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Standardized microcosms in microbial risk assessment. *Sheldon Krimsky; Roger P. Wrubel; Inger G. Naess; Stuart B. Levy; Richard E. Wetzler; Bonnie Marshall.*

Abstract: The use of microcosms for the prerelease risk assessment of genetically engineered soilborned bacteria is discussed. It is also proposed that results of microcosms studies be standardized to allow comparisons with other similar studies.

Naturally occurring microorganisms have long been exploited by humans in areas such as food chemistry, agriculture, wastewater treatment, mining, and pharmaceuticals. The rapid advancement of molecular biology has given rise to the genetic modification of microorganisms for the creation of new products that are now reaching the marketplace. For example, the transfer of a gene from the stomach cells of calves to bacteria has allowed the large-scale production by fermentation of chymosin, an enzyme essential in cheese making (Wrage 1994). Genetically engineered microorganisms are now being used in the manufacture of protein hormones to increase milk production, enzyme detergents, high fructose corn syrup, human insulin, and interferon. Interestingly, all of the live genetically engineered microorganisms approved for commercial sale have been those used in contained structures, such as fermentation tanks. The only genetically engineered microorganisms intended for environmental release that are now commercially available are killed pseudomonads, which contain and protect from degradation a bacterially derived insect toxin (Fischer 1991). To date no live genetically engineered microorganisms intended for environmental release have been approved for commercial sale in the United States.

While many environmental uses of genetically engineered microorganisms for agriculture and bioremediation have been suggested (Berry and Hagedorn 1991, Lindow et al. 1989, Miller et al. 1983, OTA 1991), relatively few small-scale field tests have been conducted (Tables 1 and 2). In contrast, approximately 1000 permits for field tests of genetically engineered plants have been granted.(1)

Many of the microorganisms approved for field testing were create,4 through the deletion or insertion of genes from species within the same genus. In other cases, regulatory sequences have been introduced or genes from other genera have been inserted as markers, to allow identification of the bacteria and viruses after release.

Since 1987 only five types of live microorganisms containing genes (other than noncoding regulatory genes or marker genes) from organisms not closely related have been released in the United States (Tables 1 and 2). One is an endophytic bacterium, Clavibacter xyli, engineered to express an insect-specific toxin gene from another bacterium, Bacillus thuringiensis (Dimock et al. 1989). Another is a vaccinia virus containing a gene from the rabies virus, which has been distributed in baits to immunize wildlife populations to combat the spread of rabies (Rupprecht et al. 1986). Finally, two species of symblotic nitrogen-fixing bacteria--Rhizobium meliloti for inoculation of alfalfa and Bradyrhizobium japonica for inoculation of soybean--have been engineered to increase nitrogen availability in their

respective host plants (Ronson et al. 1990). Recombinant R. meliloti contains genes from B. japonica and Rhizobium leguminosarum, and recombinant B. japonica contains genes from R. meliloti and R. leguminosarum.

The vaccinia-rabies vaccine was the first, and to date only, live inter-generic genetically engineered microorganism to be approved for commercial sale in the United States. On 20 April 1995 a conditional license was issued to the manufacturer for one year. After that time, to renew the license the company must present additional data on the safety and efficacy of the product. The genetically engineered vaccinia-rabies vaccine was approved for commercialization by the European Union in 1994.

In July 1995 an insect pathogenic virus containing a scorpion venom gene was released in the United States. The recombinant virus is designed to kill pest caterpillars more quickly than the wild-type virus.

In part, the reason for the slow progress in the development of genetically engineered microorganisms intended for large-scale environmental release may have to do with the uncertainty of the risk associated with such experiments (Levidow 1995, Shaw et al. 1992). Field testing of new microorganisms, even on a small scale, presents a significant problem. Once released, the microbes may establish and disperse in the environment becoming nearly impossible to eliminate (NRC 1989). Based on this observation, the US Environmental Protection Agency (EPA; Federal Register 1994a, p. 45544) has concluded that the "R & D which involves intentional testing of microorganisms in the environment should be subjected to some review."

[1]The list of permits for field tests of transgenic plants in the United States may be obtained from: Biotechnology, Biologics, and Environmental Protection, USDA-APHIS, 6505 Belcrest Rd., Hyattsville, MD 20782.

EPA specifies the data requirements for applicants to receive approval for field releases of genetically engineered microorganisms, but the methods of obtaining these data are undefined (Federal Register 1994a, b). Microbial ecologists have suggested a staged risk-assessment process that starts with a description of the genetics and natural history of the donor and host organisms and next moves to laboratory experiments, then to microcosm experiments, and finally to field tests (e.g., Alexander 1985, Fredrickson et al. 1990).

In this article, we describe the uses and limitations of microcosms for prerelease risk assessment of genetically engineered soilborne bacteria. A microcosm is "an intact, minimally disturbed piece of an ecosystem brought into the laboratory for study . . . that behaves ecologically like its counterpart in the actual field" (NRC 1989, p. 117). This research set-up contrasts to artificial laboratory systems, which select certain properties of the field while excluding others and are not subunits of aquatic or terrestrial systems. These systems are sometimes referred to as synthetic communities (NRC 1989). Microcosm studies have been proposed as an effective, straightforward means to predict the ecological effects of genetically engineered microorganisms before initiating field experiments (Frederick and Pilsucki 1991, Greenberg et al. 1988, Trevors 1988).

Table 1. Genetically engineered bacteria that have been field-tested in the United States. Donor organisms of marker genes (e.g., *lac* operon genes or antibiotic resistance genes) are only reported when the primary purpose of the field release was to determine the effectiveness of the marker.

Organism	Date	Type of genetic modification	Purpose of modification	Organization	Current status
Pseudomonas syringae (ice-nucleating bacteria)	1987–1990	Deletion of ice nucleation gene	Frost protection for crops (e.g., strawberry and potato)	Advanced Genetic Sciences (later merged with DNA Plant Technology)	Research discontinued
P. syringae	1987–1991	Deletion of ice nucleation gene	Frost protection for crops	University of California, Berkeley	Field tests discontinued
Pseudomonas fluorescens (rhizosphere bacteria)	1987	Insertion of lac operon genes *lacZ* and *lacY* (*lacZY*)	Track bacteria in field. *LacZY* is used as a marker gene.	Monsanto Co./Clemson University	Research discontinued although the *lacZY* marker

We argue in this article that data from microcosm studies should be required for regulatory approval of field releases of genetically engineered microorganisms. We also propose that regulatory agencies in collaboration with microbial ecologists encourage the standardization of methods and procedures, where possible, for using microcosms in risk assessment of soil bacteria. Without some degree of standardization, it is likely to be difficult to compare data from diverse microcosm studies. Moreover, new regulatory regimes for genetically engineered microorganisms are likely to be met with confusion without some effort to develop uniform measurement protocols, a prerequisite to ensure the replicability of results and the growth of a body of dependable knowledge.

Evaluation of genetically engineered microorganisms

Within the scientific community some contend that introducing genetically engineered microorganisms poses little or no environmental risk. This reasoning is based on several assertions described by Miller (1991), three of which are relevant to our discussion. First, genetically engineered microorganisms are not new, because the transfer of DNA among unrelated organisms has been occurring in nature for billions of years. Thus, nature has already seen all the possible genetic combinations that humans could devise.

