collected from the natural system and exchanged with the seawater in the microcosm. The volumes removed and added are equal; the water turnover time is equal to that of the natural system being simulated. Seawater is aerated by the physical motion of the stirring paddle. The rate of stirring is adjusted so that the dissolution rate of a solid material is similar to that of the natural system.

9. Equilibrium period:

a. Is laboratory equilibrium required before testing? Yes_____ No_X___

b. If so, what is the equilibration period?

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

10. Microcosm "lifespan":

a. How long are microcosm tests generally run? — Typically 30 days, but longer tests are possible.

b. What are the most important factors in establishing the lifespan of this microcosm? An adequate cleaning regime to eliminate significant fouling on the microcosm walls.

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.): Fluorescent lamps

b. Typical light intensity: Average water column irradiation = 38µE m^{-2} s^{-2}

c. Lighting control (intensity, photoperiod, means of control, etc.): Irradiation is constant during the light period of a particular season; photoperiod is seasonally-dependent and controlled by an electric timer.

GENERAL CHARACTERISTICS (CONTINUED)

12. Which of the following environmental parameters are routinely monitored?

a.	Soil moisture	____
b.	Relative humidity	____
c.	Temperature	_X_
d.	Light intensity	____
e.	Inorganic nutrients	_X_
f.	Carbon dioxide	____
g.	Dissolved oxygen	_X_
h.	Other (specify)	Water column particulates, vertical profile of oxygen in sediment

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Natural water temperatures are reproduced by placing all microcosms in a water bath which is continuously and rapidly flushed with seawater derived from the natural system. Natural temperatures could be simulated by placing a temperature control in the water bath.

14. How is water/air circulated/mixed?

Water mixing is controlled by a paddle rotating at a speed such that the dissolution rate of a known solid material is equivalent to that of the natural system.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

Yes_X_ No_____ (See Protocol Draft)

b. If so, describe containment design.

Gas phase containment over microcosms. Water bath containment by using closed circulation. Exit and entry to microcosms is by a sterilizable compartment.

c. Could containment be improved by design modification?

Yes_X_ No_____

d. If so, what is the nature of the modifications needed to improve containment?

Better filters and sterilization methodologies.

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

____ a.	Considerable resources, skill, or time.
_X_ b.	Moderate resources, skill or time.
____ c.	Minimal resources, skill or time.
____ d.	Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No____

   b. Microcosm operation? Yes_x_ No____

   c. Output analysis? Yes_x_ No____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No____

   b. Microcosm operation? Yes____ No____

   c. Output analysis? Yes____ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes____ No____

   b. Operate a microcosm? Yes____ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

2. What levels of difficulty would be involved in making the modifications in (1) above?

   ____ a. Considerable resources, skill or time.

   ____ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

   No limit to water column or surface microlayer sampling. Benthic sampling is restricted to completion of study *or* if additional replicate microcosms are used to sample before the completion of the study.

2. Is destructive sampling during the course of a test run required?

   Yes__x__ No_____ with regard to benthos only

3. Would design modifications allow the use of alternative sampling strategies?

   Yes_____ No__x__ (additional microcosms would allow measurement of benthos dynamics)

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

   ____ a. Less than $100

   ____ b. Between $100 and $500

   ____ c. Between $500 and $1000

   __x__ d. More than $1000

2. How many replicate vessels are generally used per treatment?

   3 - 5 replicates/treatment

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

   __x__ a. Less than $5000 (excluding vessel cost, for a chemical test)

   ____ b. Between $5000 and $20000

   ____ c. Over $20000

   ____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Phytoplankton - direct cell counting	_____
	ANIMALS	Zooplankton & transient larval forms: direct count	_____
	BENTHOS	Benthos - sieve (0.5 mm), stain (Rose Bengal), count	_____
	MICROORGANISMS	Surface microlayer ATP analysis	_____
	OTHER (SPECIFY)	Zooplankton age structure:juv./adult; naup./juv.	_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Relationships developed using above data	_____
	BACTERIA/PROTOZOA	same	_____
	PLANTS/HERBIVORES	same	_____
	HERBIVORES/PREDATORS	same	_____
	OTHER (SPECIFY)	same	_____
ENERGY FLOW	PRIMARY PRODUCTION	Estimated from temporal dynamics	_____
	SECONDARY PRODUCTION	Same	_____
	P/R RATIO		_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM. CYCLING	NITROGEN		_____
	PHOSPHORUS		_____
	SULFUR		_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)	Phytoplankton ident. (diatoms, bluegreen, etc.)	_____
	ANIMAL (SPECIFY)	Sediment bioturb./resusp.: radioactive microspheres	_____
	MICROBIAL (SPECIFY)		_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm

Size excludes large macrofauna from these microcosms. [However in natural systems macrofauna are usually transient in time and space.]

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS	
COMMUNITY STRUCTURE	PLANTS	H		
	ANIMALS	H		
	BENTHOS	H		
	MICROORGANISMS	H		
	OTHER (SPECIFY)			
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		X	
	BACTERIA/PROTOZOA		X	
	PLANTS/HERBIVORES	H		
	OTHER (SPECIFY)			
ENERGY FLOW	PRIMARY PRODUCTION	H		
	SECONDARY PRODUCTION	H		
	P/R RATIO		X	
	OTHER (SPECIFY)			
BIOGEOCHEM. CYCLING	NITROGEN		X	
	PHOSPHORUS		X	
	SULFUR		X	
	OTHER (SPECIFY)			
OTHER EFFECTS	PLANT (SPECIFY)			
	ANIMAL (SPECIFY)		X	Sed. resusp.,
	MICROBIAL (SPECIFY)			bioturb.
	OTHER (SPECIFY)			

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

(1) Sampling problems - Define the spatial scale of natural system being simulated.

(2) Causes for observed deviation or divergent behavior of laboratory system from natural system.

(3) Ease of measurement in field is sometimes difficult in laboratory and vice-versa.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
1. Has your microcosm response to this factor been compared to field data?	Yes ___ No _x_	Yes ___ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	___	___
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No _x_	Yes___ No _x_
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON BENTHIC-PELAGIC MICROCOSM

Dr. Kenneth Perez
U.S. Environmental Protection Agency
Environmental Research Laboratory
South Ferry Road
Narragansett, RI 0288
(FTS) 838-6056

Dwyer, R.L., and K.T. Perez. 1983. An experimental examination of ecosystem linearization. Am. Nat. 121:305-323.

Experimental marine microsom test protocol and support document: Measurement of the ecological effects, fate and transport of living micro-organisms in a site-specific marine ecosystem. U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI. Preliminary Draft. 42 p.

Federal Register. 1987. §797.3100 Site-specific aquatic microcosm test. 52(187):36352-36360.

Perez, K.T., E.W. Davey, N.F. Lackie, G.E. Morrison, G.G. Murphy, A.E. Soper, and D.L. Winslow. 1983. Environmental assessment of a phthalate ester, di(2-ehtylhexyl) phthalate (DEHP), derived from a marine microcosm. *In:* W.E. Bishop, R.D. Cardwell, and B.B. Heidolph (eds.), Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802, American Society for Testing and Materials, Philadelphia, pp. 180-191.

Perez, K.T., G.E. Morrison, E.W. Davey, N.F. Lackie, G.E. Soper, R. J. Blasco, D.L. Winslow, R.L. Johnson, P.G. Murphy, and J.F. Heltshe. 1991. Influence of size on fate and ecological effects of Kepone in physical models. Ecological Applications. (3):237-248.

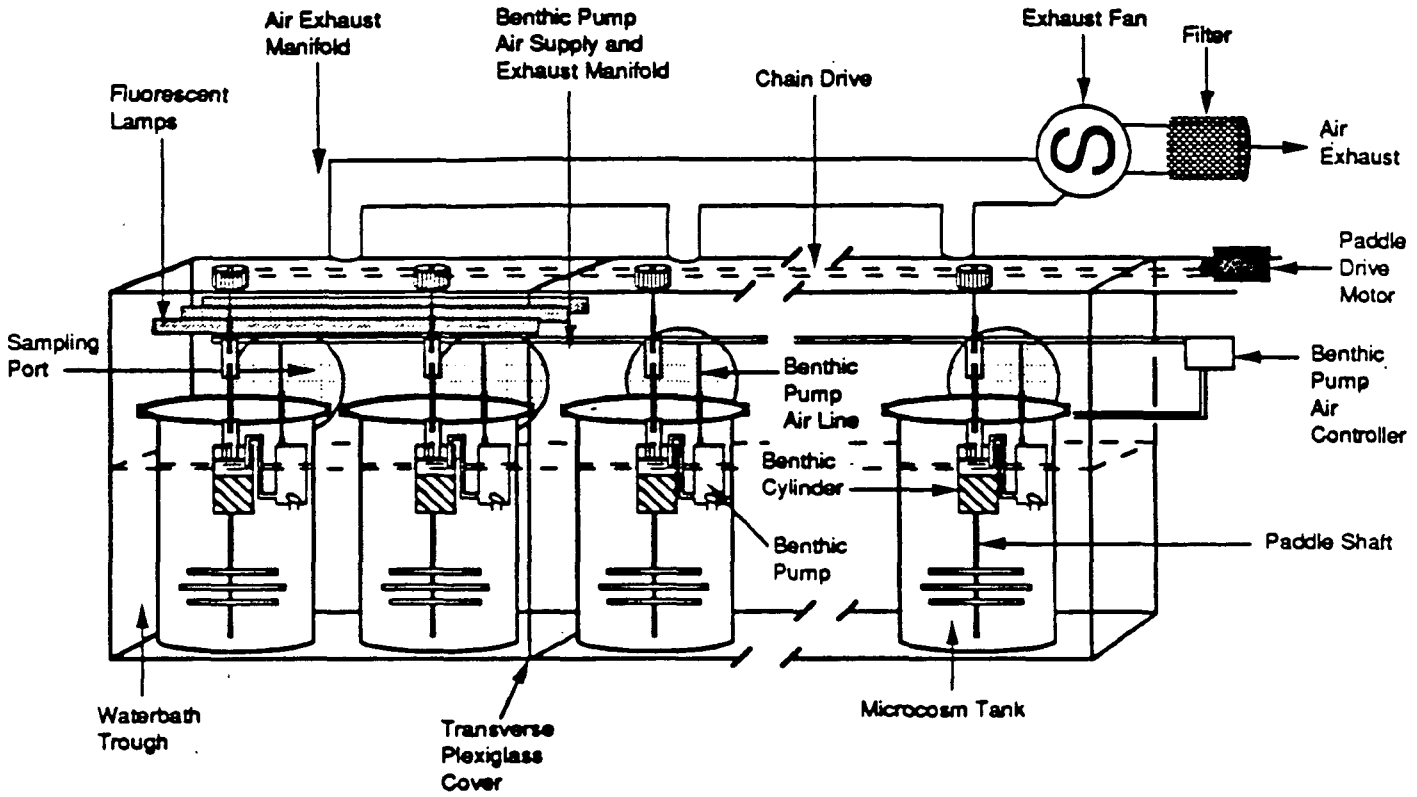


Figure 1. Benthic pelagic microcosm unit.

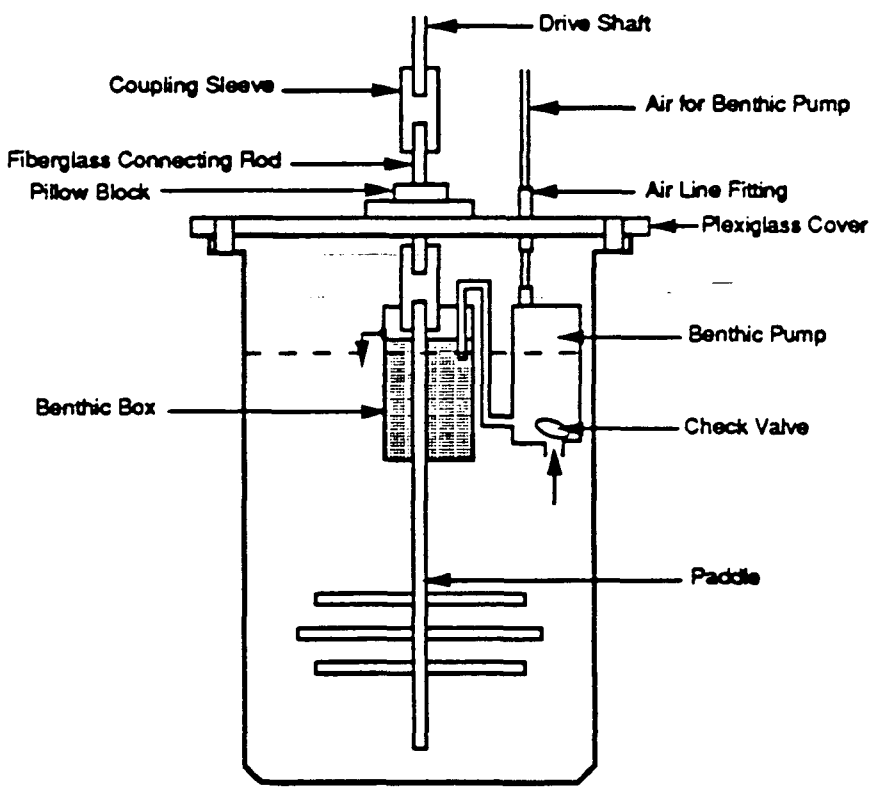


Figure 2. Benthic pelagic microcosm facility.

COMPARTMENTALIZED LAKE MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: N. KROER

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Microcosm consists of 3 units: algal and herbivore (216 and 27-l glass aquariums), and benthic community (sediment core(s) in plexiglass tube(s)). A Peristaltic pump recycles water through silicone tubing at 2.5 L/h (algal unit →→herbivore unit →→ sediment cores in series →→ algal unit). A 150 μ nylon screen prevents escape of zooplankton from herbivore unit but allows movement of smaller organisms. Flow between units can be adjusted to control grazing and geochemical cycling. Water volume to sediment-surface ratio may be adjusted.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No____	Bact., flagel., diatoms
Primary producers (specify)	Yes_x_ No____	Phytoplankton
Invertebrates (specify)	Yes_x_ No____	Zooplankton, benthic
Vertebrates (specify)	Yes____ No_x_	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Sediment: Intact cores.
Water: Water in algal unit flushed through a 150 μm sieve to remove zooplankton. The water in the herbivore unit is unfiltered and contains the zooplankton removed by sieving water for the algal tank.

4. If environmental media are used, how is the environment sampled?

Intact sediment cores are collected in clear plexiglass tubes. Water is collected in plastic carboys. Water for the algal unit is filtered through a 150 μm sieve to remove zooplankton. The zooplankton is placed in the herbivore unit (with unfiltered water). The microcosms are set up within 4-5 h of sampling.

5. What habitats are represented?

   a. Typically:

Benthic and pelagic (water column)

   b. What factor(s) limit the habitats that could be represented?

GENERAL CHARACTERISTICS (CONTINUED)

6. Microcosm size:

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
a. Typically:	Algal unit: 60x60x60 Herbivore unit: 30x30x30	216 L 27 L	Depends on number and size of sediment cores

b. What factor(s) limit these size characteristics?

The size of the units may be varied, but relatively large units may be preferable to properly scale surface area to volume.

c. How much space is required per microcosm unit?

Space for a rack with 3 shelves; overall dimensions: 200 cm (H) x 80 cm (W) x 80 cm (D)

7. For what purpose was the microcosm originally designed?

For testing GEMs

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

10% of the water in the algal unit is replaced on a daily basis (workdays). The percentage may either be increased or decreased to simulate the natural water residence time. The microcosms are not aerated.

9. Equilibrium period:

a. Is laboratory equilibrium required before testing?

Yes_____ No__x__

b. If so, what is the equilibration period?

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

10. Microcosm "lifespan":

a. How long are microcosm tests generally run?

3-4 weeks

b. What are the most important factors in establishing the lifespan of this microcosm?

Wall growth on the sides of the microcosm may limit the lifespan. However, no effects on the bacterial community due to wall growth have been observed.

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	The algal unit is illuminated by 12 Phillips TLD fluorescent tubes. The herbivore unit and the sediment cores are not illuminated.
b. Typical light intensity:	Max 350 $\mu E\ m^{-2}\ sec^{-1}$ measured at water surface. Light intensity may be regulated by turning off individual tubes.
c. Lighting control (intensity, photoperiod, means of control, etc.):	Light cycles are controlled by a PC. Every week the photoperiod is adjusted to the average light/dark ratios for that week.

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	____
b. Relative humidity	____
c. Temperature	__X__
d. Light intensity	__X__
e. Inorganic nutrients	__X__
f. Carbon dioxide	__X__ (for primary production measurements)
g. Dissolved oxygen	__X__
h. Other (specify)	__X__ pH

13. How is temperature controlled (constant temperature room, water bath, etc.)?	The microcosms are housed in a cold room at approx. 5-10°C. The algal tank and the water-bath with the sediment cores are heated with immersed heating elements.
14. How is water/air circulated/mixed?	Water in algal units is mixed by a Teflon®-coated stainless steel paddle adjustable to various speeds. Paddle is 40 x 17 cm with 42 holes (1.5 cm diameter).

CONTAINMENT

1. a. Is containment with current microcosm design microcosm design adequate for working with GEMs?

   Yes_x_ No_____

   b. If so, describe containment design.

   All units are placed in stainless steel pans that drain into a 300-L container in the event of breakage. Extra-strength glass is used in aquaria. Seals and a glass cover prevent escape of aerosols. HEPA filters are used to filter environmental chamber air. Some containment problems may arise while cleaning the zooplankton filter or sampling water.

   c. Could containment be improved by design modification?

   Yes_____ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   ____ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_____ No_x_

   b. Microcosm operation? Yes_____ No_x_

   c. Output analysis? Yes_____ No_x_

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes_x_ No_____

   b. Microcosm operation? Yes_x_ No_____

   c. Output analysis? Yes_x_ No_____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes_____ No_____ A manuscript is in preparation.

   b. Operate a microcosm? Yes_____ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

Replacing the herbivore unit with a large volume of water above the sediment in the sediment cores probably will make it easier to conduct microcosm tests. At the same time the ratio of surface area to water volume would be reduced (less effect of wall growth)

2. What levels of difficulty would be involved in making the modifications in (1) above?

____ a. Considerable resources, skill or time.

____ b. Moderate resources, skill or time.

__x__ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Currently, 10% of the water (i.e., 25 L) is removed for sampling and replaced with new filtered water daily (workdays). More (or less) water could probably be replaced. Given the microcosm size, there is almost no limit to repetitive sampling.

