Chapter 8

Scientific Issues: Risk Assessment and Risk Management

Photo credit: Grant Heilman, Inc.

Contents

Page

INTRODUCTION 225
RISK ASSESSMENT 225
- Concerns and Postulated Environmental Risks of Biotechnology 225
- Major Risk Assessment Reports 227
- Biotechnology Ecological Risk Assessment 231
- Applicability of Diverse Bodies of Knowledge to Assessments of Large-Scale Commercial Release 235
- Commercial Release Issues 240

RISK MANAGEMENT 247
- Design of Science-Based Regulation 247
- Generic v. Case-by-Case Approach 247
- Relative Risks Compared to Traditional Practices 248
- Cost-Benefit Analyses 248
- Small-Scale v. Large-Scale Issues 248

SCIENTIFIC METHODS OF MANAGING RISK 249
- Promoters Turned On or Off by Specific Stimuli 249
- Suicide Genes 249
- Prevention of Gene Transfer 250
- Combinations of Genes 250

AGRONOMIC METHODS OF MANAGING RISK 250
- Physical Barriers 250
- Spatial Barriers 251
- Temporal Barriers 251

SUMMARY POINTS 251
CHAPTER PREFERENCES 252

Boxes

Box — *Page*

8-A. Ecological Risk Assessment Questions 233
8-B. Learning by Doing: Successive Field Releases 238
8-C. Monitoring Microorganisms 245
8-D. Relevant Research Fields 246

Figures

Figure — *Page*

8-1. Alternative Risk Analysis Approaches 229
8-2. Risk Assessment Framework for Environmental Introductions 232

Table

Table — *Page*

8-1. Comparison of Traditional and Developing Biotechnology 248

Chapter 8
Scientific Issues: Risk Assessment and Risk Management

INTRODUCTION

The large-scale commercial use of agricultural biotechnology gives rise to several questions. Does the release of large numbers of genetically engineered organisms into the environment pose special risks'? If so, what is the order of magnitude of these risks compared to the risks of traditional agricultural practices'? What benefits offset such risks?

Generally, concerns about genetic engineering focus on:

- possible ''escape" of a genetically engineered organism, such that it invades new ecological niches or outcompetes naturally occurring organisms and becomes a pest;
- possible disruption of a delicately balanced ecosystem;
- possible direct risks to humans or wildlife;
- possible problems of gene stability and of gene transfer to unintended recipient organisms;
- possible impact on evolution; and
- the sheer "newness" of the technique.

This chapter addresses these concerns and describes the range of scientific views on biotechnology and risk. A consensus has developed that risk assessment is desirable and feasible. Risk assessment in general is founded on principles and methodologies that can apply to biotechnology. We know what questions to ask in assessing ecological risks of planned introductions. A knowledge base already exists pertinent to these questions and risk assessment studies on this topic are proliferating. Science-based risk management builds on this technical knowledge and on our capabilities for risk assessment.

Risk assessment methodologies and our technical knowledge base make it possible to conduct effective risk assessments of specific introductions and to manage risks of acceptable introductions. Science-based regulations are central to effective management of risk. A variety of scientific and agricultural methods can be used to manage risk in particular situations.

RISK ASSESSMENT

Concerns and Postulated Environmental Risks of Biotechnology

General Concerns

Questions arise concerning the impact of introduced genetically engineered organisms: What is the likelihood that such organisms will persist in the environment"? What is the likelihood that they will spread, constituting an invasion into the ecological community'? Will they become pests, with a deleterious effect on other species'? Will the expression of the gene itself lead to an unwanted effect on the ecosystem'?

Other questions have to do with the recombinant gene itself (38): What avenues exist for gene transfer within and between various species in nature'? How probable are such exchanges and at what rates would they occur. if at all? If introduced genes are transferred to genomes existing in nature. how well— and how stably—will the functions for which they code be expressed?

Finally, broader, more fundamental questions can be posed: Are we in fact dealing with a phenomenon so novel that we have no way of predicting outcomes, of Performing adequate risk assessment? Do we have a moral right to manipulate still further the species and the ecology of our planet'? Are we losing an intangible, aesthetic quality to our lives by so doing'? Can we at-ford to say no to the benefits that this technology can confer on agriculture

Concerns About Plants

Specific concerns relating to genetically engineered plants include the possibility that transgenic plants will persist and become serious agricultural weeds; that the transgenic plants will invade natural habitats and disrupt local ecological interactions; and that the pollen of transgenic plants will act as a vector. bringing the introduced genes to other species that may then themselves become problem weeds. The likelihood of such possibilities occurring remains somewhat controversial, underscoring the importance of information from field trials and research. It is noteworthy, however, that transfer of genes from conventionally bred crop plants to noncrop plants has not created obvious problems in the past, and that traditional crop plants rarely have invaded natural ecosystems (I 4).

lnvasions of plants (by seeds. fruits. or vegetatively reproducing units) involves dispersal, persistence. and establishment: all three stages must be ''successful if' engineered plants are to become weeds. For transgenes (introduced genes) to move from crop plants and cause or contribute to a weed problem, hybridization with a reproductively compatible species must occur. For tiny

given crop species, only a small number of the wild relative species that are reproductively compatible are actually likely to present serious weed problems; however, it is theoretically possible for a plant to become a weed in a novel environment *(43).*

One specific concern posed frequently by some environmentalists, among others (32), is that genes for herbicide tolerance might be transferred from crop plants to weeds. If this were to occur, natural selection could favor the trait in weedy neighbors of crops treated with the herbicide. With any use of herbicides, furthermore, increased selection pressure is put on wild species for any herbicide tolerance traits they might already possess. Such developments might lead eventually to increased use of chemical herbicides. A fundamental debate has arisen between industry scientists who maintain that crops can be genetically engineered to be tolerant of particularly ''environmentally friendly' herbicides and some environmentalists who say. essentially, that no new technology should be used to favor continued use of chemicals in the environment.

Concerns About Microorganisms

In part because they are invisible and relatively "unknowable, " microorganisms tend to elicit more concerns on the part of the public than do plants. Parameters of concern related to genetically engineered microorganisms include the possibility of gene transfer and recombination. the possibility of movement into new environments, and the possibility of infection of nontarget organisms. Questions asked include: Will genetically engineered microorganisms give rise to biological risks for humans or other species? Will they give rise to environmental problems'? Do we have the technical understanding to evaluate and predict any such problems'?

Whether bacteria, fungi. viruses, or baculoviruscs, microorganisms suffer from a bad reputation at the broadest level of public perception: they are. after all often equated with"germs. " One specific concern raised with regard to genetically engineered organisms is the possibility of genetic material from such organisms being transferred to human gut bacteria. The risk of infection of humans. or other deleterious effects, is clearly going to be examined for planned introductions of microorganisms. For example. among the questions raised by Monterey County staff considering the Advanced Genetic Sciences (AGS) proposal to field test Frostban[R] was whether or not the *Pseudomonas fluorescens could* ' 'sensitize or aggravate existing health conditions among sensitive human populatitons living near the proposed test site' (66).

To assess risk of problematic infection of humans by genetically engineered organisms. information must be available on exposure level. This hinges on such factors as bioavailability or likelihood of absorption into cells or tissues, specificity, and level of interaction possible of the microorganisms or their chemical products with nontarget (human) tissues; and potential of the microorganisms for colonization or infectivity. The degree of pathogenicity must be considered as well. Some relevant factors include virulence. Possession of toxins, host range, and relative susceptibility. Generally. risk assessment will factor in predictability of the behavior of the recombinant DNA identified microorganisms based on their parent organisms, as well as knowledge of specific recombinant techniques used (40).

Scientists' concerns focus less on pathogenicity and more on the possible impacts of genctically engineered microorganisms on the environment. Suggested impacts include possible influences on: indigenous population size. diversity of species. the ecological community. natural cycles, and evolution of the introduced organisms (76). Microbial environments are complex. By one estimate some 10^9 microorganisms, representing a variety of taxonomic groups, inhabit one gram of soil. Uncetanties exist as to possible consequences of sudden introductions on balanced microbial ecosystems (46). Microbial diversity in the soil is high (88). This limits the niches available to introduced mirorganisms (86). While introduced microorganisms may thus compete poorly. they may persist in low-density populations. A key issue is whether or nor an unexpected later resurgent bloom or population expansion from a low-density population can be reasonably envisioned (84).

Since microorganisms can and do change location. questions of dispersal—and possible subsequent reproduction in nontargeted ecological sites—also are raised. The ability of a particular strain to transfer genes to other specics will affect the likelihood of other microorganisms being affected in new. nontarget areas. All questions bearing on survival, multiplicaton, and dispersal of genetically enginered microoganisms: on possible exchange of genes between introduced and indigenous microorganisms: and ultimately on issues of environmental and public safety, are engaging attention of academic and industrial scientists, the public, and governmental regulators alike (22).

Views Held in the Scientific Community

Particularly in the early days. the issue of planned introductions of genetically engineered organisms sparked

a range of views on safety even among scientists (50). In the mid-eighties, microbiologist Winston Brill argued that. for centuries, traditional breeding has altered animals and plants without negative consequence: and that microorganisms, including pathogenic species, have been added to the soil in hopes 0ft beneficial impacts. also without negative consequences (7). His conclusion that these observations alone formcd a basis for risk assessments of' organsms that have had one or a few genes added drew fire from a group of ecologists (lo). These critics pointed out that mutations that increase an organisms niche range can be ecologically significant. and that some ramifications of an organism's impact on the environment are not predictable from knowledge of its introduced genes alone. Casc-by-case quantitative risk assessment for deliberate release was recommended.

In 1987, *Science* published side-by-side articles by Frances Shw-pies (75) and Bernard Davis (15). Sharples. an ecologist. reaffirmed the need for casc-by-case assessments, given the complexity of any organism's interactions with the environment. Molecular biologist Davis suggested that the experience of ecologists with introductions of higher organisms is less pertinent to risk assessment of engineered microorganisms than are the insights of fields mom concerned with the specific properties of those microorganisms: population genetics. bacterial physiology. epidemiology. and the study of pathogenesis.

The range of possible views on safty runs from "zero risk' to catastrophic risk'; those who presume " small-risk, pending research occupy the middle of the spectrum. In the mid-eighties, molecular biologists tended to stress the relavance of the safty record of laboratory biotechnology and graviated toward the ''zero-risk" end of the spectrum. Ecologists, who tended to stress the complexities of the natural environment. were less sanguine about potential risks. but stopped short of the cat astrohic-risk position taken by certain envronmentalists. An important distinction exists between ecologists and evironmentaists. The former are:

- scientists concerned with the fundamental properties, proccssscs, and components of ecological systems .

The latter,

- by definition, are concerned with various sociopolitical aspects of environmental quality and management. They may or may not be experts in understanding ecological processes and the organization of ecological systems (63).

Some environmentalists, keenly aware of problems posed by past technologies, argue that the proposed user of new technologies bears the burden of proving safety. Biotechnology proponents, in contrast, argue that any risks are to date hypothetical. so that the burden of proof should rest with the doomsayer (5 I).

In the late 1980s and early 1990s. discussion has increasingly centered around developing appropriate risk-assessment parameters and frameworks and designing regulator-y treatment according to risk. The current ''operational' approach is in agreement with analyses in key reports that will be described in the next section (50). ''Presumed small risk. or risk in exceptional cases. with research or risk assessment required. is becoming more of a common theme. Arguments are tending to become more refined. revolving about such issues as legitimacy of risk-assessment parameters; the degree to which lessor-is from past field trials can be generalized: correct assessment procedures for casc-by-evaluations; development of predictive science related to these issues; science-btised regulations; and scientific mainagement of risk. Today the imminence of large-scale release is bringing all these discussions into sharp focus.

Major Risk Assessment Reports

Introduction to Risk Assessment

Why Risk Assessment Is Needed-Society *today* has been ''sensitized' to technology the public, in all its many forms, looks at past technnologies—those of the chemical or nuclear industries for example-and sees negative outcomes that were not thoroughly considered prior to implementation of the technologies. Along with skepticism is a strong strain of environmentalism. a growing uneasiness that far too often, for our convenience. we carelessly and permantly harm the environment. Furthermore, however unrealistic it may be, a desire for ''zero-risk seems to underliie many responses to technology and to life in general today.

For these reasons as well as to achieve the fundamental objective of promoting safety it behooves regulatrs and other responsible parties to conduct reasonable risk assessments of new technologics. Biotechnology, in particular planned introductions of recombinant DNA-modified orgainisms. is among the technologies for which risk assessment is now done. This is necessary for regulators. important to the public's sense of cconfidence, and useful to ''users' of biotechnology'. including researchers in academdia, industry. and government.

Principles of Risk Assessment—' *'Risk"* can be defined as the potential for negative or adverse consequent to arise from an activity or an event (23). Risk also can be defined as the probability of an event occurring multiplied by the cost of its occurrence (44). Risk assessment can be viewed as ''the process of obtaining quantitative or qualitative measures of risk levels. including estimates of possible health effects and other consequences as well as the degree of uncertainty in those estimates' (23).

Risk assessment simply is an analytical tool that pulls together a great deal of diverse data in order to estimate a potential risk from an event or a process (81). Often, historical data on possible adverse consequences are difficult or impossible to obtain, making risk assessment ''an inexact process that attempts to characterize and quantify uncertainty, but never completely eliminates it. " Nonetheless, despite the limitations and challenges, use of risk assessment principles makes it possible to organize and interpret knowledge so as to improve the prediction of possible outcomes and ultimately to manage risk (23).

Risk assessment has been defined as a five-stage process:

1. *Risk identification—* defining the nature of the risk, source, mechanism of action, and possible adverse consequences;
2. *Risk-source* chracterization—characterizing the source of potential risk;
3. *Exposureassessment—* assesing the intensity. frequency and duration of human or environmental exposures to risk agents;
4. *Dose-response assessment—* assessing the relationship between dose of the risk agent and health or environmental consequences; and
5. *Risk estimation—* intergrating a risk-source characterization with an assessment of exposure and dose-response, leading to overt measures of the level of the health, safety or environmental risk involved (59, 92).

Clearly these stages can be adapted to fit a variety of kinds of risks, and the entire process can take several different forms. (See figure 8-1.)

The choice of an approach to risk assessment depends in large part on the extent and quality of available knowledge, degree of expected precision, and importance attached to outcomes at a low probability. Where the knowledge base is large and little uncertainty exists, a risk or hazard may be described quite readily and a more precise "deterministic consequence analysis" might even be performed. On the other hand, when less knowledge is available and the level of uncertainty is high. a qualitative risk screening may be all that is possible. perhaps leading to a more quantitative 'probabilistic risk assessment.

