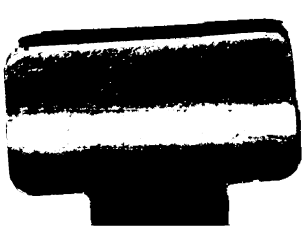
IICA

GUIDELINES FOR THE RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED ORGANISMS

ORGANIZATION OF AMERICAN STATES
INTERNATIONAL OFFICE OF EPIZOOTICS

PROGRAM II:
TECHNOLOGY GENERATION AND TRANSFER



ISSN-0534-5391

GUIDELINES FOR THE RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED ORGANISMS

ORGANIZATION OF AMERICAN STATES
INTERNATIONAL OFFICE OF EPIZOOTICS

PROGRAM II:
TECHNOLOGY GENERATION AND TRANSFER

MISCELLANEOUS PUBLICATIONS
SERIES

ISSN-0534-5391
A1/SC-91-13

June, 1991
San Jose, Costa Rica

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE/
CANADIAN INTERNATIONAL DEVELOPMENT AGENCY PROJECT

The general objective of the IICA/CIDA Project is to strenghten the conceptual and operational development of IICA's five Programs of action, in the technical areas having high priority in the Institute's Medium Term Plan and the PLANLAC. Through the IICA Programs, the IICA/CIDA Project, with the collaboration of Agriculture Canada, supports the efforts of the countries to reactivate and modernize their agricultural sectors, in a framework of ever-improving relations between Canada and the countries of Latin America and the Caribbean.

INDEX

PROLOGUE

Advances in molecular biology continue to proceed at a fast rate. More and more genes responsible for specific diseases are being identified and the first actual gene therapy program in man has been authorized in the U.S. Genes have been introduced into several plants which make them resistant to herbicides and insects. Even chicken resistant to Marek's disease have been produced, by inserting DNA from the virus into the germline.

These feats are but a few of the examples which can be accomplished though biotechnology and they show how near the market place some of them are. Actually, several genetically modified organisms obtained through biotechnology have already been submitted to the regulatory process required for large scale applications and commercial production. Several transgenic plants are expected to clear the regulatory requirements by next year.

The regulatory assessment of biotechnology products, specially live organisms, has become one of the more important and costly phases of the development of commercial products. This even when the safeguards and requirements have been simplified and reduced significantly, as experience has shown that the initial fears regarding the safety of DNA recombinant technologies and others may have been exaggerated.

No biotechnology regulations exist in Latin America and the Caribbean, with the exception of a few research institutes that have established internal biosafety assessment procedures for work with biotechnological techniques. This is not only because of the small research effort currently being undertaken in the region, but also because of the lack of political or public pressure to establish these regulations. But with the rapid advent of commercial live products obtained through biotechnology, it is urgent to establish in each country of the region adequate mechanisms and norms to safeguard public health and the environment from any foreseeable and significant risks. The guarantee of standards equal to the ones applied in developed countries is an important objective, so as to maintain the confidence of the scientists and the general public in the new technologies. This is in the best interest also of companies and research institutes which need clear guidelines for their work. The rapid access to the latest technology by Latin American

and Caribbean countries, essential for maintaining and increasing the productivity and competitiveness of their agriculture and industry, will depend heavily on the existence of this regulatory framework.

The policy and approaches towards biosafety in developed countries are not necessarily the most adequate for developing countries. There is therefore a need for adapting them to the local circumstances, on the basis of the experience of the more advanced countries. This can be achieved best on a regional basis, due to the lack of national expertise and resources, as the cooperative action of the Inter American Institute for Cooperation on Agriculture, the Pan American Sanitary Bureau, the Organization of American States, the International Office of Epizootics and the U.S. Department of Agriculture in this matter shows.

The first meeting of the Interamerican Study Group on The New Biotechnologies, created by these organizations, held in San Jose, Costa Rica in 1988, produced the Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology, widely distributed in the region. The second meeting, held in Brasilia, May 30 to June 1st 1990, wrote and recommended the present guidelines for the release into the environment of genetically modified organisms. They represent the consensus view of the distinguished group of experts which attended the meeting, and constitute a solid base for the establishment of any national regulatory approach in the region.

The molecular methods are exciting and powerful and allow the movement of genes across biological barriers. At the same time, information on the ecology of such organisms and experience on their introduction into the environment is limited. These ecological uncertainties must be addressed scientifically. Possible adverse effects can be minimized or eliminated by appropriate measures to assess and confine the initial introduction to a specific environment. There follows in the Guidelines a framework for evaluating these risks prior to limited or general release of products into the environment. We trust that the treatment of these issues agreed upon by the Inter-American Study Group, provide a basis for sound decision making processes in the region.

Dr. Carlyle Guerra de Macedo
Director
Pan American Sanitary Bureau
PAHO/WHO
Washington, D.C.

Dr. Martin Piñeiro
Director General
Inter-American Instutute for Cooperation on Agriculture
San Jose, Costa Rica

Dr. Miguel Laufer
Director
Department of Scientific and Technological Affairs
Washington, D.C.

Dr. Louis Blajan
Director General
International Office of Epizootics (OIE)
Paris, France

1. INTRODUCTION

The release of genetically modified organisms into the environment has currently drawn much attention in many countries. In many cases research and development in biotechnology have been advanced up to the stage of field tests of the modified organisms, phase previous to the final stage of commercialization of these products. Concern with potentially adverse effects these modified organisms may have on the environment and on public health have been raised. In many countries, this concern has lead to regulations of field tests and larger scale introductions of these organisms into the environment.

Efforts on research and development of the new biotechnologies in Latin America has been significantly smaller than efforts undertaken by countries belonging to the OECD. Nevertheless, as noticed in a recent survey conducted by IICA, there are at least eight projects in the region whose goal is to develop transgenic organisms (plants and microorganisms), i.e. organisms containing foreign genetic material introduced by means of genetic engineering techniques. On the other hand, multinational companies are already interested in conducting tests of their transgenic products in various countries of the region. Since many of these products will soon be approved by regulatory agencies in their home countries, it may not be too long before they will become available in the international market. As an example, as of 1990, the United States Department of Agriculture had approved 78 field tests of transgenic plants. These two facts justify the need for the rapid introduction of appropiate mechanisms to be used when assessing and approving of field tests, and granting import and commercialization licenses for these new products.

The present guidelines wish to offer the competent authorities as well as scientists and businessmen from Latin America and the Caribbean, a detailed technical and operational framework which can be used by them to assess the environmental risks associated with the release of genetically modified organisms in the environment. These guidelines are based on the world's most recent experience on this matter, particularly on an approach followed by Canada. The Canadian approach was analized and modified to fit regional requirements during a meeting of the Interamerican Study Group on the New Biotechnologies conducted in Brasilia on May, 1990. These guidelines are based on the tenet that any

evaluation should focus on the product independently of the process by which it was obtained. Given the little international experience presently available, they advice that any judgements and decisions should be made on a case by case basis. This, in turn, suggests that a maximum degree of flexibility should be maintained so that changes and novel concepts, expected to arise with the advent of new developments in this topic, may be incorporated.

The following methodology was used in the elaboration of these guidelines. A first draft of the guidelines, made by IICA, was submitted to the Interamerican Study Group on the New Biotechnologies. This group was divided into four working units (human health, animal health, plants, and microorganisms) who were to analize these guidelines for two days. Each subgroup's recommendations and conclusions were presented in a Plenary session, during which additional recommendations were made. Walter Jaffé and Jerry Callis incorporated all these conclusions and recommendations to the first draft. A second draft of the guidelines was sent to each of the members of the Study Group for their review. The current and final draft includes all the observations made during this second revision.

With the sole exception of one case in Mexico, in Latin America and the Caribbean there are no mechanisms, nor is there any expertice, on the evaluation and regulation of the release of genetically modified organisms into the environment. We expect that the present guidelines will represent a common and coordinated framework to be used in the regulation of this important phase in the development of products obtained through the new biotechnologies in Latin America and the Caribbean. This chance to develop an harmonious policy will ease the integration and free market exchange of biotechnology products and enterprises between countries of the region.

2. GENERAL ASPECTS OF THE REGULATION OF BIOTECHNOLOGY

DEFINITIONS

2.1. For the purpose of this document regulation is defined in the broadest possible sense as an oversight term, which covers principles, guidelines and standards, as well as legislation.

2.2. "Plants" include: Gymnosperms, Angiosperms, and lower plants, including fungi which form sporophores, algae and microalgae, excluding cyanobacteria.

2.3. "Microorganisms" include: viruses, bacteria including cyanobacteria, protozoa and fungi, excluding fungi which form sporophores, and other unicellular non-photosythetic organisms.

2.4. Veterinary biologics include animal vaccines, diagnostic reagents and monoclonal antibodies used in the treatment of diseases and include those produced by conventional and new techniques of biotechnology.

OBJECTIVES OF REGULATION

2.5. Primary considerations to be taken into account in the development of regulatory policies, guidelines and directives include:

- protection of public health and the environment
- addressing the legitimate concerns of the general public regarding the safety of biotechnology
- promotion of international trade and cooperation
- the development of national capabilities in biotechnology, including research and development, training and industry.