2. Is destructive sampling during the course of a test run required?

Yes____ No__x__

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No____ (e.g., sediment sampling)

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

____ a. Less than $100

____ b. Between $100 and $500

____ c. Between $500 and $1000

__x__ d. More than $1000 (<$2000)

2. How many replicate vessels are generally used per treatment?

Three

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000

____ b. Between $5000 and $20000

____ c. Over $20000

__x__ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by 14C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	chl *a* extraction from phytoplankton	
	ANIMALS		X
	BENTHOS		X
	MICROORGANISMS	Bacteria-AODC; flagellates/ciliates-primulin stain	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Turnover of free amino acids; DOC concentration	
	BACTERIA/PROTOZOA	Grazing by flagellates/ciliates: filtration	
	PLANTS/HERBIVORES		X
	HERBIVORES/PREDATORS		X
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION	^{14}C-carbonate uptake	
	SECONDARY PRODUCTION	^{3}H-thymidine incorp.; bac./flag. production in filtered water	
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN	Analysis: NH_4^+, NO_3^- concentrations	
	PHOSPHORUS	Analysis of PO_4^{-3} concentrations	
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

Fish, clams etc. are excluded as they may change the behavior of the microcosm by eating zooplankton or filtering the water (affecting phytoplankton and microheterotroph populations). A larger volume of water would be required if these organisms are to be included

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	L-I (phytoplankton)	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	H	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	I	____
	BACTERIA/PROTOZOA	I-H	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	L-I	____
	SECONDARY PRODUCTION	H	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	H	____
	PHOSPHORUS	H	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

Light limitation seems to reduce the algal biomass (chl *a*) and primary production. However, this is not reflected in the microbial community. Variability due to organisms filtering the water (zooplankton and benthic invertebrates) tend to be less relative to a single container with water and sediment. The reason is probably that zooplankton and clam/polychaete grazing is limited by the flow rate between units. A manuscript is in preparation that will discuss the problems in more detail.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes ____ No _x_	Yes ____ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes _x_ No ____	Yes _x_ No ____
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON COMPARTMENTALIZED LAKE MICROCOSM

Mr. Niels Kroer
National Environmental Research Institute
Department of Marine Ecology and Microbiology
Frederiksborgvej 399
P.O. Box 358
DK-4000 Roskilde, DENMARK
(45 46 30 13 88)

Coffin, R., N. Kroer, and N. Jorgensen. 1990. Heterotrophic microbial dynamics in aquatic microcosms: Design considerations and field validation. *In:* Review of Progress in the Biotechnology-Microbial Pest Control Agent Risk Assessment Program, EPA/600/9-90/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Cornallis, OR and Environmental Research Laboratory, Gulf Breeze, FL, pp. 137-138

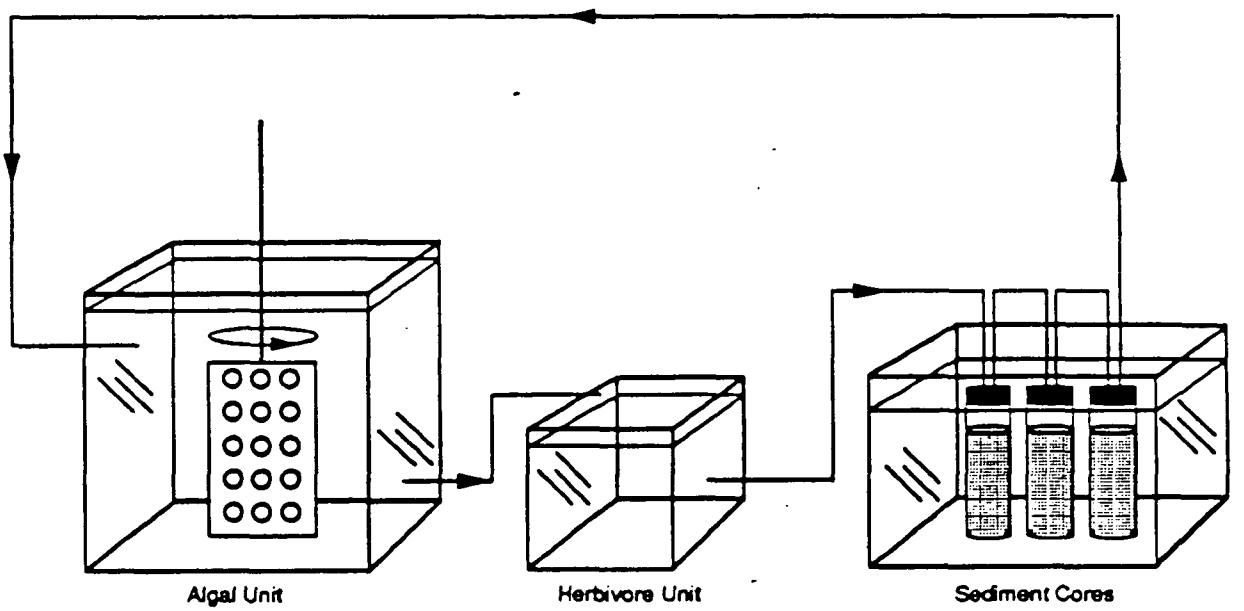


Figure 3. **Compartmentalized lake microcosm.**

MIXED FLASK CULTURE MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: L. SHANNON

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Mixed flask culture (MFC) microcosms are relatively small systems consisting of 50 ml of sand sediment, 900 ml of nutrient medium and 50 ml of inoculum (stock community collected from natural ponds) in 1 L beakers. The beakers are covered with a large petri dish to prevent contamination. The test typically consists of 4 treatment groups, each containing 5 replicate microcosms.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	genera unknown variety of green and blue
Primary producers (specify)	Yes_x_ No___	green algae and diatoms, cladocerans, copepods, rotifers, amphipods
Invertebrates (specify)	Yes_x_ No___	chironomid larvae, snails
Vertebrates (specify)	Yes___ No_x_	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Communities are established from a mixed stock culture derived from samples collected from a variety of natural ponds.
"Wild" samples are allowed to "co-adapt" in the laboratory for 3 months before use.

4. If environmental media are used, how is the environment sampled?

Samples collected in small buckets, mixed in 40-L aquaria. Nutrient medium (T82) is added and systems equilibrated for 3 months.

5. What habitats are represented?

   a. Typically:

Small eutrophic ponds

   b. What factor(s) limit the habitats that could be represented?

(1) Size is the main limiting factor. Because of their small size these systems would be probably be poor surrogates for large pelagic systems. (2) Since these are static they could not represent lotic systems.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	Volume (L)	Soil/Sediment Surface Area (cm^2)
6. Microcosm size:			
a. Typically:	1 L beaker 10 cm dia. 14.5 cm height	1 L	78.5 cm^2
b. What factor(s) limit these size characteristics?	The upper limit is a function of incubator space. These microcosms could not be much smaller or they would be unable to support zooplankton populations.		
c. How much space is required per microcosm unit?	Approximately 2088 cm^3		
7. For what purpose was the microcosm originally designed?	This test system was designed to provide data on the effects of chemicals or microorganisms introduced into a freshwater environment. It can also be used to monitor survival of introduced microorganisms.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	There is free exchange with the air. These are static systems with replacement for evaporative loss.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes__x__ No_____		
b. If so, what is the equilibration period?	Three months for the initial stock culture which can then be maintained for many months (we have maintained some for 1-1/2 to 2 years). The microcosms are allowed to equilibrate for 6 weeks prior to treatment.		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	To allow time for development of algae and zooplankton populations. Equilibration is determined on the basis of primary production (oxygen gain) and zooplankton population density.		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	Usually 42 days; although they have been run over 1 year.		
b. What are the most important factors in establishing the lifespan of this microcosm?	Ability to maintain algae and zooplankton populations.		

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	"cool light" fluorescent tubes.
b. Typical light intensity:	~ 500 foot candles.
c. Lighting control (intensity, photoperiod, means of control, etc.):	12:12, L:D.

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	____
b. Relative humidity	____
c. Temperature	__x__ (controlled @ 20°C)
d. Light intensity	__x__ (controlled)
e. Inorganic nutrients	__x__
f. Carbon dioxide	____
g. Dissolved oxygen	__x__
h. Other (specify)	__x__ (pH, Eh)

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Microcosms are kept in environmental chamber.

14. How is water/air circulated/mixed?

Fans circulate air in the environmental chamber. Water is not mixed.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

   Yes_x_ No_____

   b. If so, describe containment design.

   Each beaker is covered with a large petri dish cover. All beakers are contained in a growth chamber.

   c. Could containment be improved by design modification?

   Yes_x_ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   Add appropriate filters to the air intake and exhaust ports on the growth chamber.

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   ____ b. Moderate resources, skill or time.

   _x_ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No_____

   b. Microcosm operation? Yes_x_ No_____

   c. Output analysis? Yes_x_ No_____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes_____ No_____

   b. Microcosm operation? Yes_____ No_____

   c. Output analysis? Yes_____ No_____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes_____ No_____

   b. Operate a microcosm? Yes_____ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

Sediments are currently being modified to provide substrate for a richer, more diverse microbial community.

2. What levels of difficulty would be involved in making the modifications in (1) above?

____ a. Considerable resources, skill or time.
____ b. Moderate resources, skill or time.
_x_ c. Minimal resources, skill or time.
____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Generally, population sampling is accomplished by withdrawing subsamples (50 ml for zooplankton, 13 ml for microorganisms, 2 ml for protozoa). The 50 ml zooplankton subsamples are replaced. The others are not. These systems are generally able to withstand the removal of 50 mL per week with no ill effects. The volume removed each week is replaced with deionized H_2O.

2. Is destructive sampling during the course of a test run required?

Yes____ No_x_ (although it might be used in some tests)

3. Would design modifications allow the use of alternative sampling strategies?

Yes_x_ No____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

_x_ a. Less than $100
____ b. Between $100 and $500
____ c. Between $500 and $1000
____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Five

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000
_x_ b. Between $5000 and $20000
____ c. Over $20000
____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Algae counted (microscope) in Palmer-Maloney cell	_____
	ANIMALS	Direct count or microscopic count of subsamples	_____
	BENTHOS	Direct count	_____
	MICROORGANISMS	plate count/DAPI stain/^{14}C-gluc. degrad./selec. media	
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	density/activity of bact. funct. groups in sediment	_____
	BACTERIA/PROTOZOA	protozoan vs bacterial functional group densities	_____
	PLANTS/HERBIVORES	algal taxa vs zooplankters, snails, insect density	_____
	HERBIVORES/PREDATORS	Usually not measured: few predators in the system	_____
	OTHER (SPECIFY)		_____
ENERGY FLOW	PRIMARY PRODUCTION	oxygen gain	_____
	SECONDARY PRODUCTION	zooplankton counts	_____
	P/R RATIO	oxygen gain/oxygen loss	_____
	OTHER (SPECIFY)	Total carbon, total dissolved carbon	_____
BIOGEOCHEM. CYCLING	NITROGEN	auto analyzer: NO_3^-, NO_3^-, NH_3	_____
	PHOSPHORUS	auto analyzer: ortho- and total phosphate	_____
	SULFUR		_____
	OTHER (SPECIFY)	Silica	_____
OTHER EFFECTS	PLANT (SPECIFY)		_____
	ANIMAL (SPECIFY)		_____
	MICROBIAL (SPECIFY)		_____
	OTHER (SPECIFY)	pH, Eh	_____

Reasons that a parameter cannot be addressed in your microcosm

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	H(algae); L(macrophy.)	
	ANIMALS	H(zooplank.); I(insect)	
	BENTHOS	I(snails); L(insects)	
	MICROORGANISMS		X
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		X
	BACTERIA/PROTOZOA		X
	PLANTS/HERBIVORES		X
	OTHER (SPECIFY)		X
ENERGY FLOW	PRIMARY PRODUCTION	I	
	SECONDARY PRODUCTION	I	
	P/R RATIO	H	
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN		X
	PHOSPHORUS		X
	SULFUR		X
	OTHER (SPECIFY)		X
OTHER EFFECTS	PLANT (SPECIFY)		X
	ANIMAL (SPECIFY)		X
	MICROBIAL (SPECIFY)		X
	OTHER (SPECIFY)		X

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

Studies are currently being conducted. This microcosm and a new "aquatic core" microcosm are being compared to 9 natural ponds. Parameters being compared include pH, production, respiration, P/R, nutrients and nutrient cycling rates, and populations of : (1) microbial functional groups, (2) algae (3) zooplankton (4) insects, (5) molluscs.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes_x_ No ___	Yes _x_ No ___
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	_H_	_H_
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No ___	Yes___ No ___
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.	Need improved methods for monitoring the organism; resolution with current techniques is not as fine as would be desired.	
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON MIXED FLASK CULTURE MICROCOSM

Dr. Lyle Shannon
University of Minnesota
Biology Department
Duluth, MN 55812
(218) 726-8000

Flum, T.F. and L.J. Shannon. 1987. The effects of three related amides on microecosystem stability. Ecotoxicol. Enviorn. Saf. 13:239-252.

Shannon, L.J., T.E. Flum, R.L. Anderson, and J.D. Yount. 1989. Adaptation of mixed flask culture microcosms for testing the survival and effects of introduced microorganisms. *In:* U.M. Cowgill and L.R. Williams (eds.), Aquatic Toxicology and Hazard Assessment: 12th Volume, ASTM STP 1027, American Society for Testing and Materials, Philadelphia, pp. 224-239.

Shanon, L.J., T.E. Flum, and J.D. Yount. 1989. Draft Protocol for a Mixed Flask Culture Microcosm Toxicity Test.

Yount, J.D. and L.J. Shannon. 1988. State changes in laboratory microecosystems in response to chemicals from three structural groups. *In:* J. Cairns, Jr., and J.R. Pratt (:eds.) Functional Testing of Aquatic Biota for Estimating Hazards of Chemicals, ASTM STP 988, American Society for Testing and Materials, Philadelphia, pp. 86-96.

POND MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: J. GIDDINGS

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

The system consists of glass aquaria (generally 80-L, although 8-L and 120-L systems have also been used), containing natural pond water and a 5- to 10-cm sediment layer. The microcosm contains the natural macrophytic, pelagic and benthic communities.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	Pelagic, benthic
Primary producers (specify)	Yes_x_ No___	Algae, macrophytes
Invertebrates (specify)	Yes_x_ No___	Zooplankton, benthos
Vertebrates (specify)	Yes___ No_x_	(Fish could be included)
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Pond water and sediment are collected from natural sources and placed into aquaria. Macrophytes (community from natural sources) are planted. Community may be supplemented by zooplankton or macroinvertebrates from natural sources or from cultures.

4. If environmental media are used, how is the environment sampled?

Sediment collected with shovel or dredge. Water collected with pump, sampling bottle, or depth-integrated column sampler. Macrophyte communities collected *en masse* by hand.

5. What habitats are represented?

   a. Typically:

System normally includes aerobic and anaerobic sediment, macrophyte, and free-swimming habitats corresponding to typical littoral freshwater environments.

   b. What factor(s) limit the habitats that could be represented?

Shallow depth, absence of circulation and water renewal. Lotic or deep pelagic systems cannot be simulated except in general sense.

GENERAL CHARACTERISTICS (CONTINUED)

6. Microcosm size:

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
a. Typically:	60 x 30 x 40(D) or 60 x 30 x 60(D)	80-120	2000

b. What factor(s) limit these size characteristics?

Lab space (controlled light and temperature) is only limitation. Systems less than 80 L possible but harder to sample, more variable.

c. How much space is required per microcosm unit?

Less than 4 m^2 for 12 to 20 replicates.

---

7. For what purpose was the microcosm originally designed?

Measuring fate and effects of toxicants on typical freshwater ecosystems.

---

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

Aeration can be provided but usually isn't; macrophytes supply plenty of oxygen. Pond water added to replace water removed in sampling; distilled water added to replace water lost by evaporation.

---

9. Equilibrium period:

a. Is laboratory equilibrium required before testing?

Yes__x__ No_____

b. If so, what is the equilibration period?

6-8 weeks

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

Criteria: Photosynthesis/respiration ratio should be approximately one (as determined by D.O. concentrations). The pH usually levels off at ~ 8-9. Macrophytes become well-established.

Purpose: Achieve representative productivity; reach relative stability (conditions relatively constant day-to-day); replicates become more uniform.

---

10. Microcosm "lifespan":

a. How long are microcosm tests generally run?

6-12 months.

b. What are the most important factors in establishing the lifespan of this microcosm?

Eventually, macrophytes senesce (nutrient limitation?) and replicates diverge.

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	Sun-simulating fluorescent lights
b. Typical light intensity:	150-250 $\mu E\ m^{-2}\ sec^{-1}$ (about 1/3 full sunlight)
c. Lighting control (intensity, photoperiod, means of control, etc.):	12:12 photoperiod

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	____	
b. Relative humidity	____	
c. Temperature	X	
d. Light intensity	X	
e. Inorganic nutrients	X	(N, P)
f. Carbon dioxide	X	(pH, alkalinity)
g. Dissolved oxygen	X	
h. Other (specify)	X	conductivity, organic carbon, suspended solids

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Environmental chamber (usually)

14. How is water/air circulated/mixed?

Not done.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

   Yes_____ No__x__

   b. If so, describe containment design.

   c. Could containment be improved by design modification?

   Yes__x__ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   Use filters, anteroom in environmental chamber.

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   ____ b. Moderate resources, skill or time.

   __x__ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes__x__ No_____

   b. Microcosm operation? Yes__x__ No_____

   c. Output analysis? Yes_____ No__x__ (Standard ANOVA or regression analysis is sufficient)

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes_____ No_____

   b. Microcosm operation? Yes_____ No_____

   c. Output analysis? Yes__x__ No_____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes__x__ No_____

   b. Operate a microcosm? Yes__x__ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

Develop sampling techniques for sediment.
Apply microbiological techniques to benthic and planktonic communities.

2. What levels of difficulty would be involved in making the modifications in (1) above?

____ a. Considerable resources, skill or time.
____ b. Moderate resources, skill or time.
__x__ c. Minimal resources, skill or time. (If microb. tech. exist).
____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Sediment sampling would be limited by quantity of sediment available (roughly 10-20 L). Repeated destructive sampling would disturb ecological conditions. Otherwise, there are few practical limits. Repeated sampling and monitoring are normal.

2. Is destructive sampling during the course of a test run required?

Yes__x__ No__x__ (Yes, for enumeration/monitoring of benthic or pelagic communities.)

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

__x__ a. Less than $100
____ b. Between $100 and $500
____ c. Between $500 and $1000
____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Three

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000
____ b. Between $5000 and $20000
__x__ c. Over $20000
____ d. An estimate has not been made

(Main cost is labor for monitoring which varies depending on test objectives.)