Second, natural habitats are saturated with indigenous populations of microorganisms that, through natural selection, are highly adapted to their environments. Consequently, when genetically engineered microrganisms are released into new environments their survival and dispersal are likely to be limited by the indigenous microorganisms (Campbell 1991, Miller 1991). Third, the recombinant techniques used to create a genetically engineered organism are considered precise modifications of single genes whose functions are known and can be excised from one organism and transferred to another. Accordingly, the effect of the new gene on the functioning and behavior of the genetically engineered microorganism can be predicted with a high degree of certainty (Miller 1991). Thus, dangerous and safe combinations can be distinguished without elaborate and costly risk assessment.

Table 2. Genetically engineered viruses that have been field-tested in the United States. Donor organisms of marker genes (e.g., *lac* operon genes or antibiotic resistance genes) are only reported when the primary purpose of the field release was to determine the effectiveness of the marker.

Organism	Date	Type of genetic modification	Purpose of modification	Organization	Current status
Autographa californica nuclear polyhedrosis virus (lepidopteran pathogen)	1989	Deletion of polyhedrosis gene	Limit the persistence of a recombinant virus in the environment to increase the safety of releases	Boyce Thompson Institute for Plant Research	Technology licensed to AgriVirion
Orthopox vaccinia (pox virus)	1990–1995	Insertion of glycoprotein gene from *Lyssavirus rabies* (rabies virus)	Vaccine for wildlife rabies	Rhone-Merieux, Inc., and Wistar Institute	Rhone-Merieux, Inc., issued conditional license for use and sale of vaccine April 1995–April 1996
Lymantria dispar nuclear polyhedrosis virus (gypsy moth pathogen)	1993	Deletion of polyhedrosis gene and insertion of *lacZ* gene from *E. coli*	Determine persistence and track movement of the viruses in field	Boyce Thompson Institute for Plant Research	Monitoring of site continuing, to track virus, through 1995. No commercial value at this time.
A. californica	1993–1994	Deletion of polyhedrosis gene	Determine infectivity and persistence of the virus for control of *Trichoplusia ni* (cabbage looper). The ultimate goal is to develop a virus that will die off soon after the infected insect dies. Then insect-specific toxin genes could be inserted into the virus without the risk of unwanted toxicity to nontarget insects.	AgriVirion, Inc.	Research continuing
A. californica	1993	Deletion of the *egt* virus gene. Insertion of a scorpion venum.	Improve efficacy of virus for control of caterpillars. Deletion of the egt gene from the virus to make the caterpillar cease feeding more quickly after infection. Insertion of a scorpion venom gene to kill the caterpillar more quickly than the wild-type virus.	American Cyanamid	Research continuing

These propositions may be true for many microorganisms and habitats (e.g., Liang et al.

1982, Walter et al. 1987), but they are not necessarily true for all organisms in all cases (Curtiss 1988). Ecologists have warned against such a generic approach to safety assessment (Tiedie et al. 1989). According to Regal (1994), many of the earliest, socalled generic safety models, such as those that presume nature will reject or keep in balance genetically engineered organisms, are now widely considered to be based on outdated scientific thinking. The fate and effects of a genetically engineered microorganism are likely to depend on its phenotypic and genotypic properties, the characteristics of the receiving environment, and the interactions between them.

Accordingly, regulatory agencies have based risk assessments on practical case-by-case experiments rather than on a priori analysis, which assumes an understanding of the behavior of the genetically engineered microorganism in the field. For example, Holmes and Ingham (1994) reported that a genetically modified strain of the common decomposer bacterium Klebsiella planticola, designed to synthesize ethanol from the breakdown of agricultural residues, shows evidence of adversely affecting a soil food web. The ethanol-producing K. planticola were found to destroy mycorrhizal fungi, which are essential to the growth of some plants, including important agricultural crops. Extrapolating from one species of microorganism to another or from tests conducted under one set of environmental conditions to another may not be appropriate and is often unreliable (Berry and Hagedorn 1991).

The principle of case-by-case evaluation of genetically engineered organisms has been accepted by the 25 member countries, including the United States, of the Organization for Economic Co-operation and Development (OECD), as well as EPA (Federal Register 1994a, b, OECD 1995). Once familiarity with specific genetically engineered organisms or groups of organisms is gained, regulatory requirements can be streamlined as has been proposed by EPA for B. japonicum and R. meliloti (Federal Register 1994a, Gaugitsch and Torgersen 1995). One goal of OECD is to establish mutual acceptance of data between members, which would facilitate cooperation on research and marketing of genetically engineered microorganisms (OECD 1995). To reach such a goal, there is a need to expand the agreement on principles to include the specific methods for testing.

The difficulty of predicting the consequences of releases of genetically engineered microorganisms has raised serious public concerns (Alexander 1985, Krimsky 1991). The uncertainty over the release of genetically engineered microorganisms presents a difficult risk-assessment and management problem for regulatory agencies responsible for ensuring that the human health or ecological impacts of such releases are minimized. Public concerns and regulatory uncertainties taken together may present a significant obstacle in the commercial development of these products by deterring researchers from pursuing field experiments (Regal et al. 1989).

Evaluation of genetically engineered microorganisms must include analyses that quantify their survival, their competitiveness with indigenous organisms, their reproduction rates, and their dispersal abilities by both passive (e.g., with subsurface water movement) and active (e.g., oriented movement via flagella) transport. Further requirements for risk assessment include the possibility of vector-mediated movement (e.g., attachment to mobile soil-inhabiting invertebrates), the probability of transfer of transgenes to other microorganisms, and the capability of transgenic microorganisms to persist in a resting state (Federal Register 1994b).

Because microbes can actively move and reproduce, more organisms may be found distant from the release point than were introduced in the inoculum. This possibility is in contrast to the increasing dilution of chemicals from this point of release and highlights the difference in risk assessment of chemicals from genetically engineered microorganisms. Although there is a sizeable body of knowledge pertaining to the genetics and ecology of microorganisms, little is currently known about its relevance for predicting the behavior of genetically engineered microorganisms under the variety of abiotic and biotic environmental conditions that they may encounter.

Because containment is largely impractical, escape of microorganisms is a function of the inherent survival and dispersal characteristics of the organism (Fuxa 1991). Thus, it is important to develop methods to gather as much reliable data on the characteristics of genetically engineered microorganisms, including their effects on flora and fauna, before field releases are approved. The OECD countries have recognized this need and have agreed to assess the behavior of genetically engineered microorganisms "in simulated natural environments such as microcosms, growth rooms, greenhouses, insectaries. . ." (OECD 1994). Prerelease evaluation could be used to eliminate potentially hazardous releases and to provide information to design the protocols for the initial environmental release of genetically engineered microorganisms.