A much-used framework to assess risk is that developed for the evaluation of health effects associated with chemicals in the environment. This was endorsed by a National Academy of Science report (67) and refined at the Environmental Protection Agency (EPA). This chemical risk-assessment framework sometimes has been adapted for evaluation of planned introductions of recombinant DNA-modified organisms into the environment (13. 16. 30).

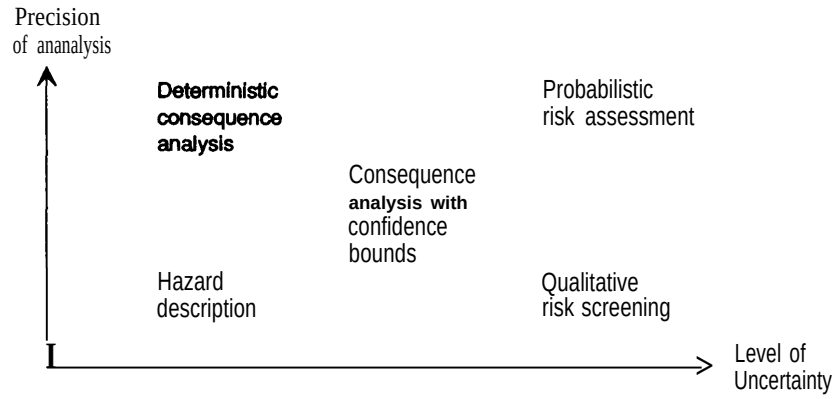
The National Research Council (NRC) and the Ecological Society of America (ESA) (69, 85) developed in 1989 risk assessment frameworks designed for recombinant DNA-modified organisms. But they were quite different from the chemical approach. The NRC procedure takes account of the degree of "familiarity"of a planned introduction; the ESA uses a risk attributes categorization; both lead towards the determintaion of an appropriate level of concern. While differing somewhat in perspective. the two approaches nonetheless resemble each other in basic conclusions and therefore together provide a solid framework for risk assessment of planned introductions. Clearly, choice of framework for risk assessment will influence the kinds of data required for evaluation and for permit applications (50). The two reports described below have had significant impact on the recent framing of discussions about planned introductions. Even proponents of chemical risk-assessment procedures point out that these procedures can be used to determine whether or not a particular organism should be evaluated intensively using an analogue of a chemical risk assessment (81).

National Research Council Report

Background—In late 1989, the National Research Council published *Field Testing Genetically Modified Organisms: Framework for Decisions.* This was requested by the Biotechnology Science Coordinating Committee (BSCC) on behalf of its member regulatory agencies. The report covered:

- plants and microorganisms,
- field-test introductions (but not large-scale commercial applications and related issues),
- environmental (but not human health) effects,

Figure 8-l—Alternative Risk Analysis Approaches



SOURCE: J. Fiksel and V.T. Covello, "The Suitability and Applicability of Risk AssessmentMethods for Environmental Applications of Biotechnology" in *Biotechnology Risk Assessment: Issues and Methods for Environmental Introductions (New* **York, NY: Pergamon Press, 1986), pp 1–34-**

- scientific issues principally (but not regulatory policy).
- field test conditions in the conterminous United States, and
- general procedures for determining categories (not specific case recommendations).

A fundamental principle underlying the study. and first introduced in an earlier National Academy of Science document (68), is that safety assessments of a recombinant organism "should be based on the nature of the organism and the environment into which it will be introduced, not on the method by which it was modified. A related point is that ''no conceptual distinction exists between genetic modification of plants and microorganisms by classical methods or by molecular methods that modify DNA and transfer genes.

Topics analyzed for the 1989 report include: relevant biological characteristics of genetically modified plants; experience with genetic modification and introductions of plants modified ' 'traditionally" and by molecular genetic techniques; potential weediness: the features of the genetic modification in microorganisms; phenotypic characteristics of the parent organism and of its genetically modified derivatives: and relevant features of the environment into which the organism will be introduced.

Findings-The report recommends that the impacts of' genetic modification on the phenotype of the organism and the mobility of the altered gene be assessed. In some cases. when persistence of' the modified orgtanism is not wanted or when uncertainty exists as to effects on the immediate environment. risk assessment should emphasize the phenotypic properties relating to the persistence of the organism and its modification. Questions to be considered include: fitness of the genetically modified organism; its tolerance to physicochemical stresses; its competitiveness range of available substrates; and. if applicable. pathogenicity, virulencc. and host range. The report describes the long historry of safety in the useful employment of' plants and microorganisms. and underscores the need for field tests to increase the capability to assess any risks of large-scale introductions.

Specific scientific conclusionss of the report pertaining to plants include:

1. The current means for making evaluations of introductions of traditionally bred plants are appropriate (on the basis of experience with field tests of hundreds of millions of genotypes over decades).
2. Crops altered by molecular and cellular techniques should pose risks no different from those posed by crops modified by traditional genetic methods for similar traits.
3. The potential for enhanced weediness is the principal risk to the environment seen from introductions of genetically moditied plants. although the likelihood of this occurring is low.
4. Confinement by biological. chemical spatial, physical. environmental and temporal means is the principal means of maintaining the safety of field introductions of classically modified plants.
5. Experimental plants grown in field confinement rarely if ever escape to cause problems in the environment.
6. Established confinement options are equally applicable to field introductions of plants modified with

molecular or cellular methods and to plants modified with classical genetic methods.

Conclusions concerning microorganisms included:

1. Many molecular techniques make possible genetic changes in microbial strains that can be fully characterized.
2. The molecular techniques are powerful in their capability to isolate genes and transfer them across biological barriers.
3. Field experience has given rise to a great deal of information about some microorganisms; nonetheless, less information exists on microbial ecology and less experience with planned introductions of genetically modified microorganisms than there is for plants. No adverse effects have been noted from microbial introductions to date; a field test should go forward when sufficient information is available for its safety evaluation.
4. The probability of adverse effects can be minimized or eliminated by appropriate means of confining the microorganism to the environment into which it was introduced; one example would be the use of "suicide genes.

The framework for evaluating risk developed in the report is structured around the following questions:

1. **Are we** *familiar* **with the properties of the organism and the environment into which it may be introduced?**
2. **Can we** *confine or control* **the organism effectively?**
3. **What are the probable** *effects* **on the environment should the introduced organism or a genetic trait persist longer than intended or spread to nontarget environments? (69)**

The familiarity criterion is key to this report and has reappeared consistently in risk assessment discussions since. Familiarity means having sufficient information on which to base a reasonable assessment of safety or risk. Thus, as our information base increases, so does the scope of "familiarity." When the familiarity criterion is not met. the possibility of confining or controlling the organism and the potential consequences of failing to control it must be evaluated.

The report is intended to provide a basis for a "flexible, scientifically based. decisionrnaking process. The classification of an introduced organism into a particular risk category is made possible by the framework for evaluating field tests (69).

The 1989 NRC report is often cited and has provided a conceptual framework for many approaches to risk assessment of planned introductions of genetically engineered organisms into the environment. Its level of detail made it more palatable to technical audiences than the 1987 pamphlet, which was at times criticized for making assertions without documentation (11, 50).

The Ecological Society of America Report

Another seminal assessment was published in 1989, *The Planned Introduction of Genetically Engineered Organisms: Ecological Consideratons and Recommendations (85).* This report was prepared for the Public Affairs Committee of the Ecological Society of America (ESA) and also has been broadly disseminated and cited. Dr. James Tiedje chaired a workshop committee in April 1988, examining ecological aspects of planned environmental introductions of genetically engineered organisms. The Workshop Committee's initial draft was reviewed at great length by the ESA Public Affairs Committee, the ESA Executive Committee, and other ecologists. The report

> supports **the use of advanced** biotechnology **for the development of environmentally sound products. and states that the phenotype of a transgenic organism, not the process used to produce it, is the appropriate focus of regulatory oversight. Ecological risk assessment of proposed introductions must consider the characteristics of the engineered** trait, **the parent organism, and the environment that will receive the introduced organism (85).**

Like the NRC report, the ESA report emphasizes product, rather than process, as the appropriate focus of evaluation and regulation. Thus, ''genetically engineered organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them" (85). Yet the report acknowledges the potential for novelty and consequent likelihood of evaluation inherent in the new techniques. The report acknowledges, however, that ''because many novel combinations of properties can be achieved only by molecular and cellular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques. Moreover, it recognizes that even precise genetic characterization of transgenic organisms does not necessarily allow scientists to predict all ecologically important expressions of phenotype in the environment.

The ESA report emphasizes the importance of considering a variety of ecological factors in ecological ramifications of planned introductions. Among these are

survival, reproduction, interactions with other organisms, and effects on ecosystem function and dynamics. Potential undesirable impacts must he weighed in evaluations. While explicitly calling attention to the complexities of ecological risk assessment, the report supports the position that "ecological oversight of planned introductions should be directed at promoting effectiveness while guarding against potential problems. Thus, the authors observe that most cases will present a minimal risk to the environment and provide a set of specific scientific criteria for ''sealing the level of oversight to individual cases. The four categories of criteria included:

1. attributes of genetic alteration,
2. attributes of the parent organism,
3. phenotypic attributes of the engineered organism in comparison with the parent organism. and
4. attributes of the environment.

Specific attributes are grouped according to level of risk presented and corresponding level of scientific risk assessment needed. Coming as it did from a group of ecologists, the ESA report is often cited as a touchstone for those wishing to balance the positive potential of biotechnology with a sensitivity to the environmental consequences of actions.

Biotechnology Ecological Risk Assessment

Introduction

A central goal of ecological risk assessment of planned introductions of recombinant DNA-modified organisms is to ''make a reasonably accurate prerelease prediction of the behavior an organism is likely to exhibit in its new ecological context and given its particular genetic modification, and to be able to detect and avert potential problems before they occur" (76).

Most scientists seem to concur that the focus of risk assessment should be on a particular organism, with its characteristics (genetically modified or not) and the genes that code for them, in a particular environment. Experimental protocols for ecological risk assessments need to be refined to screen out potentially problematic introductions before release (l4).

While scholars argue as to which risk assessment model would best apply to environmental introductions of recombinant DNA-modified organisms, all agree that the complexity of ecological factors renders biotechnology risk assessment particularly challenging. Living organisms can change locution, reproduce, and perhaps exchange genes. Once released into the environment. they will interact in a dynamic fashion with other species. They are indeed different from chemicals.

Ecological risk assessment is a still young methodology, and not standardized. Some argue that directly relevant data are scarce enough, and ecological phenomena are sufficiently complex that resasoned qualitative judgments are more feasible than more precise quantitative assessments. In practice, expert review panels using good scientific judgment and common sense. along with guidelines of points to consider, achieve qualitative assessments of the riskiness of various combinations of factors. As experience is gained. codification of the principles of review should evolve for application to future cases. Augmentation of" human judgment with knowledge system technology has been suggested as a means of facilitating the process (24, 66).

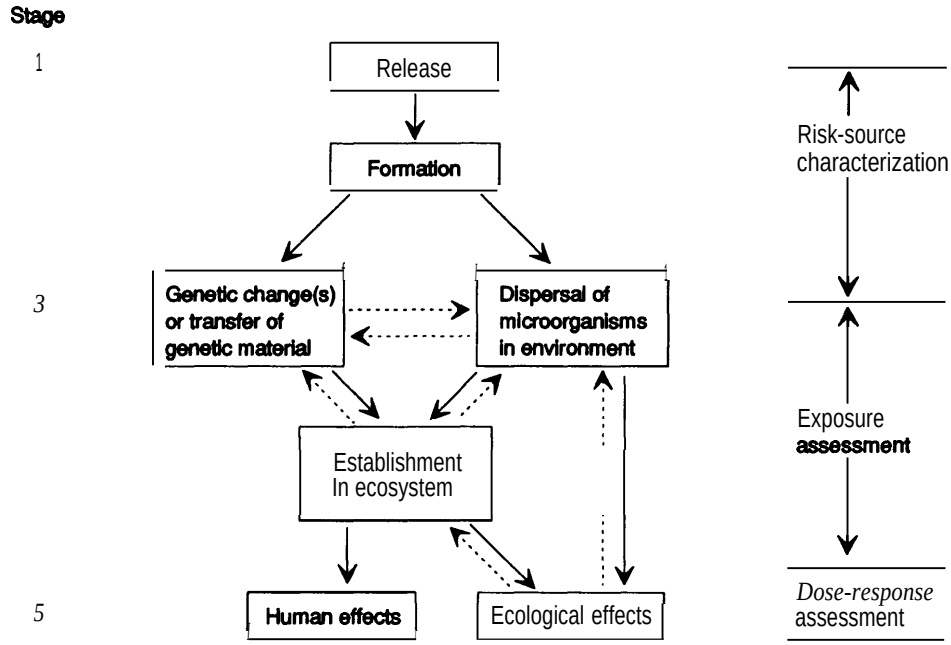
One way of conceptuall y applying risk assessment procedures to planned introduction of recombinant DNA-modified organisms into the environment is to match the three classic risk assessment stages (A. risk-source characterization: b. exposure assessment: and c. dose-response assessment) with the five stages involved in planned introductions. (See figure 8-2.) Information about stage one. *formation of a* recombinant DNA-modifiedd organism. and stage two, its deliberate release or accidental escape into the environment contributes to risk-source characterization. Exposure assessment would take into account data on stage three. *proliferation of* the organisms, including dispersal and possible exchange of genetic material, as well as stage tour. their *establishment* in an ecosystem. Stage five, human and ecological effects, relate quite directly to dose-response assessment (23).

Another way of looking at risk assessment of planned introductions is to consider the defination of "risk" as the product of' ''exposure' and "hazard." Exposure is related to the possibility of escape of the arganism, its survial. reproduction. and spretd. as well as to the gene transferred and the vector, if present. Assessment of the hazard, or potential environmental impact. depends on the ultimate fate of the introduced organism-whether it becomes extinct. establishes a balance with indigenous species. or overruns the recipient environment (53).

Specific objectives of ecological risk assessment for plants. for example. include:

1. determination of the potential for crops to persist and spread in a variety of habitats,
2. discovery of the range of species that can cross-pollinate with various transgenic crops.

Figure 8-2—Risk Assessment Framework for Environmental Introductions



SOURCE: Office of Technology Assessment, 1992.

3. investigation of the ecological performance of hybrid plants produced, and
4. development of protocols making it possible for crop breeders to carry out ecological risk assessments on new transgenic plants in the future.