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	pigment analysis; periphytometers; macrophyte observed	_____
	ANIMALS	zooplank. collect.; macroinvert. obs. final harvest	_____
	BENTHOS	macroinvertebrate obs.; final harvest (sieving)	_____
	MICROORGANISMS	any applicable ecological techniques	_____
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	litter bags & glucose uptake have been measured	_____
	BACTERIA/PROTOZOA		_____
	PLANTS/HERBIVORES	Not studied; could use enclosures/repeated sampling	_____
	HERBIVORES/PREDATORS		_____
	OTHER (SPECIFY)		_____
ENERGY FLOW	PRIMARY PRODUCTION	Diurnal D.O., ^{14}C	_____
	SECONDARY PRODUCTION	Diurnal D.O	_____
	P/R RATIO	Diurnal D.O	_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM.	NITROGEN	Any applicable ecological techniques; water anal.	_____
	CYCLING PHOSPHORUS	Same	_____
	SULFUR	Same	_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)		_____
	ANIMAL (SPECIFY)	Fish survival and growth; on site bioassays	_____
	MICROBIAL (SPECIFY)		_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	H	
	ANIMALS	I	
	BENTHOS	I	
	MICROORGANISMS	H	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	I	
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES		
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION	H	
	SECONDARY PRODUCTION		
	P/R RATIO	H	
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN	I	
	PHOSPHORUS	I	
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization Yes ____ No _X_	Environmental Mobility (Specify organism or gene) Yes ____ No _X_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ____ No ____ Possibly	Yes____ No ____
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON POND MICROCOSM

Dr. Jeffrey Giddings
Springborn Bionomics, Inc.
790 Main Street
Wareham, MA 02571
(508) 295-2550

Franco, P.J, J.M. Giddings, S.E. Herbes, L.A. Hook, J.D. Newbold, W.K. Roy, G.R. Southworth, and A.J. Stewart. 1984. Effects of chronic exposure to coal-derived oil on freshwater ecosystems: I. Microcosms. Environ. Toxicol. Chem. 3:447-463.

Giddings, J.M. 1986. A microcosm procedure for determining safe levels of chemical exposure in shallow-water communities. *In:* J. Cairns, Jr. (ed.), Community Toxicity Testing, ASTM STP 920, American Society for Testing and Materials, Philadelphia, pp.121-134.

Giddings, J.M., and P.J. Franco. 1985. Calibration of Laboratory bioassays with results from microcosms and ponds. *In:* T.P. Boyle (ed.), Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, ASTM STP 865, American Society for Testing and Materials, Philadelphia, pp. 104- 119.

SEDIMENT CORE MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: PRITCHARD/CLARK

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Three borosilicate glass vessel designs have been used, each with an intact sediment core and an overlying water column: Ecocore uses 35 mm (diam.) x 40 cm glass tubes; Ecocore II uses 3 or 4 L reaction kettles (Corning 6947) or 27-L Jars (Corning 6942-27L), or Seagrass Communities of clear acrylic tubes (16 cm diam. x 50 cm) with flat, acrylic bottoms.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	Bacteria, protozoa
Primary producers (specify)	Yes_x_ No___	Phytoplankton, seagrasses
Invertebrates (specify)	Yes_x_ No___	Benthic, epibenthic
Vertebrates (specify)	Yes___ No_x_	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Natural assemblages of water column plankton are added to microcosms containing intact sediment cores with their associated benthic and/or seagrass communities.

4. If environmental media are used, how is the environment sampled?

Water is collected in a carboy, and sediment in acrylic or glass coring devices.

5. What habitats are represented?

   a. Typically:

Usually salt marsh or shallow estuarine bay, vegetated or barren substrates. Freshwater systems (including a eutrophic lake) have been simulated.

   b. What factor(s) limit the habitats that could be represented?

Scaling considerations for deep bodies of water.

GENERAL CHARACTERISTICS (CONTINUED)

6. Microcosm size:

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
a. Typically:	3.5 (diam) x 40	0.175	9.6
	13 (diam) x 24/32	3.0/4.0	133/133
	29 (diam) x 45	27	660
	16 (diam) x 50	10	200

b. What factor(s) limit these size characteristics?

Lower limit: sampling frequency and volumes, inclusion of larger animals/plants.
Upper limit: Decontamination of vessels, effluent and containment considerations.

c. How much space is required per microcosm unit?

Ecocore: 25 cm^2
Ecocore II: 0.3 m^2
Seagrass system: 0.2 m^2

7. For what purpose was the microcosm originally designed?

Ecocore and Ecocore II (reaction kettles): to determine the fate of xenobiotic compounds; 27-L system: GEM Risk Assessment; seagrass microcosm: ecological effects of test chemicals.

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

Ecocore: static operation, aerated (and mixed) with a long stainless steel needle; Reaction kettle: both static and flow-through (40 ml/h) modes; 27-1 system: daily batch replacement (10%); seagrass community: flow-through design (7 L/h) with airstone for mixing and aeration

9. Equilibrium period:

a. Is laboratory equilibrium required before testing?

Yes__x__ No_____

b. If so, what is the equilibration period?

At least overnight.

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

Primarily to allow settling of particulates suspended as a result of sampling.

10. Microcosm "lifespan":

a. How long are microcosm tests generally run?

Usually, 2 to 6 weeks

b. What are the most important factors in establishing the lifespan of this microcosm?

Wall growth and food/nutrients limitations if operated in a static mode.

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	Earlier tests: fluorescent (40-W, cool white; 250-W GE Power Groove).
b. Typical light intensity:	900 Einsteins $m^{-2} s^{-1}$, measured at water surface, with two 400-W Multi-Vapor lamps.
c. Lighting control (intensity, photoperiod, means of control, etc.):	Timer controls photoperiod, typically 14:10 (Light:Dark).

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	____
b. Relative humidity	____
c. Temperature	__x__
d. Light intensity	____
e. Inorganic nutrients	__x__ (NH_3 concentration)
f. Carbon dioxide	____
g. Dissolved oxygen	__x__
h. Other (specify)	__x__ Salinity

13. How is temperature controlled (constant temperature room, water bath, etc.)?	Clear acrylic bath, with refrigerated circulator attached.
14. How is water/air circulated/mixed?	Ecocore: Aeration through needle. Ecocore II: 300 rpm motor and glass stirrer. Seagrass Community: Water flow and air stone.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs? Yes_____ No__x__

   b. If so, describe containment design.

   c. Could containment be improved by design modification? Yes__x__ No_____

   d. If so, what is the nature of the modifications needed to improve containment? An enclosure with HEPA filters would be required, and the effluent would have to be treated. Sealed tops may be added to the microcosm vessels.

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   __x__ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_____ No__x__

   b. Microcosm operation? Yes_____ No__x__

   c. Output analysis? Yes_____ No__x__

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes__x__ No_____

   b. Microcosm operation? Yes__x__ No_____

   c. Output analysis? Yes__x__ No_____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes__x__ No_____

   b. Operate a microcosm? Yes__x__ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

None

2. What levels of difficulty would be involved in making the modifications in (1) above?

_____ a. Considerable resources, skill or time.

_____ b. Moderate resources, skill or time.

_____ c. Minimal resources, skill or time.

_____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Static systems (i.e., Ecocore) are limited by the relatively small volume of water and sediment, while the larger systems which use periodic water replacement or flow-through design do not share these problems. All systems can be replicated (more easily with smaller systems) and may be destructively sampled, however.

2. Is destructive sampling during the course of a test run required?

Yes_____ No__x__ (But is desirable for Ecocore)

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No_____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

__x__ a. Less than $100 (Ecocore, Seagrass com.)

__x__ b. Between $100 and $500 (Reaction kettle, 27-L jar)

____ c. Between $500 and $1000

____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Two for small systems, up to eight for seagrass systems

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000

____ b. Between $5000 and $20000

____ c. Over $20000

__x__ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Plant composition for seagrass, epiphytes abundance	_____
	ANIMALS	Epifauna colonizing seagrass	_____
	BENTHOS	Sieving, Rose Bengal staining, and sorting	_____
	MICROORGANISMS	AO Direct Counts; CFU; bact. diversity by morphol.	_____
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	5-amino acid total pool/turnover	_____
	BACTERIA/PROTOZOA	Selective filtration, staining, and counting	_____
	PLANTS/HERBIVORES		_____
	HERBIVORES/PREDATORS		_____
	OTHER (SPECIFY)	Leaf litter loss rate	_____
ENERGY FLOW	PRIMARY PRODUCTION	Phytoplankton ^{14}C-uptake; macrophyte-growth	_____
	SECONDARY PRODUCTION	Thymidine uptake; leucine uptake	_____
	P/R RATIO	24-hour dissolved oxygen cycle	_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM. CYCLING	NITROGEN	Ammonia concentration	_____
	PHOSPHORUS	Phosphate concentration	_____
	SULFUR		_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)	Thalassia-chl *a*; epiphyte: chl *a*, dry wt	_____
	ANIMAL (SPECIFY)		_____
	MICROBIAL (SPECIFY)	Gene exchange	_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm

Large vertebrates or invertebrates may not be appropriate due to small vessel size, or flow of water necessary to provide planktonic food.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	L	____
	ANIMALS	L	____
	BENTHOS	L	____
	MICROORGANISMS	Diversity-L; ADOC-H; CFU-H	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	I	____
	BACTERIA/PROTOZOA	I	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	L	____
	SECONDARY PRODUCTION	Thiamine uptake-H; glut. assim./min.-H	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	L pH	____
BIOGEOCHEM. CYCLING	NITROGEN	L Ammonia	____
	PHOSPHORUS	I Phosphate	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

Statistical problems (i.e., how many samples, what sampling intervals, choice of statistical tests, etc. to detect significant differences), selection of sensitive endpoints, and interpretation (what do differences mean?).

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization Yes ____ No _x_	Environmental Mobility (Specify organism or gene) Yes ____ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes _x_ No____ Possibly	Yes_x_ No ____
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON SEDIMENT CORE MICROCOSM

Dr. P.H. Pritchard
U.S. EPA
Environmental Research
Laboratory
Gulf Breeze, FL 32561
(FTS) 228-9340

Dr. James Clark
Environmental Toxicology Division
Exxon Biomedical Sciences, Inc.
Mettlers Road, CN 2350
East Millstone, NJ 08875-2350
(908) 873-6039

Clark, J.R., and J.M. Macauley. 1990. Comparison of the seagrass *Thalassia testudinum* and its epiphytes in the field and in Laboratory test systems. *In:* W. Wang, J.W. Gorsuch, and W.R. Lower, (eds.), Plants for Toxicity Assessment, ASTM STP 109-1, American Society for Testing and Materials, Philadelphia, pp. 59-68.

Coffin, R., N. Kroer, and J. Jorgensen, 1990. Heterotrophic microbial dynamics in aquatic microcosms: Design considerations and field validation. *In:* Review of Progress in the Biotechnology-Microbial Pest Control Agent Risk Assessment Program, EPA/600/9-90/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR, and Environmental Research Laboratory, Gulf Breeze, FL, pp. 137-138.

Cripe, C.R., and P.H. Pritchard. 1990. Aquatic test systems for studying the fate of xenobiotic compounds. *In:* Aquatic Toxicology and Risk Assessment: Thirteenth Volume, ASTM STP 1096, W.G. Landis and W.H. van der Schalie (eds.), American Society for Testing and Materials, Philadelphia, PA, pp. 29-47.

Kroer, N., and R.B. Coffin. Microbial trophic interactions in aquatic microcosms developed for testing genetically engineered micro-organisms: A field comparison. In press. Microbial Ecology.

Macauley, J.M., J.R. Clark, and A.R. Pitts. 1990. Use of *Thalassia* and its epiphytes for toxicity assessment: Effects of a drilling fluid and tributyltin. *In:* W. Wang, J.W. Gorsuch, and W.R. Lower, (eds.), Plants for Toxicity Assessment, ASTM STP 1091, American Society for Testing and Materials, Philadelphia, pp. 255-266.

Morton, R.D., T.D. Duke, J.M. Macauley, J.R. Clark, W.A. Price, S.J. Hendricks, S.L. Owsley-Montgomery, and G.R. Plaia. 1986. Impact of drilling fluids on seagrasses: An experimental community approach. *In:* J. Cairns, Jr. (ed.), Community Toxicity Testing, ASTM STP 920, American Society for Testing and Materials, Philadelphia, pp.199-212.

O'Neill, E.J., C.R. Cripe, L.H. Mueller, J.P. Connolly, P.H. Pritchard. 1989. Fate of Fenthion in Salt-marsh Environments: II. Transport and Biodegradation in Microcosms. Environ. Toxicol. Chem. 8:759-768.