The role of microcosms

The purpose of prerelease microcosm studies, as well as of simpler laboratory experiments, is to determine whether any genetic modifications introduced into the microorganism are likely to create unacceptable risks before it is released into the environment. For example, if the genetically engineered microorganism is not expected to outcompete its parental strain, but microcosm tests indicate that it does, moving to field tests may be contraindicated before the system is better understood. Microcosms are more likely than synthetic systems to reveal how organisms behave under field conditions.

The notion of addressing ecological questions with microcosms is derived from a basic problem in field ecology: How can the contributions of the variables of interest in complex ecosystems be identified? By moving a small part of the ecosystem into the laboratory, experimental variables can be controlled more effectively than in the actual ecosystem. The purpose of a microcosm is to add a level of ecological reality to laboratory experiments while maintaining many of the advantages of the controlled environment. US regulatory agencies have begun to cite the use of microcosms in their data requirements (Federal Register 1994a). Under EPA's proposed rules for releasing microbial products of biotechnology, experimental release applications under the Toxic Substances Control Act (1976) require information on survival and dissemination according to relevant environmental conditions. While some of these data may be obtainable in microcosms more readily than in the field, the primary advantage of microcosms is that they provide a means of identifying potentially hazardous genetically engineered microorganisms before they are released into the environment. In this respect, microcosms may function for genetically engineered microorganisms in the same way that in vitro assays serve as a screen for chemicals before they are approved for commercial or industrial use. The chemical assays are not a substitute for animal experiments or human epidemiological data, but they serve as a preliminary screen.

The experiments of Holmes and Ingham (1994) highlight the advantages of using

microcosms containing the indigenous soil biota and plants before actually releasing genetically engineered microorganisms in the field. Microcosms containing the K. planticola genetically engineered to synthesize ethanol from organic wastes showed reduced plant growth, reduced colonization of plant roots by mycorrhizae, and increased populations of plant parasitic nematodes compared to treatments containing the unmodified parental strain. Field testing this genetically engineered microorganism without conducting the appropriate prerelease microcosm experiments might have resulted in establishment of an ecologically destructive agent. The unintended effects of the organism on a complex soil food web might not have been observed in synthetic-community experiments. The researchers noted that current EPA protocols do not test microbes under realistic conditions and would have missed the potential ecological effects of the modified Klebsiella(2) (Gillis 1994).

Varieties of microcosms

Ideally, the results of microcosm experiments, for selected variables, provide a reasonable facsimile of what occurs in the larger and more complex ecosystem. However, microcosms are still isolated structures, and the material fluxes that are part of all ecosystems are impossible to replicate in a small closed system. In addition, because of the physical structure of microcosms, they have abnormally high surface-to-volume ratios and are thus subject to edge effects, which may skew results compared to those that might be obtained in the field (Lacy and Stromberg 1990).

One of the difficulties in comparing the results of data from microcosms is that there exists no consensus within the scientific community about the most appropriate size and shape of a soil microcosm, how it should be designed, and the methods for using it. Varied sizes and shapes of microcosms used in research have included test tubes 18 x 150 mm, containing 2 g of soil (Stotzky et al. 1990); soil cores (17.5 cm diameter x 60 cm; Bolton et al. 1991a, b); and plastic trays (5 x 27 x 55 cm; Armstrong et al. 1987). Microcosm studies have employed a variety of soils including sterile soil, homogenized mixtures of field soil, and intact soil cores in which the soil is disturbed as little as possible.

In the 1970s, growing concern about the effects of pesticides and other xenobiotics on ecosystems stimulated the use of microcosms as model systems for risk assessment. Previously, researchers had relied on simple laboratory experiments or entire ecosystems for basic research on the behavior of single organisms or the interactions among organisms. Microcosms allowed researchers to standardize and replicate experiments to a degree not possible in the environment. In addition, radiolabeling--a technique scientists used to trace the movement of chemicals--could be applied in microcosms without the risk of releasing radioactive materials into the environment. Relatively simple microcosms enable investigators to assess rapidly the fate and effects of selected pesticides and other pollutants in soils (Cole et al. 1976, Gillett and Gile 1976, Metcalf 1977). Many of these experiments used a standardized soil mixture that did not contain the natural complement of indigenous biota found in native soils. Employing standardized soil mixtures rather than in situ soil is convenient and allows for uniform manipulation of environmental factors such as pH, water content, and nutrients. But usefulness of such microcosms in determining survival and dispersal of genetically engineered microorganisms in the environment is questionable. Succeeding generations of microcosms have been designed with the intent of replicating natural conditions more closely.

An understanding of the importance of the physical, chemical, and biological characteristics

of soils in the functioning and interactions of soil biota has led to the design of microcosms that attempt to preserve soils in their natural state as much as possible (Berry and Hagedorn 1991, Hicks et al. 1990). Hollow cores are driven into the ground and then pulled up with the soil remaining inside. The intact soil core can then be used as the microcosm. Ideally the method used to obtain the intact soil core causes minimal disturbance to the soil structure. In instances, the soil is transferred from the corer to the microcosm, causing more disruption that may have biological effects. For example, the movement of microorganisms in microcosms using repacked field soils is reduced compared with intact soil cores, which contain a higher proportion of macropores (large air pockets) and connecting channels (NRC 1989).

Intact soil cores containing the native biota have been used to study chemical effects (Jackson et al. 1979, O'Neill et al. 1977). These experiments were directed at measuring soil biotic activity as an indicator of the overall effect of the chemicals on soil microorganisms (i.e., rates of respiration, decomposition, and/or nutrient cycling), rather than to understand the effects of the chemicals on the population dynamics and community structure of the soil microorganisms.

Microcosms may be divided into two types: those designed to replicate, as near as possible, the complexity of the environment, and simpler microcosms used to assess a specific interaction between a chemical or microorganism and some environmental parameter. The former microcosms can be expensive and labor intensive to construct and manage, while the latter may suffice for research questions involving fewer variables (Greenberg et al. 1988, Lacy and Stromberg 1990).

A standard microcosm for testing the fate and effects of xenobiotics was established in 1987 by the American Society for Testing and Materials (ASTM 1988), based on the work of Van Voris (1985). This microcosm is an intact soil core of intermediate size, 17 cm diameter by 60 cm depth. It has been used in experiments to test the efficacy of microorganisms used both as degradation agents and as indicators of toxicity. Plants can be grown in this microcosm, allowing assessment of another important variable that affects microorganisms and ecosystem function. Although detailed analysis of microbial interactions has not been a focus of these experiments, some studies using the standard microcosm examined different groups of microorganisms such as mycorrhizal fungi, algae, nitrogen-fixing bacteria, and rhizosphere bacteria (Bolton et al. 1991a, Fredrickson et al. 1989). Experience with the standard microcosm has underscored the importance of the soil's physiochemical factors in relation to the effects that chemical compounds have on the soil biota. Soil characteristics such as the type and concentration of clay minerals, the proportion of organic matter, pH, temperature, and redox potential have been shown to modify the toxicity of xenobiotics and to affect the interaction of microorganisms with toxic chemicals (Hicks et al. 1990).