SCOPE AND GENERAL PRINCIPLES OF REGULATION

2.6. This document applies to genetically modified organisms in general, that is, organisms obtained by classical and by molecular techniques of genetic modification.

2.7. No conceptual distinction exists between genetic modifications of plants and microorganisms by classical methods or by molecular methods that modify the DNA and transfer genes.

2.8. From the point of view of the introduction into the environment, no conceptual distinction exists between a genetically modified

organism and an exotic one (organisms not present in an ecosystem or geographical location). These Guidelines apply therefore to both cases.

2.9. Genetic engineering activities done at the laboratory, in contained conditions, are regulated in many countries by a voluntary notification to some competent authority at the organization or national level. Genetic engineering activities in non-contained conditions, such as field tests of genetically engineered organisms, are regulated in some countries by legally competent institutions, under existing laws, and in a few instances by special laws. This regulation is generally through permits for field tests or the licensing of products.

2.10. The use of recombinant DNA or other genetic engineering techniques does as such, represent special risks which cannot be handled under the good laboratory practices concepts. The regulation of biotechnology, therefore should address the products obtained with these techniques. Safety assessment of a recombinant DNA (rDNA) modified 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.

REGULATION OF PLANT BIOTECHNOLOGY

2.11. Transgenic plants, irrespective of where they were produced, should be evaluated on safety aspects to ascertain the possible impacts to man and the environment.

2.12. Most Latin American and Caribbean countries have plant quarantine systems and regulations to deal with plant introductions which need to be supplemented both in infrastructure and training to approach the new biotechnologies.

2.13. Latin American and Caribbean countries should seek a consensus on their regulation to facilitate the assessment of these impacts.

2.14. Latin American and Caribbean countries are strongly advised to obtain all information necessary for the evaluation of transgenic plants from the institutions -locally or internationally- from which these plants were obtained, on a government to government

basis, before any tests or releases are made.

REGULATION OF VETERINARY BIOTECHNOLOGY

2.15. In most instances the regulation of veterinary biotechnology products is covered by current legislation. It is therefore appropriate to develop guidelines for regulating the biotechnology products instead of proposing new legislation.

2.16. Based on the biological characteristics of the new products and on safety aspects, biotechnological veterinary biologics can be classified as follows:

Class I:

Inactivated rDNA derived viral vaccines
Inactivated rDNA derived bacterial vaccines
Viral, bacterial, cytokines or other products
Monoclonal antibodies (hybridoma) products
Vaccines containing live organisms modified by gene deletion or insertion (no foreign DNA).

Class II:

Vaccines using a live vector to carry recombinant derived foreign genes
Vaccines containing live organisms modified by gene insertion or deletion (introduction of foreign DNA).

The definition of each of these products is presented in Appendix I.

3. DEFINITION AND GENERAL PRINCIPLES OF PLANNED RELEASES INTO THE ENVIRONMENT

DEFINITION OF PLANNED RELEASE

3.1. A planned release is considered to be any experimental trial, commercial production or use of product which involves the use

of organisms (organisms new to the ecosystem, genetically modified or otherwise), in non-contained conditions such as:

i. In open fields, paddocks and natural ecosystems;

ii. In enclosed facilities which are not contained, e.g. shade house, animal pens;

3.2. These guidelines are intended also to encompass work which is not intended for releases as such, but which is to be performed in non-contained facilities or restricted field locations, since such work may allow incidental releases to the environment.

3.3. Stages in the introduction of organisms:

Laboratory/Greenhouse
Small scale field research
Large scale field research
Commercial production and distribution

In the case of live biotechnology veterinary products:

Laboratory research and development
Controlled containment experiments
Limited field trials using target species
Licensing of the product

In the case of research in humans, the following stages are common:

Laboratory trials
Preclinical studies
Clinical studies Phase I, Phase II and Phase III.

REGULATION OF RELEASES

3.4. The limit for each stage must be defined on a case by case basis, depending on the nature of the organism. Case-by-case means an individual review of a proposal against assessment criteria which are relevant to the particular proposal.

3.5. Governments are advised to put mechanisms in place for the oversight of the various stages, e.g. laboratory/greenhouse, field research, scale-up and commercialization. Governments should decide the level of oversight for each stage

3.6. The oversight procedure should be taken in accordance with the level of the risk involved in each case, based on the nature of the organisms, the scale of the trial and whether clinical trials in humans are involved.

3.7. Review of potential risks should be conducted on a case-by-case basis, prior to application; this is not intended to imply that every case will require review by national or other authorities since various classes of proposals may be excluded.

3.8. The regulatory conditions to be met for each stage in the case of biotechnological veterinary biologics are summarized in Appendix II.

3.9. It is recommended that in the cases of clinical trials involving human beings the ethical code of the World Health Organization (WHO) be applied.

3.10. The review and oversight of releases into the environment should be the responsibility of the Institutional Biosafety Committees (IBC) on the level of the institutions, and of the National Biosafety and Technical Advisory Committee (NBTAC) on a national level.

3.11. For the definition and terms of reference of the IBC and the NBTAC the Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology, prepared by the Inter American Study Group on the New Biotechnologies and published by IICA/PAHO/OAS/OIE should be used.

4. SMALL SCALE FIELD TRIALS

PRINCIPLES AND PRACTICES OF FIELD RESEARCH: GOOD DEVELOPMENTAL PRACTICES

4.1. In the case of small scale field trials with plants and micro-organisms the concept of Good Developmental Practices for Small Scale Research with Genetically Modified Plants and Microorganisms, proposed by the OECD, should be used.

4.2. The Good Developmental Practices (GDP) are intended as scientific guides for the performance of low or negligible risk field research for whatever purposes, including basic and applied research. They are not intended to preclude individual national approaches to regulation of field research with plants and microorganisms.

4.3. The concept embodied in GDP is that a set of experimental conditions can be identified under which small scale field research of low or negligible risk can be conducted with a specific genetically modified organism. The key factors in determining the safety of any specific experiment are:

i. the characteristics of the organism(s) used;
ii. the characteristics of the research site; and
iii. the use of appropriate, scientifically acceptable, and environmentally sound experimental practices.

4.4. The first working assumption for GDP is that certain scientific principles relate to the organism, the research site, and the experimental practices have varied relative importance in determining whether an experiment is of low or negligible risk.

A second assumption is that a conclusion regarding risk of an experiment can be reached by evaluating the relevant factors and their interaction under the conditions of the experiment.

The third assumption is that the interaction of these factors is easier to address in small-scale field experiments because of their limited scope.

4.5. Certain organisms may have characteristics such that their use under a broad range of conditions would be considered to be of low or negligible risk. Other organisms with known adverse effects may be acceptable for field experiments provided the experimental design presents a situation in which it is possible to maintain control of these adverse effects by mitigation methods and/or confinement of the research organism or its genetic material to a restricted research site.

4.6. A number of assumptions are also made concerning the key factors which determine the safety of any specific experiment. These assumptions are described below for the characteristics of the organism; the characteristics of the site; and the experimental practice.

Characteristics of Organisms

4.7. Plants - The plants most likely to be tested are domesticated, can be reproductively isolated, and are not likely to persist in a noncultivated environment or even in a test plot. Characteristics of plants which would be considered include:

i. the reproductive biology of the plant, such as its flowers, pollination requirements and seed characteristics, and an extended history of controllable reproduction with lack of dissemination and establishment in an environment comparable to the research site;

ii. the possibility of hybridization between genetically modified plants and their wild relatives. A list of known and suspected relatives should be requested;

iii. the mode of action, persistence, and degradation of any newly acquired toxic compound;

iv. the nature of biological vectors used in transferring DNA to plants.

4.8. Microorganism - As distinct from plants, tests with microorganisms usually involve large populations, some portion of which

may persist. The individual organisms in these populations cannot always be genetically isolated, e.g. the possibility of horizontal DNA transfer cannot always be excluded in microorganisms.

Microorganisms must be thought of in statistical terms that consider the probability of an event occurring in a given population/environment.

Characteristics of microorganisms which would be considered include those affecting:

i. dispersal, survival and multiplication;

ii. interactions with other species and/or biological systems

iii. potential for gene transfer.

Characteristics of the Research Site

4.9. The research site can be chosen both to design field trials of low or negligible risk, and to meet the objectives of the research. The term "site" is intended to include the research plot proper and an appropriate part of the surrounding environment.

4.10. At the small scale stage of research, since the affected environment is generally more localized than at other stages, it should be possible for the investigator to choose a research site most suitable from the safety aspect by identifying for example:

i. important ecological and/or environmental considerations relative to safety in the specific geographical location (e.g. highwater table, heavy field run-off, etc.);

ii. climatic conditions;

iii. size, e.g. physical area;

iv. an appropriate geographical location in relation to proximity to specific biota that could be affected.

The safety of research can be augmented by choosing a site

comparable to one in which there is an extended history of relevant research and where dissemination and establishment have not been observed beyond the site.

Experimental Practice

4.11. Like any other research, scientifically acceptable and environmentally sound field research requires careful experimental design, e.g. formulation of an hypothesis and statement of objectives; development of specific methodologies for the introduction of organisms, monitoring, mitigation; a precise description of the design of experiments, including planting density and treatment patterns; and the description of specific data to be collected, and of methods of analysis to test for statistical significance.