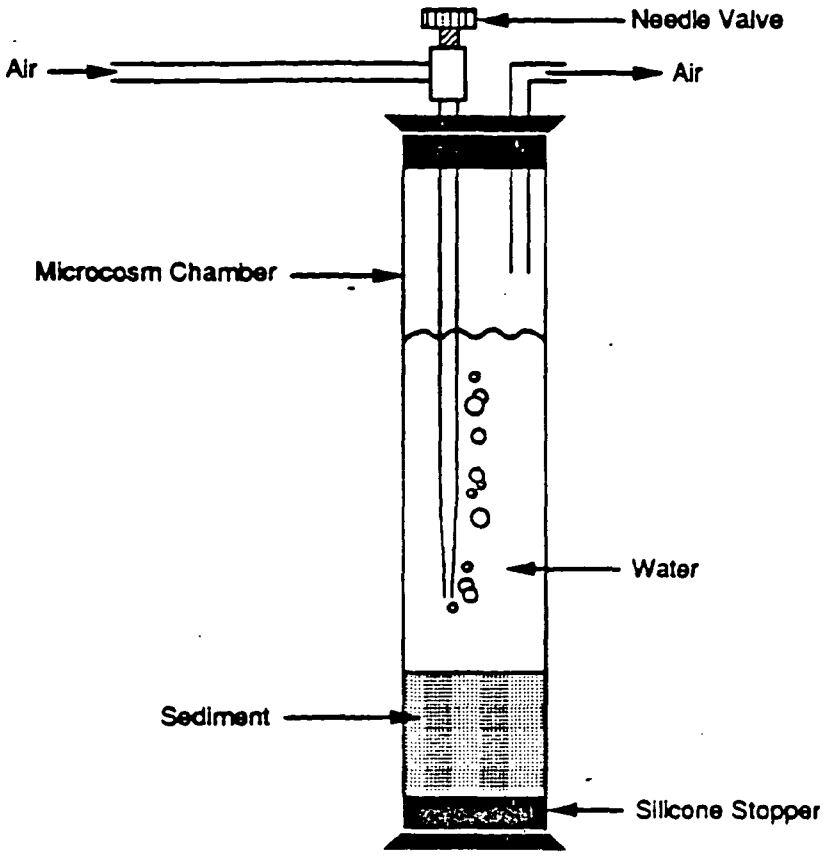


Figure 4. **Ecocore microcosm.**

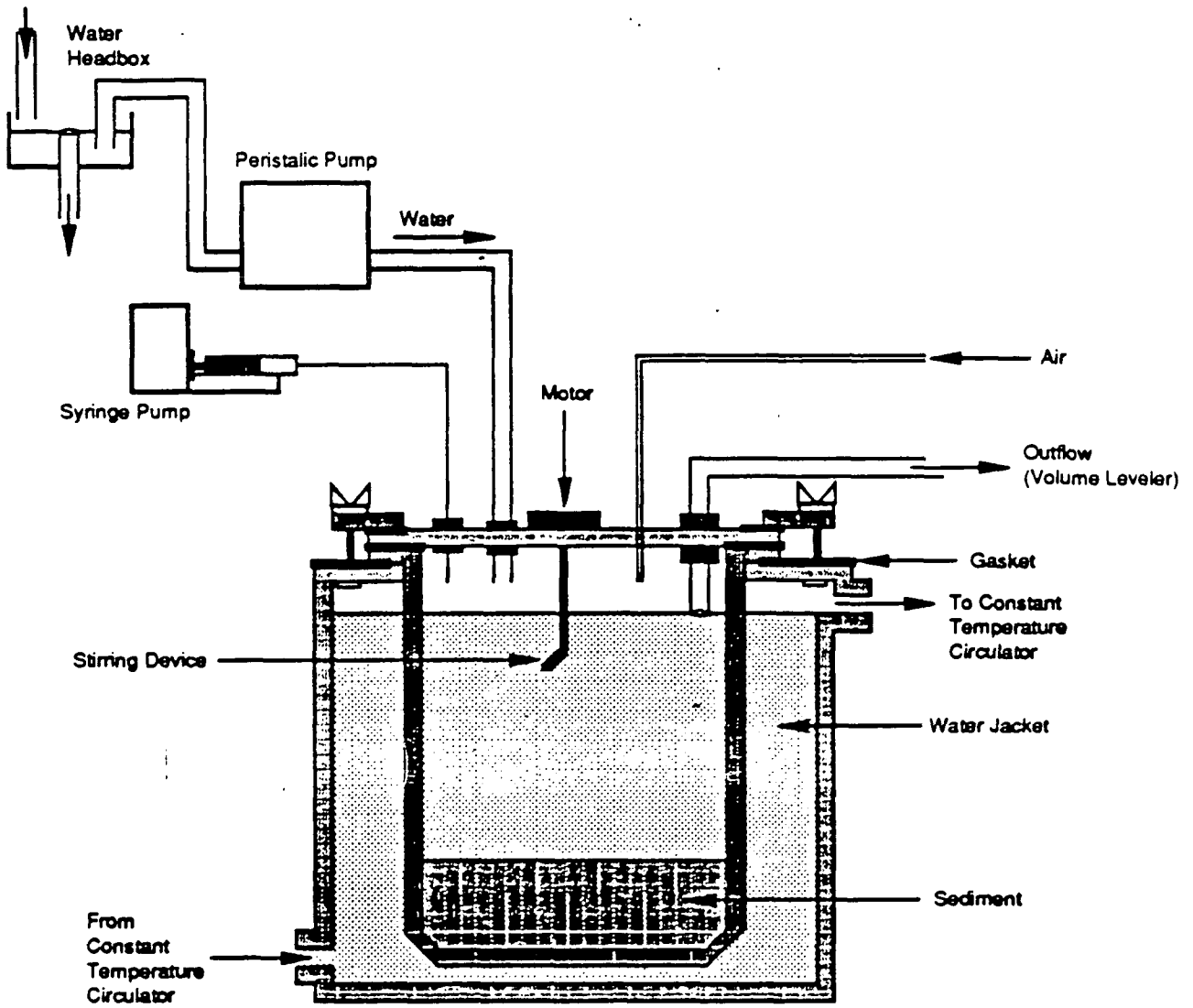


Figure 5. **Ecocore II microcosm.**

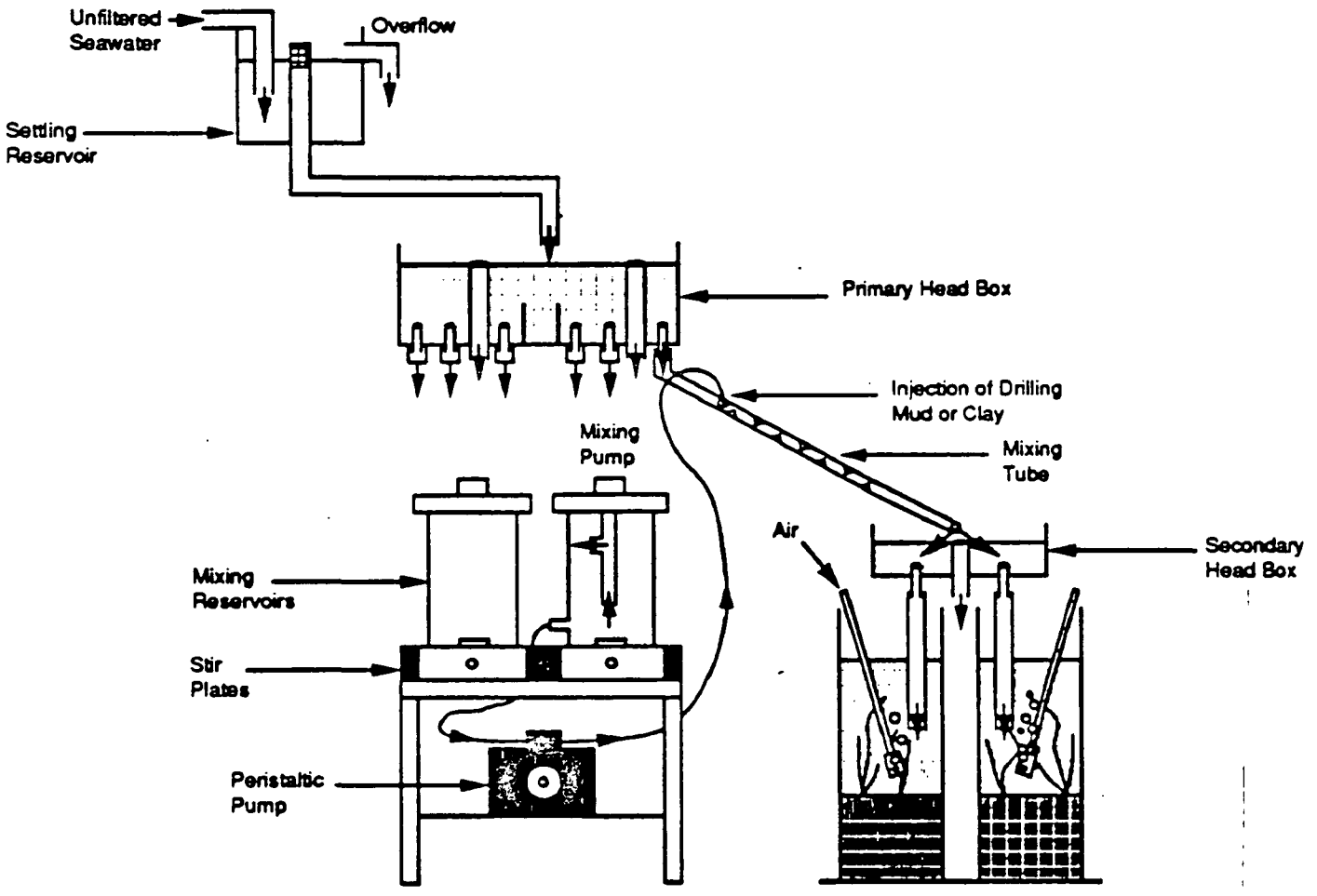


Figure 6. **Seagrass community.**

STANDARD AQUATIC MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: F. TAUB

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Each microcosm consists of a 4-L glass container, covered with a petri dish. Substrate is washed sand plus chitin and cellulose. Medium is distilled water and reagent grade salts. Algae and invertebrates are added from laboratory cultures.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	_____
Primary producers (specify)	Yes_x_ No___	10 species of algae_____
Invertebrates (specify)	Yes_x_ No___	5 species_____
Vertebrates (specify)	Yes___ No_x_	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Laboratory cultures are the source for the organisms. Reinoculation of organisms is done once per week at numbers below the detection limit (are likely to be counted only if reproduction occurs). This allows populations to develop after temporary periods of toxicity or random extinction.

4. If environmental media are used, how is the environment sampled?

N/A

5. What habitats are represented?

   a. Typically:

Early spring through summer of a temperate aquatic community, e.g., pond.

   b. What factor(s) limit the habitats that could be represented?

Size is a limitation; large carnivores cannot be included. Preliminary work was done on a marine system.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	Volume (L)	Soil/Sediment Surface Area (cm^2)
6. Microcosm size:			
a. Typically:		3 L	314.2
b. What factor(s) limit these size characteristics?	convenience, number of replicates		
c. How much space is required per microcosm unit?	A typical SAM microcosm experiment using 24-30 microcosms can be run on a 2.6 x .85 meter table in a temperature controlled room or reach-in incubator.		
7. For what purpose was the microcosm originally designed?	This microcosm was designed to measure ecological effects of a test chemical or to explore the potential of a novel organism to invade and become established, and its effects such as changes in nutrient cycling or species displacement.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems; describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	The petri dish cover allows some exchange with the atmosphere, especially when it is removed for sampling the community. Aeration is avoided because dawn-night-dawn oxygen measurements are used to estimate net photosynthesis and respiration.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes_x_ No_____		
b. If so, what is the equilibration period?	7 days		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	The growth of algae and reproduction of animals are checked and outlier(s) (if any) or cracked microcosms are eliminated.		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	SOP is 63 days, but some have been maintained for up to a year.		
b. What are the most important factors in establishing the lifespan of this microcosm?	Volume removed in twice-weekly sampling.		
11. What kind of lighting is used?			
a. Type of lights (wattage, model, source, etc.):	Two 8-foot (high intensity, warm white) fluorescent tubes (GE F96PG17WW).		
b. Typical light intensity:	80 $\mu E\ m^{-2}\ sec^{-1}$ (850-1000 ft-c).		
c. Lighting control (intensity, photoperiod, means of control, etc.):	12:12 L:D photoperiod		

GENERAL CHARACTERISTICS (CONTINUED)

12. Which of the following environmental parameters are routinely monitored?

a.	Soil moisture	____
b.	Relative humidity	____
c.	Temperature	_X_
d.	Light intensity	_X_
e.	Inorganic nutrients	_X_
f.	Carbon dioxide	____
g.	Dissolved oxygen	_X_
h.	Other (specify)	_X_ NO_3^-, NO_2^-, NH_3, pH, O_2 (3 point), pH

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Environmental chamber, or temperature controlled room.

14. How is water/air circulated/mixed?

Manually, before sampling.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

Yes____ No_X_

b. If so, describe containment design.

c. Could containment be improved by design modification?

Yes_X_ No____

d. If so, what is the nature of the modifications needed to improve containment?

Unbreakable containers (e.g., change from glass to plastic). Sampling procedures would require change.

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

____ a. Considerable resources, skill, or time.

____ b. Moderate resources, skill or time.

_X_ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No____

   b. Microcosm operation? Yes_x_ No____

   c. Output analysis? Yes_x_ No____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No____

   b. Microcosm operation? Yes____ No____

   c. Output analysis? Yes____ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes_x_ No____

   b. Operate a microcosm? Yes_x_ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

   Define the microbial community (concurrently, algae—including blue-greens—and protozoa, rotifers, etc. are enumerated), but not (usually) specific bacterial, fungal species.

2. What levels of difficulty would be involved in making the modifications in (1) above?

   ____ a. Considerable resources, skill or time.

   _x_ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Sampling of algae, protozoa and rotifers requires removing a few ml.

Sampling of pH and O_2 currently involves electrode introduction; perhaps these could be chemically decontaminated after use.

Sampling of zooplankton (remove, pour subsamples, return) would have to be modified. Photography is a possibility.

2. Is destructive sampling during the course of a test run required?

Yes____ No__x__

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

__x__ a. Less than $100

____ b. Between $100 and $500

____ c. Between $500 and $1000

____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Five or Six

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000

__x__ b. Between $5000 and $20000

____ c. Over $20000

____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Algae: count (10 sp.); dominance, diversity index	_____
	ANIMALS	Count 5 species of animals; species dominance,	_____
	BENTHOS	Ostracod and amphipods are part of system	_____
	MICROORGANISMS	CFU select. media; Electron Transport System; ATP	_____
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		_____
	BACTERIA/PROTOZOA	CPU and microscopic protozoan counts	_____
	PLANTS/HERBIVORES	Algal counts and herbivore counts	_____
	HERBIVORES/PREDATORS	(might use invertebrate predators/small fish)	_____
	OTHER (SPECIFY)		_____
ENERGY FLOW	PRIMARY PRODUCTION		_____
	SECONDARY PRODUCTION		_____
	P/R RATIO		_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM. CYCLING	NITROGEN	Analysis NO_3^- (plant uptake), NO_2^-, NH_3 (from zooplankton)	_____
	PHOSPHORUS	Algal uptake and recycling by zooplankton	_____
	SULFUR		_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)	Changes in algal dominance, species diversity	_____
	ANIMAL (SPECIFY)	Changes in animal dominance, species diversity	_____
	MICROBIAL (SPECIFY)	Antibiotic resistance	_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm	System is too small for fish population. Small fish, such as juvenile Medaka would be a possibility.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	I	
	ANIMALS	I	
	BENTHOS	I	
	MICROORGANISMS		
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES	I	
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION	I	
	SECONDARY PRODUCTION		
	P/R RATIO	I	
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN	I	
	PHOSPHORUS	I	
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

A dissertation by F. Joan Hardy (1984, "Responses of naturally-derived aquatic microcosms to selective chemical stress," doctoral dissertation, University of Washington, Seattle, WA, 276 p.) compared the responses of indoor and outdoor microcosms derived from Lake Washington and Green Lake to the "Standardized Aquatic Microcosm" during two sequential years. Although the test utilized streptomycin as a stressor, comparison of the controls should provide information relevant to field calibration of this system.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
1. Has your microcosm response to this factor been compared to field data?	Yes ___ No _x_	Yes ___ No ___
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	Yes ___ No ___	Yes ___ No ___
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes _x_ No ___ Depends on funding	Yes ___ No ___
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?	Copper, insecticide, and streptomycin effects.	

FURTHER INFORMATION ON STANDARDIZED AQUATIC MICROCOSM

Dr. Frieda Taub
School of Fisheries, HF-15
University of Washington
Seattle, WA 98195
(206) 685-2115

ASTM, 1991. Practice for Standardized Aquatic Microcosms: Fresh Water. 1991 Annual Book of ASTM Standards, Vol. 11.04, American Society for Testing and Materials, Philadelphia.

Conquest, L.L., and F.B. Taub. 1989. Repeatability and reproducibility of the Standardized Aquatic Microcosm: Statistical properties, p. 159-177. In U. Cowgill (ed.) Aquatic Toxicology and Hazard Assessment, 12th Volume ASTM STP 1027, American Society for Testing and Materials, Philadelphia, PA.

Haley, M.V., E.L. Vickers, T.-C. Cheng, J. DeFrank, T.A. Justus, and W.G. Landis. 1990. Biodegradation and reduction in aquatic toxicity of the persistent riot control material 1,4-Dibenz-Oxazepine. *In:* Aquatic Toxicology and Risk Assessment: Thirteenth Volume, ASTM STP 1096, W.G. Landis and W.H. van der Schalie (eds.), American Society for Testing and Materials, Philadelphia, PA, pp. 60-76.

Harrass, M.C., and F. B. Taub. 1985. Comparison of laboratory microcosms and field responses to copper. *In:* T.P. Boyle (ed.), Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems, ASTM STP 865, American Society for Testing and Materials, Philadelphia, pp. 57-74.

Swartzman, G.L., F.B. Taub, J. Meador, C. Huang, and A.C. Kendig. 1990. Modeling the effect of algal biomass on multispecies aquatic microcosms response to copper toxicity. Aquat. Toxicol. 17: 93-118.

Taub, F.B., P.L. Read, A.C. Kindig, M.C. Harrass, H.J. Hartmann, L.L. Conquest, F.J. Hardy, and P.T. Munro. 1983. Demonstration of the ecological effects of streptomycin and malathion on synthetic aquatic microcosms. *In:* W.E. Bishop, R.D. Cardwell, B.B. Heidolph (eds.), Aquatic Toxicology and Hazard Assessment: Sixth Symposium, ASTM STP 802, American Society for Testing and Materials, Philadelphia, pp. 5-25.

Taub, F.B., and Read, P.L. "Standard aquatic microcosm protocol," Draft final report, U.S. Food and Drug Administration Contract No. 223-83-7000 with FDA, Washington, DC 20204 (1986). Available from Dr. B.L. Hoffmann, U.S. FDA, HFF-304, Rm. 511157, 200 C Street SW, Washington, DC 20204.

STREAM MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: T. BOTT

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Each microcosm is constructed of vinyl coated heavy gauge steel with plexiglass end plates. A drilled, plexiglass buffer plate is used to establish laminar flow. Surface sediments (2 cm) from the stream are placed into 40 plastic trays (0.1 m square x 0.051 m deep) with the bottoms removed and replaced with 400-μm mesh nylon screen. This allows for exchange of water, dissolved nutrients, and biota between the surface sediments and those under the trays and reduces the likelihood of generating anaerobic conditions. Microcosms are housed in a greenhouse.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	Bacteria/fungi/algae/protozoa
Primary producers (specify)	Yes_x_ No___	Algae
Invertebrates (specify)	Yes_x_ No___	Insects, snails, meiofauna
Vertebrates (specify)	Yes___ No_x_	___
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Seeding from natural "parent" stream.

4. If environmental media are used, how is the environment sampled?

Surface sediments are removed from White Clay Creek with a shovel and transferred to a pail, brought to a greenhouse, and placed in trays. Coarser sediments underneath are collected similarly and placed in the microcosms. The trays are then placed on top.

5. What habitats are represented?

a. Typically:

Flowing stream (presently simulates a slow run); specialized habitats such as leaf packs, rocks or pools can be added to the system.

b. What factor(s) limit the habitats that could be represented?

Size and slope limit flow to moderate velocity; fast ripple would be hard to duplicate; size also limits number of habitats included when sample replication is factored in.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	Volume (L)	Soil/Sediment Surface Area (cm^2)
6. Microcosm size:			
a. Typically:	223 cm (L) x 20.3 cm (W) x 12.7 cm (D)		
b. What factor(s) limit these size characteristics?			
c. How much space is required per microcosm unit?	c. Greenhouse is 3.69 m wide x 4.62 m long.		
7. For what purpose was the microcosm originally designed?	Testing effects of introduced bacteria on benthic community and stream ecosystem parameters.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Water from the creek is pumped to a 140 L header tank from which it is distributed to water jackets and microcosms. The water from each microcosm is collected through five 2.54 cm i.d. tubes into 20 L collection tank (also in a water jacket) from which it is recycled to the head of each microcosm. Water is discharged to the parent stream after filtration (cartridge filters) and treatment by ultraviolet radiation (Sanitron Sterilizer).		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes__x__ No_____		
b. If so, what is the equilibration period?	4 weeks		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	*Purpose:* To let sediments resettle and surface communities to reestablish. Criteria were not established or used - but would involve testing for chlorophyll a concentrations, algal species occurrence, insect species occurrence.		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	1 to 4 months; several years may be possible.		
b. What are the most important factors in establishing the lifespan of this microcosm?	1) Construction material. 2) Sediment build-up from repeated storms (water coming in carries silt from parent stream during storms which settles out because microcosm flow rate is always the same).		

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	Ambient solar radiation
b. Typical light intensity:	
c. Lighting control (intensity, photoperiod, means of control, etc.):	

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	
b. Relative humidity	
c. Temperature	X
d. Light intensity	X
e. Inorganic nutrients	X
f. Carbon dioxide	
g. Dissolved oxygen	
h. Other (specify)	

13. How is temperature controlled (constant temperature room, water bath, etc.)?	Water jackets and the use of vinyl coated metal maintains near-ambient streamwater temperatures.
14. How is water/air circulated/mixed?	Water (35L) is recirculated through the systems with the addition of 0.9 L of new water/min. This can be varied. Overflow is returned to stream after treatment (see 8). Teel Pumps (IP677A) are used for recirculation from collection tanks to the top of the microcosm stream.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

   Yes_____ No__x__ Partially

   b. If so, describe containment design.

   See 8 (above) for treatment of discharge water.

   c. Could containment be improved by design modification?

   Yes__x__ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   Increase isolation of each stream. Cement greenhouse floor (presently gravel). Filter air in greenhouse and use negative pressure. Need larger collection pool in event of pump failure.

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   __x__ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_____ No__x__

   b. Microcosm operation? Yes_____ No__x__

   c. Output analysis? Yes_____ No__x__

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes_____ No__x__

   b. Microcosm operation? Yes_____ No__x__

   c. Output analysis? Yes_____ No__x__

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes__x__ No_____

   b. Operate a microcosm? Yes__x__ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

Make slightly deeper and enlarge exit ports to allow for greater water velocity and simulation of faster flows in riffles.

2. What levels of difficulty would be involved in making the modifications in (1) above?

____ a. Considerable resources, skill or time.

_x_ b. Moderate resources, skill or time.

____ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Removal of trays from system for measures of photosynthesis and respiration in respirometers followed by destructive sampling of sediments for analyses of ATP, chlorophyll *a*, total bacterial densities, densities of added bacterial population, enzyme activities, protozoa and meiofaunal densities (if desired), uptake of radio-actively tagged nutrients, bacterial productivity measurements. Number of trays limits sampling of the system.

2. Is destructive sampling during the course of a test run required?

Yes_x_ No____

3. Would design modifications allow the use of alternative sampling strategies?

Yes____ No_x_

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?	___ a.	Less than $100
	_x_ b.	Between $100 and $500 (Includes recirculation, not water supply)
	___ c.	Between $500 and $1000
	___ d.	More than $1000
2. How many replicate vessels are generally used per treatment?	Two	
3. What is the estimated minimal cost of a complete microcosm test, including vessels?	___ a.	Less than $5000
	___ b.	Between $5000 and $20000
	___ c.	Over $20000
	_x_ d.	An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Algal biomass by chlorophyll *a*, spp. by microscopy	
	ANIMALS		
	BENTHOS	Sieve, sort, count, weigh, identify, ATP	
	MICROORGANISMS	FA/DAPI/AO counts; biochem. markers-FAME/lipid-P/ATP	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Radio-substrate incorp.; DOC change; POC: wgt., chem.	
	BACTERIA/PROTOZOA	Feeding studies; fluores.-labeled bact.; bact. den.	
	PLANTS/HERBIVORES		
	HERBIVORES/PREDATORS		X
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION	D.O. change; ^{14}C-bicarbonate uptake	
	SECONDARY PRODUCTION		(X)
	P/R RATIO	D.O. change in flowing water respirometers	
	OTHER (SPECIFY)	Leaf litter decomp.: leaf pack wt. change over time	
BIOGEOCHEM. CYCLING	NITROGEN		
	PHOSPHORUS		
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		(X)
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

Herbivores, predators: Size and water velocity might limit the inclusion of some herbivores and/or predators.

Secondary production: Size and water velocity limitations for some organisms.