Microcosm experiments on nonengineered and genetically engineered microorganisms have provided data on a number of important risk factors including microbial survival (Bentjen et al. 1989, Recorbet et al. 1992,(3) van Elsas 1991(4)), transport and migration (Bashan 1986, Bentjen et al. 1989, Harvey et al. 1989, Kemp et al. 1992,(5) Parke et al. 1986, Paterson et al. 1993,(6) Trevors et al. 1990,(7) van Elsas et al. 1991,(8) Walter et al. 1991), gene exchange among microorganisms (Armstrong et al. 1987,(9) Richaume et al. 1989, Smit and van Elsas 1992, Trevors 1988 and citations within, Walter et al. 1991), and interactions of microorganisms with the rhizosphere (Bentjen et al. 1989, Dijkstra et al. 1987a, b, Fredrickson et al. 1989, Heijnen et al. 1993, Morel et al. 1989,(10) Scher et al. 1984, 1985,

van Elsas et al. 1992).

Limitations of microcosms

The usefulness of the microcosm studies for risk assessment of genetically engineered microorganisms has been restricted by several factors. First, testing vector-mediated movements in a microcosm can be problematic particularly if the vectors of microbes are unknown, which is often the case. Some vectors may be large organisms, such as insect larvae, earthworms, and vertebrates (e.g., Heijnen and Marinissen 1995). These organisms may be hard to work with in the contained microcosm environment. Microcosm results may then not reflect true movement patterns in the field, because the boundary requirements of the microcosm limit longer movements that might occur in the field.

Second, microcosm studies are usually conducted under static environmental conditions. Diurnal and seasonal changes are often ignored. When environmental fluctuations are included in the experimental protocol, they are frequently limited in scope. The relative importance of and links between environmental variables such as temperature, light, hydration, and cropping remain unclear. The particular challenge is to distinguish experimentally the impacts of environmental and habitat variation upon such microbial responses as survival, reproduction, and dispersal.

Third, recent evidence provides strong support that naturally occurring bacteria frequently exist in a viable but dormant state (Roszak and Colwell 1987). These bacteria have proven to be unculturable using the simple and relatively inexpensive traditional plating methods and therefore have remained undetected. More than 90% of viable indigenous soil flora are not culturable, and introduced organisms readily enter this state (Roszak and Colwell 1987). Thus, it is troublesome for risk assessment conducted in microcosms or the field if genetically engineered microorganisms enter dormancy and become unidentifiable. High specificity may be attained by using newer detection methods such as monoclonal antibody tagging, protein product, or rRNA fingerprinting and gene probes; however, these procedures are relatively laborious and expensive and may have low sensitivity for samples that cannot be concentrated (Colwell et al. 1988). Purification of DNA directly from soil has yielded variable results, often complicated by the coprecipitation of humic polymers that impede subsequent DNA manipulation (Kluepfel 1993). Recently, a sensitive method has been developed that reportedly extracted DNA from as few as ten bacterial cells from a sandy loam soil without DNA amplification (Selenska and Klingmuller 1991). While unable to distinguish between live, dead, and dormant cells, such methods have the advantage of tracking total target DNA, which previously had eluded detection.

[3]E. Ingham, 1995, personal communication. Oregon State University, Corvallis, OR.

Finally, most microcosm studies have failed to calibrate their findings with the corresponding patterns that microorganisms exhibit in the field. Calibration refers to the process of determining the degree of similarity of results obtained from the microcosm to those obtained from the field (Pritchard and Bourquin 1984). While it is not realistic or necessary that microcosms exactly predict field phenomena, it is essential to demonstrate that they exhibit trends analogous to those of field processes. For microcosms to be effective risk-assessment tools for evaluation of genetically engineered microorganisms, definition of the acceptable limits of analogous trends in the calibration of microcosms must be set by a consensus of researchers and regulators. The assignment of limits is a normative process and reaching concordance may not be simple.

Functional ecological attributes, such as respiration, nutrient cycling, and primary productivity, and/or structural attributes, such as the survival and growth of species of interest or community composition, can be used to calibrate microcosms (Bolton et al. 1991a, Pritchard and Bourquin 1984). Once calibration with the field is obtained for a given system and test organism, microcosms can be used as the basis for environmental studies as suggested by Stotzky et al. (1990). Recalibration of a microcosm is necessary if a genetically engineered organism is to be released into a new environment, because the unique physiochemical features of each soil are important determinants of the fate and effects of the introduced microorganism. However, once a larger number of genetically engineered soil bacteria have been evaluated in microcosms and released in diverse environments, generalizations among release sites might be possible, obviating the need for recalibration for each release.

New approaches

Bolton et al. (1991b) examined the reproducibility of field and soil microcosm tests, relative to the behavior of microorganisms, by comparing microcosms in the laboratory under ambient conditions, growth chambers with simulated field temperature fluctuations, field lysimeters (encased, intact soil cores replaced in the field), and field plots. All were planted with winter wheat and inoculated with a wheat root-inhibiting Pseudomonas sp. Bacterial survival varied in the different treatments as a function of time. However, if all were examined at the same stage of wheat growth (which occurred at different times in the four assays), no significant differences in Pseudomonas sp. populations were observed. This observation underscores the importance of calibrating the microcosms with the field parameters and indicates that plant growth stage might be a better calibration point than total elapsed time. Bolton et al. (1991b) also discovered the necessity of sampling at different depths and noted the presence of what is called a container effect, which not only alters root growth patterns, and consequently bacterial survival patterns, but possibly contributes to enhanced water movement through the confined soil volume.

Levy and his colleagues(11) have been conducting experiments designed to evaluate the survival and dispersal pattern of wild-type and mutant Pseudomonas sp. under field conditions and in microcosms maintained in a greenhouse. The microcosm, an intact soil core that measures 15 cm diameter x 30 cm, is left in the field for two months to equilibrate before being moved to the greenhouse. Simulated rainfall is provided in the greenhouse at levels similar to field patterns. Replicated samples are taken from the center of the microcosm, where the bacteria are introduced, and at several concentric annull in three directions from the center. Samples extracted from the soil surface and at several depths provide data on survival and movement in three dimensions (Figure 1). Important in their design 1 the inoculation of paired organisms in the same core: the wild-type parent, indigenous to the soil, along with its genetically engineered mutant. The relative survival of the wild type and mutant is assessed in each microcosm. The fate of the wild-type parent acts as an internal standard and allows for the evaluation of the reproducibility of the system. Comparisons between field and microcosm data are likely to indicate how well the microcosm predicts the survival and movement of the mutant and wild-type pseudomonads in the field. The influence of soil water content on the interaction of wild-type and mutant strains can also be assessed, as well as any differences in rainfall effects between field and microcosm.

The approaches employed by Bolton et al. (1991a, b) and Levy and his colleagues(12)

increase the confidence researchers can have in microcosms as risk-assessment tools, to be employed before field release of microorganisms.

Desirability of standardization

While the case-by-case approach to risk assessment of genetically engineered microorganisms must be maintained, standardization of the types of variables tested and the methods used to conduct the experiments is likely to allow data to be shared and compared among researchers and regulators. Thus, if microcosms are to be effective tools in assessing risks of genetically engineered microorganisms for release into the environment, some form of standardization of experimental methods and design would be desirable. Standardization would allow data to be mutually acceptable among the OECD countries, possibly obviating the need to repeat risk-assessment experiments for genetically engineered microorganisms already approved by member countries.