4.12. Environmental sound practices for such research include: choosing an appropriate geographical location in relation to proximity to significant biota that could be affected; characterizing the research site including, for example, size and preparation, climatic features; designing introduction protocols including quantity and frequency of applications; choosing methods of site preparation and cultivation; choosing methods for confinement, decontamination, monitoring, mitigation; designing treatments applicable to the research; developing suitable safety and handling procedures for application of contingency plans in the event of the need for early termination of an experiment.

OPERATION OF GDP

4.13. This section outlines the operation of GDP to conduct safely small-scale field research with genetically modified plants and microorganisms, and to assist in designing low or negligible risk field experiments. GDP is intended to provide general concepts that allow flexible national approaches to the design and evaluation of field research.

Appropriate Experimental Practices

4.14. This part presents a set of practices which should be carefully considered when conducting small scale field research with plants and microorganisms. Researchers designing and conducting

these field experiments should develop protocols and codes of practice showing how they intend for example to:

1. Keep numbers of the modified organism to the lowest practicable level appropriate for the experiment.

2. Exercise measures to limit dispersal and establishment from the source and to supplement these measures when appropriate.

3. Monitor adequately the organism within the research site, and be prepared to apply control or mitigation measures if appropriate and necessary to avoid unintended adverse environmental effects during or at the termination of the experiment.

4. Test for the presence of established organisms or, where appropriate, transferred genetic information, outside of the primary research site;

5. Apply control or mitigation measures (see Appendix III) if appropriate and necessary to avoid adverse environmental effects outside of the primary research site.

6. Develop procedures for termination of the experiment and waste disposal.

7. Provide appropriate safeguards/education and training for all personnel involved in research.

8. Maintain records regarding the results and conduct of their trials.

Experiments with Plants

4.15. The safety of small scale field research with plants can be determined by analyzing the characteristics of the organism and the research site; and by employing appropriate, scientifically acceptable, and environmentally sound experimental practices. The following discussion of GDP for plants includes characteristics of the organism and assumes the prudent choice of research

site and experimental practices.

4.16. The intent of GDP is to design field experiments so that: (1) the experimental genetically modified plants remain reproductively isolated from the gene pool represented by sexually compatible plants outside the experimental site; (2) genes or genetically modified organisms will not be released into the environment beyond the research site; or (3) plants are used which, even without reproductive isolation, will not cause unintended, uncontrolled adverse effects.

4.17. The scientific principles of GDP for plants, as presented in Appendix IV, are derived from experience gained in field research with new plant varieties obtained by conventional and new plant breeding techniques.

4.18. GDP can be achieved in one or both of the following ways:

(1) The experimental design allows for control of reproduction.

(a) An experiment restriction or intrinsic biological limitation makes the plant incapable of reproduction; or

(b) Reproductive isolation or the functional equivalent minimizes the likelihood of reproduction outside of the experimental plot.

(2) The experimental design limits the likelihood of harm to (or a significant impact on) the environment.

(a) There is minimal likelihood that the plant will survive, disperse or become established beyond the research site;

(b) Any toxic compound newly acquired or enhanced by the plant has a minimal likelihood of detrimental effects on managed or natural ecosystems; or

(c) Gene transfer vectors that present a risk of injury, disease or damage to the plant have been adequately disarmed and/or eliminated from the plant.

Experiments with Microorganisms

4.19. The safety of small scale field research with microorganisms can be determined by analyzing the characteristics of the organisms and the research site, and by employing appropriate experimental practice.

4.20. The intent of GDP is to design field experiments so that: (1) transfer of genetic material of interest is controlled; (2) dissemination of microorganisms containing that genetic material is controlled; or (3) there are no unintended, uncontrolled adverse effects on other organism even though transfer and dissemination may occur.

4.21. Dissemination comprises the concepts of "movement/dispersal" and "establishment" beyond the test site.

4.22. The ability of a microorganism to disseminate into the environment and to transfer genetic material to other organisms, and the availability of suitable, reachable habitats/niches in the vicinity of the research site will, thus, be important factors in evaluating safety. Appendix V specifically discusses scientific principles for microorganisms.

4.23. GDP can be achieved in the following ways:

(1) The experimental design allows for control of transfer of genetic material and dissemination beyond the research site:

(a) The biology of the organism minimizes the probability of horizontal gene transfer or measures are taken to prevent or minimize it to the extent possible; and

(b) The biology of the organism limits its ability to compete; and

(c) Measures are taken to minimize movement/dispersal of the microorganism from the test site; or

(d) Measures are taken to prevent or mitigate (see Appendix III) establishment beyond the test site if

necessary.

(2) The experimental design limits the likelihood of harm to (or significant impact on) areas beyond the research site;

(a) There should be no adverse environmental effects beyond the research site, even if the microorganism should disseminate from the site, as shown by knowledge and previous experience (e.g. biology of the organism, environmental conditions, results from contained studies and previous field trials as assessed within the framework set out in "Recombinant DNA Safety Considerations" 1986 OECD report); and

(b) The experiment should be designed to monitor for effects on other organisms (e.g. plant or animal health, microbial communities, ecosystem processes, other biological systems) and to control or mitigate such effects, should they occur.

REVIEW AND OVERSIGHT

4.24. It is recommended that the planning of small scale field trials be in accordance with the Recombinant DNA Safety Considerations and the Good Developmental Practices produced by the OECD.

4.25. Applications for small scale field trials should be reviewed and endorsed in the first instance by the IBC. The Committee can consult with and request approvals from the NBTAC, if required. It should also inspect that the procedures and practices specified in these Guidelines are followed, ensure that all the involved personnel have sufficient training and experience, and maintain a file on these trials.

4.26. The NBTAC reviews the proposals of small scale field research trials submitted by an IBC, consults with relevant government agencies and other organizations as appropriate and reports on the trials to the responsible government agency.

4.27. While it is advisable that ICBs and oversight committees be established as outlined above, when this is not practical or feasible,

It is suggested that advice be sought, as desired, from other regional or international organizations.

ASSESSMENT

4.28. For the assessment of small scale field trials the following framework, proposed by the National Research Council of the U.S. (US NRC) should be used:

Are we familiar with the properties of the organism and the environment into which it may be introduced?

Can we confine or control the organism effectively?

What are the probable effects on the environment should the introduced organism or a genetic trait persist longer than intended or spreed to non-target environments?

4.29. The major concerns with plants produced by genetically engineering techniques are "weediness", the toxicity of edible plants, the production of plants with undesirable characteristics and the effects of transferring undesirable traits to other plants. Confinement is the primary condition to assure safety of the trial under this category.

4.30. In the case of microorganisms the influence of the genetic alteration on their phenotype and the mobility of the modified trait are aspects which should be assessed with special care.

4.31. In the case of plants, the following information for the review and assessment of small scale field trials is required (presented in more detail in Appendix VI):

1. Characteristics of plant material.

2. Gene donor, insert specification, expression vector and gene product.

3. Transformation system.

4. Results of laboratory and greenhouse assays, when

applicable.

5. Proposed test site(s).

6. Reproductive isolation or biological containment measures.

7. Harvest and site monitoring.

8. Post harvest land treatment and site monitoring.

4.32. In the case of microorganisms, the following information for the review and assessment of small scale field trials is required (presented in more detail in Appendix VII):

1. Wild-type or recipient identification and characterization.

2. Characterization of inserted genetic material or genetically engineered microorganism.

3. Environmental impact assessment.

4. Pathogenicity and toxicity assessment.

5. Quality assurance/quality control.

6. Field monitoring.

4.33. In the case of biotechnological veterinary biologics, small scale field trials using target species call for an environmental assessment which is described in Appendix VIII entitled Environmental Assessment Requirements.

MONITORING

4.34. In the case of plants, the small scale field trials should be monitored considering the following factors:

1. Isolation of the genetically altered crop from related species by either physical or cultural means.

2. The presence of similar species and weedy relatives in the test area.

3. The efficacy of monitoring procedures used during the trial.

4. The general appearance and conditions of the trial.

5. LARGE SCALE FIELD RESEARCH OR SCALE-UP FIELD TESTS

5.1. Scale-up field tests should be reviewed based on the results of small scale field tests. The conditions will be determined on a case by case basis.

5.2. In the case of microorganisms, the basic principles of Good Industrial Large Scale Practice (GILSP), proposed by the OECD, can be extended or applied to industrial scale-up and commercial production.

5.3. The hazards associated with rDNA microorganisms can be assessed and managed in a similar way to those associated with other organisms. It should be recognized that, for organisms considered to be of low risk, only minimal controls and containment procedures are necessary. This will be the case for the vast majority of rDNA organisms used in industrial large scale production. For this reason, we endorse the concept of Good Industrial Large-Scale Practice (GILSP) for organisms which may be handled at a minimal level of control.

For organisms manipulated by rDNA techniques, criteria forallowing use of GILSP (see appendix IX) can be identified for the parental (host) organism, for the rDNA-engineered organism, and for the vector/insert employed:

1. The host organism should be non-pathogenic; should not contain adventitious agents; and should have an extended history of safe industrial use, or have built-in environmental limitations that permit optimum growth in the industrial setting but limited adverse consequences in the

environment.

2. The rDNA-engineered organism should be non-pathogenic; should be safe in the industrial setting as host organism, and without adverse consequences in the environment.