Animals: Size and water velocity will limit the study of riffle organisms and fish.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	I Algae(chl *a*)	x (Chl *a*, bact. dens.)
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	H	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	H Community Respir.	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	x (Litter decomp.)
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

Major problem. Differing storm effects in microcosms and the parent stream. In microcosms, sedimentation of the silt load occurs; in the parent storm there is scour, and no scour occurs in the micro-cosm because flow rates are constant.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
1. Has your microcosm response to this factor been compared to field data?	Yes ____ No _x_	Yes ____ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes _x_ No ____	Yes ____ No ____
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON STREAM MICROCOSM

Dr. Thomas Bott
Stroud Water Research Center
Division of Environmental Research
Academy of Natural Sciences
R.D. #1 Box 512
Avondale, PA 19311
(215) 268-2153

Bott, T.L., and L.A. Kaplan. 1990. Cellulytic bacteria as surrogates for a genetically engineered microorganism: Microcosm studies of persistence and effects in streambed sediments. *In:* Review of Progress in the Biotechnology-Microbial Pest Control Agent Risk Assessment Program, EPA/600/9-90/029, U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR and Environmental Research Laboratory, Gulf Breeze, FL, pp. 139-143.

WASTE TREATMENT MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: M. GEALT

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

This microcosm simulates a municipal waste facility with four replicates of each type of holding tank. Materials are primarily plexiglass, PVC, glass, Tygon, and epoxy. Medium (see below) is pumped from a holding tank to the primary settling tanks (ST1) with a peristaltic pump; liquid flows by gravity to aerator tanks and then to secondary settling tanks (ST2). Sludge from ST2 is pumped back to the aerator tanks. The final effluent from the ST2's goes to a 100-L tank to which bleach is added.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	
Primary producers (specify)	Yes___ No___	Depends on medium used
Invertebrates (specify)	Yes___ No___	Depends on medium used
Vertebrates (specify)	Yes___ No_x_	___
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Authentic Wastewater from raw wastewater, settling tank, etc., may be used to supply the growth medium and culture. *Artificial Medium* consisting of a synthetic wastewater or 0.03% nutrient broth, can be used with either a combination of pure cultures from wastewater, etc. (*characterized*) or bacteria derived from primary or raw sewage (*uncharacterized*).

4. If environmental media are used, how is the environment sampled?

Uncharacterized bacteria are obtained as a grab sample from raw wastewater, settling tank, etc. Authentic wastewater, when used, is pumped from a municipal treatment facility into 200- to 300-L holding tanks (enough for a 5 - 6 day test) and maintained at room temperature until used.

5. What habitats are represented?

   a. Typically:

Waste treatment system.

   b. What factor(s) limit the habitats that could be represented?

No limitations as long as microcosm is applied to waste treatment systems.

GENERAL CHARACTERISTICS (CONTINUED)

6. Microcosm size:

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
a. Typically:	Settling tank (each)	7 L	N/A
	Aerator (each)	5 L	
	Lines (total)	1 L	
	Lagoons (if used)	10 L	

b. What factor(s) limit these size characteristics?

Dimensions for one complete system: 500 cm (L) x 300 cm (H) x 200 cm.
Room size, getting the common feed, etc. to function, and engineering so that sampling is physically possible.

c. How much space is required per microcosm unit?

Each replicate system requires 7.5 m^3; four replicates require 30 m^3

7. For what purpose was the microcosm originally designed?

To model GEM survival and gene transfer in a waste treatment system.

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

The microcosm is not directly connected to the environment. Medium is pumped according to the desired system retention time. Aerators use "house" compressed air and aquarium air stones to produce continuous bubbling (like a boil) in the tank.

9. Equilibrium period:

a. Is laboratory equilibrium required before testing?

Yes__x__ No_____

b. If so, what is the equilibration period?

1-2 days without test organisms (GEMs) but with wastewater organisms.

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

Uncertain. We assume it allows biofilm to form which aids in gene transfer mechanisms.

10. Microcosm "lifespan":

a. How long are microcosm tests generally run?

6 days (beyond 2 day acclimation period)

b. What are the most important factors in establishing the lifespan of this microcosm?

Nutrient level. High nutrients lead to high growth which tend to clog return activated sludge lines.

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	Room light (Two 100 or 150 watt) overhead.
b. Typical light intensity:	
c. Lighting control (intensity, photoperiod, means of control, etc.):	Generally on during day and off at night.

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	____
b. Relative humidity	____
c. Temperature	__x__
d. Light intensity	____
e. Inorganic nutrients	____
f. Carbon dioxide	____
g. Dissolved oxygen	__x__
h. Other (specify)	__x__ Optical density (for cell growth)

13. How is temperature controlled (constant temperature room, water bath, etc.)? Air-conditioned room maintained at 20-25° C.

14. How is water/air circulated/mixed? Peristaltic pump (one for medium flow, one for return activated sludge)

CONTAINMENT

I. a. Is containment with current microcosm design adequate for working with GEMs?

Yes_x_ No_____

b. If so, describe containment design.

Plexiglass covers on tanks contain aerosols. Environmental chamber has its own AC and exhaust system. To facilitate cleaning, floor and walls are made of ceramic tile, and there is a floor drain.

c. Could containment be improved by design modification?

Yes_x_ No_____

d. If so, what is the nature of the modifications needed to improve containment?

1. Tight-fitting lids with air exchange filters.
2. Time-controlled chlorine bleach addition to waste holding tank.
3. Automatic sampling devices not requiring removal of the tank tops for sampling.

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

____ a. Considerable resources, skill, or time.

_x_ b. Moderate resources, skill or time.

____ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

a. Microcosm construction? Yes____ No_x_

b. Microcosm operation? Yes____ No_x_

c. Output analysis? Yes____ No_x_

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

a. Microcosm construction? Yes_x_ No____

b. Microcosm operation? Yes_x_ No____

c. Output analysis? Yes_x_ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

a. Construct a microcosm? Yes_x_ No____

b. Operate a microcosm? Yes_x_ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

1. Automatic sampling and monitoring, e.g., temperature, D.O., pH, etc.
2. Restructure activated sludge return lines to decrease clogging (using larger diameter tubing, different pump heads, etc.)

2. What levels of difficulty would be involved in making the modifications in (1) above?

___ a. Considerable resources, skill or time.

_x_ b. Moderate resources, skill or time.

___ c. Minimal resources, skill or time.

___ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

5 ml samples can be obtained from any or all of the following:

ST1, ST2: influent, settled solids, and effluent.
Aerator (return sludge container).
Lagoon

2. Is destructive sampling during the course of a test run required?

Yes___ No_x_

3. Would design modifications allow the use of alternative sampling strategies?

Yes_x_ No___

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

___ a. Less than $100

___ b. Between $100 and $500

_x_ c. Between $500 and $1000

___ d. More than $1000

2. How many replicate vessels are generally used per treatment

Four

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

___ a. Less than $5000

_x_ b. Between $5000 and $20000

___ c. Over $20000

___ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS		X
	ANIMALS		X
	BENTHOS		X
	MICROORGANISMS	Standard methods	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Standard methods (BOD, TOC, suspended solids)	
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES		X
	HERBIVORES/PREDATORS		X
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION		
	SECONDARY PRODUCTION		
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM CYCLING	NITROGEN		
	PHOSPHORUS		
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	N/A	
	ANIMALS	N/A	
	BENTHOS	N/A	
	MICROORGANISMS		X
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		X
	BACTERIA/PROTOZOA		X
	PLANTS/HERBIVORES	N/A	
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION		
	SECONDARY PRODUCTION		
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN		
	PHOSPHORUS		
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)	N/A	
	ANIMAL (SPECIFY)	N/A	
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY) Wastewater operation parameters	H	

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes ___ No _x_	Yes ___ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	___	___
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No ___	Yes ___ No ___
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON WASTE TREATMENT MICROCOSM

Dr. Michael Gealt
Dept. of Bioscience & Biotechnology
Drexel University
32nd and Chestnut Streets
Philadelphia, PA 19104
(215) 885-5092

Mancini, P., S. Ferreis, D. Nave, and M.A. Gealt. 1987. Mobilization of plasmid pHSV106 from *Escherichia coli* HB101 in a laboratory-scale waste treatment facility. Appl. Environ. Microbiol. 53:665-671.

Sagik, B.P., and C.A. Sorber. 1979. The survival of host-vector systems in domestic sewage treatment plants. Recombinant DNA Bull. 2:55-61.

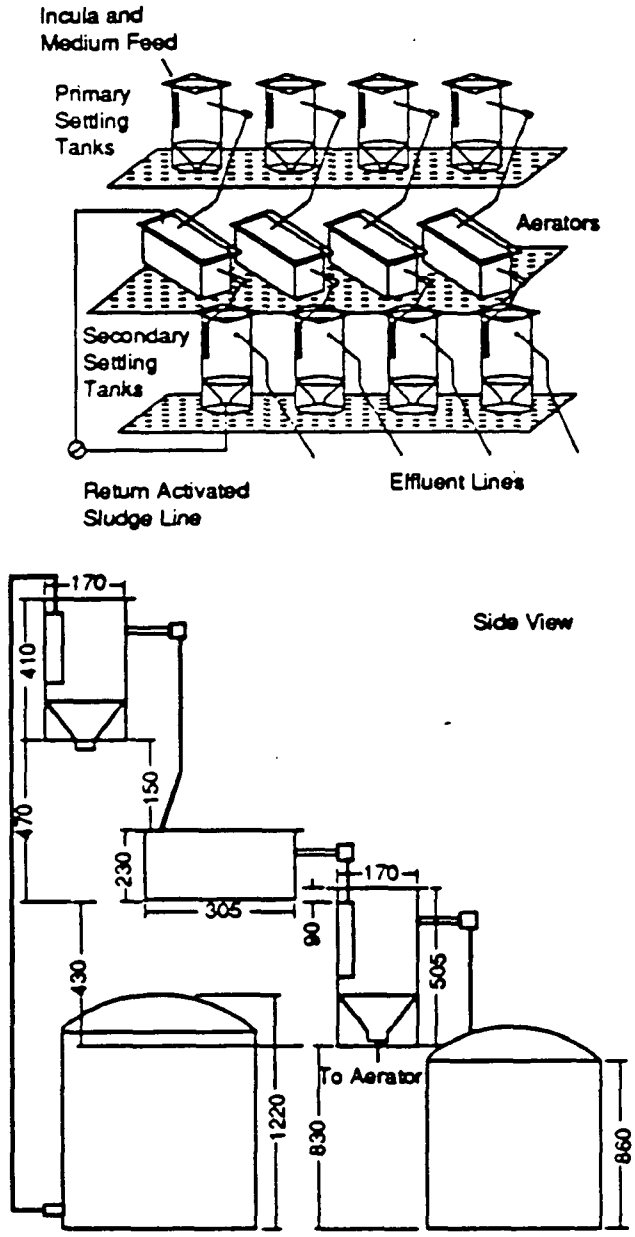


Figure 7. **Laboratory waste treatment facility.**

Appendix C
Terrestrial Microcosms

ROOT MICROCOSM SYSTEM

GENERAL CHARACTERISTICS

DEVELOPER: D. KLEIN

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Seeds are sterilized (20% Chlorox) and germinated on sterile 1/10 strength nutrient agar. Noncontaminated plants are transferred to a 1-liter Pyrex jar containing autoclaved, fritted clay covered with 2 cm of sand. Hoaglands's solution (1/4 strength, 400 ml) buffered to pH 7 with Sorensens phosphate is added, and the jar is sealed with a lid containing 3 holes: 1 for sterile air input, 1 for sterile nutrient input, and one for the plant (surrounded by silicone sealant). For nonsterile treatments, a 10 ml mixture of rhizosphere organisms can be added.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	Soil microbiota_____
Primary producers (specify)	Yes_x_ No___	Plant seedlings_____
Invertebrates (specify)	Yes___ No_x_	Could be included
Vertebrates (specify)	Yes___ No_x_	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Sterile seedlings are transplanted. Natural mixed inocula, or specific single or combined microbial isolates can be added.

4. If environmental media are used, how is the environment sampled?

Autoclaved, fritted clay is used.

5. What habitats are represented?

   a. Typically:

Grass and forb systems

   b. What factor(s) limit the habitats that could be represented?

The plants must be limited in size. Small trees could possibly be used, but only in scaled-up root microcosm system.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
6. Microcosm size:			
a. Typically:	Approx. 12 x 12 cm	1L	Approx. 100 cm^2
b. What factor(s) limit these size characteristics?			
c. How much space is required per microcosm unit?			

7. For what purpose was the microcosm originally designed?

To measure plant root and microbial respiration, and to separate the two processes.

8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.

With the tubing connections, air and water in the Root Microcosm Systems can be exchanged when desired.

9. Equilibrium period:

a. Is laboratory equilibrium required before testing?

Yes_____ No__x__

b. If so, what is the equilibration period?

c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.

10. Microcosm "lifespan":

a. How long are microcosm tests generally run?

Approximately 90 days.

b. What are the most important factors in establishing the lifespan of this microcosm?

Plant establishment and viability, and lack of system contamination.

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):

Standard greenhouse or growth chamber conditions; depends on the environmental conditions to be duplicated.

b. Typical light intensity:

c. Lighting control (intensity, photoperiod, means of control, etc.):

GENERAL CHARACTERISTICS (CONTINUED)

12. Which of the following environmental parameters are routinely monitored?

These depend on experimental design

a. Soil moisture ____

b. Relative humidity ____

c. Temperature __X__

d. Light intensity __X__

e. Inorganic nutrients __X__

f. Carbon dioxide __X__

g. Dissolved oxygen __X__

h. Other (specify) ____ __X__ Dissolved organic matter

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Constant temperature room or growth chamber.

14. How is water/air circulated/mixed?

A syringe is used to exchange water in each individual unit to slowly pass the liquid through the filters.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

Yes__X__ No_____

b. If so, describe containment design.

Physical barrier on top of unit.

Membrane filters on gas and water inlet and outlet.

c. Could containment be improved by design modification?

Yes_____ No__X__

d. If so, what is the nature of the modifications needed to improve containment?

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

____ a. Considerable resources, skill, or time.

____ b. Moderate resources, skill or time.

__X__ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No____

   b. Microcosm operation? Yes_x_ No____

   c. Output analysis? Yes_x_ No____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No____

   b. Microcosm operation? Yes____ No____

   c. Output analysis? Yes____ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes_x_ No____

   b. Operate a microcosm? Yes_x_ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

   Improvement of ability to sample plant growth matrix before completion of an experiment, and to remove root sub-samples.

2. What levels of difficulty would be involved in making the modifications in (1) above?

   ____ a. Considerable resources, skill or time.

   ____ b. Moderate resources, skill or time.

   _x_ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Gas and liquid sampling, and microbial sampling of liquid medium. Periodic sampling of solid material can be accomplished by setting up replicate units which can be taken apart at desired intervals.

2. Is destructive sampling during the course of a test run required?

Yes____ No_x__

3. Would design modifications allow the use of alternative sampling strategies?

Yes_x__ No____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

_x_ a. Less than $100 (Approx. $5/unit)

____ b. Between $100 and $500

____ c. Between $500 and $1000

____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Three to four

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

_x_ a. Less than $5000

____ b. Between $5000 and $20000

____ c. Over $20000

____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS		_____
	ANIMALS		_____
	BENTHOS		_____
	MICROORGANISMS	Microscopic and viable populations; lipid analyses	_____
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Microscopic and viable pop.; exudate analysis	_____
	BACTERIA/PROTOZOA		_____
	PLANTS/HERBIVORES		_____
	HERBIVORES/PREDATORS		_____
	OTHER (SPECIFY)		_____
ENERGY FLOW	PRIMARY PRODUCTION	Plant growth and respirometry	_____
	SECONDARY PRODUCTION	Microbial responses in the rhizosphere	_____
	P/R RATIO		_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM. CYCLING	NITROGEN	Chemical analysis	_____
	PHOSPHORUS	Same	_____
	SULFUR	Same	_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)	Growth responses (dry weight) and respirometry	_____
	ANIMAL (SPECIFY)		_____
	MICROBIAL (SPECIFY)	Community structure and function characteristics	_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm

With appropriate construction and sampling modifications, it should be possible to sample a full range of plant/microbe interactions in smaller plant systems.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	____	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	____	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes ____ No _x_	Yes ____ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes _x_ No ____	Yes _x_ No ____
	If funding is available.	
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.	The major variables tested to date have been nitrogen level, plant type and microbial inoculation presence in the plant root zone.	
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON ROOT MICROCOSM SYSTEM

Dr. Donald Klein
Colorado State University
Dept. of Microbiology
Fort Collins, CO 80523
(303) 491-6947

Klein, D.A., B.A. Frederick, M. Biondini, and M.J. Trlica. 1988. Rhizosphere microorganism effects on soluble amino acids, sugars, and organic acids in the root zone of *Agropyron cristatum*, *A. smithii* and *Bouelona gracilis*. Plant Soil. 110:19-25.

Biondini, M., D.A. Klein, and E.F. Redente. 1988. Carbon and nitrogen losses through root exudation by *Agropyron cristatum*, *A. smithii* and *Bouelona gracilis*. Soil Biol. Biochem. 20:477-482.