It would be useful if a set of parameters determined to be salient to the behavior of genetically engineered microorganisms were included in all microcosm studies. For Soil-inhabiting microorganisms the list of key parameters might include:

* soil type characterization;

* soil nutrients determination;

* temperature, pH, water content, and oxygen content;

* plant presence;

* rainfall amounts and patterns;

* toxicants or antibiotics;

* microbial vectors (macrofauna).

It is also important to establish a uniform system for measuring survival, competition, dispersal, and gene transfer. A set of methodological considerations for standardizing the use of microcosms in risk assessment might include:

* field soil selection;

* soil microcosm preparation (placement of,soil in microcosm);

* microcosm soil-sampling techniques;

* calibration methods;

* microbial dispersal analysis;

* gene transfer determination;

* assay methods: either plate counting or DNA extraction;

* watering regime.

For example, it is known that newly released soil microorganisms are especially subject to movement with water compared to established microbes adhering to soil particles(13) (NRC 1989). Thus, a standardized procedure could be established for providing hydration as a variable in all risk assessments using microcosms. Variables such as microcosm size and methods of sampling microorganisms could also be standardized.

Environmental parameters must be accounted for in all experimental designs, but the specific environmental conditions are likely to vary according to the characteristics of the proposed release site. For example, while rainfall should be included in experiments, the amount and timing of rainfall would vary to reflect differences in the environments into which the genetically engineered microorganisms are intended for release. While all microcosms should use intact soil cores prepared in a uniform manner, the actual soil must be characteristic of the site of intended release.

Microcosms cannot eliminate the need for small-scale field tests, but they can and should be used to screen out high-risk releases. If, for example, an organism was tested in a microcosm and surprisingly showed the capacity to produce a chemical that was toxic to other soil organisms, then its release could be proscribed.

Thus, microcosms standardized in design and method of application can have several benefits for risk assessment of genetically engineered microorganisms. First, if potentially dangerous organisms can be screened out before field tests, it is likely to save time and money for industry and regulatory agencies. Second, firms could have a set of uniform standards and testing protocols that can be adapted to different organisms and different field conditions. Third, the public may also gain greater confidence in a regulatory process that makes explicit the criteria for the approval of releasing any genetically engineered microorganism in the field.

[3]Genetically engineered microorganisms were used in these microcosm studies.
[4]See footnote 3.
[5]See footnote 3.
[6]See footnote 3.
[7]See footnote 3.
[8]See footnote 3.
[9]See footnote 3.
[10]See footnote 3.

Conclusions

Obtaining the most relevant prerelease data on how genetically engineered organisms are likely to perform once released into the environment is of paramount importance because physical containment in the field is unrealistic. Compared to simple laboratory assays, microcosm studies can provide more realistic risk-assessment data on the genetically engineered organisms' survival, reproductive capacity, competitive ability, and effects on nontarget organisms. The added time and costs associated with effective microcosm studies are likely to be beneficial if any dangerous releases are avoided.

We recommend that regulatory authorities require applications for field release of genetically engineered microorganisms to include microcosm-generated ecological data. Further, it would be desirable for a lead agency like EPA or OECD to convene a workshop of experts to determine the extent to which microcosm experimental design can be standardized. Making the results of microcosm studies comparable would provide mutually acceptable data and would lead to an increase in the applicability of the information obtained from each risk assessment.

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(1) The list of permits for field tests of transgenic plants in the United States may be obtained from: Biotechnology, Biologics, and Environmental Protection, USDA-APHIS, 6505 Belcrest Rd., Hyattsville, MD 20782. (2) E. Ingham, 1995, personal communication. Oregon State University, Corvallis, OR. (3) Genetically engineered microorganisms were used in these microcosm studies. (4) See footnote 3. (5) See footnote 3. (6) See footnote 3. (7) See footnote 3. (8) See footnote 3. (9) See footnote 3. (10) See footnote 3. (11) SK Levy, BM Marshall, and RE Wetzler, 1995, manuscript in preparation. Tufts University, Boston, MA. (12) See footnote 11. (13) See footnot 11.

References cited

Alexander M. 1985. Ecological consequences: reducing the uncertainties. Issues in Science and Technology 1: 57-68.

[ASTM] American Society for Testing and Materials. 1988. Standard guide for conducting a terrestrial soil-core microcosm test. ASTM Designation E: 1197-87. Annual book of ASTM standards 11.04: 801-803.

Armstrong JL, Knudsen GR, Seidler RJ. 1987. Microcosm method to assess survival of recombinant bacteria associated with plants and herbivorous insects. Current Microbiology 15: 229-232.

Bashan Y. 1986. migration of the rhizosphere bacteria Azospirillus brasilense and Pseudomonas fluorescens towards wheat roots in the soil. Journal of General Microbiology 132: 3407-3414.

Bentjen SA, Fredrickson JK, Li SW, Van Voris P. 1989. Intact soil-core microcosms for evaluating the fate and ecological impacts from the release of genetically engineered microorganisms. Applied and Environmental Microbiology 55: 198-202.

Berry DF, Hagedorn C. 1991. Soil and groundwater transport of microorganisms. Pages 57-73 in Ginzburg LR, ed. Assessing ecological risks of biotechnology. Boston (MA): Butterworth-Heinemann.

Bolton H Jr., Fredrickson JK, Thomas JM, Li SW, Bentjen SA, Smith JL. 1991a. Field calibration of soil-core microcosms: ecosystem structural and functional comparisons. Microbial Ecology 21: 175-189.

Bolton H Jr., Fredrickson JK, Bentjen SA, Workman DJ, Li SW, Thomas JM. 1991b. Field calibration of soil-core microcosms: fate of a genetically altered rhizobacterium. Microbial Ecology 21: 163-173.

Campbell AM. 1991. Microbes: the laboratory and the field. Pages 28-44 in Davis BD, ed.

The genetic revolution. Baltimore MD): The Johns Hopkins University Press.

Cole LK, Metcalf RL, Sanborn JR. 1976. Environmental fate of insecticides in terrestrial model ecosystems. International Journal of Environmental Studies 10: 7-14.

Colwell RR, Somerville C, Knight 1, Straube W. 1988. Detection and monitoring of genetically-engineered microorganisms. Pages 47-60 in Sussman M, Collins CH, Skinner FA, Stewart-Tull DE, eds. The release of genetically-engineered microorganisms. New York: Academic Press.

Curtiss R III. 1988. Engineering organisms for safety: what is necessary? Pages 7-20 in Sussman M, Collins CH, Skinner FA, Stewart-tull DE, eds. The release of genetically-engineered micro-organisms. New York: Academic Press.

Dijkstra AF, Govaert JM, Scholten GHN, van Elsas JD. 1987a. A soil chamber for studying the bacterial distribution in the vicinity of roots. Soil Biology and Biochemistry 19; 351-352.

Dijkstra AF, Scholten GHN, van Veen JA. 1987b. Colonization of wheat seedling (Triticum aestivum) roots by Pseudomonas fluorescens and Bacillus subtilis. Biology and Fertility of Soils 4: 41-46.