3. The vector/insert should be well characterized and free from known harmful sequences; should be limited in size, as much as possible, to the DNA required to perform the intended function; should not increase the stability of the construct in the environment unless that is a requirement of the intended function; should be poorly mobilizable; and should not transfer any resistance markers to microorganisms not known to acquire them naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture.

6. LICENSING OF GENETICALLY MODIFIED PRODUCTS

GENERAL PRINCIPLES

6.1. Licensing will depend on large scale field tests and will be reviewed on a case by case basis.

6.2. A new product coming into a country which is approved elsewhere or a product developed within the country must meet the existing regulatory requirements of that country before commercial use.

6.3. Mechanisms that exist in the countries for licensing of products may be adequate for genetically modified organisms, when there is an acceptable degree of familiarity with the proposal.

6.4. The familiarity of the proposal can be determined using the decision framework proposed by the US NRC, shown in Appendix X for plants and in Appendix XI for microorganisms.

INFORMATION REQUIREMENTS

6.5. For licensing of biotechnological veterinary biologics, the submission must include, in addition to the information for environmental assessment, results from the restricted field trials to demonstrate that the product is pure, safe, potent and efficacious. These requirements are listed in Appendix XII along with those for physical requirements of the facility, sanitation and security.

6.6. In the case of transgenic plants containing or expressing toxins at levels that may be hazardous to man or animal health, labelling and packaging precautions must be accordingly provided.

6.7. The national regulatory institution should be informed on the licensing or registration of a product in the country of origin, when this product is seeking registration in a determined country.

6.8. The exporting countries of a genetically modified organism that was not allowed field testing in that country, should provide the reasons and information for that position to another country where it is intended to be exported, when required by it.

NEED FOR LOCAL TESTS

6.9. Local tests and assessments may be needed to grant a license or permit the commercial use of an organism to be introduced in a determined country. This should be decided on a case by case basis. The assessment framework proposed by the US NRC can be used to decide if these local tests are needed.

ROLE OF RESEARCH INSTITUTES AND INTERNATIONAL ORGANIZATIONS

6.10. Research institutes should play no role at this stage unless required by a regulatory authority.

6.11. Regional organizations should play a role in harmonizing principles and procedures for commercialization and licensing of organisms.

6.12. Where there is no regulatory infrastructure advise should be sought, as desired, from other regional or national authorities.

7. BIBLIOGRAPHY

AGRICULTURE CANADA (1989). Guidelines for the Regulation of Veterinary Biologics produced by Biotechnology, Nepean, Ontario, Canada.

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE (1988). Guidelines for the Use and Safety of Genetic Engineering Techniques or Recombinant DNA Technology, Wash. D.C.

KALOUS M.J.; DUKE L.H. (1989). The Regulation of Plant Biotechnology in Canada, Part 2, The Environmental Release of Genetically Altered Plant Material, Seed Division, Agriculture Canada, Ottawa, Ontario, pp. 20-26.

MAJOR D.W.; HART D.R.; LUSH D.L. (1988). Release of Genetically Engineered Microorganisms into the Environment, Beak Consultants Limited, Canada.

NATIONAL RESEARCH COUNCIL (1989). Field Testing Genetically Modified Organisms: Framework for Decisions, Wash. D.C.

OECD (1986). Recombinant DNA Safety Considerations, Paris.

OECD (1990). Good Developmental Practices for Small Scale Research with Genetically Modified Plants and Microorganisms, Paris.

APPENDIX I

CLASSIFICATION OF BIOTECHNOLOGY VETERINARY BIOLOGICS

Source: Agriculture Canada (1989), Guidelines for the Regulation of Veterinary Biologics produced by Biotechnology.

CLASS I

- Inactivated rDNA derived viral vaccines.
- Inactivated rDNA derived bacterial vaccines.
- Viral, bacterial, cytokines or other subunits.
- Monoclonal antibody (hybridoma) products.
- Vaccines containing live organisms modified by gene insertion or deletion (no introduction of "foreign" DNA).

CLASS II

- Vaccines using a live vector to carry recombinant derived foreign genes.
- Vaccines containing live organisms modified by gene insertion or deletion (introduction of "foreign" DNA).

Class I includes products prepared from recombinant derived inactivated organisms such as viruses, bacteria, bacterin-toxoids, virus subunits or bacterial subunits, cytokines, monoclonal antibody products used prophylactically, therapeutically or components of diagnostic kits. These nonviable products pose no risk to the environment and present no new or unusual safety concerns. Live products in this class, containing organisms resulting from deletions, single base changes, and rearrangements within a single gene, may need data to establish environmental safety. These products are almost equivalent to modified live vaccines which have been used without any unexpected risks for decades.

Class II includes products containing live microorganisms that have been modified and involve introduction of DNA from different organisms or different strains of the same organism; this class consists

of products using live vectors to carry one or more recombinant derived foreign genes that code for immunizing antigens and/or immune stimulants. Live vectors may carry multiple recombinant derived foreign genes and are capable of efficiently infecting and immunizing host animals. Deleted genes in Class I or Class II live products may code for virulence, oncogenicity, enzyme activity, or other biochemical functions. Added genes may result in the expression of unique marker antigens or the production of novel biochemical by-products.

Precautions must be taken to ensure that this addition or deletion of specific genetic information does not impart increased virulence, pathogenicity, or survival advantages in these organisms, greater than those found in natural or wild-type forms. Modification must not impart undesirable new or increased adhesive or invasive factors, colonization properties, or different survival within the host. It is important that genes added or deleted do not compromise the safety characteristics of these organisms. In most cases their safety characteristics are improved, so that they cannot pose any new threat to humans, or other animal species, or to the environment.

The genetic information to be added or deleted must consist of well characterized DNA segments. Required licensing data may include base pair analysis, sequence information, restriction endonuclease sites, as well as phenotypic characterization of the altered organism. A comparison is also required between the engineered organism and its parent strain with respect to biochemical pathways, virulence traits, or other factors affecting pathogenicity. When used as live vectors of foreign genes, the new rDNA organisms must be fully recharacterized and compared with the parent virus. Concerns for safety to humans and animals, and impact on the environment, must be addressed in an environmental assessment or environmental impact study to be evaluated by and *Ad Hoc* Committee before live products can be considered for limited field trial or licensing.

APPENDIX II

REQUIREMENTS FOR DEVELOPMENT, FIELD TESTING AND LICENSING OF LIVING, GENETICALLY MODIFIED ORGANISMS AS VETERINARY VACCINES

Source: Agriculture Canada (1989), Guidelines for the Regulation of Veterinary Biologics produced by Biotechnology.

STAGE I

Laboratory Research and Development follow OECD-Safety Guidelines.

STAGE II

Controlled containment experiments using target and non-target animal species,

STAGE III

Limited field trial on target species,

Submission of data for environment assessment and field trial to Regulatory Agency.

Negotiation on case-by-case basis may need *Ad-Hoc* Committee evaluation.

STAGE IV

Complete submission including field trial results for product license.

License issued if requirements are met.

The authorization of procedures and guidelines for the review of application for experiments at stages 2-4 will be evaluated on a case-by-case basis by the appropriate regulatory agency.

The physical containment facilities for the second stage will be almost equivalent to those for Stage I but the conditions can be negotiated upon the review of the data generated at Stage I.

The movement of the live-recombinant DNA product from Stage II to Stage III level, for a limited field trial outside the controlled containment facilities, will require a complete submission of all available data on the product, including safety data from Stage I and Stage II experiments. At this stage, it will be decided whether an *Ad-Hoc* Committee is needed to evaluate this data and assess environmental impact

of the authorization of such a trial. Appropriate data must be submitted by the manufacturer so that the regulators and members of the *Ad-Hoc* Committee can determine that the conditions for the proposed field trial are safe for the environment and adequate to prevent the spread of disease.

APPENDIX IV

SCIENTIFIC PRINCIPLES FOR FIELD RESEARCH WITH PLANTS

Source: OECD (1990), Good Developmental Practices for Small Scale Field Research with Genetically Modified Plants and Microorganisms, Paris

The following sections give the scientific principles underlying GDP conditions for field research with genetically modified plants. The size of field experimental plots will more than likely be determined by the characteristics of the experimental plants (i.e., orchard crops will require larger experimental plots, while grain crops could be adequately evaluated using smaller experimental plots). While selective plant breeding has been practiced in some form for thousands of years, it was only after the rediscovery of Gregor Mendel's work in 1900 that the type of scientific genetic modification now practiced by plant breeders became widespread. Observations made by scientists, based on a knowledge of plant genetics, plant morphology, plant reproductive biology and plant physiology have resulted in the practices now used by plant breeders to ensure the genetic integrity of their experimental material. This experience and that gained from the controlled field tests of genetically modified plants helps to identify plant characteristics and experimental conditions that allow the safe conduct of small scale field research.

1. Small scale field research with genetically modified plants is analogous to the small scale field research already conducted by plant breeders in evaluating potentially useful new varieties. The genetic modifications achieved through conventional plant breeding techniques have produced single or multiple gene mutations and changes in chromosome number through: chemical treatment or ionizing radiation; crosses between cultivars of a crop species; and interspecific crosses, including crosses between cultivated species and crosses between cultivated species and related noncultivated species. When conducting conventional plant breeding research, attention is often given to preventing possible genetic interactions between plants in the research plot and any nearby sexually compatible plants. Natural transfer of genetic material from plants to other organisms has not been demonstrated.