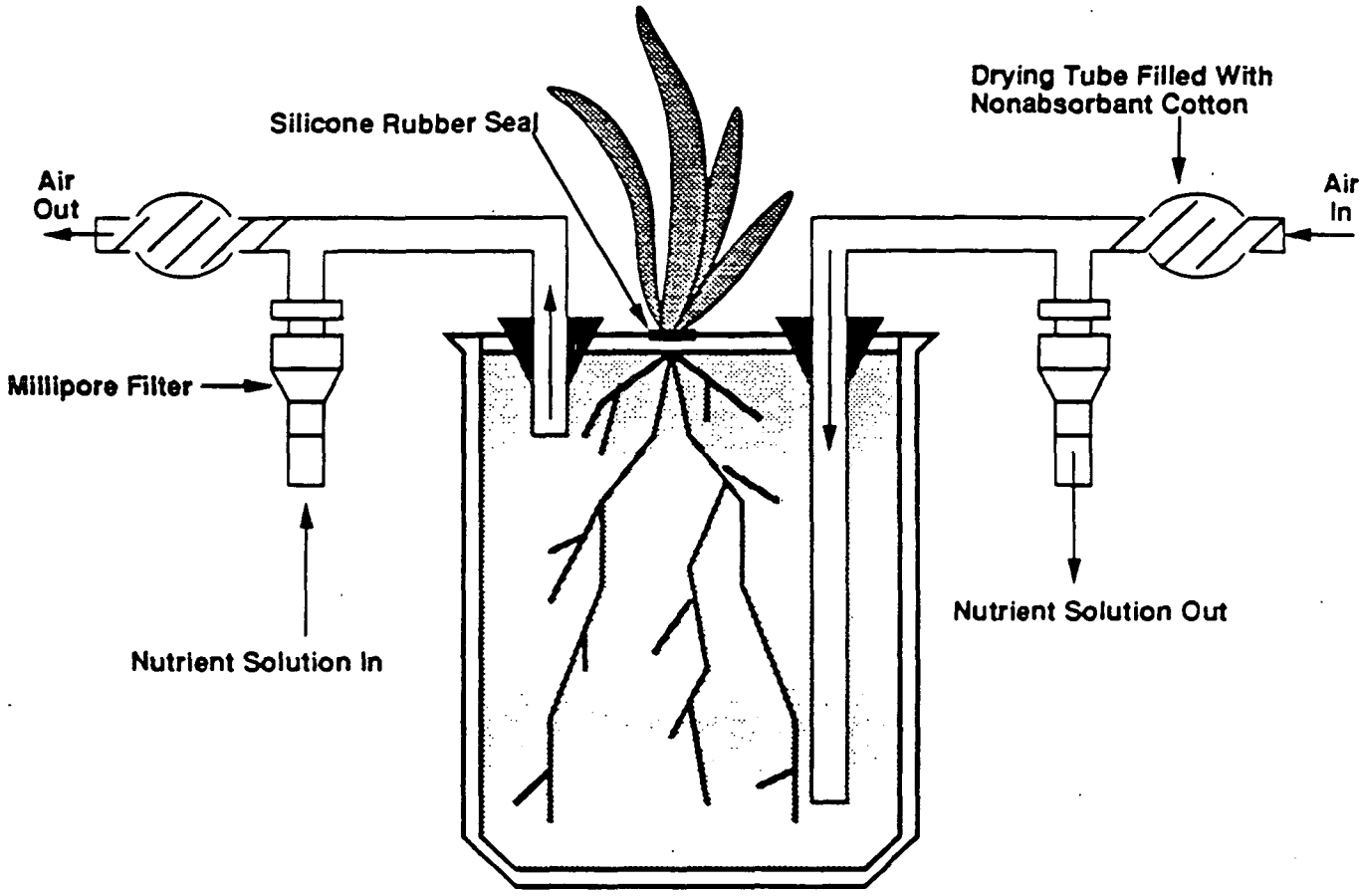


Figure 8. Root microcosm system.

SOIL CORE MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: J. FREDRICKSON

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

The 60-cm-deep by 17-cm-diameter microcosm consists of a 17-cm-diameter tube of Driscopipe (polyethylene pipe) containing an intact soil core (40 cm) covered by homogenized topsoil (20 cm). The natural grassland microcosm is an intact, totally undisturbed 17-cm-diameter by 60-cm-deep test system. This tube sits on a Buchner funnel that is covered by a thin layer of glass wool. Six to eight microcosms are typically contained in a moveable cart, which is packed with insulated beads or a comparable material to reduce drastic changes in temperature profile.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No____	indigenous soil microflora
Primary producers (specify)	Yes_x_ No____	plants w/size, time limits
Invertebrates (specify)	Yes_x_ No____	soil microfauna_____
Vertebrates (specify)	Yes____ No_x_	possibly small mammals___
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

They are "pre-established" as the microcosm consists of an intact soil core it harbors indigenous communities. Plants, microorganisms, microfauna etc., can be readily introduced.

4. If environmental media are used, how is the environment sampled?

A steel coring tube is driven into soil and extracted to obtain an intact core housed in a Driscopipe liner.

5. What habitats are represented?

   a. Typically:

Limited to terrestrial environments, mainly soils and unsaturated sediments.

   b. What factor(s) limit the habitats that could be represented?

Physical limitations for saturated sediments and water.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
6. Microcosm size:			
a. Typically:	60 (depth) x 17 (diam)		
b. What factor(s) limit these size characteristics?	Physical ability to extract an intact core. They can be quite large if the proper heavy equipment is available.		
c. How much space is required per microcosm unit?	~ 1 $Ft.^3$		
7. For what purpose was the microcosm originally designed?	Toxicological studies of impacts of chemicals on soil biota and nutrient cycling processes.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Moisture: Water characterized using ASTM D19, Test Methods for Water Quality Analysis. Microcosms are leached at least once before dosing and once every two or three weeks after dosing, based on natural rainfall amounts. Leachate is collected in 500-ml flasks attached to the Buchner funnel.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes x No		
b. If so, what is the equilibration period?	Soil is saturated and allowed to drain. The length of time varies with soil texture but can be < 24 h for a coarse grained soil to 3-4 days for a clay soil.		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	In general, one pore volume of water is leached through the core to remove initial concentrations of nutrients. Following this initial leaching, no additional time is required for equilibration.		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	Microcosms generally operated over 2-3 week periods although there is essentially no restraint on lifespan.		
b. What are the most important factor in establishing the lifespan of this microcosm?	Microcosms have been operated for up to 8 months without plants.		

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	Light for the test system can be natural or artificial, depending on the use of a growth chamber or a greenhouse.
b. Typical light intensity:	400 μEinsteins m^{-2}
c. Lighting control (intensity, photoperiod, means of control, etc.):	That which is optimal for plant growth or mimics specific field photoperiod.

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	X
b. Relative humidity	
c. Temperature	X
d. Light intensity	X
e. Inorganic nutrients	X
f. Carbon dioxide	
g. Dissolved oxygen	
h. Other (specify)	

13. How is temperature controlled (constant temperature room, water bath, etc.)?	Microcosms in insulated carts or other devices are kept in a greenhouse or environmental chamber where temperature and light can be controlled.
14. How is water/air circulated/mixed?	Air is circulated via greenhouse or growth chamber fans.

CONTAINMENT

1. a. Is containment with current microcosm design microcosm design adequate for working with GEMs?

   Yes_x_ No_____

   b. If so, describe containment design.

   Microcosms are contained in a greenhouse or in a growth chamber within a laboratory.

   c. Could containment be improved by design modification?

   Yes_x_ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   An improved HEPA-filtered containment chamber for housing the soil-cores. Such a chamber has been designed and a prototype was constructed. Designs are available.

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   _x_ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No____

   b. Microcosm operation? Yes_x_ No____

   c. Output analysis? Yes_x_ No____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No____

   b. Microcosm operation? Yes____ No____

   c. Output analysis? Yes____ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes____ No____

   b. Operate a microcosm? Yes____ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

1) Development of a system that would allow maintenance of soil water potential. This could be done either (a) manually by weighing cores every day and adding water to a pre-determined constant weight or (b) automatically by developing a computer-controlled system that would add water when the weight of a core dropped below a certain value.
2) Use in a programmable environmental chamber that spans the temperature-humidity values in the field.

2. What levels of difficulty would be involved in making the modifications in (1) above?

_____ a. Considerable resources, skill or time.

__x__ b. Moderate resources, skill or time.

_____ c. Minimal resources, skill or time.

_____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Sampling plants without destruction is difficult but can be done.

Subsampling soil is accomplished easily but can destroy the physical integrity of the core for transport (leaching) studies.

2. Is destructive sampling during the course of a test run required?

Yes_____ No__x__ (in general, but is dependent on nature of the experiment)

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No_____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?	_x_ a. Less than $100 ___ b. Between $100 and $500 ___ c. Between $500 and $1000 ___ d. More than $1000
2. How many replicate vessels are generally used per treatment?	A minimum of three replicates
3. What is the estimated minimal cost of a complete microcosm test, including vessels?	___ a. Less than $5000 _x_ b. Between $5000 and $20000 ___ c. Over $20000 ___ d. An estimate has not been made (Depends on the complexity of the experiment, the analyses required and the institution conducting the test.)

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS		
	ANIMALS		X
	BENTHOS		
	MICROORGANISMS	introduced indigenous soil microbes (bacteria, fungi)	
	OTHER (SPECIFY)	earthworms, aphids, corn borers (GEM vectors)	
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	mineralization of ^{14}C-labeled cellulose	
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES	aphids & corn borers on plants	
	HERBIVORES/PREDATORS		
	OTHER (SPECIFY)	bacterial colonization, nodulation of plant roots	
ENERGY FLOW	PRIMARY PRODUCTION	plant biomass (root & shoot), microbial respiration	
	SECONDARY PRODUCTION	soil microbial biomass	
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN	^{15}N uptake, mineralization, pool partitioning	
	PHOSPHORUS	Plant assimilation, leaching	
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		X
	MICROBIAL (SPECIFY)	rhizome pop./divers.; enzymes: dehyd./glucosid./perox.	
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

Animals in soil-core microcosms generally cause out-of-scale problems (e.g., excess grazing of plants)

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	H	
	ANIMALS		
	BENTHOS		
	MICROORGANISMS	M-H	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	H	
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES		
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION	M-H	
	SECONDARY PRODUCTION	H	
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN	M-H	
	PHOSPHORUS		
	SULFUR		
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)*	L-H	
	OTHER (SPECIFY)		

* rhizosphere & soil populations: diversity/enzyme activities.

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes _x_ No ___	Yes _x_ No ___
	Pseudomonas sp. and *Streptomyces lividan*	An *Azospirillum* and a *Pseudomonas*
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	H	I-H
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No ___	Yes___ No ___
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.	Growth chamber favored microbial growth & function over field. Comparability better between plant growth stages than on actual time basis. Field temperature and humidity changes were difficult to simulate.	
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?	Effects: ^{14}C-Cellulose mineralization; rhizosphere populations; enzyme activity (dehyd./glucosid.) ^{14}N transformation; microbial biomass; transport by leaching, root growth, earthworms; nutrient uptake & leaching.	

FURTHER INFORMATION ON SOIL CORE MICROCOSM

Dr. James Fredrickson
Battelle Pacific NW Laboratories
P.O. Box 999
Richland, WA 99352
(509) 375-3908

Federal Register. 1987. 797.3775 Soil-core microcosm test. 52(187):36363-36371.

E1197 Guide for Conducting a Terrestrial Soil-Core Microcosm Test. *In* 1991 Annual Book of ASTM Standards, Vol. 11.04, American Society for Testing and Materials, Philadelphia, PA.

Bentjen, S.A., J.K. Fredrickson, P. Van Voris, and S.W. Li. 1989. Intact soil-core microcosms for evaluating the fate and ecological impact of the release of genetically engineered microorganisms. Appl. Environ. Microbiol. 55:198-202.

Fredrickson, J.K., S.A. Bentjen, and H. Bolton, Jr., S.W. Li, and P. Van Voris. 1989. Fate of Tn5 mutants of root growth-inhibiting *Pseudomonas* sp. in intact soil-core microcosms. Can. J. Microbiol. 35:867-873.

Fredrickson, J.K., H. Bolton, Jr., S.A. Bentjen, K.M. McFadden, S.W. Li, and P. Van Voris. 1990. Evaluation of intact soil-core microcosms for determining potential impacts on nutrient dynamics by genetically engineered microorganisms. Environ. Toxicol. Chem. 9:551-558.

Bolton, H. Jr., J.K. Fredrickson, J.M. Thomas, S.W. Li, D.W. Workman, S.A. Bentjen, and J.L. Smith. 1991. Field calibration of soil-core micro microcosms: Ecosystem structural and functional comparisons. Microb. Ecol. 21:175-189.

Bolton, H. Jr., J.K. Fredrickson, S.A. Bentjen, D.W. Workman, S.W. Li, and J.M. Thomas. 1991. Field calibration of soil-core microcosms: Fate of a genetically altered rhizobacterium. Microb. Ecol. 21:161-173.

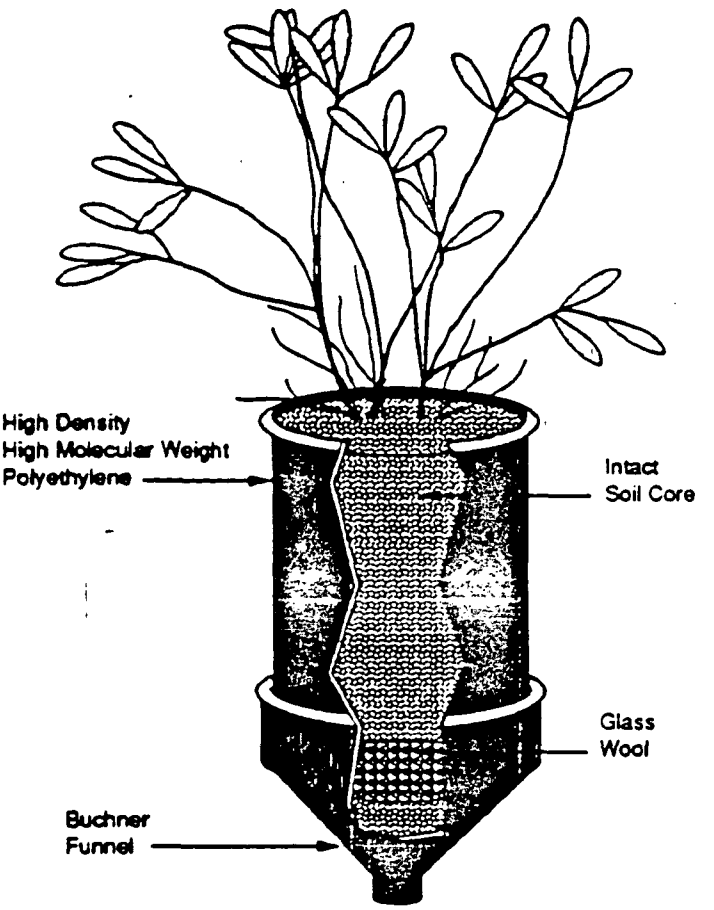


Figure 8. Soil core microcosm.

SOIL IN A JAR MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: G. STOTZKY

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Sieved (1 cm) soil is stored in a greenhouse or laboratory. Two weeks before use, soil water tension adjusted to -33 kPa, and soil is mixed with a glucose solution (1% wt/wt) and ca. 20 mg fresh garden soil g^{-1} soil. 50-g (oven-dry equivalent) of sieved (2 mm) soil adjusted to -33 kPa water tension is added to 8 - 10 100-ml glass vials, which are placed in a 1-gal wide-mouth jar. A manifold, attached to a scrubber system (to saturate air with water and remove oil, CO_2, nitrogen compounds, and other contaminants), provides air to the jar. CO_2 in exiting air is trapped and quantified.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes x No ___ soil microbiota
Primary producers (specify)	Yes ___ No x
Invertebrates (specify)	Yes x No ___ soil microinvertebrates
Vertebrates (specify)	Yes ___ No x
Other (specify)	

3. Describe how communities of organisms are established in the microcosm.

Sieved soil (1 cm mesh) from the top 5 cm of a field contains microbiological and microinvertebrate communities.

4. If environmental medium are used, how is the environment sampled?

Soil is collected from the surface of a field.

5. What habitats are represented?

Soil from a tilled or untilled field

   a. Typically:

   b. What factor(s) limit the habitats that could be represented?

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	Volume (L)	Soil/Sediment Surface Area (cm^2)
6. Microcosm size:			
a. Typically:	16 x 26 cm	~ 3.8 L	
b. What factor(s) limit these size characteristics?	Convenience		
c. How much space is required per microcosm unit?	Approximately 26 cm^2		
7. For what purpose was the microcosm originally designed?	Used for soil microbiological research, testing the effects of pollutants (e.g., heavy metals, acid precipitation, pesticides) on microbial activity in soil.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Soil containers are continuously flushed with water-saturated air.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes____ No_X_		
b. If so, what is the equilibration period?			
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.			
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	Days, weeks, or months		
b. What are the most important factors in establishing the lifespan of this microcosm?	Design and purpose of study; maintenance of soil at -33 kPa water tension		
11. What kind of lighting is used?			
a. Type of lights (wattage, model, source, etc.):			
b. Typical light intensity:			
c. Lighting control (intensity, photoperiod, means of control, etc.):	Constant darkness or light/dark cycle may be used		

GENERAL CHARACTERISTICS (CONTINUED)

12. Which of the following environmental parameters are routinely monitored?

a.	Soil moisture	_x_ (at beginning and end of test)
b.	Relative humidity	_x_ (maintained constant)
c.	Temperature	_x_ (maintained constant)
d.	Light intensity	_____
e.	Inorganic nutrients	_x_ (at beginning & perhaps end of test)
f.	Carbon dioxide	_x_
g.	Dissolved oxygen	_____
h.	Other (specify)	_x_ (pH, species diversity, enzyme activity, and survival of GEMs at beginning and end of test)

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Constant temperature incubator or room.

14. How is water/air circulated/mixed?

Continuous flushing with water-saturated, CO_2-free air.

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

Yes_x_ No_____

b. If so, describe containment design.

Soil contained in glass vessels is autoclaved before disposal

c. Could containment be improved by design modification?

Yes_____ No_x_

d. If so, what is the nature of the modifications needed to improve containment?

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

____ a. Considerable resources, skill, or time.

____ b. Moderate resources, skill or time.

____ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes_x_ No____

   b. Microcosm operation? Yes_x_ No____

   c. Output analysis? Yes_x_ No____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No____

   b. Microcosm operation? Yes____ No____

   c. Output analysis? Yes____ No____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes____ No____

   b. Operate a microcosm? Yes____ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

   Greater degree of automation for measuring CO_2 evolved (e.g., capacitance measurements; automatic sampling for titration)

2. What levels of difficulty would be involved in making the modifications in (1) above?

   ____ a. Considerable resources, skill or time.

   _x_ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Repetitive sampling is limited by the number of soil vials within the master jar.

2. Is destructive sampling during the course of a test run required?

Yes__x__ No____ (For analyses in #12 above; no, if only respiration is measured.)

3. Would design modifications allow the use of alternative sampling strategies?

Yes____ No__x__

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

__x__ a. Less than $100 (without titrator, etc.)

____ b. Between $100 and $500

____ c. Between $500 and $1000

____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Three to five

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

__x__ a. Less than $5000 (without labor)

__x__ b. Between $5000 and $20000 (with labor)

____ c. Over $20000

____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS		X
	ANIMALS		X
	BENTHOS		X
	MICROORGANISMS	Species diversity by selective media; probes	
	OTHER (SPECIFY)		X
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Addition of specific substrates	
	BACTERIA/PROTOZOA	Selective media	
	PLANTS/HERBIVORES		X
	HERBIVORES/PREDATORS		X
	OTHER (SPECIFY)		X
ENERGY FLOW	PRIMARY PRODUCTION		X
	SECONDARY PRODUCTION	Can use ^{14}C-labeled substrates	
	P/R RATIO		X
	OTHER (SPECIFY)		X
BIOGEOCHEM. CYCLING	NITROGEN	Soil anal.-perfusion apparatus (EPA/600/3-90/011)	
	PHOSPHORUS	Soil anal	
	SULFUR	Soil anal	
	OTHER (SPECIFY)	Soil anal	
OTHER EFFECTS	PLANT (SPECIFY)		X
	ANIMAL (SPECIFY)		X
	MICROBIAL (SPECIFY)	Addition of GEMs	
	OTHER (SPECIFY)		X

Reasons that a parameter cannot be addressed in your microcosm

System is limited to soil. It could be modified to include plants, but this would not be practical.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	____	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	____	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

Not conducted

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
1. Has your microcosm response to this factor been compared to field data?	Yes ___ No _X_	Yes ___ No _X_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	___	___
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No _X_	Yes ___ No _X_
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON SOIL IN A JAR

Dr. Guenther Stotzky
New York University
Department of Biology
1009 Main
Washington Square
New York, NY 10003
(212) 998-8266

Stotzky, G. 1965. Microbial Respiration. In *Methods of Soil Analysis*, C.A., Black et al., (eds), American Society of Agronomy, Inc., Madison, WI. pp. 1550-1570.