Box 8-A illustrates the sorts of specific questions that can be asked and answered about plant introductions based on field observations, field experiments, and contained experiments (14).

Risk assessment pertaining to genetically modified (or nonmodified) viruses used in weed biocontrol, as an additional example, would include:

1. information on virus attributes such as virulence, host range, vector specificity, survival, and dispersal characteristics;
2. information on desirable and undesirable virus attributes and on the stability of these attributes;
3. information on the virus' effect on the target weed's genetic stability; and
4. information on the release site and how a variety of ecological variables affect infection, dispersal, population dynamics, and safety (87).

In summary, key features of ecological risk assessments of planned introductions include properties of the introduced organism (not the method by which it was produced) and of the recipient environment. including the demographic characteristics of the organism, the genetic stability and likelihood of gene transfer, and the interactions between the species and the physical and biological parameters of the environment. Scale and frequency of introductions should also be factored into risk assessments. Furthermore, since recapture or recall of introduced organisms usually will not be feasible, assessments should also consider possible means of containment, monitoring, and possible mitigation if adverse consequences occur (74).

Research Needs and Promise of Risk Assessment

The current interest in effective risk assessment of the products of biotechnology has stimulated workshops, conferences, discussions. and articles. More and more frequently. insights from the fields of ecology. population biology, population genetics, and evolution are being recast into the language of risk assessment (31, 50, 52. 62). Additional research needs to be undertaken on a variety of fronts to facilitate risk assessment. For example, a need exists to develop models and use data from field tests to predict the rate of spread of introduced organisms in various situations (54).

Box 8-A—Ecological Risk Assessment Questions

Field observations	Field experiments	Contained experiments
Persistence		
What is the survival of the vegetative parts of the plant under a range of climatic conditions, on soils of different kinds with different categories of drainage? How is perennation affected by the introduced genes? What factors influence plant mortality outside arable fields and how are these influenced by the novel genes? What is the nature of seed dormancy under different environmental conditions, and how does the introduced genetic change influence triggering, duration, and hardiness during dormancy?	What is the fate of seeds sown into a range of plant communities, including other arable crops, forage crops, permanent grasslands, and natural habitats? What is the fate of transplanted seedlings in different habitats? What is the fate of transplanted mature plants (or rootstock) in different vegetation types? How long does experimentally planted seed remain dormant but viable in a range of soil types?	How is pollen viability affected in transgenic plants? How is seed dormancy affected? How do transgenic plants perform in competition experiments with crop plants and with selected native plants?
Spread of the vegetative plant		
What is the seed production of the plant when grown in a crop and in natural vegetation? Is seed production limited by the rate of pollination? What is the germination rate of seeds in soil? What is the mortality of seeds and seedlings in arable soils and beneath native vegetation? What is the phenology of seedling emergence and growth? What are the natural enemies of the seedlings? What is the role of vertebrate and invertebrate herbivores in crop and noncrop habitats? What is the mechanism of seed dispersal? How far are seeds dispersed and how does this vary with environmental conditions? Do the seeds produced by plants grown outside arable fields give rise to a second generation of plants?	What is the vegetative growth rate on different substrates and with different competing species? Is the thinning rule (i.e., density-dependent plant mortality) similar for transgenic and nontransgenic plants? What kind of compensatory growth is exhibited (e.g., gap-filling)?	Is seed size or morphology different in transgenic plants, and how might this affect seed dispersal? Do transgenic plants present greater risks of spread by vegetative fragments?

(continued on next page)

Box 8-A—Ecological Risk Assessment Questions—Conthuecf

Field observations	Field experiments	Contained experiments
If the plant were to prove invasive, at what rate would it spread and which habitats would it occupy?		
Which plant species (if any) are displaced when (and if) the plant is established in natural habitats?		
Which plant species are responsible for the competitive suppression of the plant in different natural habitats?		
Horizontal gene transfer through pollen		
How much pollen is produced?	What is the fate of labeled pollen?	Which plant species allow pollen germination on their stigmas?
What is the phenology of pollen production and what is the phenology of stigma receptivity of other plant species growing in the neighborhood of crops (i.e., within 500-1,000 m)?	How much pollen reaches the stigmas of other wild plants under different conditions?	How is pollen dispersal affected in transgenic plants?
Over what distance is pollen dispersed under different meteorological conditions?	Which insects carry the pollen?	Which plant species form viable, hybrid seed and at what rate is this seed produced?
Which is the pollen deposited, on which species, and in what numbers?	How far away from the crop can an individual, potted crop plant be pollinated and how does the rate of pollination fall off with distance under a range of habitat conditions?	What is the germination rate of hybrid seed?
Where is the pollen deposited, on which species, and in what numbers?	What plants make the most efficient 'pollen barriers' for the construction of guard rows; is it nontransgenic members of the same species or plants that form physical barriers to pollen flow or to insect flight?	What phenotypes are exhibited by hybrid individuals?
What is the geographic distribution of closely related wild plants in the vicinity of centres of crop cultivation and what is their small-scale (100's m) distribution as weeds within arable fields and on land adjoining field foundaries?		What is the performance of hybrid plants in competition experiments with crop plants and with selected native plants?
What natural habitats are found within 1,000 m of arable fields, in those areas where the crops are grown, and what flora is supported by these habitats?		What is the nature of perennation and vegetative dormancy in hybrid and transgenic plants?

SOURCE: Michael J. Crawley, "The Ecology of Genetically Engineered Organisms: Assessing the Environmental Risks," Introduction of *Genetically Modified Organisms into the Environment,* Harold A. Mooney and Giorgio Bernardi (eds.) (New York, NY: John Wiley and Sons, 1990).

Achieving predictive capabilities in extrapolating from field tests to large-scale introductions is an additional goal. Along with further research, data from field tests and research then can feed into the design of future field tests and large-scale introductions. our scientific understanding pertinent to ecological risk assessment should increase exponentially over the next few years.

This explosion of knowledge not only can improve safety but also the effectiveness of introduced organisms in various habitats. There seems to be general agreement, even among ecologists and environmentalists, that most biotechnology products will not be harmful. However, because uncertainty does exist, for instance, as to which applications might be harmful. reasonable caution and willingness to assess risk are appropriate (76).

Risk assessment prior to introductions is a reasonable and necessary step, consensus dictates. More research can sharpen our powers of prediction and build on an already sol id foundation of information. Eventually, criteria can be developed to match individual cases with appropriate risk categories. In the meantime, as a broader knowledge base is being built, the safety of each introduction needs to be judged, basically, on a case-by-case basis (51). Understanding gained from case studies and other relevant research can be employed in the current transition to risk assessments of large-scale introductions.

Applicability of Diverse Bodies of Knowledge to Assessments of Large-Scale Commercial Release

Introduction

In all approaches to risk assessment, the key question is predictability. Do we have sufficient information to make a reasonable prediction as to what will occur for a particular release'? Can we in fact legitimately draw on knowledge gained from agricultural experience, laboratory tests, past field tests of recombinant DNA-modified organisms, and accumulated knowledge of genetics, microbiology, molecular biologics, and ecology? Are the characteristics of any individual large-scale release *familiar* enough that we can bring such knowledge to bear on the risk assessment'?

Species Introductions

Those interested in the evaluation of risks from biotechnology sometimes turn to the experience base with introduced ''exotics, species accidentally or deliberately released in a completely new environment. Dutch elm disease is often-cited as a consequence of the accidental introduction of a fungus; kudzu vine. running rampant in the South after being brought in as a roadside ground cover, is pointed to as a deliberate introduction gone awry.

One viewpoint holds that species invasions may be useful analogues of planned introductions of genetically engineered species, i.e., an invasion is an invasion. Thus. experience with analyses of key properties of 'successful invaders, as well as of vulnerable environments. theoretically can be brought to bear in evaluating planned introductions (63).

Most scientists agree, however, that invasions by exotics have limited applicability to planned introductions of genetically modified species. For example, introduced exotic plants that have caused problems come with many traits that enhance weediness; whereas genetically modified plants, by contrast. are modified in only a few characteristics (69). The distinction between the introduction of modified genotypes of crop organisms and the introductions of totally new exotics-whether or not they are genetically engineered—is, in fact, generally regarded as an important one (14). Even so. lessons learned as to the ecological parameters of ''invading species' and recipient environments may be useful in categorizing degrees of risk for a specific planned introduction of a recombinant DNA-modified organism. For example, comparisons can be made between the characteristics of such an organism and the characteristics often found in very successful invading species. Habitat characteristics can also be compared to help assess site for vulnerability or resistance to invasion (63).

Agriculture

Perhaps the oldest analogue to planned introductions of genetically modified species is agriculture itself. For much of human history, new forms of crops and domesticated animals have been introduced to the environment. Major crops have been bred by the millions for centuries; all these field tests and commercial releases provide a substantial experience base. Throughout this vast experience, no significant harm to human or animal health has occurred due to these introductions per se, nor have major crop plants become bad weeds. Normal selection procedures have eliminated plants with problems. Furthermore, "recalls" of crop varieties are common under the laws of supply and demand. In short, no evidence exists in the United States that plant breeding leads to ecological problems (6).

The NRC report's call for ● 'familiarity" as a criterion for risk assessment makes drawing on the experience base

Photo credit: Monsanto Co.

Genetically engineered tomato plants are shown being planted by researchers at a Monsanto-leased farm in **Jersey County, IL.**

of agriculture logical for most planned introductions of genetically modified agricultural organisms. A specific example of how the agricultural experience can be applied to biotechnology risk assessment is the 80 years of usage of Bt *(Bacillus thuringiensis* with its toxin) as a natural insecticide; its history of safe use is often regarded as evidence that transferring the gene for a Bt toxin would be environmentally safe (6). The 100-year experience base with vaccines, rhizobial bacteria, and other biological controls provides information applicable to large-scale microbial introductions (20, 29, 62, 90). As a final example, corn breeders have significantly changed the corn genome and have conducted planned introductions into the environment of these modifications for the past 70 years, without negative ecological experience. Breeders have gained experience in protecting the purity of these genomes, calculating the likelihood that the modifications will spread to other plants, deploying the modified genomes, and maximizing their strengths and minimizing their weaknesses (18).

Although there are limitations to the analogy between seed purity and gene transfer to weeds (notably, the risks associated with weed genes contaminating seed for planting crops are quite different from those associated with engineered genes getting into a weed population), this analogy does represent a useful starting point for risk assessment in controlled release.

Although some observers emphasize the novelty of gene combinations that can be brought about through biotechnology, a key difference between traditional crop breeding and the "new biotechnology" is that changes in genomes are more precise using biotechnology. With genetic engineering, one gene is moved at a time; by contrast, huge numbers of genes are recombined in crosses that lead to new plant varieties. It is nonetheless true that ecological effects of a changed phenotype sometimes may not be predictable even with precise changes in genotype (85).

Certainly, risk assessments are needed of individual cases involving particular genes. For example, forage crops such as alfalfa, which are not so dependent on cultivation practices, may have higher—and perhaps problematic—survival capabilities outside of the farm than others (6).

Two of the chief concerns about planned introduction of genetically modified species have no analogs in traditional agriculture. With the exception of some introduced crops that become weeds in tropical countries, crop plants have not invaded natural habitats. Furthermore. no obvious problems have arisen due to transfer of genes from traditionally bred crops to wild plants (14).

Laboratory Testing

Results of laboratory tests have been drawn on by those interested in risk assessment of genetically engineered microorganisms in particular. Various studies of microbial genetics, as well as use of soil microcosms (or laboratory model ecosystems) that mimick the natural environment, have provided useful information.

A great many reported laboratory tests involve investigations of mechanisms and likelihoods of gene transfer. For example, transformation (the uptake of naked DNA into a competent or receptive cell) is a form of gene transfer well understood in the laboratory, but not well described in natural settings. Laboratory records on transduction (the transfer of genes between bacterial strains by virus particles) have led to theoretical models predicting the possibility and frequency of transduction from an introduced genetically modified microorganism to a natural species. Another mechanism of horizontal gene transfer studied in the laboratory is conjugation, the process of genetic exchange between bacterial cells. Finally, transposition, the process by which mobile genetic sequences change positions within a genome can be associated with gene transfer.

Soil microcosms, even with sterile soil, are a feasible way of assessing what kind of gene transfer mechanisms *can* occur in nature; they are therefore a useful tool in risk assessment (38, 70). Research has now been done using more realistic soil microcosms, with the objective of learning more about the impact of conjugation on

introduced genetically modified microorganisms. For example, some experiments have been done using nonsterile soils, in an attempt to produce a closer analogue to nature.

Another set of questions that laboratory tests can help address is related to population biology. Relative fitness of genetically modified microorganisms in the laboratory, for example, pertains directly to establishment and possible spread of introduced organisms in an environment; some information toward quantitative risk assessments can be gained from contained laboratory testing in chemostats (44). Laboratory tests also can help illuminate the role played by various soil environments in successful introductions (93).

Of course, constraints exist on the applicability of laboratory tests, having to do with feasibility and with the impossibility of reproducing the full complexity of a natural environment. Some important parameters relevant to introductions are, for example, the relative fitness of the introduced recombinant DNA-modified organism in the new environment with its multiple dimensions of biological, chemical, and physical features, including competition with other microorganisms; microbial population density, which may vary over time and space; population dynamics; and availability of habitats (5). The dynamic complexity of many such features makes it impossible for a laboratory test to mimic reality completely. Work is beginning on testing for effects such as pathogenicity or toxicity in more realistic multispecies systems or microcosms (26).

Perhaps the principal lessons learned from laboratory research have to do with the potential to work creatively with soil microcosms. The more realistic the soil microcosm used, the higher the predictive value of the laboratory tests is likely to be, particularly where extrapolation from the laboratory to the field is relatively well understood. It has been suggested that mesocosms (larger contained walk-in chambers, the environmental parameters of which can be controlled) could provide more realistic complexity than soil microcosms. This added realism might improve risk assessment (93).

Small-Scale Field Tests

Field tests of conventionally produced crop varieties represent part of a step-wise progression toward full-scale commercialization; the same is true of field tests of recombinant DNA-modified organisms. Initially. new varieties are assessed in a laboratory or greenhouse; then they are observed in small-scale field plots where they are evaluated according to various protocols, statistical

Photo credit: Monsanto Co.

Researchers begin test of tomato plants carrying the Bt toxin gene in test plant.

procedures, and analytical methods. Large-scale tests and commercialization complete the process. Each stage provides information for the next stage (53). For the most part. principles and procedures useful in small-scale field tests are also relevant at the large-scale test and commercialization stages as well (36). Field testing and monitoring constitute ''real world empirical methods' that are important components of risk assessment (23).