Dimock MB, Beach RM, Carlson PS. 1989. Endophytic bacteria for the delivery of crop protection agents. Pages 88-92 in Roberts DW, Granados RR, eds. Proceedings of biotechnology, biological pesticides, and novel plant-pest resistance for insect pest management. Ithaca (NY): Boyce Thompson Institute for Plant Research.

Federal Register. 1994a. Microbial products of biotechnology; proposed regulation under the toxic substances control act; proposed rule. Federal Register 59: 45526-45585.

--1994b. Microbial pesticides; experimental use permits and notifications; final rule. Federal Register 59: 45600- 45615.

Fischer LM. 1991. New pesticides that nobody hates. The New York Times July 17: D1, D7.

Frederick RJ, Piluski RW. 1991. Nontarget species testing of microbial products intended for use in the environment. Pages 32-63 in Levin M, Strauss H, eds. Risk assessment in gentic engineering. New York: McGraw Hill.

Fredrickson JK, Bentjen SA, Bolton H Jr., Li SW, Van Voris P. 1989. Fate of Tn5 mutants of root growth-inhibiting Pseudomonas sp. in intact soil-core microcosms. Canadian Journal of Microbiology 35: 867-873.

Fredrickson JK, Van Voris P, Bentjen SA, Bolton H Jr. 1990. Terrestrial microcosms for evaluating the environmental fate and risks associated with the release chemicals or genetically engineered microorganisms to the environment. Hazard Assessment of Chemicals 7: 157-202.

Fuxa JR. 1991. Release and transport of entomopathic microorganisms. Pages 83-113 in Levin M, Strauss H, eds. Risk assessment in genetic engineering New York: McGraw-hill.

Gaugitsch H, Torgersen H. 1995. Streamlining regulations, keeping high safety standards. Ambio 24: 47-50.

Gillett JW, Gile JD. 1976. Pesticide fate in terrestrial laboratory ecosystems. International Journal of Environmental Studies 10: 15-22.

Gillis AM. 1994. Sullying soil webs. Bioscience 44: 734-735.

Greenberg EP, Poole NJ, Pritchard HAP, Tiedje J, Corpet DE. 1988. Use of microcosms. Pages 266-274 in Sussman M, Collins CH, Skinner FA, Stewart-tull DE, eds. The release of genetically-engineered micro-organisms. New York: Academic Press.

Harvey RW, George LH, Smith RL, Leblanc DR. 1989. Transport of microspheres and indigenous bacteria through a sandy aquifer: results of natural and forced gradient tracer experiments. Environmental Science and Technology 23: 51-56.

Heijen CE, Marinissen JCY. 1995. Survival of bacteria introduced into soil by means of transport by Lumbricus rubellus. Biology and Fertility of Soils 20: 63-69.

Heijnen CE, Hok-a-Kin CH, van Elsas JD. 1993. Root colonization by Pseudomonas fluorescens introduced into soil amended with bentonite. Soil Biology and Biochemistry 25: 239-246.

Hicks RJ, Stotzky G, Van Voris P. 1990. Review and evaluation of the effects of xenobiotic chemicals on microorganisms in soil. Advances in Applied Microbiology 35: 195-253.

Holmes TM, Ingham ER. 1994. The effects of genetically engineered microorganisms on soil food webs. Supplement to the Bulletin of the Ecological Society of America 75/2. Abstracts of the 79th Annual ESA Meeting, Science and Public Policy. Knoxville, Tennessee, 7-11 Aug 1994.

[11]SK Levy, BM Marshall, and RE Wetzler, 1995, manuscript in preparation. Tufts University, Boston, MA.

[12]See footnote 11.

Jackson DR, Ausmus BS, Levin M. 1979. Effects of arsenic on nutrient dynamics of grassland microcosms and field blots. Water Air and Soil Pollution 11: 13-21.

Kemp JS, Paterson E, Gammack SM, Cresser MS, Killham K. 1992. Leaching of genetically modified Pseudomonas fluorescens through organic soils: influence of temperature, soil pH, and roots. Biology and Fertility of Soils 15: 308-314.

Kluepfel DA. 1993. The behavior and tracking of bacteria in the rhizosphere. Annual Review of Phytopathology 31: 441-472.

Krimsky S. 1991. Biotechnics and society: the rise of industrial genetics. New York: Praeger Publishers.

Lacy GH, Stromberg VK. 1990. Pre-release microcosm tests with a genetically engineered plant pathogen. Pages 81-98 in MacKenzie DR, Henry SC, eds. Biological monitoring of genetically engineered plants and microbes. Bethesda (MD): Agricultural Research Institute.

Levidow L. 1995. The Oxford baculovirus controversy. Bioscience 45: 549-555.

Liang LN, Sinclair J, Mallory L, Alexander M. 1982. Fate in model ecosystems of microbial species of potential use in genetic engineering. Applied and Environmental Microbiology 44: 708-714.

Lindow SE, Panopoulos NJ, McFarland BL. 1989. Genetic engineering of bacteria from managed and natural habitats. Science 244: 1300-1306.

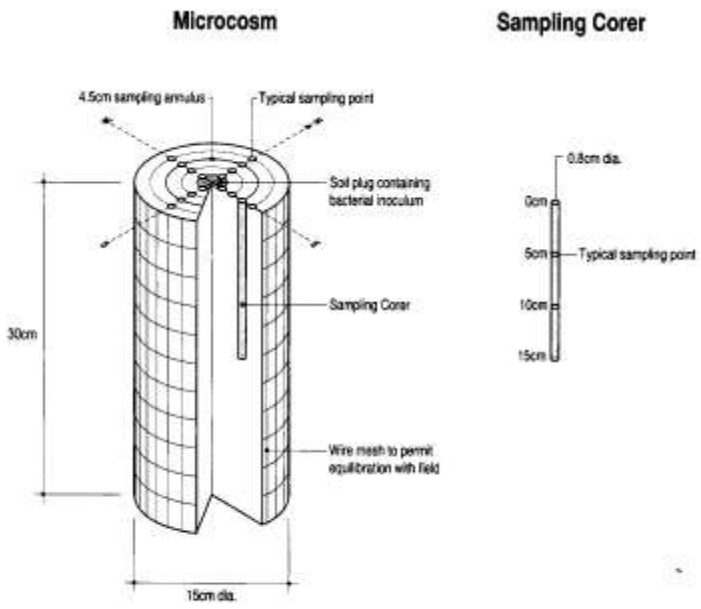
Metcalf R. 1977. Model ecosystem approach to insecticide degradation: a critique. Annual Review of Entomology 22: 241-261.

Miller HI. 1991. Regulation. Pages 196-211 in Davis BD, ed. The genetic revolution. Baltimore (MD): The Johns Hopkins University Press.

Miller LK, Lingg AJ, Butla LA Jr. 1983. Bacterial, viral, and fungal insecticides. Science 219: 715-721.

Morel JL, Bitton G, Chaudhry GR, Awong J. 1989. Fate of genetically modified microorganisms in the corn rhizosphere. Current Microbiology 18: 355-360.