2. Conventional small scale field research evaluates the characteristics of a new plant variety and its interaction with the environment. Field experiments of new plant varieties produced by conventional plant breeding methods have shown that most new plants are usually inferior to, or, at best, no better than the parent varieties. Many plants are of no practical use and are discarded, with no effect on either the environment or on subsequent plant breeding. Only a very small proportion of new

germ plasm lines produced by plant breeders warrants further research or eventual commercial release.

3. There have been some instances where the intentional or accidental introduction of a foreign plant species into a new environment has had an adverse environmental impact. Examples include Johnsongrass (*Sorghum halepense*) introduced into South Carolina, U.S., as a forage plant in the 1830s, and waterhyacinth (*Eichhornia crassipes*) introduced into Florida, U.S., as an aquatic ornament. Many other important weeds (Canada thistle, yellow starthistle, field bindweed), now present in the United States, are the result of the accidental introductions of foreign plant species. In Europe, there have been similar problems as a result of intentional or accidental introductions of foreign plant species such as sunflower (*Helianthus annuus*) and common ragweed (*Ambrosia artemisiifolia*). These examples involve the uncontrolled release of a complete genome rather than the controlled transfer into plants of single or few genes which is the current case with genetically modified organisms. Therefore, the field testing of genetically modified plants conducted under the principles described for GDP should not be considered analogous to uncontrolled introductions of foreign plants into entirely new environments.

REPRODUCTIVE ISOLATION OF GENETICALLY MODIFIED PLANTS

4. To prevent effects on the environment, conventional plant breeding experiments have reproductively isolated plants in the research plots in addition to limiting the size of the plots. Employing practices that ensure reproductive or genetic isolation of the modified plants is an excellent method for preventing the inadvertent dissemination of genetic material from the test plant into other members of the same or related species.

5. In considering natural mechanisms for reproductive or genetic isolation on the evolution of plant species, Stebbins (1950) emphasized those characteristics identified as "prezygotic" (occurring prior to mating), since they can usually be controlled by manipulating the experimental plants or the environment into which the plants are to be introduced. Plants manipulated in this way can be made incapable of producing and/or disseminating

any genetic material (via pollen, seeds, etc.) that would allow new genes to become permanently incorporated in the gene pool of the species.

6. To provide some guidance in determining the types of practices that are appropriate for reproductive isolation, a list of examples is provided at the end of this appendix. When reviewing these examples of practices currently used to achieve genetic isolation, consideration should be given as to how, in each instance, a particular practice compensates in some way for a characteristic of either the plant or the field research environment. The end result of using such practices will be that experimental genetically modified plants are reproductively isolated.

7. The practice of maintaining a considerable degree of reproductive isolation is currently used by plant breeders in order to conduct meaningful plant breeding experiments, and by certified seed producers to produce genetically pure seeds. In these practices, the emphasis is on preventing the contamination of the test or breeding plants with extraneous genetic material (in most cases via pollen) to maintain the genetic purity of the experimental or breeding plant population. Although the practices used to protect the genetic purity of a breeding line differ from those used in field research, where the emphasis is on controlling dispersal of the genetic material of experimental plants from the test plot, the same principles apply. These principles can be employed to successfully control dispersal of genetic material from the experimental plot.

8. The practices currently employed by plant breeders and certified seed producers offer useful models for reproductive isolation in field research involving genetically modified plants. These practices result in the spatial, mechanical, temporal, and genetic isolation that evolutionary biologists use to define reproductively isolated plant populations. In most cases, if field research is conducted so that experimental genetically modified plants remained reproductively isolated from the pool of sexually compatible plants outside the experimental site, the objectives of GDP would be achieved. Using GDP, small scale field research with genetically modified plants may be conducted with a reasonable assurance of having no significant adverse effects on

the environment.

9. Although reproductive isolation is likely to be the main safety concern for most small scale field tests, there may be cases in which additional measures to ensure reproductive isolation as well as other factors would be considered. For example, the plants to be field tested may have been modified to contain or express toxins, or to contain biological vectors capable of transferring genetic material. The following two sections outline the nature of the problems that may be encountered in the cases of toxins and of some biological vectors, and provide factors to be evaluated when these types of field tests are anticipated.

PLANTS GENETICALLY MODIFIED TO CONTAIN OR EXPRESS TOXINS

10. Many plants contain toxic compounds. Some serve as defenses against pathogens and predators. Genetic modification techniques can enhance or decrease a plant's defense mechanisms or can add new defense components to the plant. It may be desirable to develop plant varieties that contain toxic compounds or to cause toxic compounds native to the plant to be expressed at much higher than naturally occurring levels. In many cases, field research involving plants expressing these toxins will be safe because enough will be known about an introduced toxin, its mode of action, the potential effects of the toxin on target and non-target organisms, and the techniques for incorporating the gene or genes coding for the toxin into the plant.

11. There is some possibility of environmental risk in small scale field research involving plants modified to contain toxins, even if the plant's genetic material remains confined to the experimental site, since these plants might affect organisms entering the site, or have some residual, unintended effects on non-target organisms that were exposed to these plants or their products after the plants themselves have been removed from the field experiment site. It is possible to conduct research safely with plants genetically modified to contain some toxic compound or to express some native toxic compound at higher levels. There should be sufficient information about issues such as the mode of action, persistence, and degradation of the toxin to be able to

limit the effects of the toxin to the target organisms at the test site. Additional precautions may be as simple as fencing the site, or as complex as planting the test plot at an isolated location, caging the plants involved in the field test, or instituting strict measures to account for all plant material produced in the field research.

PLANTS GENETICALLY MODIFIED THROUGH THE USE OF BIOLOGICAL VECTOR SYSTEM

12. Various physical, chemical, and biological means are available to transform plants with new genetic material. These techniques include the use of electroporation, microinjection, ballistic microprojectiles, biological organisms, or portions of biological organisms (plasmids). The first three techniques cited above are mechanical procedures that are unlikely to increase the probability of inadvertent transfer of genetic material at any time other than at the initial insertion. With biological vectors, however, there is the possibility that the vector could subsequently act as an infectious agent unless the vector becomes biologically inactive and/or is eliminated from the transformed plant.

13. The safety of small scale field research with plants that have been transformed through the use of biological vectors is enhanced when the vector system is unlikely to transfer genetic material after the initial transformation has occurred. If the vector presents a plant pest risk (i.e. a risk of injury, disease, or damage), that risk must be adequately eliminated. In most cases the vector should be eliminated from the plant or inactivated once the transformation has been completed. DNA that is to be used in developing a genetically modified plant should be: (a) well characterized and unlikely to be transmitted after entering the plant (disarmed *Agrobacterium tumefaciens* Ti plasmid meets this specification); (b) transferred from the same or closely related species (as the recipient plant); and/or (c) transferred from nonpathogenic prokaryotes or nonpathogenic lower eukaryotic plants; and/or (d) transferred from plant pathogens only if the sequences capable of production of disease or damage in plants have been deleted.

14. Currently, the vector system most widely used to transfer DNA into a plant cell is naturally present in the bacterium *Agrobacterium tumefaciens* and is commonly referred to as the TI plasmid. There is now a considerable body of evidence based on experiments conducted under laboratory and greenhouse conditions establishing the safety of this vector system. In most of the field research with genetically engineered plants conducted to date, the vector systems derived from *A. tumefaciens* have had the genes associated with the pathological response to infection physically deleted. In addition, the transformations have been conducted in such a way that no vector sequences involved in pathogenicity are present in the transformed plant, and the vector agent, the bacterium, does not survive. In this way, the possibility of the vector being able to cause any transfer of genetic material from the modified plant has been eliminated.

15. The following are examples of current experimental practices which are used to maintain reproductive isolation in plants:

    i. The most common method used to isolate plants from sexually compatible plant populations is spatial separation. Most requirements for growing certified seed include some specification as to the distance the field must be from any field containing plants of the same species. The specific distance required will depend on the biology of the species in question. Self-pollinated species with fragile pollen will require relatively short distances, while some open-pollinated species with hardy pollen will experience some degree of contamination when separated from compatible plants by as much as several miles.

    ii. In the case of some dioecious plants, removal of the male or female reproductive structure(s) may allow plants to be safely grown in close proximity to compatible plants. An example of the use of this method is mechanical detasseling in seed corn production. By removing the tassel (containing the pollen producing male flowers) it is possible to entirely eliminate the source of genetic material from the male that can be transferred via pollen.

iii. A variation of the technique discussed above involves the incorporation into the plants in question of a cytoplasmic male sterility trait. When this trait is present, almost no viable pollen is produced, and the plant will virtually remain reproductively and biologically isolated.

iv. It may be possible to grow the plants in question in such a way that flowering will occur either earlier or later than it would be expected to occur in plants of nearby compatible crops and/or wild plant species. This use of temporal reproductive isolation can potentially be as effective as spatial separation in limiting the movement of genetic material. Pollen dissemination may also be prevented by physical covering of flowers (bagging) prior to anthesis.

v. When the objectives of a field test do not require that seed be produced, such as when forage qualities of alfalfa are being evaluated, it may be possible to harvest plants prior to flowering. In this case, reproductive isolation could be achieved in some crops that for some reason (normally insect pollinated) might otherwise be difficult to isolate.