Small-scale field tests can be used to elucidate characteristics that will be factored into risk assessments of possible large-scale planned introductions. For example, survival and spread of particular recombinant bacteria in a particular soil environment, as well as efficacy of function and stability of an introduced gene. can be estimated in field tests (1, 3, 47). Field tests also can be used to assess ''invasiveness' of transgenic crops (73). Data from field tests can be integrated into quantitative predictive models of gene flow and gene spread (39).

Field tests also provide agronomically significant information, including data on the expression or performance of the introduced gene and on the overall growth and vigor of the genetically modified plant (64). For example, 1990 field tests of insect-resistant cotton plants have allowed such agronomic traits as yield, fiber length, fiber strength, fiber quality, seed composition, and quality to be evaluated by Monsanto, which is planning for commercial introduction in 1994 or 1995 (28).

Well-designed, well-monitored field tests of increasing scale and complexity also should allow undesirable impacts to be observed while there is still an opportunity to correct them (43). A ''stepwise progression in test

design" is seen as an approach to field trials that will reduce complexity and otherwise benefit later I urge-scale efforts (47). (See box 8- B.) An important stage is expansion from single-site into multisite field testing, which allows sites to undergo different conditions, such as weather, and thus provides information on the variation possible in performance and impact *(73)*. Testing over more than 1 year can provide information on the consistency of measured characteristics such as survival and efficacy. Such information will have significant implications for commercial scale planned introductions. Good. statistically sound experimental design can be important in facilitating effective transitions from the field test to commercial-scale introduction (57). For agronomic and risk assessment purposes. scale-up from field tests is a useful and informative process.

There are, however. a few constraints on the applicability of small-scale field tests to large-scale tests or commercialization. An important one is the emphasis often placed on containment in small-scale field tests involving recombinant DNA-modified organisms. Containment is, of course, the antithesis of uncontained, large-scale introduction (36). Bagging plants, for example, prohibits pollination and, furthermore. would not be feasible at a large-scale (53).

When a product is commercialized. it will be far more widespread in the environment than it was in the days of its field test; many more ''nontarget species will be exposed to it (26). As people increasingly use transgenic plants. the chance for errors will increase because some users may not follow safety procedures (43).

Despite these limitations, field tests are providing the datai about agronomic qualities and risk assessment considerations needed for the design of' large-scale tests and commercialization. Detection and monitoring techniques are improving. A step-by-step progression from individual field tests through multisite field tests to large-scale testing to commercialization is being followed for recombinant DNA-modified organisms as it has been for conventionally produced organisms. without problems. Research still needs to be done to identify important distinctions between small-scale and large-scale tests; this should improve experimental design and efficiency (53).

Deliberations on Field Tests and on Large-Scale Release

Over the past several years. field tests have made important contributions to risk assessments for large-scale release of DNA-modfied organisms. The data from field tests provide the most directly relevant basis for predic-

Box 8-B—Learning by Doing: Successive Field Releases

Crop Genetics International (CGI) is a company that has used a "stepwise progression in test design" as it has moved from an initial field test to later tests. The focus was the delivery of biopesticidal gene products by endophytic bacteria inoculated into seeds. First tested was a bacterial endophyte *(Clavibacter xyli* subsp. cynodontis) genetically modified to produce low levels of the delta-endotoxin of ***Bacillus thuringiensis*** (Bt) subsp. *kurstaki*, and inoculated into corn seed. CGI developed a strategy for multiple risk assessment studies of field releases. The focus of the field release studies was twofold: performance of plants grown from endophyte-inoculated seed; and persistence and spread of the genetically modified strain under different environmental conditions. The first two releases were used to develop a profile of the recombinant strain's behavior in the environment. In 1989, the test design was extended to multiple sites in four States to examine its behavior overdiversified environmental conditions. This **was** the first release to take place in multiple States of a viable microorganism genetically modified to produce a biopesticide. In 1990, a new recombinant strain selected for its activity against the target pest (European corn borer) was incorporated readily into the well-established testing procedures and program, with the objective of determining efficiency. As the study progressed between 1988 and 1990, by agreement with regulators, levels of containment were gradually lowered as data on safety were obtained. In fact, the early tests were specifically designed to address risk assessment issues such that future small-scale introductions could be made with less rigid containment and such that containment requirements could be eliminated in large-scale field tests. Efficacy studies now can be done under reduced containment requirements. Multiple-site field testing of the improved strains is the next logical step toward large-scale tests and commercialization. Stepwise progression of tests is a rational strategy from a company's point of view, as well as from a regulator's point of view.

SOURCE: Stanley J. Kostka, "The Design and Execution of Successive Field Releases of Genetically Engineered Microorganisms," *Biologicd Monitoring of Genetically Engineered Plants and Microbes*D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27-30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 167-176.

(ion as to the safety of large-scale release, particularly in cases where a small-scale field test is itself scaled-up to a large-scale introduction. Equally important, scientists in many disciplines have been gaining practice through field testing in the process of risk assessment. Now that applications for large-scale release are imminent, researchers familiar with comparable evaluations at a small-scale can begin to integrate their experience and apply it to the new assessment task at hand.

Several recent conferences have helped to define approaches to the risk assessment of large-scale introductions. Commonalities arc emerging, suggesting that a state of readiness for large- scale introductions is in fact being reached.

Several biological principles with implications for assessment of large-scale introductions emerged from the International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms (November 27–30, 1990. Kiawah Island. South Carolina). For example:

- The integration of genes into the chromosomes of recombinant DNA-modified organisms has proven to be predictably stable.
- Gene transfer frequencies of recombinant DNA-modified organisms are consistent with patterns recorded for natural populations.
- The frequencies of transposon relocations in recombinant DNA-modified organisms are consistent with those of natural populations.
- Some microorganism detection methods are extremely sensitive. and this contributes to better understanding of the fate of a microorganism in the environment.
- Background microbial populations have been characterized as complex. and thus the release of genetically modified microbes may be insignificant by comparison..

The symposium also highlighted the strong foundation of conventional knowledge in crop improvement, microbial testing. and food processing that is available to support safe commercialization of biotechnology products. Research needs cited included: detection methods, sampling methodologies. monitoring protocols and modeling techniques, and empirical data for improved design and evaluation of experiments (53).

A workshop on transgenic plants conducted by the Maryland Biotechnology Institute and the USEPA Office of Pesticide Programs (June 18–20, 1990) evaluated the human and environmentall impacts that could result from the ‘‘widespread. full scale”use of plants genetically modified to produce a pesticidal substance. Workgroups discussed: 1) studies and information needed for assessment; 2) scientific rationale for determining the occasional need for specialized studies; and 3) availability and test protocols for developing risk assessment information.

The consensus of all groups was that such transgenic plants posed concerns and possible effects that are not unique, and risk assessment issues can be addressed through readily obtainable information on possible effects of the plant or of the pesticidal substance (89).

The USDA-sponsored ‘‘Workshop on Safeguards for Planned Introductions of Transgenic oilsecd Crucifers’ (October 9, 1990, Cornell University’) was held to identify agricultural biosafety issues relevant to oilsecd rape (or canola) as soon as possible. Unlike most crops, oilseed rape has weedy relations in North America. The potential for. and possible results of, gene transfer are therefore of concern. The workshop group agreed that with mill ions of acres planted. gene transfer will occur. Therefore, an ‘‘ecological map’ of wild species was called for, so that the location of field trials could be planned to deliberately minimize proximity and hence possibility for gene transfer. Experimental trials and research were recommended to quantify risks. as were studies of the factors influencing gene transfer potential- i .e.. travel of pollen. effective fertilization, the production of viable seed, and the plant reaching reproductive age and passing on its new set of genes. The group agreed that studies should emphasize the conditions under which transfer and expression of the transferred gene take place. and the consequenses-relative risk—of such events (61). A comparable meeting was held for maize and wheat (Keystone, Colorado, December 6–8, 1990); another is planned for rice.

Summary

A long history of agriculture provides an immense bunk of data relevant to risk assessment: diverse scientific fields contribute principles and knowledge. Data from small-scale field tests of recombinant DNA-modified oganisms not only provide specifics necessary for the evaluation of large-scale counterparts. they also provide a risk assessment testing ground. Each risk assessment of a field test adds to the regulator's experience base in adopting risk assessment methodologies to planned introductions. This learning through experience is a natural part of the evolution of oversight as we move from small-scale to large-scalec introductions.

Commercial Release Issues

A variety of issues relevant to planned introductions of recombinant DNA-modified organisms are receiving heightened attention as large-scale commercial releases become imminent. Principal concerns focus on the fitness of the engineered organism (defined as overall genetic contribution to future generations, usually quantified as number of offspring produced) and its potential to become established as a weed or a pest, the stability of the engineered gene, the potential for gene transfer, and impact on other organisms and the environment. Basically, these concerns are the same ones raised with regard to small-scale field tests of genetically engineered organisms. Large-scale agricultural uses involve large numbers of organisms that are usually less contained than their less numerous counterparts in field trials.

Fitness and Potential to Become Established

For a species to become established in a natural community, its relative fitness must be such that it competes successfully with other species. The lack of weediness on the part of most major crops illustrates a direct contrast between domestication and what is useful for survival in the wild (14. 43). Many traits necessary for successful weediness either have never existed in or have been deliberately bred out of crop plants to maximize productivity in a cultivated setting. One analysis showed that serious weeds tend to have on average 10 to 11 ''weedy characteristics'; crop plants have on average only 5 of these characteristics (42). Thus, the chances of any crop plant simultaneously undergoing five to six relevant gene changes to become a weed are vanishingly small (37).

Features of organisms that ecologists identify with weediness include broad ecological tolerance, ability to exploit an under-utilized resource. or ''readaptation' to a new habitat to which the organism is well-suited and in which controlling biological agents *do not exist* (76). Other characteristics that help to make a plant thrive as a weed include the following:

- rapid growth to a flowering stage,
- continuous seed production as long as growing conditions allow,
- high seed output.
- long-lived seed,
- pollination by wind or unspecialized insects,
- high competitive ability,
- broad environmental tolerance,
- seed dispersal over short and long distances, and
- vegetative persistence and propagation.

The probability of successful establishment of a recombinant DNA-modified organism as compared to its unmodified counterpart will naturally be dependent on the nature and phenotypic expression of the specific genotypic modification made, along with the rest of the organism's phenotype, in relation to these ecological criteria. Different kinds of engineered genes will vary in the degree and nature of their impact on the phenotype of the engineered organism. Also, engineered genes may vary in terms of the conditions under which they will be expressed. For example, if a gene is only induced to be expressed under specialized conditions, then its phenotypic impact will be negligible the rest of the time.

It has been well established from studies of induced mutations that most dramatic phenotypic changes in an organism result in reduced fitness (2, 14). Engineered genes that affect the growth, resource allocation, or some other aspect of an organism may convey added economic value, but may also produce a maladapted plant that is unlikely to survive outside of cultivation. On the other hand. genes that have relatively little effect on the overall phenotype, such as genes induced only on certain occasions for disease or pest resistance, might confer a real fitness advantage, even in natural populations. It is generally assumed that genes for disease resistance present a physiological cost that reduces fitness in the absence of disease, although the importance of that cost has been challenged (7 I). However, sometimes if the gene is not expressed, such costs go down, contributing to its potential long-term persistence.

Assessments of the risks of introduced organisms becoming pests must take these factors into account as well as others. For example, introducing a character into an organism whose ecological properties are otherwise well-known, or taking a particular property associated with terrestrial bacteria and introducing it into another terrestrial bacterium, enables some prediction of how that character might respond in that target ecological setting. Thus, in assessing the potential risk associated with a particular phenotypic modification, the target environment should be considered.

If a species became established as a pest, existing communities would be disrupted; fortunately, the likelihood of either a genetically modified plant or a microorganism becoming a pest is relatively low. Most crop varieties produced through conventional means do not become pests (6). Experiments to date indicate that genetically modified microorganisms in some cases may not persist at significant levels (3) and therefore may

often be unlikely to proliferate and disrupt existing com munities composed of vast numbers and numerous species of microorganisms (19. 86). So for all organisms modified in any way. emphases in risk assessment of microorganisms should be placed on the specific product. Until more is known about consequences of large-scale use of genetically modified plants. a deliberate approach rather than complacency seems warranted.

Gene Stability

The stability of an engineered gene is important to risk assessments of planned introductions of recombinant DNA-modified organisms. A gene that has become a stable component of the transgenic organism is more predictable in its function, expression. and possible mobility than one that has not. one aspect of gene stability is persistence. An engineered gene construct usually consists of several components, all of which must be present and intact for the gene to function. In addition to the structural gene that codes for the desired gene product. a promoter gene is needed for it to be expressed—to be turned ‘‘on’ or ‘‘off. Such constructs maybe broken apart by natural genetic recombination. A promoter separated from its structural gene is useless; the structural gene without the promoter remains unexpressed.

The stability of a particular gene also may be directly influenced by the vector used to introduce it into the engineered organism. Bacterial plasmids or DNA-carrying bodies, are potentially the most mobile of the vectors used to insert genes. Plasmids function by inserting themselves into the bacterial chromosome. carrying an engineered gene along with them. Insertion sites for such plasm ids are nonrandom; they are specific sequences that could be recognized by other plasmids, which may pick up the inserted gene and carry it along to another organism. on the other hand, it also is often true that insertion of a particular plasmid will immunize the cell against insertion of similar plasmids.

Genes directly inserted into chromosomes are more stable than genes carried by plasmids. However, chromosomes are complex structures, and the manner in which particular genes express or recombine is determined by their relative positions on chromosomes. An engineered gene inserted in some parts of the chromosome may be more exposed to recombination than genes on other parts of the chromosome. The relative stability of an engineered gene in a plant species can be increased by inserting it into portions of chromosomes subject to lower levels of recombination.

To summarize. a gene’s stability depends on the nature of the gene itself and on the means of introducing it into the recipient organism. Either of these can be manipulated deliberately to increase stability.