Stotzky, G. 1989. Methods to measure the influence of genetically engineered bacteria on ecological processes in soil. U.S. Environmental Protection Agency, EPA/600/3-90/011. 36 pp.

Stotzky, G. 1991. Evaluation of selected biochemical and ecological methods to assess effects of recombinant bacteria in terrestrial ecosystems. U.S. Environmental Protection Agency. In press. 170 pp.

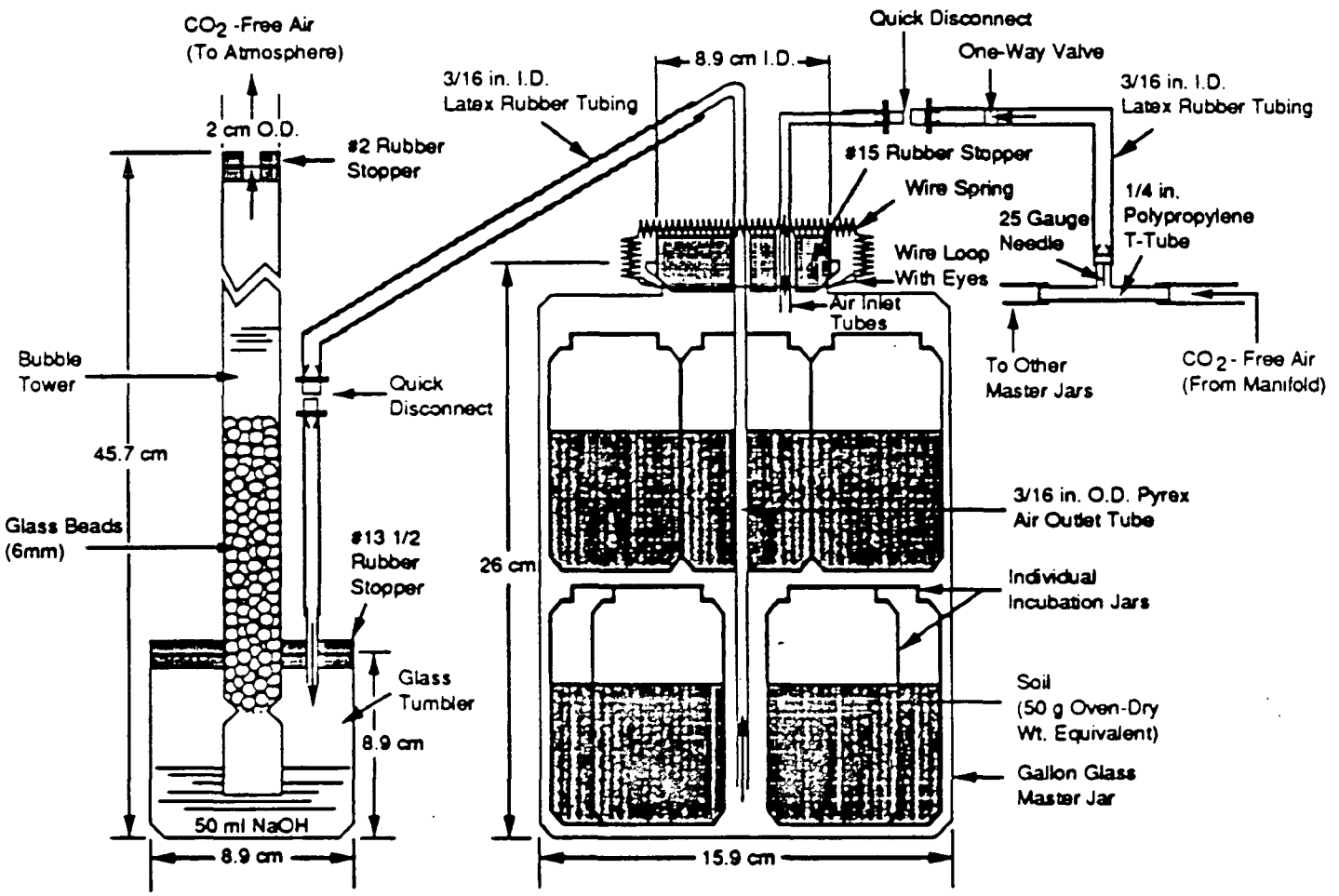


Figure 10. **Soil in a jar.**

TERRESTRIAL MICROCOSM CHAMBER

GENERAL CHARACTERISTICS

DEVELOPER: J. GILLETT

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

The Terrestrial Microcosm consists of a chamber (1 x 0.75 x 0.75 m) constructed of glass plate, plexiglass, a UV-transparent glass top cover, and removable side panels (with glove openings). This chamber rests on a polyethylene box (1 x 0.75 x 0.55 m). It contains soil and a variety of biota, including seedlings. Soil is mixed and sieved through a coarse (1 cm) screen to remove rocks, roots and other debris; then it is sieved through a 2 mm screen after being tumbled in a portable cement mixer. Each system requires about 200 to 300 kg of sieved soil which is added in 5-cm layers saturated with water and packed by application.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No____ Indigenous
Primary producers (specify)	Yes_x_ No____ Indig. or agric. plants
Invertebrates (specify)	Yes_x_ No____ Indig. & earthworms
Vertebrates (specify)	Yes_x_ No____ Voles/quail with 730 cm soil
Other (specify)	

3. Describe how communities of organisms are established in the microcosm.

Through the use of unsterilized soil.

4. If environmental media are used, how is the environment sampled?

Air: polyurethane foam filters and direct air sampling; soil: coring; water: leachate sampling.

5. What habitats are represented?

a. Typically:

a. Agroecosystems

b. What factor(s) limit the habitats that could be represented?

b. Plant size, temperature means and extremes.

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
6. Microcosm size:			
a. Typically:	Upper chamber: 100(L)x75(W) x50(D) Lower chamber: 100x75x55	Air: 563 Soil: 375	7500
b. What factor(s) limit these size characteristics?	Soil depth must be < 50 cm		
c. How much space is required per microcosm unit?	Footprint is about 2 m x 1.5 m, but additional space is taken up by air supply pipes.		
7. For what purpose was the microcosm originally designed?	Designed for assessing the influence of the environment on fate and effects of pesticides and toxic substances.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Air passing through the chamber is controlled by manual valves and is exhausted through a HEPA filter.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes__x__ No_____		
b. If so, what is the equilibration period?	2-3 weeks		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	Depends on measures employed. Leachate NO_3-N concentration should be at background. Ca or Fe in leachates		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	2-17 weeks		
b. What are the most important factors in establishing the lifespan of this microcosm?	Crop "life"; if vole is used, consumption of crop.		

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	1000-watt sylvania metal halide lamp, positioned 55 cm above the chamber.
b. Typical light intensity:	
c. Lighting control (intensity, photoperiod, means of control, etc.):	18:6 L:D cycle

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	X
b. Relative humidity	X
c. Temperature	X (Soil and air)
d. Light intensity	
e. Inorganic nutrients	X (In leachate)
f. Carbon dioxide	
g. Dissolved oxygen	
h. Other (specify)	X (Water output: leachate & air moisture)

13. How is temperature controlled (constant temperature room, water bath, etc.)?	A constant temperature room containing the chambers is heated and cooled to ± 1°C.
14. How is water/air circulated/mixed?	Negative flow through baffled filters

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

   Yes__x__ No_____

   b. If so, describe containment design.

   Glove box

   c. Could containment be improved by design modification?

   Yes__x__ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   ____ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   __x__ d. Can't estimate at this time. (Depends on desired level of containment)

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction?

   Yes__x__ No_____ (Note, however, that the purpose of this system is to have flexibility in protocols)

   b. Microcosm operation?

   Yes__x__ No_____

   c. Output analysis?

   Yes__x__ No_____

---

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction?

   Yes_____ No_____

   b. Microcosm operation?

   Yes_____ No_____

   c. Output analysis?

   Yes_____ No_____

---

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm?

   Yes__x__ No_____

   b. Operate a microcosm?

   Yes__x__ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

2. What levels of difficulty would be involved in making the modifications in (1) above?

_____ a. Considerable resources, skill or time.

_____ b. Moderate resources, skill or time.

_____ c. Minimal resources, skill or time.

_____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

(1) Soil cores are replaced with "control plugs."

(2) No limits to air and water sampling.

2. Is destructive sampling during the course of a test run required?

Yes_x_ No_____

3. Would design modifications allow the use of alternative sampling strategies?

Yes_____ No_x_

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

_____ a. Less than $100

_____ b. Between $100 and $500

_____ c. Between $500 and $1000

_x_ d. More than $1000

2. How many replicate vessels are generally used per treatment?

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

_____ a. Less than $5000

_____ b. Between $5000 and $20000

_x_ c. Over $20000

_____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by 14C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Biomass measurements	
	ANIMALS	Soil sampling and enumeration	
	BENTHOS		NA
	MICROORGANISMS	Soil sampling and enumeration	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		
	BACTERIA/PROTOZOA		
	PLANTS/HERBIVORES	Consumption	
	HERBIVORES/PREDATORS	Predation rate	
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION		
	SECONDARY PRODUCTION		
	P/R RATIO		
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN Leachate analysis		
	PHOSPHORUS	Same	
	SULFUR	Same	
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		
	ANIMAL (SPECIFY)		
	MICROBIAL (SPECIFY)		
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

Destructive sampling for a number of parameters limits repetitive observations over time.

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	____	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	____	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes ___ No _x_	Yes ___ No _x_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	___	___
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ___ No _x_	Yes ___ No _x_
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?	Chemical fate and mass balances are very close in field results, but no specific studies using GEMs have been made.	

FURTHER INFORMATION ON TERRESTRIAL MICROCOSM CHAMBER

Dr. James Gillett
16 Fernow Hall
ICET Cornell University
Ithaca, NY 14853 3001
(607) 255-2163

Gillett, J.W., and J.D. Gile. 1976. Pesticide fate in terrestrial laboratory ecosystems. Intern. J. Environ. Stud. 10:15-22.

Gile, J.D., J.C. Collins, and J.W. Gillett. 1982. Fate and impact of selected wood preservatives in a terrestrial model ecosystem. J. Agric. Food Chem. 30:295-301.

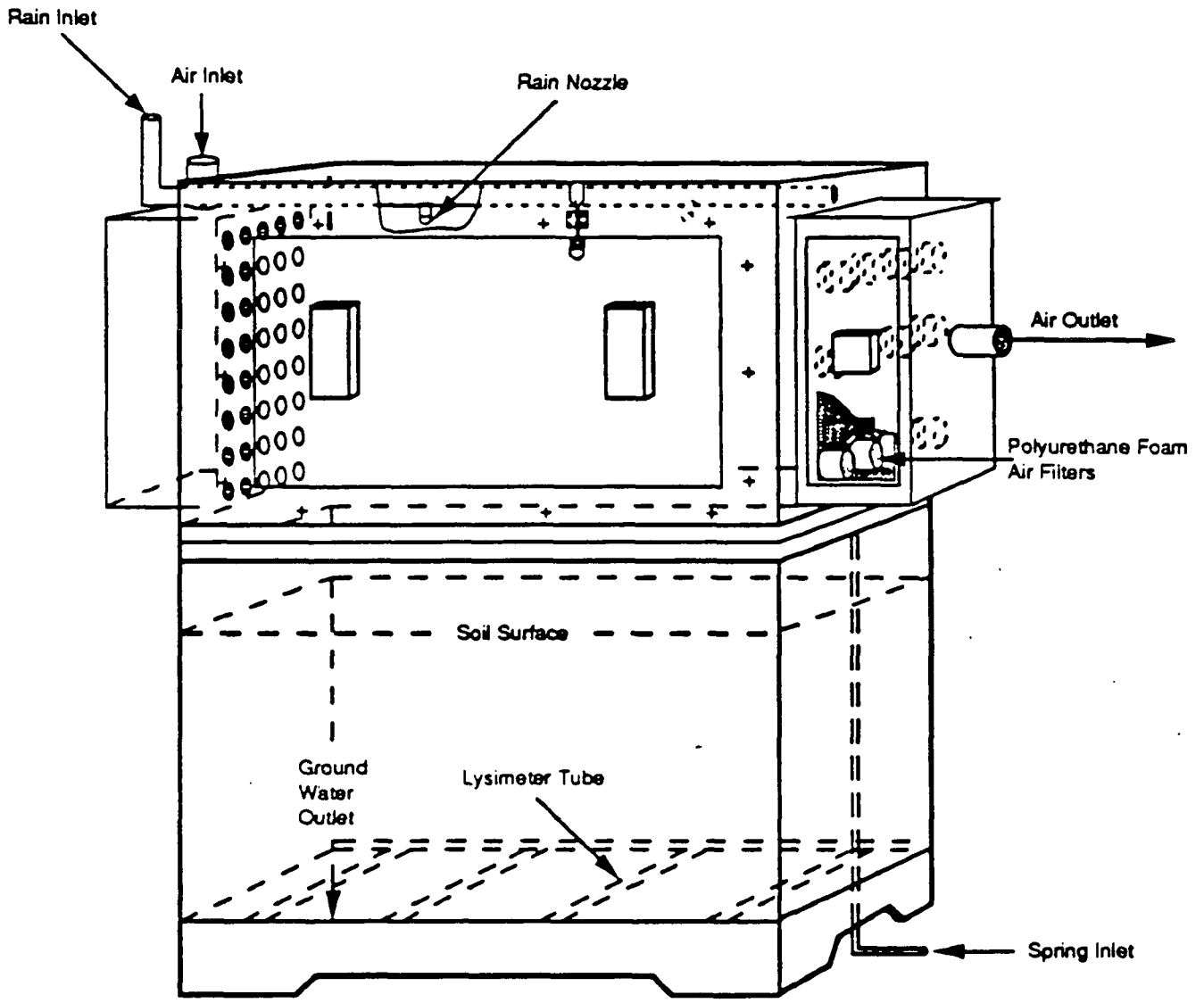


Figure 11. **Terrestrial microcosm chamber.**

TERRESTRIAL MICROCOSM SYSTEM

GENERAL CHARACTERISTICS

DEVELOPER: SEIDLER/ARMSTRONG

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

The Terrestrial Microcosm makes use of the Gillett microcosm (See Terrestrial Microcosm Chamber) for small scale experiments. Each chamber holds 2 wooden trays (47 x 37 x 7 cm) lined with polyethylene bags, supported by a metal rack in the chamber, 50 cm above the floor. Trays are planted with beans or other selected indigenous plants. A humidifier is located below the trays, and single-pass air is forced through the chambers and exhausted through HEPA filters.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_x_ No___	Indigenous bacteria, fungi
Primary producers (specify)	Yes_x_ No___	Indigenous; planted seeds
Invertebrates (specify)	Yes_x_ No___	Indigenous; cutworms, etc.
Vertebrates (specify)	Yes___ No___	_____
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Indigenous species collected with soil sample. Insects are introduced from lab cultures. Seeds are planted.

4. If environmental media are used, how is the environment sampled?

Soil is collected with shovel and put in wooden trays lined with plastic bags.

5. What habitats are represented?

   a. Typically:

Agricultural field

   b. What factor(s) limit the habitats that could be represented?

Size of the trays that hold soil and plants

GENERAL CHARACTERISTICS (CONTINUED)

	Dimensions (cm)	Volume (L)	Soil/Sediment Surface Area (cm^2)
6. Microcosm size:			
a. Typically:	Upper chamber: 100 x 75 (w) x 75 (d) Lower chamber: 100 x 75 x 55		3480
	Each microcosm contains two wooden trays (47 x 37 x 7 cm) holding soil and plants.		
b. What factor(s) limit these size characteristics?	Size of larger chamber holding the trays and convenience of lifting trays with soil.		
c. How much space is required per microcosm unit?	About 3 m x 3 m x 4 m (overhead) for plastic box that contains the trays.		
7. For what purpose was the microcosm originally designed?	Effects of toxic chemicals on ecosystem processes.		
8. Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Air passing through chamber is controlled by manual valves and is exhausted through a HEPA filter. Each chamber contains an industrial grade humidifier below the trays, and water accumulating on the chamber floor is suctioned through a tube, collected in a 5 gal container and disinfected with bleach.		
9. Equilibrium period:			
a. Is laboratory equilibrium required before testing?	Yes__x__ No_____ May not be required		
b. If so, what is the equilibration period?	Three days to allow plants/soil to acclimate to air temperature, relative humidity, and light/dark cycle.		
c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.	To acclimate contents of trays to chamber environment		
10. Microcosm "lifespan":			
a. How long are microcosm tests generally run?	3-4 weeks		
b. What are the most important factors in establishing the lifespan of this microcosm?	Loss of plants due to destructive sampling		

GENERAL CHARACTERISTICS (CONTINUED)

11. What kind of lighting is used?

a. Type of lights (wattage, model, source, etc.):	A 1000-watt GTE Sylvania metal halide lamp is centered over each system.
b. Typical light intensity:	Unknown
c. Lighting control (intensity, photoperiod, means of control, etc.):	Light/dark cycle - variable with timer

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	X
b. Relative humidity	X
c. Temperature	X
d. Light intensity	X (on/off)
e. Inorganic nutrients	
f. Carbon dioxide	
g. Dissolved oxygen	
h. Other (specify)	

13. How is temperature controlled (constant temperature room, water bath, etc.)?

No control—totally determined by lights and temperature of room (room is heated/refrigerated to ± 2°C)

14. How is water/air circulated/mixed?

Air passage through system by fan controlled manually with valve

CONTAINMENT

1. a. Is containment with current microcosm design adequate for working with GEMs?

   Yes_x_ No_____

   b. If so, describe containment design.

   Plastic doors with glove-box type design. HEPA filter traps particles/ bacteria before exhausting to outdoors

   c. Could containment be improved by design modification?

   Yes_x_ No_____

   d. If so, what is the nature of the modifications needed to improve containment?

   Depends on what experiments are to be done (e.g., could put whole chamber into negative pressurized room)

   e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

   ____ a. Considerable resources, skill, or time.

   ____ b. Moderate resources, skill or time.

   ____ c. Minimal resources, skill or time.

   _x_ d. Can't estimate at this time. (Depends on experimental design)

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication) been developed covering:

   a. Microcosm construction? Yes_____ No_x_

   b. Microcosm operation? Yes_x_ No_____

   c. Output analysis? Yes_x_ No_____

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes_____ No_____

   b. Microcosm operation? Yes_____ No_____

   c. Output analysis? Yes_____ No_____

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes_____ No_____

   b. Operate a microcosm? Yes_____ No_____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

Install computerized control of environment so chambers can *mimic* variability of outdoor conditions. Suggest controls for RH, air and soil temperature, soil moisture, and light intensity.