APPENDIX V

SCIENTIFIC PRINCIPLES FOR FIELD RESEARCH WITH MICROORGANISMS

Source: OECD (1990), Good Developmental Practices for Small Scale Field Research with Genetically Modified Plants and Microorganisms, Paris

APPLICATION IN THE ENVIRONMENT

1. One of the primary assumptions in GDP is that limited small scale field trials present a situation where the issues to be addressed are constrained by the relatively small size of the experimental plot as compared to large scale testing or use, or with unlimited application. In small scale research, the frequency of application and the number of organisms applied would generally be lower than during large-scale testing or commercial use, and research would normally occur at only a single or a few geographic locations. The results of research on biological control agents as a means of controlling agricultural pests, indicate that the scale and frequency of introduction appear to be important factors in determining whether the microorganism will become established and the effect of the introduced microorganism on the environment.

2. In a limited small scale field experiment, the potentially affected environment is, in general, more localized, and it is therefore easier to identify the important ecological/environmental considerations which should be evaluated to devise a safe experiment. Moreover, because of the small size of the experiment, procedures and experimental designs to confine the experimental organisms may be effectively used.

3. The methods for applying the organism and the amount of inoculum are important considerations in determining the safety of field research. "The location and nature of the site of application, and the magnitude of the application are important for assessing safety" (OECD, 1986).

4. Microorganisms are generally applied in small scale field research as soil amendments, as foliar "sprays", or as inocula introduced into the vascular tissues of plants. While organisms may be introduced using other methods, the process for evaluating relevant safety considerations is expected to be similar in all cases. Therefore, the discussion of scientific principles can focus on these few as the most commonly used.

5. Greater dispersion of the microorganisms from the field plot would be expected with those application methods that involve

creation of aerosols. Consequently, relatively larger border areas (buffer strips of land) might be part of the field research design for an experiment involving foliar sprays. Alternatively, aerosol formation may be minimized by the choice of drip, rather than spray applications or irrigation.

DISSEMINATION, INCLUDING SURVIVAL AND MULTIPLICATION IN THE ENVIRONMENT

6. "The relative ability of the organism to survive and multiply in the environment in which it is applied and to be disseminated to new environments is an important consideration for assessing the safety of the release" (OECD, 1986).

7. Most of the data that form the basis for a discussion of the following considerations and, consequently, for the development of an appropriate field research design, are based on principles derived from the studies of pathogens (either plant or mammalian) or *Rhizobium* species. Limited information is available on the dissemination of saprophytic organisms (except for some that interact with plant pathogens, e.g. *Agrobacterium rhizogenes*).

8. These studies show that dissemination depends on three factors: (1) the rate of population growth (i.e. survival and multiplication); (2) the movement/dispersal properties of the population; and (3) the availability of suitable habitats or niches. "Dissemination" is composed of the concepts of "movement/dispersal" and "establishment". "Establishment" encompasses "survival and multiplication", as well as "movement/dispersal".

9. In evaluating field research, it is not possible to completely separate the concept of "dispersal" from the concept of "establishment". Rather these concepts must be considered in concert. For example, if it is accepted that an organism will not become established, dispersal from the experimental plot would be of lesser concern and methods of controlling dispersal assume a position of lesser importance. On the other hand, if dispersal from the experiment plot is low, either because of the characteristics of the experimental organism or because measures to control movement/dispersal have been

implemented, the probability of establishment may be less.

a. Rate of Population Growth

10. The rate of population growth of an experimental microorganism is dependent on a number of factors. At this time, it is not possible to describe all the factors influencing the rate of growth of a microorganism in the environment. However, some predictions of likely behavior can be made based on existing knowledge and empirical observations generated from a number of sources; greenhouse testing, microcosm testing, knowledge of the behavior of closely related organisms (e.g., parental organisms) and the intended function of the introduced trait if the experimental organism is genetically modified.

11. Whether the microorganism can increase in numbers at the site of application is an important factor influencing dissemination. A microorganism that is not able to increase its numbers several orders of magnitude at the research site, will not likely have a high probability of being able to disseminate to other sites.

12. This prediction is based on the assumptions that the microorganism must be in sufficiently high numbers at the research site to disperse minimum effective inoculum to other sites, and that some dilution of inoculum will occur as the microorganism leaves the research site. Dilution would probably increase as the microorganism moves further from the test site without encountering a suitable habitat. These assumptions appear to be supported by plant pathology studies which have shown that dissemination is directly proportional to the size of the source-pool (in this discussion, the source-pool is considered to be equivalent to the number of microorganisms of the test strain in the original research site).

13. It should be noted that the number constituting minimum effective inoculum can vary considerably from organism to organism, and thus no single standard number of organisms can be cited as a minimum effective inoculum. It can be assumed that for some organisms, a small number of them would be an effective inoculum, while for other organisms very large numbers are necessary.

The number of organisms constituting a minimum effective inoculum varies for a number of reasons. In some cases, for example, competition or other pressures (e.g., predation) can be overcome only by a large incoming population. What would constitute a minimum effective inoculum must, thus, be determined on a case-by-case basis.

14. Instituting measures to lower the number of microorganisms leaving the research site, however, would lower the probability that a number of organisms sufficient for a minimum effective inoculum would arrive at other sites. An experimental plan designed to use such measures can be implemented for small scale field research.

b. Movement/Dispersal Properties

15. The rate of dissemination is extremely sensitive to the effectiveness of movement/dispersal. It appears that, in general, the more effective movement/dispersal, the faster dissemination can occur.

16. Effectiveness of movement/dispersal generally depends on several factors. These include: mode of movement/dispersal, mechanism of achieving transport (including ability to adhere to soil or other particles); ability to infect vectors; ability to adhere to potential means of mechanical transport (e.g., animals, humans and their tools); ability to survive transport. These factors are dependent on the biological characteristics of the experimental organism. Biological characteristics of the test microorganism must thus be considered in evaluating the safety of field research.

17. Microorganisms are transported by a variety of routes: (1) by wind; (2) by water; (3) by mechanical means (e.g., humans, insects, animals); and (4) by biological vectors.

18. While some microorganisms are dispersed by several means, others may be restricted to one or a few modes of movement. In general, the more highly adapted a microorganism is to movement by one route, the poorer are its chances of movement

by other routes. An understanding of potential routes of movement/dispersal, and knowledge and implementation of methods of limiting movement/dispersal along these routes can be used to design safe field research and underlines the need for monitoring.

(1) Wind

19. Effectiveness of aerial dispersal is influenced by several factors. These include: mechanisms of entering the atmosphere (take-off) and particle ability to adhere to soil and other particles. Some microorganisms have adaptations which permit them to disperse aerially. The pre-eminent examples of microorganisms well-adapted for wind-borne dispersal may be found in the fungi. Many fungi typically produce propagules in the form of spores which protect against injury, are easily lofted into the air whenever a small amount of energy is furnished, and provide sufficient stores of energy for penetration and infection once the propagule has reached a suitable target. Fungi typically have structural adaptations enabling them to enter the atmosphere and to be dispersed by wind.

20. These adaptations are diverse, varying from passive processes such as being shed under gravity to being propelled long distances. Other microorganisms are dispersed aerially through passive means. For example, some microorganisms adhere to soil particles. Rafts of soil or dust particles are raised by wind when the ground is heated by solar radiation. The microorganisms attached to these soil particles are transported as the soil is blown by the wind. Some microorganisms adhere to insects or mites which can then be dispersed by wind currents.

21. The positioning of a field research plot can be used to address and limit potential transport through the aerial route. For example, consideration can be given to situating the experimental site so that natural features of the landscape such as trees, hills, windbreaks, or fences can be used to influence wind currents.

22. Other procedures, such as ensuring that the soil contains sufficient moisture to prevent rafts of soil particles being raised

by wind, can also be employed at a test site.

(2) Water

23. In water, dispersal is influenced primarily by the transport properties of the suspending medium. Thus, the hydrology of soil water and groundwater flow, and proximity of open bodies of water (e.g., lakes, rivers, streams) and water supplies for irrigation are among the primary physical determinants of waterborne dispersal from a terrestrial experimental plot.

24. Rain or irrigation water can also serve as a means of transport. Bacteria, viruses, and spores, sclerotia, and mycelial fragments of fungi can be dispersed by rain or irrigation water that washes the surfaces of plants or moves over or through the soil.

25. Rain splashes can throw droplets, potentially microorganism-laden, from plant surfaces into the air. Splash dispersal occurs when water droplets impinge on plant surfaces covered with microorganisms. For example certain plant pathogenic bacteria such as *Xanthomonas malvacearum* can be spread for kilometers by driving rain.

26. The research plot can be designed to address and limit dispersal through these potential routes. For example, buffer areas around the research site can be used to isolate plants within the research plot and thus prevent microorganisms contained in splash generated droplets from encountering suitable habitats proximal to the test plot. Design features such as avoidance of an overhead irrigation system or the inclusion of tile drains in the test plot can be implemented.

27. Moreover, the research plot can be situated so as to limit access of the test microorganism to groundwater or open bodies of water under either average or exceptional climatic conditions.