Gene Transfer

Another appropriate focus for risk asessments of planned introduction of recombinant DNA-modified organisms is the possibility) that novel genes may become incorporated into related wild species. Such transfers, it is argued, might lead to harmful bacteria or weeds with an " improved" characteristic such as resistance to pest attack: this might make them more difficult to control. Three key questions to be considered are: What is the probability that a gene will move from an agricultural organism to wild species’? What can be done to lower the probability? What would be the consequences of such gene transfer on agricultural and natural communities<? (37)

The probability of gene transfer from a recombinant DNA-modified organism to a wild relative depends on the introduced organism and the nature of the original gene transfer mechanism. For microorganisms such as bacteria the primary means of genetic transformation is by vectors that, as noted above. are readily incorporated into organisms and mobile between organisms. This opens up the prospect of horizontal transfer of modified genes.

In addition to vector-mediated gene transfers, or transduction, genetic transfer in bacteria can occur by transformation, in which DNA freely existing in the environment is incorporated into living cells; and conjugation, in which DNA is transferred by direct organism to organism contact (27). These mechanisms are well known from in vitro studies of microorganisms under laboratory conditions; indeed, transduction has become a common tool in the introduction of engineered genes into bacteria (55). However, little is known of the properties of these transmission mechanisms in nature (80). Due to the complexity of the bacterial environment, the scope for bacteria to bacteria contact or for mobility of bacteriophages and bacteria are much more restricted in soil than in laboratory culture.

Risk assessment of gene transfer in natural bacteria populations is also problematic because species composition and potential for gene transfer among species is poorly understood. Only a small fraction of the bacterial species growing in soil occur in sufficient numbers to be recognized by standard isolation techniques (48). It has been argued that slow-growing organisms occur in sufficiently low numbers that their potential interactions and any subsequent possible risks are negligible.

On the bright side, a number of recently developed techniques exist that can greatly facilitate studies of bacterial interactions in natural substrates (48), including flow cytometry (a technique that involves the use of laser-activated fluorescence of stained particles) and polymerase chain reaction (PCR) (65), involving the amplification of a particular gene contained at low concentration in soil to sufficiently high concentrations that it can be detected by standard DNA analysis. PCR can be used to monitor the movements of introduced genes in natural substrates (79). This allows the population dynamics of the engineered organism to be more closely monitored, the transmission of the engineered gene to background organisms to be quantified, and potential risks to be evaluated. Also, the introduced population can be "tagged' with a specific but nonfunctional DNA sequence such that the growth or decline of that population in the soil can be monitored independently of the engineered gene(s).

Actual probabilities of gene transfer of various kinds among microorganisms are still being researched. Although differing opinions certainly exist, one school of thought is that the order of magnitude of microorganisms present in the natural community, and the probable frequency with which they exchange genes, renders the potential impact of most recombinant genes being transferred relatively low.

For higher organisms. vector-mediated transfer of engineered genes is not a major concern. For example, a widely used vector for dicotyledormus plants, *Agrobacterium tumefaciens* (crown gall virus) can be readily screened out of transformed organisms before they are released. Furthermore. for many important crop species, notably cereal crops, vectors for gene transfer are not used: rather ballistic incorporation of genetic material into tissue-cultured cells (using '"gene guns') is the method currently in development. Using this method there is no chance of vector-mediated gene transfer. This leaves gene transfer through hybridization of crops and reproductively compatible (i. e., closely related) weeds as a possibility.

In higher plants, the main risk associated with gene transfer from transgenics into surrounding populations is. in fact, that of hybridization. Modified genes potentially could be transferred from transgenic plants and incorporated into the genome of a weedy species through introgressive hybridization, whereby genes are transmitted through pollen in sexual reproduction. However, working against this possibility are limited viaility of pollen, distance and physical barriers to pollination. genetic dissimilarities (i. e., incompatible fertilization processes), and failure to produce viable, fertile offspring.

Most crops grown on a large scale in temperate regions, such as corn and wheat, are grown outside of their geographic region of origin; consequently there typically are no related weed species growing in association with them. Therefore, for most crop species in the United States, pollen-mediated transfer of modified genesis only of theoretical concern. However, there are several important crop species for which closely related weed species have become introduced. Specifically. many crops in the family Brassicaceae. such as canola (oil-seed rape) and radishes, have co-occurring weedy relatives (21). Sunflowers had their center of origin in the United States and have related weedy species here as well.

Most major crop species originated in what arnow regarded as developing countries. For example, corn was developed in Central America, wheat was first cultivated in the Middle East, rice in Southeast Asia. and potatoes in South America (77). Consequently, introduction of genetically engineered crops into such regions should be handled with particular attention to the probability of gene transfer into background populations.

Additional concern focuses on the potential impact of introduced genes on the genetic structure of natural populations of plants related to important crop species. These populations represent the genetic heritage of the crop and are an irreplaceable reservoir of diverse genetic variation that may be needed in future development of the crop (8). If, because of a novel gene effect, one strain or lineage became a super weed it might outcompete and therefore eliminate other lineages; genetic variation potentially useful for crop development could be lost. More generally, biodiversity is intrinsically valued by many (12).

Pollen-mediated transfer of novel genes from crops into related weeds might also result in weeds becoming similar to the crop species. A number of well-known instances exist where selection pressures exerted by traditional agronomic practices have caused weedy species to evolve to resemble the crop species. Such weeds cannot be eliminated by standard control practices (4). Thus, weeds are capable of a wide range of genetic adaptation even without the introduction of novel genes. Although there could clearly be problems associated with potential gene transfer from transgenic plants into weed populations. there is also a large experience base in agricultural and natural populations on which to draw for predictions in this area.

A great deal is known about pollen transfer in plants **(35)** and associated Likelihoods of gene transfer. In the past few years, there has been a growing interest in tracking pollen in natural populations through ''paternity analysis, " a technique directly analogous to human paternity analysis (58, 82). The development of such approaches provides a useful means of evaluating the potential spread of modified genes, as well as a means of testing the efficacy of various measures to prevent pollen spread into wild relatives.

Gene flow in many crop species has also been studied extensively in order to determine necessary distances for genetic isolation of different plots to reduce genetic contamination of seed crops in conventional agriculture. For example, genetic contamination of seed in plantations of conifers can reach levels of 30 to 50 percent and is an extensively studied problem (78). A review of gene transfer from corn to related species concluded that the prospects for introgressive hybridization in corn were limited (17). However. it is unwise to dismiss completely consideration of gene transfer because genes transmitted at low levels could be rapidly enhanced through natural selection if they confer an advantage to their recipients. A study of hybridization among six different rice cultivars developed through conventional agriculture and the related weed *red* rice (Oryza sativa L.) found widely varying rates of hybridization with the different cultivars. The hybrids generally showed evidence of convergence towards the crop, thus opening the possibility of generating a particularly noxious weed that closely resembles the crop (49).

For specific applications of biotechnology, it is possible to articulate potential risks of gene transfer and evaluate their probability. Furthermore, long-standing agricultural practices (e. g., isolation of crops for seed certification) can be useful in managing this risk. For the few U.S. crops with weedy relatives (i.e., canola), and for other countries where crops have multiple related species, careful risk assessment should lead to reasonable risk management. It is important to remember that successful cross hybridization is in fact a complex multistep process and does not usually lead to viable, fertile hybrids. unless the species are closely related.

Evolutionary Pressures Placed on Other Organisms

Evolutionary pressures on indigenous organisms can arise in several ways. Novel organisms in a biotic community may provide new levels of competitive interactions; they may impose direct selection pressures on the native organisms; they may also enhance one species at the expense of others. Thus the assessment of risks (and benefits) associated with the planned introduction of recombinant DNA-modified organisms must consider the engineered organisms' probable interactions with the target biotic community.

Many such interactions occur in convolution of a cultivated species and its associated pathogens. pests. and weeds. One interaction that should be beneficial in terms of controlling crop pathogens involves a pathogen's response to "resistance factors." Factors conferring resistance to pathogens can be conventionally bred or genetically engineered into plants. It is well established that the introduction of pathogen resistance factors imposes selection pressures on pathogens to overcome these factors by evolving greater virulence (34). Using conventional breeding methods, it can take longer to introduce a resistance factor into a crop species than it does for pathogens to respond. Genetic engineering promises greatly to reduce the time frame for introducing resistance factors. This ''buys" the crop some lead time before the pathogen evolves a response.

Strong selection pressures also are exerted on pest species to evolve counter measures to control technologies. The use of *Bacillus thurigiensis (* Bt. for example, is an effective means of controlling insect pests that could become overutilized and thus rendered ineffective. The bacterium itself often is used in broadcast spray applications to control insect pests, and the gene for toxic agents in *Bacillus* thuringiensis has been cloned. The gene now is being incorporated into crop species in field tests. This will exert even stronger selection pressure on insect pests. Several approaches may help to diminish selection pressure and thus slow down the rate of evolution of resistance. (See ch. 6.) It may be possible, for example, to introduce the Bt gene in such a way that it is only turned on during certain stages of development, only in certain parts of the plant. or only at times of insect attack, thus decreasing its impact. Scientists from several agricultural companies have formed a Bt **resis**tance ''club' to discuss how to slow the evolution of resistance to Bt.

Another concern is that use of genetically engineered crops for herbicide resistance may result in overuse of specific herbicides and thus impose strong selection on weeds to evolve resistance to those herbicides. For example. if even ''environmenttally friendly" herbicides are overused in conjunction with transgenic monoculture, weeds might evolve resistance fairly rapidly. This may lead to a "desperate" use of far more damaging herbi-

cides. Management strategies for slowing the development of resistance may be needed. (See ch. 6.)

The convolution of a cultivated species and its associated pathogens and weeds is a quite predictable process if one genetic locus for one resistance factor is considered. The sequential introduction of resistance factors in a crop species ultimately can lead to the so-called ''gene for gene' condition in which each gene for some resistance factor in the host is matched by a gene for virulence in the pathogen. One way to break this cycle is simultaneously to introduce multiple resistance factors, thus impeding the pest's evolutionary response. Similarly, different resistance factors might be cycled from year to year so that the pest never fully responds to any one resistance factor (34). The use of genetic engineering techniques could greatly facilitate such strategies because it provides a tool for rapid generation of new lines containing different combinations of resistance factors.

Monitoring

Assessing the potential risks of environmental introductions of recombinant DNA-modified organisms, and evaluating how best to manage these risks, entails spatial and temporal monitoring of the organisms and of their introduced genes. Monitoring contributes to risk assessment and management in two ways. First. in a specific situation, it tracks indicators of gene transfer or spread of introduced organisms so that action can be taken if needed. Beyond this, monitoring adds to our database, so that risk assessments of subsequent introductions are even more accurate. Monitoring of field tests can provide information pertinent to subsequent field tests and to large-scale introductions. For example, presence or amount of gene transfer from transgenic crops to related or nonrelated weedy species could be estimated from monitoring species surrounding a test field containing a recombinant DNA-modified crop. These data can be used in future field tests or large-scale introductions involving similar crop/weed complexes. Monitoring also can help elucidate any spread of introduced microorganisms. As the ecology of their spread is understood more fully, risk assessments of new introductions can be improved. Thus, monitoring has an important role to play in the natural evolution of science-based. risk-based regulatory oversight. Highly sensitive monitoring techniques are developing rapidly. (See box 8-C.)

The following is an example of the kind of data that the Animal and Plant Health Inspection Service (APHIS) can require from monitoring (in this case recombinant entomocidal or insect-killing bacteria were field tested). Required monitoring provided data on:

1. plant colonization by the recombinant bacteria at 4 weeks after inoculation;
2. colonization of all plant parts by the recombinant bacteria monthly for 4 months;
3. dispersal, natural and mechanical, in the field of the recombinant bacteria after 60 days;
4. presence in run-off water of the recombinant bacteria;
5. presence in soil of recombinant bacteria populations;
6. effect on crop yield of the recombinant bacteria;
7. effect on crop residue decomposition of the recombinant bacteria;
8. effect on vesicular-arbuscular mycorrhizae of the recombinant bacteria 3 and 6 weeks after planting; and
9. effects of the recombinant bacteria on saprophytic gram-negative bacteria in the phylloplane.

Other points of interest needed to be addressed through the ability to track the recombinant bacteria, as well (22).

The monitoring data collected enabled APHIS to assess patterns of the spread of the recombinant bacteria on the targeted plant and its various parts, the dispersal of the bacteria in the field water and soil, effects of the bacteria on crop yield and decomposition, and the effects of the recombinant bacteria on mycorrhizae and other plant bacteria. In short, required monitoring of plants and soil contributed directly to understanding of dispersal and effects of the recombinant bacteria.

Plants generally are easier to monitor than microorganisms. As techniques for monitoring improve, field test data and, soon, large-scale test data will improve our knowledge of survival and spread of recombinant DNA-modified organisms and their genes. thus aiding us in reasoned risk assessment and management.

Research Needs

For the past two decades, basic research in molecular biology has generated many novel scientific insights and products. As a result of strong government support for such research, we have reached a point where the planned introduction of recombinant DNA-modified organisms is a reality. However, the fields of ecology and evolutionary biology, which can provide the kind of information and expertise needed to predict the impacts of planned introductions, have enjoyed less support. Fortunately, ecologists are now taking a leading role in defining a research

Box 8-C—Monitoring Microorganisms

Detection and tracking (monitoring) of recombinant DNA-modified organisms and their genes makes possible quantification of persistence or spread. Highly sensitive new techniques, among them polymerase chain reaction (PCR) and antibodies, are being utilized to contribute to the efficacy of monitoring. Data resulting from monitoring in turn contribute to the knowledge base on which risk assessments of prospective small-scale and large-scale introductions can be based. In fact, regulatory agencies' request that certain parameters be monitored in field tests allows them to fine tune upcoming assessments of large-scale applications, and to make plans for their management.

The first approved environmental introduction of a living genetically modified soil-borne bacterium in the United States, in fact, had as its goal monitoring of the bacterium's population dynamics, persistence, and movement through the soil. The genes "lac Z" and "lac Y" were engineered into a root-colonizing fluorescent pseudomonas (P. *aureofaciens),* part of a bacterial group that often promotes plant growth and protects against some plant diseases. The added genes allow the bacterium to use lactose as a source of carbon and energy and result in readily discernible deep blue bacterial colonies on a petri dish, thus providing an excellent monitoring tool. Scientists from Clemson University and Monsanto studied bacterial spread, population dynamics, and persistence over three crop cycles (19 months) in a wheat field and found similar values for both the modified and the nonmodified strains. Both strains declined to below detectable limits 38 weeks after inoculation. Also monitored were the foliar tissue of the first winter wheat crop analyzed 3 weeks before harvest and found not to have either strain present; and native soil bacteria, to which the Iac Z and Iac Y genes were not found to have transferred. The study's multifaceted sampling design, use of new techniques such as chromosomal DNA fingerprint patterns, presence of a control in the form of a nonengineered strain, and followup over three crop cycles set good examples for thorough monitoring studies in other situations (45). This work also is noteworthy as the first study analyzing frequency of genetic exchange in the environment of genes inserted into bacterial chromosomes rather than plasmids. This "success" of the chromosomal approach has implications for scientific management of gene transfer in microorganisms.