2. What levels of difficulty would be involved in making the modifications in (1) above?

__x__ a. Considerable resources, skill or time.

____ b. Moderate resources, skill or time.

____ c. Minimal resources, skill or time.

____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

Leaves and soil are currently sampled.
If destructive sampling is used, sampling is limited by small number of plants.

2. Is destructive sampling during the course of a test run required?

Yes__x__ No____

3. Would design modifications allow the use of alternative sampling strategies?

Yes__x__ No____

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

____ a. Less than $100

____ b. Between $100 and $500

____ c. Between $500 and $1000

__x__ d. More than $1000

2. How many replicate vessels are generally used per treatment?

Two "boxes" are used in an experiment; replicate experiments are performed with plants and soil in pairs of boxes

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

____ a. Less than $5000

__x__ b. Between $5000 and $20000

____ c. Over $20000

____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS		_____
	ANIMALS		_____
	BENTHOS		_____
	MICROORGANISMS		_____
	OTHER (SPECIFY)		_____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA		_____
	BACTERIA/PROTOZOA		_____
	PLANTS/HERBIVORES		_____
	HERBIVORES/PREDATORS		_____
	OTHER (SPECIFY)		_____
ENERGY FLOW	PRIMARY PRODUCTION		_____
	SECONDARY PRODUCTION		_____
	P/R RATIO		_____
	OTHER (SPECIFY)		_____
BIOGEOCHEM. CYCLING	NITROGEN		_____
	PHOSPHORUS		_____
	SULFUR		_____
	OTHER (SPECIFY)		_____
OTHER EFFECTS	PLANT (SPECIFY)		_____
	ANIMAL (SPECIFY)		_____
	MICROBIAL (SPECIFY)		_____
	OTHER (SPECIFY)		_____

Reasons that a parameter cannot be addressed in your microcosm

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	____	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	____	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide an indication of extrapolation potential.

Questions	*FACTOR*	
	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
1. Has your microcosm response to this factor been compared to field data? If so, please cite the reference(s), and, if possible, enclose a copy.	Yes ____ No _X_	Yes ____ No _X_
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ____ No _X_	Yes____ No _X_
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival, colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON POND MICROCOSM

Dr. Ray Seidler
U.S. EPA
200 S.W. 35th Street
Corvallis, OR 97333
(FTS) 420-4708

Dr. John L. Armstrong
U.S. EPA
200 S.W. 35th Street
Corvallis, OR 97333
(FTS) 420-4718

Armstrong, J.L. Protocol for application of microcosms to study of fate and survival of recombinant bacteria associated with plants and herbivorous insects. U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR. Preliminary Draft. 16 p.

VERSACORE MICROCOSM

GENERAL CHARACTERISTICS

DEVELOPER: W. HOLBEN/ J. JANSSON

1. Briefly describe the physical design including microcosm vessel material. If possible, include a labeled diagram.

Constructed of clear-cast acrylic tubing (O.D. 9.5 cm, wall thickness 0.48 cm) cut into 2.5 cm sections and reassembled to the desired height by taping them together with waterproof tape. Each microcosm contains 550 g of sieved (2-mm) Capac loam soil, packed to a bulk density of approximately 1.3 g/cm^3. Cores are planted with 5 wheat seeds, rinsed with 1.5% sodium hypochlorite and rinsed with filter sterilized water, or 3 corn seeds, pretreated with captan and methoxychlor.

2. Which of the following trophic levels are normally represented?

Microorganisms (specify)	Yes_X_ No___	Added or indigenous
Primary producers (specify)	Yes_X_ No___	(Planted)
Invertebrates (specify)	Yes___ No_X_	(Scale probably too small)
Vertebrates (specify)	Yes___ No___	
Other (specify)		

3. Describe how communities of organisms are established in the microcosm.

Cores are packed with soil, inoculated, planted (wheat/ corn) and maintained in an environmentally controlled chamber.

4. If environmental media are used, how is the environment sampled?

Sand cores contain sieved, packed soil, planted with wheat or corn seeds.

5. What habitats are represented?

a. Typically:

Generic soil.

b. What factor(s) limit the habitats that could be represented?

Scale: System is not appropriate for large scale experiments with vertebrates, etc.

GENERAL CHARACTERISTICS (CONTINUED)

		Dimensions (cm)	*Volume (L)*	*Soil/Sediment Surface Area (cm^2)*
6.	Microcosm size:			
	a. Typically:	9.5 cm (O.D.) tubes		57 cm^2
	b. What factor(s) limit these size characteristics?	Could be scaled-up if desirable		
	c. How much space is required per microcosm unit?	1 cubic foot		
7.	For what purpose was the microcosm originally designed?	To monitor transport, survival, and gene exchange of bacterial populations, in soil (bulk or rhizosphere).		
8.	Discuss any provisions for exchanging air and water in your microcosm with the environment. For aquatic systems, describe aeration and water exchange (static, static-replacement, flow-through); for terrestrial systems, indicate air exchange and addition of water.	Cores are brought to field capacity (approx. 23% moisture) by setting them in distilled water overnight. Cores were drained, both ends covered with Parafilm®. After germination, Parafilm is removed from the top of the core and moisture is maintained by daily weighings and additions of water.		
9.	Equilibrium period:			
	a. Is laboratory equilibrium required before testing?	Yes_____ No_X__		
	b. If so, what is the equilibration period?			
	c. If required, what is the purpose of the equilibrium period and what criteria are used to determine when it is equilibrated.			
10.	Microcosm "lifespan":			
	a. How long are microcosm tests generally run?	1-3 weeks		
	b. What are the most important factors in establishing the lifespan of this microcosm?			
11.	What kind of lighting is used?			
	a. Type of lights (wattage, model, source, etc.):	Fluorescent light.		
	b. Typical light intensity:			
	c. Lighting control (intensity, photoperiod, means of control, etc.):	Constant light.		

GENERAL CHARACTERISTICS (CONTINUED)

12. Which of the following environmental parameters are routinely monitored?

a. Soil moisture	x
b. Relative humidity	x
c. Temperature	
d. Light intensity	
e. Inorganic nutrients	
f. Carbon dioxide	
g. Dissolved oxygen	
h. Other (specify)	

13. How is temperature controlled (constant temperature room, water bath, etc.)?

Incubation room maintained at desired temperature and humidity.

14. How is water/air circulated/mixed?

Environmental chamber provides air exchange.

CONTAINMENT

1 a. Is containment with current microcosm design adequate for working with GEMs?

Yes x No

b. If so, describe containment design.

Small scale allows benchtop work in lab so that regular laboratory containment practices can be followed.

c. Could containment be improved by design modification?

Yes No x

d. If so, what is the nature of the modifications needed to improve containment?

e. If modifications would improve containment, what degree of difficulty would be encountered in making these modifications?

___ a.	Considerable resources, skill, or time.
___ b.	Moderate resources, skill or time.
___ c.	Minimal resources, skill or time.
___ d.	Can't estimate at this time.

PROTOCOLS

1. Has a detailed protocol (e.g., standard operating procedures, publication, etc.) been developed covering:

   a. Microcosm construction? Yes____ No__x__

   b. Microcosm operation? Yes____ No__x__

   c. Output analysis? Yes____ No__x__

2. If the answer to *any* of the above (1a, 1b, or 1c) is "no," do you expect to develop protocols within the next 2 years covering:

   a. Microcosm construction? Yes____ No__x__

   b. Microcosm operation? Yes____ No__x__

   c. Output analysis? Yes____ No__x__

3. If the answer to *any* of the above (1a, 1b, or 1c) is "no," could a competent technician, with the aid of literature descriptions:

   a. Construct a microcosm? Yes__x__ No____

   b. Operate a microcosm? Yes__x__ No____

MICROCOSM MODIFICATION POTENTIAL

1. List any additional modifications (other than containment) that you would recommend to improve the effectiveness of this microcosm for GEM risk assessment use (e.g., additional trophic levels, reduction of analytical time/costs, etc.).

   The system is pretty well suited to small scale studies and large numbers of replicates can be handled effectively.

2. What levels of difficulty would be involved in making the modifications in (1) above?

   ____ a. Considerable resources, skill or time.

   ____ b. Moderate resources, skill or time.

   __x__ c. Minimal resources, skill or time.

   ____ d. Can't estimate at this time.

SAMPLING

1. What sampling strategies are currently possible without design modification, and what are the limits for repetitive sampling?

   Total destructive sampling

   Collect and plate leachate

   Take small diameter "minicores" through profile leaving bulk of microcosm largely untouched

   Disassemble in 2.5 cm increments to sample vertically through the profile

2. Is destructive sampling during the course of a test run required?

   Yes____ No_x_

3. Would design modifications allow the use of alternative sampling strategies?

   Yes____ No_x_

COST FACTORS

1. What is the relative capital cost of a single complete microcosm unit (i.e., one vessel, stirrer, etc., without temperature control, flowing water, etc.)?

   _x_ a. Less than $100

   ____ b. Between $100 and $500

   ____ c. Between $500 and $1000

   ____ d. More than $1000

2. How many replicate vessels are generally used per treatment?

   Three

3. What is the estimated minimal cost of a complete microcosm test, including vessels?

   _x_ a. Less than $5000

   ____ b. Between $5000 and $20000

   ____ c. Over $20000

   ____ d. An estimate has not been made

APPLICABILITY FOR EVALUATING ECOLOGICAL PARAMETERS

Indicate which of the following parameters have been measured in your microcosm by briefly listing the technique (i.e., benthos by sieving, Rose Bengal Staining, and sorting; microorganisms by lipid analysis; bacteria/protozoa interactions by selective filtration, staining, and counting; primary pro-ductivity in phytoplankton by ^{14}C-carbonate uptake or in macrophytes by measuring plant growth; an aspect of nitrogen cycling by measuring ammonia concentrations or fluxes, etc.). Also indicate if an endpoint could not be used in your microcosm, and if not why.

ENDPOINT	PARAMETER	TECHNIQUE	COULD NOT BE STUDIED IN THIS MICROCOSM
COMMUNITY STRUCTURE	PLANTS	Usually 1-3 seedlings are used	
	ANIMALS		X
	BENTHOS		X
	MICROORGANISMS	DNA probes, selective plating, direct counts	
	OTHER (SPECIFY)		
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	Substrate depletion analyses (e.g. HPLC)	
	BACTERIA/PROTOZOA	Sterile/nonsterile systems; eukaryotic inhibitors	
	PLANTS/HERBIVORES		X
	HERBIVORES/PREDATORS		X
	OTHER (SPECIFY)		
ENERGY FLOW	PRIMARY PRODUCTION		
	SECONDARY PRODUCTION	Could study label uptake by microbes from plants	
	P/R RATIO		X
	OTHER (SPECIFY)		
BIOGEOCHEM. CYCLING	NITROGEN		X
	PHOSPHORUS		X
	SULFUR		X
	OTHER (SPECIFY)		
OTHER EFFECTS	PLANT (SPECIFY)		X
	ANIMAL (SPECIFY)		X
	MICROBIAL (SPECIFY)	Gene exch.; transport thru soil, population inputs	
	OTHER (SPECIFY)		

Reasons that a parameter cannot be addressed in your microcosm

Scale is too small for many of these parameters.

Biogeochemical cycling has not been tested but probably is not appropriate at this scale

FIELD CALIBRATION OF ECOLOGICAL PARAMETERS

Field calibration tests compare the responses of ecological parameters in microcosms with the field in the absence of stress agents, and may provide an indication of extrapolation potential. If a field calibration test has been performed with your microcosm for any of these parameters, please signify high, intermediate, or low comparability with the field. If you have not field-calibrated a parameter but plan to do so in the next 3 years, please indicate this, also.

FACTORS	PARAMETERS	PARAMETER HAS BEEN STUDIED; COMPARABILITY WITH FIELD WAS: H=HIGH; I=INTERMEDIATE; L=LOW	PARAMETER HAS NOT BEEN FIELD CALIBRATED BUT IS EXPECTED TO BE WITHIN 3 YEARS
COMMUNITY STRUCTURE	PLANTS	____	____
	ANIMALS	____	____
	BENTHOS	____	____
	MICROORGANISMS	____	____
	OTHER (SPECIFY)	____	____
TROPHIC INTERACTIONS	SUBSTRATE/BACTERIA	____	____
	BACTERIA/PROTOZOA	____	____
	PLANTS/HERBIVORES	____	____
	OTHER (SPECIFY)	____	____
ENERGY FLOW	PRIMARY PRODUCTION	____	____
	SECONDARY PRODUCTION	____	____
	P/R RATIO	____	____
	OTHER (SPECIFY)	____	____
BIOGEOCHEM. CYCLING	NITROGEN	____	____
	PHOSPHORUS	____	____
	SULFUR	____	____
	OTHER (SPECIFY)	____	____
OTHER EFFECTS	PLANT (SPECIFY)	____	____
	ANIMAL (SPECIFY)	____	____
	MICROBIAL (SPECIFY)	____	____
	OTHER (SPECIFY)	____	____

If comparability studies have been conducted, briefly discuss major problems encountered in making comparison, cite the reference(s), and include a copy, if possible.

FIELD VERIFICATION OF MICROBIAL FATE

Field verification tests with GEMs or microbes used as surrogates for GEMs may be conducted to compare the survival, colonization, and microbial/gene mobility observed in microcosms with the field. These tests may provide and indication of extrapolation potential.

Questions	*FACTOR*	
1. Has your microcosm response to this factor been compared to field data?	Survival/ Colonization	Environmental Mobility (Specify organism or gene)
	Yes ____ No _x___	Yes ____ No _x___
2. If the answer to 1a. (above) is "yes," please rate the degree of comparability (H=High; I=Intermediate; L=Low).	____	____
3. If the answer to 1a. (above) is "no," do you plan to conduct field verification studies with microbes in the next three years.	Yes ____ No _x___ Depends on funding availability.	Yes____ No __x__
4. If field verification studies have been conducted with microbes, briefly discuss major problems encountered in making the comparisons.		
5. Please discuss any factors other than survival colonization or microbial gene mobility potential that have been field verified in your microcosm?		

FURTHER INFORMATION ON VERSACORE

Dr. William Holben
Michigan State University
East Lansing, MI 48824
(517) 355-9282

Jansson, J.K., W.E. Holben, J.M. Tiedje, and B.K. Chelm. 1989. The fate of recombinant pseudomonads in modified soil-core microcosms (Versacores). In J.K. Fredrickson and R.J. Seidler (eds.), Evaluation of Terrestrial Microcosms for Detection, Fate, and Survival Analysis of Genetically Engineered Microorganisms and Their Recombinant Genetic Material. Report (PNL-6828) prepared for U.S. EPA, Environmental Research Laboratory, Corvallis, by Pacific Northwest Laboratory, Richland, WA. Pp. 3.1-3.23.

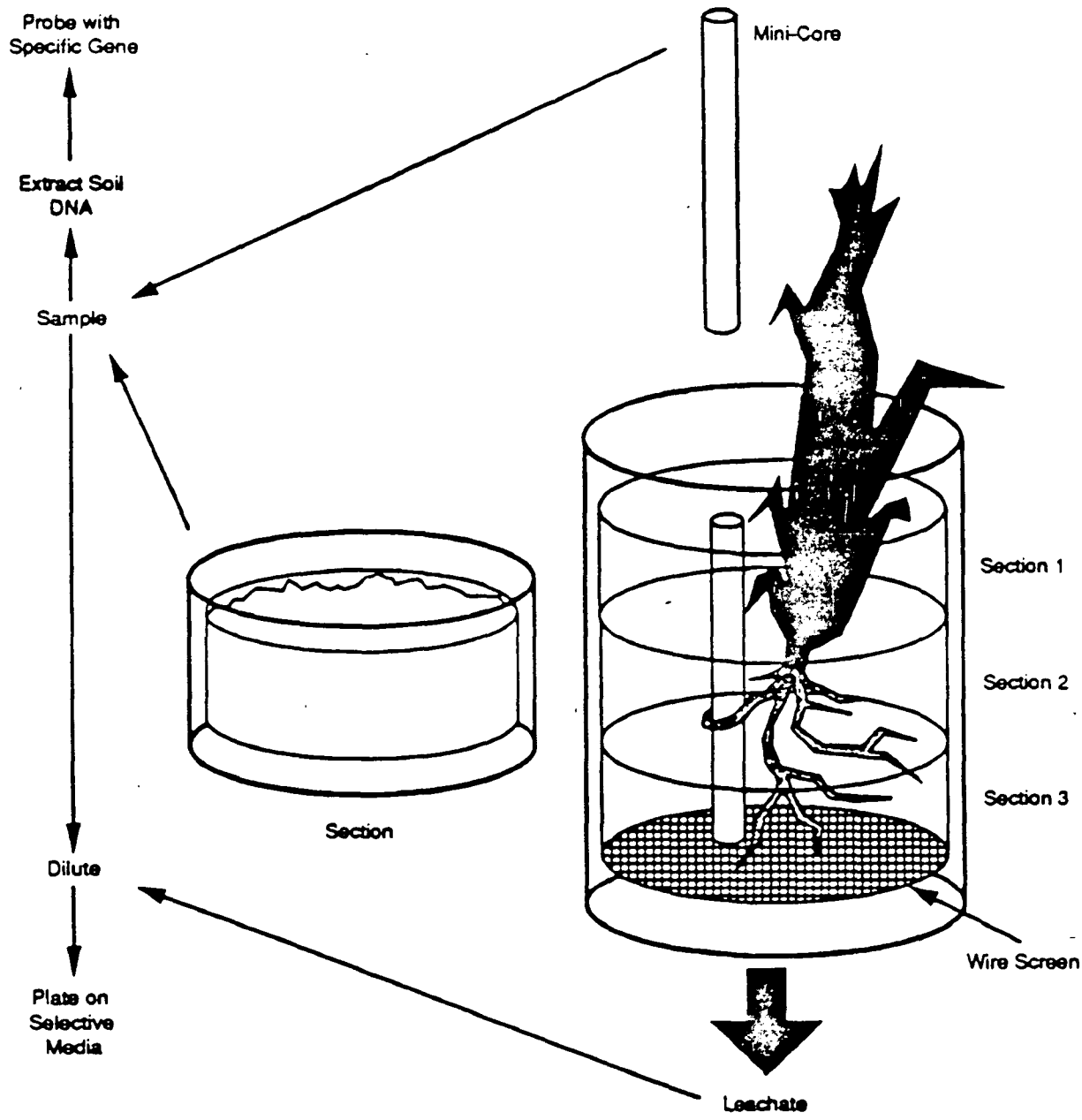


Figure 12. **Versacore.**