(3) Mechanical Means

28. Human Activities. Humans disperse all kinds of microorganisms over short and long distances in a variety of ways. Within a field, humans disperse microorganisms through the successive

handling of plants, through the use of contaminated tools and other equipment, through the transport of contaminated soil, plants, seeds and nursery stock.

29. Mechanical disturbances such as tillage may loft "rafts" of soil bearing clumps of microorganisms into the air. These rafts may then settle downwind of the test plot. Likewise, any activity that generates aerosols can also create a potential route of dispersal for microorganisms contained in the aerosol droplet.

30. In small scale field research, care can be taken to limit dispersal of microorganisms by human activities. For example, access to the test plot can be restricted to those individuals trained in procedures appropriate for limiting dispersal. Mechanical disturbances can be limited in a number of ways, such as by the choice of crop (e.g., no-till varieties) or procedures. Finally, the transport of contaminated materials can be restricted by use of appropriate procedures.

31. Animals. In nature, a variety of animals may come into contact with and serve as vectors for microorganisms. For example, bacteria may be transported by browsing and burrowing mammals, soil arthropods, earthworms, and soil clods adhering to duck feet.

32. In small scale field research, appropriate measures can be taken to limit the access of animals to the test area. This might include, for example, a screening or fencing of the experimental site.

33. Other. Insects can transport microorganisms phoretically. Their bodies can become smeared with bacteria or sticky fungal spores, and as they move between plants, the insects carry the microorganisms on the surfaces of their bodies from plant to plant. The microorganisms are then deposited on plant surfaces or in thewounds that insects make on the plants during feeding. Wounding often leads to higher establishment efficiency.

34. There are other methods by which passive dispersal can occur. For example, microorganisms that colonize flowers and buds may be dispersed by plant pollen. Because fungi and bacteria

are closely associated on plant surfaces, contamination of fungal propagules by bacteria is possible and may be a means of passive aerial dispersal for bacteria.

35. These types of potential vectors can frequently be addressed by the experimental design of the field test. For example, as noted in the section of this paper dealing with plants, a number of methods of dealing with pollen production and dispersal are available.

(4) Biological Vectors

36. Microorganisms can be transmitted by insects during feeding and movement of the insect from plant to plant. By definition, the insect vector and the microorganism establish a specific relationship. A vector carries the microorganism from one place to another and deposits it effectively (usually through wounding of the plant) where it can become established. Although there are a few exceptions, the more highly adapted and specific the vector/microorganism relationship, the less likely in general the microorganism will be moved by other vectors.

37. The relationship between the vector and the microorganism can be either persistent (circulative and propagative) or non-persistent. The persistent or circulative type of vector/microorganism relationship occurs when the insect is able to transmit the microrganism over an extended period of time, and the microorganism may multiply in the insect. Non-persistence refers to a relationship in which the vector acquires the microorganism after a short feeding period on the plant, can transmit the agent to another plant immediately after feeding and then rapidly (minutes) loses the microorganism.

38. The common insect vectors are aphids and leafhoppers, but white flies, mealy bugs, beetles, dipterans, psylla, thrips, mites and others have also been documented as vectors. Aphids and leafhoppers are by far the most important vectors of plant viruses and mycoplasmas (bacteria without cell walls).

39. Insects can vector microorganisms for both short and long distances. Insects like leafhoppers are strong fliers. Some

insects, such as mites, cannot fly but can be carried passively by wind. Even insects which are not strong fliers can disperse microorganisms over long distances since these airborne insects can be carried hundreds of kilometers by wind.

40. Field research design can be used to address potential vectoring of test microorganism by insects. For example, if it is known that the test microorganism is transmitted by aphids, a judicious choice of site might locate the test at an altitude where aphids are not present or when the aphid population is low. Using aphid repellents or denying vectors access to plants by netting are methods that could also be employed in the experimental design.

c. Availability of Suitable Habitats

41. One of the most important considerations in determining whether a microorganism will be disseminated is whether habitats and/or niches in which the microorganism will become established are available.

42. The distribution and number of potential habitats in an area to which the microorganism may be moved/dispersed are important determinants of establishment. The number, distribution, size, and susceptibility of the habitats influence the probability that a microorganism will be successful in encountering and establishing in suitable habitats.

43. If the density of potential habitats is low and the habitats are separated by relatively large distances, the probability of successful dissemination is greatly reduced, and indeed may approach zero. Strategies based on habitat density are used in agriculture to control pathogen dissemination. For example, fields can be planted with "multilines" of a crop. "Multilines" consist of several different varieties of the crop species with each variety possessing a different gene for resistance to the pathogen. Since a sufficient density of suitable habitats (susceptible plants) is not available to the pathogen, it does not disseminate in an epidemic fashion.

44. Experimental design in a small scale field trail can be used to address, to some extent, the issue of density and distribution of

potential habitats. For example, test site locations may be selected based on the distribution and size of likely potential habitats in the experimental region. This tactic is frequently employed in plant breeding field studies involving plant pathogens. Thus, the experimental design can employ the strategy of "geographic isolation".

45. Other strategies may be employed in the area proximal to the research site to help limit potential suitable habitats and thus control dissemination. For example, in one recent field experiment involving a *Rhizobium* species, wild leguminous plants which might have been suitable hosts/suitable habitats were removed from a 50 meter radius of land surrounding the research site.

d. Multiplication and Survival

46. As noted in the previous section, survival and multiplication of the experimental microorganism are important to producing a sufficiently large source-pool to permit dissemination. In order to increase its numbers at the site of introduction the experimental microorganism must be able to compete effectively against other organisms in the research site or find a new niche without competitors or containing less effective competitors.

47. The phenomena of (1) competition and (2) selection are important considerations in evaluating a submission and designing safe field research. In this paper the phenomenon of "finding a new niche" will be treated as a facet of selection.

(1) Competition

48. Negative interactions within a microbial community in a habitat are termed "competition". Competition is used here in a broad sense to include competition for available substrates and other negative interactions such as those resulting from production of toxic substances. Members of a microbial community in a habitat are able to utilize the same substrates; they occupy the same niche. Competition occurs when several populations are striving for the same resource, whether it be space, light, hosts, etc., or a limiting nutrient. In natural habitats with very low

concentrations of available sustrates, intense competition occurs.

49. Free-living Soil Microorganisms. Most of the information on free-living soil microorganisms is derived from experience with *Rhizobium* species and microbial amendments used as biological control agents. This experience shows that at the and of the growing season, the added microorganism does not usually predominate. To explain these observations, it has been hypothesized that the organisms of the microbial amendment must compete with an indigenous flora well adapted to local conditions, and are not effective in this competition.

50. A microorganism must contend with numerous factors when it is placed in the soil environment. These include: a number of well-adapted competitors (since soil is a complex matrix in which various types of organisms abound); environmental stresses (e.g., chemicals, water and temperature); various levels of predation; competition for resources; and antibiosis.

51. Microorganisms proliferate when nutrients are available and temperature and moisture levels are adequate. However, even when nutrients are in abundance, soil inhabitants must compete for them. In a situation of relative abundance, the competitive advantage lies with those having the highest growth rate. The more frequent situation is that nutrients are scarce, and organisms must frequently survive long periods of starvation. In this situation, populations with the greatest ability to survive stress conditions will generally have the competitive edge. Organisms that produce resistant structures (e.g., spores and sclerotia) are best adapted to survive the adverse conditions resulting from long periods of environmental stress and starvation. Some species have developed strategies through which they can survive for long periods of time as vegetative cells.

52. Antibiosis occurs when one microbial population produces a substance that is inhibitory to other populations. Examples of antibiosis include production to suppress competitors; and production of substances such as lactic or sulfuric acid, alcohol, acetic acid, and low-weight organic acids. Production of antibiotics probably has a significant function in competitive

interactions in microenvironments. The complementary competitive strategy would be possession of an inherent resistance to antibiotics produced by other organisms. Bacteriocins and biological toxins may also suppress populations of phytopathogens in the soil, and microbial strategies to deal with these substances probably exist.

53. Predation may also be a factor influencing microbial survival and population levels. Free-living nematodes and protozoans are present in many soils and probably are predators of microorganisms. Although the impact of such predators on microbial populations is unclear, it is likely that microorganisms have developed strategies for dealing with predation.

54. Soil is a complex matrix presenting a highly competitive environment. The interplay of the factors described above and the response of the species to them creates a balance of life in the soil which will affect the comparative competitive ability of the applied microorganism.

55. Host Obligate Microorganisms. Microorganisms that depend on a host for survival are termed host obligate microorganisms in this paper. Most available information addressing the factors affecting competitive ability in host obligate microorganisms was generated from studies of microorganisms as biological control agents, and in plant pathology as well as plant breeding.

56. In the microorganism/plant interaction, host obligate microorganisms may be epiphytic (on the surface of the plant) or endophytic (inside plant tissues) or both.

57. The endophytes have few competitors (other plant pathogens or possibly secondary invaders of diseased tissue), when compared to the epiphytic or free-living soil microorganisms. Endophytes such as viruses, viroids and some prokaryotes (e.g. rickettsia-like bacteria, mycoplasmas and spiroplasmas) exist entirely within their host or vector and rarely, if ever, survive when exposed to the outside environment. The environment in which they must compete is, thus, to a great extent determined by the host. Although they may have fewer microbial competitors, endophytes must deal with host defenses.