In future monitoring studies, the transgenic organism or the inserted gene itself might be tracked by a nucleic acid probe for a specific DNA sequence; as well as by selective media for metabolic characteristics or by antibodies to a characteristic antigen. Some tracking techniques require that bacteria be isolated and grown in the laboratory, but others are being developed that can analyze bacterial DNA as isolated from environmental samples, a capability useful in estimating the population of the introduced organisms. Still other techniques, including pulsed field electrophoresis, can be used to analyze total DNA in a simple community and possibly to then quantify different members from the sample. In communities that are more complex, higher resolution is needed and probes maybe necessary. In such cases, antibodies may give a great deal of information by tracking phenotype through detection of proteins present (19). Polymerase chain reaction methodology is an innovative technique that can be used essentially to "magnify" sensitivity of detection. Flow cytometry, a cell-sorting technique, may also have some application to monitoring.

SOURCE: Philip C. Kearney and James M. Tiedje, "Methods Used to Track Introduced Genetically Engineered Organisms," *Biiotechno/ogy for Crop Protection,* Paul Hedin, Julius Menn, Robert Hollingworth (ads.) (Washington, DC: American Chemical Society, 1988).

agendna to respond to a variety of social needs, including the planned introduction of' recombinant DNA-modified organisms (85). Since introduced species or their genes may be incorporated into natural biota, over time. a similar agenda is needed for evolutionary biology to assess the likelihood of propagation and persistence.

The likelihood of an introduced organism becoming established. competing with other organisms. spreading. exchanging genes with members of other species. indirectly affecting nontarget species, or changing over evolutionary time all need to be predicted in risk assessment. A number of fields in biology are already making contributions to these predictive capabilities. However. funding for further research is needed. (See box 8-D.)

Development of mechanisms for effective coommunication between fields is critical to meeting research needs associated with the planned introduction of recombinant DNA-modified organisms. It has been noted that interdisciplinary research is critical for the development of risk assessment and risk management pertinent to planned introductions (92). In particular. the gap between ecology and molecular biology needs to be spanned. Scientists in both areas need to be trained or encouraged to be more aware of each other's fields.

130x 8-D—Relevant Research Fields

Community Ecology

Community ecology is the study of interactions of populations of different species in a given habitat. Interspecific competition, predation, and other interactions are the province of this field. Modern community ecology is an experimental field; however, most experimental studies are limited in scope to consideration of two, or at most three interacting species. Larger experiments focusing on more realistically complex interactions, and desirable predictability of response to perturbation, will require more research. Ecological systems research on topics such as nutrient cycling can provide relevant information as well.

Population Ecology

Population ecology is the study of the dynamics and growth of populations. Such studies may emphasize properties of the species itself, such as fecundity or mortality rates, or they may emphasize effects of environmental or biotic interactions. There is a growing trend to incorporate population ecology into conservation biology. Analysis of life history can be used to determine which stages (e.g., seedling establishment versus adult survivorship) are limiting to population growth. Such analyses of sensitivity in population dynamics (9) could be useful in risk assessment of ecological impacts of recombinant DNA-modified organisms.

Population Genetics

Population genetics is the analytical study of properties of genes and changes in gene frequency over time. The mechanism by which genes are transmitted from one generation to the next and the relationship between particular genes and fitness are key to this field. This field is distinctive among biological fields because of its sophisticated theoretical framework. The theory enables some level of prediction about the behavior of genes in populations, but more emphasis on empirical studies is needed to generate useful predictive models of gene change.

Evolutionary Biology

One way to encourage empirical work in population genetics would be to place more emphasis on research in evolutionary biology. Changes over time in genetic structure—and consequent phenotypes-of populations are foci of evolutionary theory. Emphasis on dynamics of change predisposes the field towards questions of relative Spread of genes and impact of phemotypes in an ecosystem over time; these are questions that are relevant to risk assessment of planned introductions.

Systematic

The field of systematic encompasses analysis of variation of different levels of taxonomic organization. Although the ultimate goal of such analysis is taxonomic classification, this field is increasing in importance in analysis and monitoring of biotic diversity. This field could contribute to risk assessment through analysis of species relationships and species ranges to evaluate the probabilities of hybridization.

Mathamatical Modeling

Mathematical modeling entails construction of a mathematical framework to describe a process and predict outcomes from that process. Modeling has been an effective approach in risk assessment and strategic planning in agriculture, For example, models have demonstrated that allowing the existence of marginal populations of pests lets them serve as reservoirs for genes that confer susceptibility to pesticides and other means of control, such populations therefore can beneficially slow the rate of evolution of resistance (34). This seemingly counterintuitive result contraindicates a straightforward program of eradication.

Risk Assessment Methodologies

Risk assessment involves the ranking of probable outcomes from possible events. As such, in order to rank risks, one needs to first define the risks of a given practice. Development of risk assessment methodologies is an ongoing practice, and practitioners must always be ready to adapt to new problems as they arise in different situations, such as commercialization of diverse crops in a variety of environments.

SOURCE: Office of Technology Assessment, 1992.

More communication between scientists involved in basic research and applied research is also needed. Just one example is the need for communication and interaction between plant-resistance breeders and evolutionary biologists (33). Another example would be communication between farm management systems research and ecology. Many people who work in basic research are in part motivated by applied concerns. However, it does little good to generate insights on an applied problem unless there are lines of communication whereby the results of those insights are incorporated to solve the problem. Questions regarding applied problems also need to be articulated to basic researchers.

RISK MANAGEMENT

Genetically modified organisms introduced into the environment do not present us with radically novel problems. Furthermore, we have a sufficient enough base of technical knowledge and risk assessment methodologies that we can make reasonable, science-based assessments of the likely impacts of individual proposed introductions. The concerns raised do not need to paralyze agricultural progress based on biotechnology. These concerns can be respected, weighed, and addressed as necessary through science-based regulations and scientific and agronomic methods of managing risk.

Design of Science-Based Regulation

The 1986 Coordinated Framework (5 I FR 23302-23393, 1986). the more recent scope document (55 FR 147, 3118. 1990). **and other** reports attempt to create a technically sound context for biotechnology oversight. (See ch. 7.) Reviews of field trials to date have been based on technical issues of risk reduction. Technically sound evaluations of safety can provide principles for regulation and oversight. Agencies receiving proposals can add specific stipulations for risk management (66). A variety of scientific fields ranging from molecular genetics to ecology need to be brought to bear on the design or performance of oversight. As research progresses. predictability about risks and insights as to how they should be managed will improve.

The imminence of large-scale introductions underscores the need for clarification of how risk will be managed in various situations. Identification of issues. development of policy, and structure for large-scale tests and commericializations, along with modifications of the approval process for small-scale field tests, are all being requested from regulatory agencies, who are themselves grappling with the issues involved (36).

Generic v. Case-by-Case Approach

Extrapolation of results of risk assessment from one site to another still needs refining; this has ramifications for multisite, large-scale introductions. Many believe that ‘evaluation of risks must be specific to the particular application. However, attempts have been and doubtless will be made to associate individual cases with appropriate categories of risk and to manage them accordingly (51).

One key issue in the approach to risk management in planned introductions of recombinant DNA-modified organisms is whether to use a case-by-case analysis approval process or a process built on generic categories. Some, looking at the large number of applications coming down the pipeline, advocate a shift from the current case-by-case review of experiments toward more of a generic approach. Possible strategies under this approach include categorical exemptions, licensing certain categories of tests. licensing individual scientists. or delegating authority to institutions (53). Others fully expect large-scale tests and commercialization. in particular, to be reviewed on a case-by-case basis. but they do encourage the rapid appearance of protocols or some other form of guidance so that safe and effective products can be developed (36).

Advocates of a case-by-case approach point to its flexibility. As different cases arise, each can be dealt with in a manner appropriate to its nature; no one set of rules and regulations, it is argued, will cover all of the many and varied applications of biotechnologgy.

Nonetheless, over time, as our experience and research base grows it is likely that some generic approaches to certain sorts of introductions in certain sorts of environments will emerge. The criteria by which these generic approaches are defined (some requiring more attention than others) will themselves change over time (74). These developments were anticipated in the ESA report (85), which made a significant step toward scaling risks. Eventually. generic categorizations of likely risk are probable, yet each case will need to be double-checked for any idiosyncratic particularity that could trigger more focused review. It is important for the successful application of biotechnology to agriculture that sufficient long-term flexibility is built into the regulatory and oversight system

so that risk management can evolve based on improved understanding.

Relative Risks Compared to Traditional Practices

Risk management involves the weighing of costs and benefits. To put planned introductions in context, their risks could be compared to risks of traditional practices in agriculture and society. For example, risks today are associated with the widespread use of chemical pesticides; accumulation of nonbiodegradable materials; toxic wastes; agricultural practices giving rise to genetic uniformity in farm animals and crops, with loss of biological diversity; and ''natural biological calamities, ' such as the current epidemic of AIDS. Not only are risks of planned introductions put into perspective by these nonbiotechnology-related problems, but biotechnology itself may help to solve some of them. For example, biotechnology can provide alternatives to chemical pesticides, assist in the degradation of toxic wastes, provide alternatives to selective inbreeding, and contribute to development of diagnostics and vaccines for AIDs and other illnesses (74).

On a more specific level of cost/benefit comparisons, new biotechnology techniques can be compared to those associated with traditional biotechnologies. (See table 8-1 for one view of such a comparison.) Certainly controversy exists—for instance, over the relative predictability of the ecological behavior of the phenotypes of transgenic organisms even when genotype changes are precise and well-understood. On the other hand, conventional breeding changes many genes simultaneously, with consequent multiple phenotypic changes. The newer, more precise techniques may actually show up well in the comparison.

Cost-Benefit Analyses

Risk management includes the weighing of risks or of actual costs on the one hand against benefits on the other, and then trying to achieve a reasonable balance (74). Agricultural biotechnology has potential to create positive benefits for agriculture. horticulture, range management, and forestry in the 21st century (43) if it is not stalled in its developmental stages; on the other hand, it is to no one's best interests to proceed without attention to identifying and minimizing any likelihood of risks. An appropriate balance is necessary.

In a time when the expansion potential of land for agriculture is small, when labor is expensive, and when additional use of chemicals in agriculture generally is regarded as a negative, the possible exploitation of new capabilities and new information through new technologies cannot be ignored. Thus, regulations that are not science-based could exact a very real ''cost, that of not introducing an innovative, promising product.

Small-Scale v. Large-Scale Issues

As agricultural biotechnology nears the commercialization stage, risk management must take into account a number of realities, as was mentioned in the previous chapter. For example, large plots at a number of locations are needed to test a recombinant corn line. This testing needs to be done within I to 2 years of the creation of the recombinant line for a company to stay competitive in the development of new varieties. Furthermore, many hybrids will be undergoing evaluation at the same time; several of these may contain the same recombinant gene and several recombinant genes might be examined simultaneously. In short, if the recombinant material goes

Table 8-l—Comparison of Traditional and Developing Biotechnology

Characteristics	Organismal	Cellular	Molecular
Processes	Breeding	Culture - Cell - Anther - Embryo	rDNA
	Selection	Regeneration	
	Mutation	Fusion	
Control over changes	Random	Semi-random	Directed, precise
Primary changes	Unknown	Semi-known	Known
Number of variants needed	Large	Intermediate	Small, in vitro selection methods
Species restriction	Mainly within	Within & across	Within & across
Familiarity	Very high	Intermediate	Low but expanding
Ability to ask and answer risk questions	Low	Intermediate	High
Containment	Dependent on organism and independent of method; established procedures for domesticated organisms.		

SOURCE: R.W.F. Hardy, '(Large-Scale Field Testing and Commercialization: Thoughts on Issues," *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and S.C, Henry (ads.) (Bethesda, MD: Agriculture Research Institute, 1991),

successfully and quickly through testing, the breeder will soon work to combine it with other useful traits, in different genetic backgrounds, as part of genetic improvement. Large numbers of new lines will emerge from the integration of recombinant genes into conventional breeding programs so that new hybrids can be tested and commercialized.

Specific recommendations for risk management of the transition to large-scale could include: I) making geographic maps of crop relatives and placing them in an accessible database, and 2) modifying the process for approving small-scale introductions based on experience base or familiarity (36). Marshaling evidence from experiences in agriculture, laboratory tests, introductions, field tests, and current, ongoing research will make possible reasoned risk assessment and management.

SCIENTIFIC METHODS OF MANAGING RISK

The power and precision of biotechnology can be harnessed for risk management itself. Controlling the spread of introduced genes through the manner in which they are introduced is one example. Risk management can be greatly aided by using supplementary transferred genes to ensure that the ensuing recombinant DNA-modified organism only functions on certain occasions, under certain environmental conditions, or for a finite period of time. The genetic modification can be designed to: 1) constrain the potential for gene transfer (increasing the "containment' of the gene within the organism into which it was inserted), and 2) maximize its key activity while **minimizing effects** in the recipient environment (60). Mechanisms for fine-tuned technical control of this sort still are being developed; a few approaches are described briefly here. In general. in addition to turning the gene on or off under certain conditions, several approaches to containment could be considered: ''autodestruct'" mechanisms (e. g., suicide genes), engineering genes such that the host has diminished survival (as through defective regulation of metabolism), and decreasing chances of horizontal gene transfer to other organisms (as through reducing the stability or ease of inheritance of the introduced genes) (19).

Promoters Turned On or Off by Specific Stimuli

One way to limit the effect of the engineered gene itself is to attach it to a promoter that only allows expression under certain conditions (83). When a gene is not being expressed, the physiological expenditure associated with expression of the gene can be allocated to other purposes. This maintains the "efficiency" of the organism and keeps the impact of the gene's phenotype to a minimum. For example, some genes are only expressed when triggered or induced (usually through a ''promoter' gene) by a certain chemical, such as a herbicide, or in the event of local disturbance of tissue, such as a wound response resulting from chewing by insects. A gene for some form of pest resistance attached to such an ''inducible promoter' gene would have little phenotypic impact except in the presence of a pest. This is a realistic strategy with diverse applications, some of which already have been field tested. For example, a field test was conducted by Iowa State University to assess whether or not transgenic tobacco plants would respond to insect attack by turning on an inserted gene. Plants often can respond to insect attack by activating genes coding for defensive compounds. Such compounds may, for instance, block the digestive system of insects, reducing their leaf consumption. A marker gene—one used to trace the success of the recombination experiment—(chloramphenicol acetyl transferase, CAT). modified from proteinase inhibitor II genes in the tomato family, was put into tobacco to determine levels of its activation by insects under actual field conditions. Upon insect attack on foliage, the transgenic plants showed induction of the transferred proteinase inhibitor genes. This has positive implications for using the wound-inducible inhibitor promoter in biological control of insect-caused foliage damage. The potential exists for a well-managed. efficient system, in which the inserted genes function only on an as-needed basis (83).