[NRC] National Research Council. 1989. Field testing genetically modified organisms: framework for decisions. Washington DC): National Academy Press.



[OTA] Office of Technology Assessment. 1991. Biotechnology in a global economy. Report nr OTA-BA-494. Washington DC): US Government Printing Office.

O'Neill RB, Ausmus BS, Jackson DR, Van Hook RI, Van Voris P, Washburne C, Watson AP. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water Air and Soil Pollution 8: 271-277.

[OECD] Organization for Economic Co-operation and Development. 1995. Analysis of information elements used in the assessment of certain products of modern biotechnology. OECD Environment Monographs nr 100. OECD/GD (95)11. Paris: Environment Directorate, OECD.

Parke JL, Oien R, Rovira AD, Bowen GD. 1986. Soil water flow affects the rhizosphere distribution of a seed-borne biological control agent, Pseudomonas fluorescens. Soil Biology and Biochemistry 18: 583-588.

Paterson E, Kemp JS, Gammack SM, Fitzpatrick EA, Cresser MS, Mullins CE, Killham K. 1993. Leaching of genetically modified Pseudomonas fluorescens through intact soil microcosms: influence of soil type. Biology and Fertility of Soils 15: 308-314.

Pritchard PH, Bourquin AW. 1984. The use of microcosms for evaluation of interactions between pollutants and microorganisms. Advances in Microbial Ecology 7: 133-215.

Recorbet G, Steinberg C, Faurie G. 1992. Survival in soil of genetically engineered Escherichia coli as related to inoculum density, predation and competition. FEMS Microbiology Ecology 101: 251-260.

Figure 1. The microcosm design and sampling scheme used by Levy and al. (see footnote 11). Samples are taken at the intersection of the four directional indicators (N, S, E, and W) and the annuli drawn on the soil surface in this diagram. Using the sampling corer, samples are taken at the soil surface (0 cm) and at specified depths up to 15 cm.

Regal PJ. 1994. Scientific principles for ecologically based risk assessment of transgenic organisms. Molecular Ecology 3: 5-13.

Regal PJ, Klug M, Sayier G, Shapiro J, Tiedje J. 1989. Basic research needs in microbial ecology for the era of genetic engineering. Santa Barbara (CA): FMN Publishing.

Richaume A, Angle JS, Sadowsky MJ. 1989. Influence of soil variables on in situ plasmid transfer from Escherichia coli to Rhizobium fredii. Applied and Environmental Microbiology 55: 1730-1734.

Ronson CW, et al. 1990. Field release of genetically-engineered Rhizobium meliloti and Bradyrbizobium Japonicum strains. Pages 397-403 in Gresshoff PM, Roth LE, Stacy G, Newman WE, eds. Nitrogen fixation: achievements and objectives. New York: Chapman and Hall.

Roszak DB, Colwell RR. 1987. Survival strategies of bacteria in the natural environment. Microbiological Reviews 51: 365-379.

Rupprecht CE, Wiktor TJ, Johnston DH, Hamir AN, Dietzschold B, Wunner WH, Glickman LT, Koprowski H. 1986. Oral immunization and protection of raccoons (Procyon lotor) with a vaccinia-rabies glycoprotein recombinant virus vaccine. Proceedings of the National Academy of Sciences of the United States of America 83: 7947-7950.

Scher FM, Ziegle JS, Kloepper JW. 1984. A method for assessing the root-colonizing capacity of bacteria on maize. Canadian Journal of Microbiology 30: 151-157.

Scher FM, Kloepper JW, Singleton CA. 1985. Chemotaxis of fluorescent Pseudomonas spp. to soybean seed exudates in vitro and in soil. Canadian Journal of Microbiology 31: 570-574.

Selenska S, Klingmuller W. 1991. Direct detection of nif gene sequences of Enterobacter agglomerans in soil. FEMS Microbiology Letters 80: 243-246.

Shaw JJ, Beauchamp C, Dane F, Kriel RJ. 1992. Securing a permit from the United States Department of Agriculture for field work with genetically engineered microbes: a non-prohibitory process. Microbial Releases 1: 51-53.

Smit E, van Elsas JD. 1992. Conjugal gene transfer in the soil environment: new approaches and developments. Pages 79-94 in Gauthier MG, ed. Gene transfers and environment. New York: Springer-Verlag.

Stotzky G; Devanas MA, Zeph LR. 1990. Methods for studying bacterial gene transfer in soil by conjugation and transduction. Advances in Applied Microbiology 35: 57-169.

Tiedje JM, Colwell RK, Grossman YL, Hodson RE, Lenski RE, Mack RN, Regal PJ. 1989. The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70: 298-31S.

Trevors JT. 1988. Use of microcosms to study genetic interactions between microorganisms. Microbiological Sciences 5: 132-136.

Trevors JT, van Elsas JD, van Overbeek LS, Starodub M-E. 1990. Transport of a genetically engineered Pseudomonas fluorescens strain through a soil microcosm. Applied and Environmental Microbiology 56: 401-408.

van Elsas JD. 1991. Environmental risks and fate of genetically engineered microorganisms in soil. Journal of Environmental Science and Health Part A: Environmental Science and Engineering 26(6): 981-1001.

van Elsas JD, Trevors JT, van Overbeek LS. 1991. Influence of soil properties on the vertical movement of genetically-marked Pseudomonas fluorescens through large soil microcosms. Biology and Fertility of Soils 10: 249-255.

van Elsas JD, Trevors JT, Jain D. 1992. Survival of, and root colonization by, alginate-encapsulated Pseudomonas fluorescens cells following introduction into soil. Biology and Fertility of Soils 14: 14-22.

Van Voris P. 1985. Experimental terrestrial soil-core microcosm test protocol: a method for measuring the potential ecological effects. fate, and transport of chemicals in terrestrial ecosystems. Report nr EPA/600/3-85/047. Corvallis (OR): Corvallis Environmental Research Laboratory, US Environmental Protcction Agency. Available from: NTIS, Springfield, VA.

Walter MV, Barbour K, McDowell M, Seidler RJ. 1987. A method to evaluate survival of genetically engineered bacteria in soil extracts. Current Microbiology 15: 193-197.

Walter MV, Porteous LA, Prince VJ, Ganio L, Seidler RJ. 1991. A microcosm for measuring survival and conjugation of genetically engineered bacteria in rhizosphere environments. Current Microbiology 22: 117-121.