Suicide Genes

When it is important that particular recombinant DNA-modified organisms not establish viable populations, a mechanism that has been proposed for their containment is to include, along with the desired gene, a "suicide" gene that will sufficiently cripple the organism that it will not survive beyond its intended use. The suicide gene may, for example. prompt a metabolic pathway resulting in death of the cell in the presence of a specific external cue (44, 70). Another approach to containment is to introduce mutations that inactivate the transgenic organism's ability to synthesize necessary aromatic amino acids or other key metabolic pathways of the cell (19).

Alternatively. a "kill" gene can be inserted to be expressed constitutively — all the time-unless a ''protection" gene is turned on by the same promoter gene that causes expression of the key functional gene. That

297-937 0 - 92 - 9 *QL* 3

promoter can be geared to respond to some signal from the environment, such as temperature or presence of a pollutant chemical. For instance, if a protection gene for a vaccine strain is only activated above temperatures of 30 “C.. the vaccine organism will express the kill gene and die if it passes out of the host’s body (19).

The advantages of a “suicide” strategy are straightforward. Existing experimental data indicate that genetically modified microorganisms introduced into the environment usually fail to establish viable populations unless the numbers of introduced organisms are very large. To accomplish a useful effect, as in agricultural treatments or environmental clean-up, planned introductions of microorganisms generally will require inocula of large populations. Once the goal of the planned introduction has been met, a trigger factor to set off the suicide gene can be introduced that will leave behind only a small fraction of the introduced population, which may then be at too low a frequency to sustain itself.

Suicide genes are most frequently suggested for containment of microorganisms; their feasibility in plants has been questioned. With plants’ complicated physiology. difficulties could exist, for instance, in triggering the action of specific genes by any environmental cue other than some deliberate applied chemical, such as a herbicide (37). Overall, the potential effectiveness of suicide genes at this point is controversial (25). One key problem with the use of suicide genes is that natural selection would encourage the evolution of genetically based mechanisms counteracting the suicide effect.

Prevention of Gene Transfer

In the case of transgenic plants, concerns exist about the possible transfer of engineered genes to neighboring weedy populations of related species. One way to prevent gene transfer through pollen would be to shut down pollen production in the transgenic plant. This can be accomplished by introducing a male-sterility factor into the plant along with the desired trait. The use of naturally occurring male-sterility mutants has been a significant tool in traditional plant breeding. Quite recently, genes for male sterility have been cloned and reintroduced into several plant species, including canola (56). These genes were expressed in the transgenic plants and hence brought about male sterility. This strategy has a great deal of promise and currently is feasible. Its application to canola is especially pertinent because that species is among the most likel y to effect vector gene transfer to related species in North America.

For leafy crops (e.g., spinach) or root crops (e. g., sugar beets), male sterility would not be problematic. In fact, it has been suggested that male sterility used in timber tree plantations would channel more of a tree’s resources to board feet production, in lieu of reproduction. Some crops (e. g., cereals), however, require pollination, so that mixed varietal plantings of male sterile transgenic plants and male fertile. untransformed varieties could be needed (37).

Several strategies seem to have potential to decrease the risks associated with gene transfer between microorganisms. For example, a protection gene might be inserted far away from a kill gene. which itself is close to the desired gene being introduced to a host; then, if the functional gene happens to be transferred, the new recipient microorganism also would receive the kill gene, without the protection gene. Another approach might be to insert a gene for a particular active nuclease so that when a cell dies, its DNA—including the introduced fragment-released after death will have been significantly reduced. A variety of ways of inserting defects that would disrupt the host’s mobilization and conjugation systems could also cut down significantly on horizontal gene transfer (19). Engineering changes into a chromosome rather than a plasmid may decrease the likelihood of gene transfer between microorganisms; this approach also is being explored (45, 48).

Combinations of Genes

As the number of genes involved in a desired effect goes up. so does the possibility that that effect will be lost in the next and subsequent generations because of natural recombination. Thus, a possible strategy for decreasing the long-term probability of establishment of an engineered genetic effect would be to have the desired effect depend on the interaction among several separate genes.

AGRONOMIC METHODS OF MANAGING RISK

Physical Barriers

Complete containment was the preferred method of controlling risk when genetic engineering was introduced on a small scale. Examples of physical containment are “boundary strips” in the form of fences or hedgerows that can trap some large percentage of pollen. particularly that dispersed by wind. This might, however, be unfeasible to install or cause unwanted shade (37). Overall.

Photo credit: Grant Heilman, Inc.

A traditional approach to isolation of plants is to spatially separate desired plants from other plants. Similar guidelines for spatial segregation have been applied to transgenic plants as well.

a complete containment strategy has extremely limited applicability beyond small field tests. Once an organism has been placed in the field in the numbers required by agricultural production, it is likely to be exposed to a variety of biotic interactions beyond the control of reasonable physical barriers.

Spatial Barriers

The traditional approach to isolation of plants genetically improved through conventional breeding, usually for the purpose of generating a seed crop, is to isolate spatially the desired plants from other plants. Similar guidelines for spatial segregation have been applied to transgenic plants as well (64). Certainly this is feasible at the small field trial stage, and could be effective in an experimental setting to evaluate the properties of the organism as a potential pest. Some spatial separation may be feasible at the large-scale test stage, as well. Another approach to separating plants in terms of gene flow is to surround a field with flowers that will attract pollinators of the transgenic crop, so that these trap flowers rather than surrounding wild vegetation would be more likely to receive any transgenic pollen. This approach might conceivably diminish the pollinators' activity in pollinating the crop itself, however. Weed control practices using herbicides or cultivation could also decrease the chance of hybridization between the crop and wild species. A straightforward mechanism is to decrease the length of the boundary of the field and thus decrease the number of opportunities for neighbors along the boundaries to exchange genes. Large, square fields minimize these opportunities (37).

Temporal Barriers

Many problems associated with planned release could be addressed by the timing of the release. For example, if a given engineered line is released in an area with an uncultivated relative that could incorporate the engineered genes, one could manipulate the flowering (phenology) of the engineered organisms so that the crop did not flower at the same time as the weed. For example, wild relatives need short days for flowering, bush type green beans do not (72). Similarly, one could release the introduced plant at a time of year when the weed is dormant or even engineer the crops for cold tolerance, for example, to shift its flowering and production period away from that of its wild relatives. Agricultural experience and ecological understanding will play a significant role in the development of such barriers. Some agronomic practices such as irrigation can allow crop production at a time of year unfavorable for related weeds, diminishing the possibility of cross hybridization.

Crop rotation could be used to force a weed rotation. This could decrease the number of weed individuals present in the field and, therefore, the likelihood of gene transfer; it might also eliminate hybrids produced in preceding crop production periods. Crop rotation could prevent genes from being transferred to weeds outside the field for a whole season or two at a time, diminishing the chances that the gene would become established in the weed community and making it more likely to be lost due to genetic drift. The timing of harvesting could also build a barrier to cross hybridization. For some crops, such as cabbage, spinach, collards, lettuce, sugarbeets, carrots, turnips, radishes, celery, garlic, and onions, the crop product is vegetative; careful harvesting would remove the plants before their flowering, reproductive stage, thereby diminishing pollen transfer (37).

SUMMARY POINTS

Issues and concerns raised by planned introductions of recombinant DNA-modified organisms can be addressed by the integration of risk assessment methodologies with the currently existing knowledge base, continuously augmented by ongoing research and by additional data resulting from field tests. Risk management is therefore possible, with its chief compo-

nents being science-based regulation, scientific management methods, and agronomic management methods. A natural evolution of risk management and regulatory oversight is occurring as our experience base with field tests and in performing ecological risk assessments grows. This step-by-step progression in the use of recombinant DNA-modified organisms in the environment, emphasizing science-based risk assessment strikes a balance between a laissez-faire approach and a paralysis of the use of new technology. Biotechnology has the potential to contribute significantly to agriculture; scientifically sound risk assessment and management promote its acceptance as well as its safety.

CHAPTER 8 REFERENCES

1. Amarger, Noelle and Delgutte, Dominique, "Monitoring Genetically Manipulated *Rhizobium leguminosarum* bv *Viciae* Released in the Field," *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically y Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27-30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 22 1–228.
2. Antonovics, Janis and Grant, Michael, "Experimental Evidence on the Frequency of Neutral Mutations," *J. of Heredity 65: 241-242, 1974.*
3. Bakker, P. et al., *Biological Monitoring of Genetically Engineered Plants and Microbes,* D. R. MacKenzie and S.C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 201-204.
4. Barrett, S. C. H., "Crop Mimicry in Weeds," *Economic Botany* 37: *255–282,* 1983.
5. Berry, Duane F. and Hagedom, Charles, "Soil and Groundwater Transport of Microorganisms," *Assessing Ecological Risks of Biotechnology*, Lev R. Ginzburg (cd.) (Boston, MA: Butterworth-Heinemann, 1991), pp. 57–73.
6. Boyce Thomson Institute, *Regulatory Considerations: Genetically Engineered Plants,* Center for Science Information, San Francisco, CA, 1988.
7. Brill, W. J., "Safety Concerns and Genetic Engineering in Agriculture,'' *Science* 227:381–384,1985.
8. Brown, A.H. D. et al., *The Use of Plant Genetic Resources (New* York, NY: Cambridge University Press, 1989).
9. Caswell, H., Matrix Population Models, Sinauer Associates Inc., Sunderland, MA, 1989.
10. Colwell, R.K. et al. ," Letters, Genetic Engineering in Agriculture, ' *Science 229:* 11 1–1 12, 1985.
11. Colwell, R. K., ''Another Reading of the NAS Gene Report, ' *Bioscience* 38:421–423,1988.
12. Colwell, R. K., ''Natural and Unnatural History: Biological Diversity and Genetic Engineering, " *Scientists and their Responsibility,* W.R. Shea and B. Sitter (eds.) (Canton, MA: Watson International Publishing, 1989).
13. Covello, V. T., and Fiksel, J.R.(eds.), "The Suitability and Applicability of Risk Assessment Methods for Environmental Applications of Biotechnology, " Final report to the Office of Science and Technology Policy, Executive Office of the President, Report No. NSF/PRA 8502286, National Science Foundation, Washington, DC, 1985.
14. Crawley, Michael J., "The Ecology of Genetically Engineered Organisms: Assessing the Environmental Risks, " *Introduction of Genetically Modified Organisms into the Environment,* Harold A. Mooney and Giorgio Bemardi (eds.) (New York, NY: John Wiley and Sons, 1990) pp. 133-150.
15. Davis, B., "Bacterial Domestication: Underlying Assumptions, ' Science 235: 1329–1335, 1987.
16. Dean-Ross, D., "Applicability of Chemical Risk Assessment Methodologies to Risk Assessment for Genetically Engineered Microorganisms," *Rec.* DNA *Tech. Bull. 9(* 1): 16-28, 1986.
17. Doebley, J. "Molecular Evidence for Gene Flow Among *Zeq* Species, " *Bioscience 40: 443-448, 1990.*
18. Duvick, Donald N., "A Case Study: Development and Release of a Corn Hybrid for the U.S. Corn Belt, " talk presented at B1OTECH USA, Nov. 27–29, 1990, Washington, DC, 1990.
19. Dwyer, Daryl F. and Timmis, Kenneth, "Engineering Microbes for Function and Safety in the Environment, " pp. 79–98, *Introduction of Genetically Modified Organisms into the Environment,* Harold A. Mooney and Giorgio Bemardi (eds.) (New York, NY: John Wiley and Sons, 1990), pp. 167-189.
20. Ehler, L. E., "Planned Introductions in Biological Control, *Assessing Ecological Risks of Biotechnology*, Lev R. Ginzburg (cd.) (Boston, MA: Butterworth-Heinemann, 1991), pp. 21–39.
21. Ellstrand, N.C. and Hoffman, C. A., "Hybridization as an Avenue of Escape for Engineered Genes, ' *Bioscience* 40:438–442, *1990,* p. 40.
22. Faust, Robert M. and Jayaraman, Kunthala, "Current Trends in the Evaluation of the Impact of Deliberate Release of Microorganisms in the Environment: A Case Study with a Bioinsecticidal Bacterium, ' *[introduction of Genetically Modified Organisms into the Environment*, Harold A. Mooney and Giorgio Bernardi(eds.) (New York, NY: John Wiley and Sons, 1990), pp. 167-189.

23. Fiksel, Joseph and Covello, Vincent T., ''The Suitability and Applicability of Risk Assessment Methods for Environmental Applications of Biotechnology, " *Biotechnology? Risk Assessment: Issues and Methods for Environmental Introductions*, Joseph Fiksel and Vincent T. Covello (eds.) (Final Report to the Office of Science and Technology Policy, Executive Office of the President, Report No. NSF/PRA 8502286) (New York, NY: Pergamon Press. 1986), pp. 1–34.
24. Fiksel, Joseph, "Applications of Knowledge Systems for Biotechnology Risk Assessment and Management,'' *Risk Assessment in Genetic Engineering,* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 199 l), pp. 354-367.
25. Fox. J.L., ' 'Suicide as [b]''Painless' Eco-Safeguard'!' *Bio/Tech* 7:412–413, 1989.
26. Frederick, Robert J. and Pilsucki, Robert W., "Nontarget Species Testing of Microbial Products Intended for Use in the Environment, " *Risk Assessment in Genetic Engineering.* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. 32-50.
27. Fristrom, J.W. and Clegg, M. T., *Principles of Genetics,* 2d ed. (New York, NY: W.H. Freeman, 1988).
28. Fuchs. Roy L. and Serdy, Frank, S., "Genetically Modified Plants: Evaluation of Field Test Biosafety Data, ' *Biological Monitoring of Genetically? Engineered Plants and Microbes,* D. R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafet y Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27-30, 1990) (Bethesda. MD: Agriculture Research Institute, 199 l), pp. 25–29.
29. Fuxa, James R., "Release and Transport of Entomopathogenic Microorganisms, *Risk Assessment in Genetic Engineering.* Morris A. Levin and Hat-lee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1990), pp. 83–1 13.
30. Gillett, J. W., "Risk Assessment Methodologies for Biotechnology Impact Assessment, " *Potential Impacts of Environmental Release of Biotechnology Products: Assessment, Regulation, and Research Needs,* J.W. Gillett et al. (eds.), Report no. ERC-075, Ecosystems Research Center, Cornell University, Ithaca, NY, 1985.
31. Ginzburg, L.R. (cd.), *Assessing Ecological Risks of Biotechnology* (Boston, MA: Butterworth-Heinemann, 1991).
32. Goldburg, 'Rebecca et al., *Biotechnology's Bitter Harvest: Herbicide-Tolerant Crops and the Threat to Sustainable Agriculture,* Biotechnology Working Group Report, 1990.
33. Gould, F., "Genetics of Plant-Herbivore Systems: Interactions Between Applied and Basic Study, " *Variable Plant and Herbivores in Natural and Managed Systems*, R. Denno and B. McClure (eds.) (New York, NY: Academic Press. 1983), pp. 599-653.
34. Gould, F., "Evolution of Resistance to Toxic Compounds by Arthropods, Weeds and Pathogens, " commissioned background paper prepared for the Office of Technology Assessment, 1991.
35. Handel, S. N., "Pollination Ecology, Plant Population Structure, and Gene Flow, " *Pollination Biology,* L. Real (cd.) (New York, NY: Academic Press, 1983), pp. 163-211.
36. Hardy, Ralph W .F., ''Large-Scale Field Testing and Commercialization: Thoughts on Issues, " *Biological Monitoring of Genetically}' Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991, pp. 239–248.
37. Hoffman, Carol, "Exchange of Genetic Material Between Genetically Engineered Crops and Close Relatives, commissioned background paper prepared for the Office of Technology Assesment, 1991.
38. Istock, Conrad A., "Genetic Exchange and Genetic Stability in Bacterial Populations, " *Assessing Ecological Risks of Biotechnology*, Lev R. Ginzburg (cd.) (Boston, MA: Butterworth-Heinemann, 1991), pp. 123-149.
39. Kareiva, Peter, Manasse, Robin, and Morris. William, "Using Models to Integrate Data from Field Trials and Estimate Risks of Gene Escape and Gene Spread, ' *Biological Monitoring of Genetically Engineered Plants and Microbes*, D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, November 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 31–42.
40. Katz, Lori S. and Marquis, Judith K., "The Toxicology of Genetically Engineered M icroorganisms, *Risk Assessment in Genetic Engineering,* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. 51-63.
41. Kearney, Philip C. and Tiedje, James M., "Methods Used to Track Introduced Genetically Engineered Organisms, " *Biotechnology> for Crop Protection,* Paul Hedin, Julius Menn, and Robert Hollingworth (eds.) (Washington, DC: American Chemical Society, 1988), pp. 352–358.
42. Keeler, Kathleen H., "Can Genetically Engineered Crops Become Weeds'?" *Biotechnology vol. 7:* 1134–1139, 1989.
43. Keeler, Kathleen H., Turner, Charles E., "Management of Transgenic Plants in the Environment, *Risk Assessment in Genetic Engineering,* Morris A.

Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, inc., 1991), pp. 189-218.
44. Kim, Hunhyong, Ginsburg, Lev R.,andDykhuizen, Daniel E., "Quantifying(heRisks of Invasion by Genetically Engineered Organisms," *Assessing Ecological Risks of Biotechnology*, Lev *R.* Ginzburg (ed.) (Boston, MA: Butterworth-Heinemann, 1991), pp. 193-214.
45. Kluepfel, D.A. et al., "FieldTesting of a Genetically Engineered Rhizosphere Inhabiting Pseudomonad: Development of a Model System, ' *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 189– 199.
46. Kluepfel, D.A. and Tonkyn, D. W., "Release of Soil-Borne Genetically Modified Bacteria: Biosafety Implications from Contained Experiments, *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 55–65.
47. Kostka, Stanley J., "The Design and Execution of Successive Field Releases of Genetically Engineered Microorganisms, *Biological Monitoring of Genetically Engineered Plants and Microbes,* D. R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute. 1991), pp. 167-176.
48. Lacy, George H., Stromberg. Verlyn K.. ''Pre-Release Microcosm Tests with a Genetically Engineered Plant Pathogen, ' *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27-30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 81-98.
49. Langevin, S. A., Clay, K., and Grace, J. B.. "The Incidence and Effects of Hybridization Between Cultivated Rice and Its Related Weed Red Rice (Oryza sativa L.), " *Evolution 44(4): 1000-1008, 1990.*
50. Levin, Morris and Strauss, Harlee, "Introduction: Overview of Risk Assessment and Regulation of Environmental Biotechnology," *Risk Assessment in Genetic Engineering,* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. 1-17.
51. Levin, Simon, "Ecological Issues Related to the Release of Genetically Modified Organism into the Environment, ' *Introduction of Genetically Modified Organisms into the Environment,* Harold A. Mooney and Giorgio Bernardi (eds.) (New York, NY: John Wiley and Sons, 1990), pp. 151-159.
52. Levy, Stuart B. and Miller, Robert V. (eds.) *Gene Transfer in the Environment (New* York, NY: McGraw-Hill Publishing Co.), 1989.
53. MacKenzie, D.R. and Henry, Suzanne C., "Towards a Consensus, *Biological Monitoring of Genetically Engineered Plants and Microbes,* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically y Modified Plants and Microorganisms. Kiawah Island, SC, Nov. 27-30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 273–283.
54. Manasse, Robin and Kareiva, Peter, ''Quantifying the Spread of Recombinant Genes and Organisms, ' *Asses-sing Ecological Risks of Biotechnology*, Lev R. Ginzburg (cd.) (Boston, MA: Butterworth-Heinemann, 1991), pp. 215–231.
55. Maniatis, T., Fritsch, E. F., and Sambrook, J., *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1982).
56. Mariani, C. et al., "Induction of Male Sterility in Plants by a Chimaeric Ribonuclease Gene, ' *Nature* 347:737–741, 1990.
57. McIntosh, Marla S., "Statistical Techniques for Field Testing of Genetically Engineered Microorganisms, *Risk Assessment in Genetic Engineering,* Morris A. Levin and Hat-lee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. 219-239.
58. Meagher, T. R., "Analysis of Paternity Within a Natural Population of *Chamaelirium luteum, 1.* Identification of Most-Likely Male Parents, ' *Amer. Nat 128: 199-215. 1986.*
59. Merkhofer, M. and Covello, V., *Risk Assessment and Risk Assessment Methods: The State of the Art,* NSF study, Washington, DC, as cited in Fiksel and Covello, 1986.
60. Miller. Robert V. and Levy, Stuart B., "Horizontal Gene Transfer in Relation to Environmental Release of Genetically Engineered Microorganisms, ' *Gene Transfer in the Environment*, Stuart B. Levy and Robert V. Miller (eds.) (New York, NY: McGraw-Hill Publishing Co. 1989), pp. 405-420.
61. Moffat, Anne, "Workshop Discusses Biosafety Aspects of Oilseed Crucifer Field Trials, " *Genetic Engineering News:* January 1991: p. 15.
62. Mooney, Harold A. and Bernardi, Giorgio (eds.), *Introduction of'Genetically Modified Organisms into*

the Environment (New York. NY: John Wiley and Sons, 1990).

63. Mooney, Harold A. and Drake, J. A., "The Release of Genetically Designed Organisms in the Environment: Lessons from the Study of the Ecology of Biological Invasion s." *Introduction of Genetically> Modified Organisms into the Environment*, Harold A. Mooney and Giorgio Bernardi (eds.) (New York, NY: John Wiley and Sons, 1990). pp. 117–129.
64. Muench, Stephen R., ''Requirements and Considerations in Successful Field Releases of Genetically Engineered Plants, ' *Biological Monitoring of Genetically Engineered Plants and Microbes*. R. MacKenzie and S.C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov · 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 199 l), pp. 3–7.
65. Mullis, Kary B., "The Unusual origin of the Polymerase Chain Reaction,' " *Scientific American*, April 1990: 56-65.
66. Naimon, Jonathan S.. ' 'Using Expert Panels to Assess Risks of Environmental B iotechnology Applications: A Case Study of the 1986 Frostban[R] Risk Assessments, *Risk Assessment in Genetic Engineering*, Morris A. Levin and Harlee S. Strauss (cd\.) (New York. NY: McGraw-hill. Inc., 1991), pp. 319–353.
67. National Academy of Sciences (NAS), National Research Counc i]. *Risk Assessment in the Federal Government: Managing the Process (Washington,* DC: National Academy Press, 1983).
68. National Academy of Sciences. *Introduction of Recombinant DNA -Engineered Organisms into the Environment: Key Issues*, Washington, DC, 1987.
69. National Research Council, *Field Testing Genetically Modified Organisms: Framework for Decisions (* Washington, DC: National Academy Press, 1989).
70. Olson. B.H. et al., ''The Implications of Horizontal Gene Transfer for the Environmental Impact of Genetically Engineered Microorganisms, *Risk Assessment in Genetic Engineering*, Morris A. Levi n and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill. Inc., 1991), pp. 163-188.
71. Parker, M., ' 'The Pleiotropy Theory for Polymorphism of Disease Resistant Genes in Plants,'' *Evolution* 44: 1872-74, 1990.
72. Purse glove. J. W., *Tropical Crops, Dicotyledons (London.* England: Longman, 1974).
73. Rees, Mark et al., "An Ecological Perspective to Risk Assessment, *Biological Monitoring of Genetically Engineered Plants and Microbes*, D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island. SC, Nov. 27-30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991, pp. 9– 19.
74. SCOPE/COGENE, Joint Statement, Mooney and Bernard, 1990, pp. xvii–xxiv.
75. Sharples, Frances E., "Regulation of Products from Biotechnology, ' *Science* 235: 1329–1332, 1987.
76. Sharples, Frances E., "Ecological Aspects of Hazard Identification for Environmental Uses of Genetically Engineered Organisms, *Risk Assessment in Genetic Engineering,* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. **18-31.**
77. Simmonds, N. W., *Evolution of Crop Plants (New* York. NY: Longman, Inc., 1976).
78. Smith, L.B. and Adams. W.'I'., ' 'measuring Pollen Contamination in Clonal Seed Orchards With the Aid of Genetic Markers, " *Proc. 17th South. Forest Tree Improv. Conf.* Athens, GA. 1983. pp. 69-77.
79. Steffan, R.J. and Atlas, R. M., '" DNA Amplification to Enhance Detection of Genetically Engineered Bacteria in Environmental Samples.. *Applied and Environmental Microbiology* 54: 2185-2191, 1988.
80. Stotzky, G., "Gene Transfer Among Bacteria in Soil, ' *Gene Transfer in the Environment*, Stuart B. Levy and Robert V. Miller (eds.) (New York, NY: McGraw-Hill Publishing Co., 1989), pp. 165–222.
81. Strauss, Harlee S., "Lessons from Chemical Risk Assessment,'' *Risk Assessment in Genetic Engineering*, Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill. Inc.. 1991), pp. 297–318.
82. Thompson. Elizabeth, *Pedigree Analysis in Human Genetics (* Baltimore, MD: Johns Hopkins University Press, 1986).
83. Thornburg, Robert W., ''Modes of Expression of a Wound- inducible Gene in Field Trials of Transgenic Plants, ' *Biological Monitoring of Genetically Engineered Plants and Microbes*, D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafet y Results of Field Tests of Genetically Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 199 l), pp. 147– 154.
84. Tiedje, James M., ''Environmental Monitoring of Microorganisms (Appendix G), " *Prospects for Physical and Biological Containment of Genetically Engineered Organisms.* James W. Gillett (cd.) Proceedings, the Shackleton Point Workshop on Biotechnology Impact Assessment, Oct. 1–4, 1985, Ecosystems Research Center, Cornell University. 1985.
85. Tiedje, James M. et al., "The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations. " *Ecology 70(* 2):298–315, 1989 (introduction special feature by H.A. Mooney and P.G. Risser).

86. Tiedje, James M. "Ecological Principles and Experiences in the Environmental Release of Genetically Engineered Organisms, ' pp. 115– 127, *Advances in Biotechnology: Proceedings of an International Conference*, organized by Swedish Council for Forestry and Agricultural Research and Swedish Recombinant DNA Advisory Committee, pp. 115– 130, SJFR, Stockholm, 1990.

87. Tolin, Sue A., "Persistence, Establishment, and Mitigation of Phytopathogenic Viruses, " *Risk Assessment in Genetic Engineering,* Morris A. Levin and Harlee S. Strauss (eds.) (New York, NY: McGraw-Hill, Inc., 1991), pp. 1 14–139.

88. Torsvik, Viadis, Goksoyr, Jostein, and Daae, Fridalise, "High Diversity in DNA of Soil Bacteria, " *Applied and Environmental Microbiology>.* vol 56(3):782–787, *1990.*

89. Transgenic Plant Workshop. Final Report, Maryland Biotechnology Institute and USEPA Office of Pesticide Programs, Jun. 18–20, 1990.

90. Upper, Christen D. and Hirano, Susan S., *'Aerial Dispersal of Bacteria, " *Assessing Ecological Risks of Biotechnology?',* Lev R. Ginzburg (cd.) (Boston, MA: Butterworth-Heinemann, 1991, pp. 75–93.

91. U.S. Congress, Office of Technology Assessment, *Impacts of Applied Genetics: Microorganisms, Plants, and Animals, (OTA-BA-218)* (Washington, DC: U.S. Government Printing Office, 1981).

92. U.S. Congress, Office of Technology Assessment, *New Developments: Biotechnology>: Field Testing Engineered Organisms, Genetic and Ecological Issues,* (OTA-BA-350) (Washington, DC: U.S. Government Printing Office, 1988).

93. van Elsas, J. D., Heijnen, C. E., and van Veen, J. A., ''The Fate of Introduced Genetically Engineered Microorganisms (GEMs) in Soil, in Microcosm; and the Field: Impact of Soil Textural Aspects, " *Biological Monitoring of Genetically Engineered Plants and Microbes.* D.R. MacKenzie and Suzanne C. Henry (eds.) (International Symposium on the Biosafety Results of Fieldtests of Genetically y Modified Plants and Microorganisms, Kiawah Island, SC, Nov. 27–30, 1990) (Bethesda, MD: Agriculture Research Institute, 1991), pp. 67–79.