Wrage K. 1994. Move over BST: tons of "biofoods" already consumed. Biotech Reporter 11 (4): 1, 4.

ogy 21: 163–173.
Campbell AM. 1991. Microbes: the laboratory and the field. Pages 28–44 in Davis BD, ed. The genetic revolution. Baltimore (MD): The Johns Hopkins University Press.
Cole LK, Metcalf RL, Sanborn JR. 1976. Environmental fate of insecticides in terrestrial model ecosystems. International Journal of Environmental Studies 10: 7–14.
Colwell RR, Somerville C, Knight I, Straube W. 1988. Detection and monitoring of genetically-engineered microorganisms. Pages 47–60 in Sussman M, Collins CH, Skinner FA, Stewart-Tull DE, eds. The release of genetically-engineered microorganisms. New York: Academic Press.
Curtiss R III. 1988. Engineering organisms for safety: what is necessary? Pages 7–20 in Sussman M, Collins CH, Skinner FA, Stewart-Tull DE, eds. The release of genetically-engineered micro-organisms. New York: Academic Press.
Dijkstra AF, Govaert JM, Scholten GHN, van Elsas JD. 1987a. A soil chamber for studying the bacterial distribution in the vicinity of roots. Soil Biology and Biochemistry 19: 351–352.
Dijkstra AF, Scholten GHN, van Veen JA. 1987b. Colonization of wheat seedling (*Triticum aestivum*) roots by *Pseudomonas fluorescens* and *Bacillus subtilis*. Biology and Fertility of Soils 4: 41–46.
Dimock MB, Beach RM, Carlson PS. 1989. Endophytic bacteria for the delivery of crop protection agents. Pages 88–92 in Roberts DW, Granados RR, eds. Proceedings of biotechnology, biological pesticides, and novel plant-pest resistance for insect pest management. Ithaca (NY): Boyce Thompson Institute for Plant Research.
Federal Register. 1994a. Microbial products of biotechnology; proposed regulation under the toxic substances control act; proposed rule. Federal Register 59: 45526–45585.
_____. 1994b. Microbial pesticides; experimental use permits and notifications; final rule. Federal Register 59: 45600– 45615.
Fischer LM. 1991. New pesticides that nobody hates. The New York Times July 17: D1, D7.
Frederick RJ, Piluski RW. 1991. Nontarget species testing of microbial products intended for use in the environment. Pages 32–63 in Levin M, Strauss H, eds. Risk assessment in gentic engineering. New York: McGraw Hill.
Fredrickson JK, Bentjen SA, Bolton H Jr., Li SW, Van Voris P. 1989. Fate of Tn5 mutants of root growth-inhibiting *Pseudomonas* sp. in intact soil-core microcosms. Canadian Journal of Microbiology 35: 867–873.
Fredrickson JK, Van Voris P, Bentjen SA, Bolton H Jr. 1990. Terrestrial microcosms for evaluating the environmental fate and risks associated with the release chemicals or genetically engineered microorganisms to the environment. Hazard Assessment of Chemicals 7: 157–202.
Fuxa JR. 1991. Release and transport of entomopathic microorganisms. Pages 83–113 in Levin M, Strauss H, eds. Risk assessment in genetic engineering. New York: McGraw-Hill.
Gaugitsch H, Torgersen H. 1995. Streamlining regulations, keeping high safety standards. Ambio 24: 47–50.
Gillett JW, Gile JD. 1976. Pesticide fate in terrestrial laboratory ecosystems. International Journal of Environmental Studies 10: 15–22.
Gillis AM. 1994. Sullying soil webs. BioScience 44: 734–735.
Greenberg EP, Poole NJ, Pritchard HAP, Tiedje J, Corpet DE. 1988. Use of microcosms. Pages 266–274 in Sussman M, Collins CH, Skinner FA, Stewart-Tull DE, eds. The release of genetically-engineered micro-organisms. New York: Academic Press.
Harvey RW, George LH, Smith RL, LeBlanc DR. 1989. Transport of microspheres and indigenous bacteria through a sandy aquifer: results of natural and forced gradient tracer experiments. Environmental Science and Technology 23: 51–56.
Heijen CE, Marinissen JCY. 1995. Survival of bacteria introduced into soil by means of transport by *Lumbricus rubellus*. Biology and Fertility of Soils 20: 63–69.
Heijnen CE, Hok-A-Kin CH, van Elsas JD. 1993. Root colonization by *Pseudomonas fluorescens* introduced into soil amended with bentonite. Soil Biology and Biochemistry 25: 239–246.
Hicks RJ, Stotzky G, Van Voris P. 1990. Review and evaluation of the effects of xenobiotic chemicals on microorganisms in soil. Advances in Applied Microbiology 35: 195–253.
Holmes TM, Ingham ER. 1994. The effects of genetically engineered microorganisms on soil food webs. Supplement to the Bulletin of the Ecological Society of America 75/2. Abstracts of the 79th Annual ESA Meeting, Science and Public Policy. Knoxville, Tennessee, 7–11 Aug 1994.
Jackson DR, Ausmus BS, Levin M. 1979. Effects of arsenic on nutrient dynamics of grassland microcosms and field plots. Water Air and Soil Pollution 11: 13–21.
Kemp JS, Paterson E, Gammack SM, Cresser MS, Killham K. 1992. Leaching of genetically modified *Pseudomonas fluorescens* through organic soils: influence of temperature, soil pH, and roots. Biology and Fertility of Soils 15: 308–314.
Kluepfel DA. 1993. The behavior and tracking of bacteria in the rhizosphere. Annual Review of Phytopathology 31: 441–472.
Krimsky S. 1991. Biotechnics and society: the rise of industrial genetics. New York: Praeger Publishers.
Lacy GH, Stromberg VK. 1990. Pre-release microcosm tests with a genetically engineered plant pathogen. Pages 81–98 in MacKenzie DR, Henry SC, eds. Biological monitoring of genetically engineered plants and microbes. Bethesda (MD): Agricultural Research Institute.
Levidow L. 1995. The Oxford baculovirus controversy. BioScience 45: 549–555.
Liang LN, Sinclair J, Mallory L, Alexander M. 1982. Fate in model ecosystems of microbial species of potential use in genetic engineering. Applied and Environmental Microbiology 44: 708–714.

References cited

Alexander M. 1985. Ecological consequences: reducing the uncertainties. Issues in Science and Technology 1: 57–68.
[ASTM] American Society for Testing and Materials. 1988. Standard guide for conducting a terrestrial soil-core microcosm test. ASTM Designation E: 1197-87. Annual book of ASTM standards 11.04: 801–803.
Armstrong JL, Knudsen GR, Seidler RJ. 1987. Microcosm method to assess survival of recombinant bacteria associated with plants and herbivorous insects. Current Microbiology 15: 229–232.
Bashan Y. 1986. Migration of the rhizosphere bacteria *Azospirillus brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. Journal of General Microbiology 132: 3407–3414.
Bentjen SA, Fredrickson JK, Li SW, Van Voris P. 1989. Intact soil-core microcosms for evaluating the fate and ecological impacts from the release of genetically engineered microorganisms. Applied and Environmental Microbiology 55: 198–202.
Berry DF, Hagedorn C. 1991. Soil and groundwater transport of microorganisms. Pages 57–73 in Ginzburg LR, ed. Assessing ecological risks of biotechnology. Boston (MA): Butterworth-Heinemann.
Bolton H Jr., Fredrickson JK, Thomas JM, Li SW, Bentjen SA, Smith JL. 1991a. Field calibration of soil-core microcosms: ecosystem structural and functional comparisons. Microbial Ecology 21: 175–189.
Bolton H Jr., Fredrickson JK, Bentjen SA, Workman DJ, Li SW, Thomas JM. 1991b. Field calibration of soil-core microcosms: fate of a genetically altered rhizobacterium. Microbial Ecol-

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