58. Plant obligate epiphytic microorganisms may be categorized on the basis of the kind of nutritional relationship they maintain with the host. In their residency or epiphytic phase on leaves or roots, certain host obligate microorganisms exist mainly if not entirely in an apparent state of commensalism with the plant. They obtain nutrients (as leaf or root exudates) from the plant but cause no harm to it. However, given the right conditions, they can kill and destroy host tissues through the action of toxins and enzymes and then multiply in the dead tissue.

59. In a second type of nutritional relationship, the host-obligate microorganism obtains nutrients from a plant by killing the host tissue in advance of colonization.

60. Many of the factors affecting competition among free-living soil microorganisms can be seen in host-obligate microorganisms. These include competition for space, competition for nutrients, predation, environmental stress, and antibiosis. In addition to dealing with these factors, both epiphytic and endophytic host-obligate microorganisms must also find and colonize/infect suitable hosts. The need for host-obligate microorganisms to find suitable hosts is a factor which can be used in designing an experimental protocol to test these organisms safely.

(2) Selection

61. Selective pressure is exerted by the environment and favors organisms possessing adaptive features. The best known examples of selection in microorganisms is the emergence of bacterial strains resistant to antibiotics. Selection of resistant strains is promoted by the use of antibiotics by clinicians, in animal feed, and for agricultural purposes. Another example of selection is the increase in the numbers of microorganisms capable of degrading certain man-made synthetic-organic compounds (e.g., pesticides). In this instance, selection is promoted by the introduction of large amounts of these man-made compounds to the environment.

62. For the purposes of this paper, "discovery of a new niche" is treated as a form of selection. It occurs when a microorganism

develops the capability of performing a "new" function within an ecosystem. It can also occur when a microorganism performing a function which the indigenous community does not perform is introduced into an ecosystem (e.g., the introduction of *Ceratocystis ulmi* to the North American continent).

63. Selective pressures affect the ability of an organism to survive, multiply and to increase its relative proportion of the community. Selection, thus, can have an important influence on movement/dispersal and establishment, as well as on survival and multiplication.

64. Clearly, in attempting to evaluate the probability that an introduced microorganism will be an effective competitor, be favored by selection or find a new niche, a number of factors should be examined. These include, the source of the test organism and the source of the added gene, if any, and the environment in which the test will occur. In many instances, the microorganism will be experimented within the agro-ecosystem from which it or its parental microorganisms were isolated. In such a situation neither the introduced gene nor the introduced microorganism will be new or unique in that environment, although the frequency at which the gene/microorganism combination occurs in that site subsequent to application may differ from that generally observed.

65. The added gene/microorganism combination would be in competition with the indigenous population of microorganisms. While this does not guarantee that the added gene/microorganism combination will not be an effective competitor in the test environment, it does set some limit on the types of risk scenarios to be considered. In this type of research situation a knowledge of the function of the added gene and the behavior of the parental organisms can be used to predict the likely response of the gene/microorganism combination to factors such as competition for nutrients, predation and environmental stress, selection, and antibiosis.

66. Given present knowledge, however, the competitive ability of the experimental microorganism will frequently have to be tested empirically. Data generated in the laboratory, greenhouse or

microcosm may therefore form an important element in an evaluation of small scale field research.

67. That the inoculum used in limited small scale field research is frequently insignificant when compared to the indigenous population also plays a role in determining the likely fate of the gene/microorganism combination. When relatively small numbers of the gene/microorganism combination are added to an experimental site, it is probable that the competitive advantage lies with the indigenous population. In addition, when the application involves a relatively small number of organisms, the probability that sufficient genetic variation will exist from which genotypes can be selected is less.

68. In some instances, the microorganism or the added gene may be isolated from environments other than the environment of the research site. In this situation, a careful comparison of competitive ability of the gene/microorganism combination can be based on research in controlled environments such as greenhouses, microcosms etc. The intended function of the added gene and the behavior of the recipient parental microorganism are also important considerations. An appropriate environmental design would take into account these considerations.

69. To illustrate the approach, the following example is offered. In 1987/1988 in the U.S. a field experiment was performed with a *Pseudomas aureofaciens* modified to contain the genes from *Escherichia coli* K-12 that code for the production of lactose permease and beta-galactosidase (lacZY). This modification permits the organism to grow on lactose, unlike other pseudomonades. During the risk assessment of the limited field experiment, the probability that the modification would confer a competitive or selective advantage on this *Pseudomonas* was evaluated. A prediction that the modification would not confer a competitive advantage was in part based on: (1) the expectation that the inserted genes confer the ability to metabolize only a limited number of sugars (primarily lactose, with the possibility that mannose and xylose would serve as substrates); (2) the fact that few sites would favor the experimental organism (i.e., commonly available substrates would be limiting but lactose would be abundant) should the organism disperse from the experimental plot;

(3) studies documenting low levels of survival of the parental strain, and greenhouse studies indicating no difference in survival ability between test and parental strains, suggesting the modified organism would not be a highly effective competitor; and (4) the limited nature of the small scale experiment,

70. The site of the field experiment was an important consideration in evaluating competitive ability and the probability that selection might favor the lacZY *Pseudomonas*. Several conditions were placed on the field experiment in order to control potential routes of dispersal and a monitoring program was implemented.

INTERACTIONS OF THE MICROORGANISM WITH OTHER SPECIES AND/OR BIOLOGICAL SYSTEMS IN THE ENVIRONMENT

71. Experimental microorganisms in small scale field research can interact with other species in a number of ways. In the OECD "Recombinant DNA Safety Considerations", two specific kinds of interaction are noted in the outline. These are: (1) the effects of the microorganisms on target or non-target organisms and (2) the potential for and effect of horizontal transfer of genetic material. This section of GDP addresses these two types of interaction and an initial attempt is made to describe the considerations about them. These considerations can also be related to field research design.

a. Target or Non-Target organisms

72. Many of the microorganisms that are tested in field plots are intended to have effects on another organism, the target organism. For decades, plant pathologists have used microorganism that cause plant disease in the field to evaluate plants for disease resistance. Other plant pathogens have been tested in the field to gain fundamental knowledge about the biology and the pathogenicity of those microorganisms. Microorganisms used as biocontrol agents are specifically selected or modified to affect a target pest organism. Some microorganisms such as *Bacillus thuringiensis* are used routinely in the environment as biological control agents for some lepidopteran insects. Limited small scale research using unmodified microorganisms have been conducted with little ad-

verse effects on the environment even though the microorganisms have known effects on other organisms in the environment being reported. The issues that are routinely considered in these tests are instructive in testing genetically modified microorganisms.

73. When a microorganisms is experimented with, it is important not only to evaluate the expected effect on the target organism but also the effects on non-target organisms. When genetic engineering is used to modify microorganisms to act as biological control agents, the genes that are inserted may encode toxins or they may broaden the host range or increase virulence of the microorganism for a particular target organism. The effect of any new trait on the host range of the microorganism should be evaluated in the laboratory before field testing. Potential non-target organisms should be identified by experimenting with representative species under contained conditions. It is generally unlikely that the relative abundance of a species in a community or ecosystem will be significantly altered as a consequence of small scale field research if the microorganism can be effectively limited to the plot and its immediate surroundings. Yet it is important that field research be conducted so as to limit exposure to sensitive non-target species.

74. These concepts can be applied to specific examples. New strains of *B. thuringiensis* should be experimented with on a plot on which no threatened or endangered species of lepidopteran insects will be exposed to the delta endotoxin produced by the bacterium. It is essential that great care be taken in testing beneficial insects for sensitivity to the test microorganism and in limiting the exposure of significant population of sensitive beneficial insects.

b. Gene Transfer

75. The gene transfer capability of an engineered microorganism or the stability of the genetic construct will affect the microorganism's interactions with other microorganisms. Gene transfer refers to the dissemination of genetic material through natural genetic mechanisms.

76. The factors to be considered in analyzing the effects of gene transfer on the safety of a genetically modified microorganism are the following:

(1) What is the probability of horizontal transfer of the genetic material?

(2) If the gene is transferred, will the new genetic information be maintained and expressed?

(3) If known, what does the transferable material code for?

(4) If the transformed microorganism moves beyond the point of introduction, how will it affect, as a result of the transformation, the surrounding populations or communities of plants, animals, and indigenous microbes?

77. Gene transfer refers to the dissemination of genetic material through natural genetic mechanisms. The mechanisms by which plasmids and/or chromosomal genes are transferred include conjugation, transformation, transduction, and cell fusion. Although these mechanisms have been studied in the laboratory, little is known about the frequency of genetic exchange in nature. Logically, we expect that genetic transfer frequencies are lower in nature compared to the laboratory, but frequencies in nature have not been extensively studied. A few exchanges of genetic material in nature o simulated natural settings have been documented.

78. Accurate prediction of genetic dissemination or escape requires that the frequency of genetic transfer be known. However, a few generalizations can be offered concerning genetic transfer in simulated or natural habitats. Genetic transfer occurs at lower frequency in soil and water habitats than in vitro culture systems.

79. Other factors that may affect transfer are the presence or absence of: (1) large bacterial densities that enhance mating; (2) free DNA that may promote transformation; and (3) clay

materials or minerals that may promote growth and plasmid transfer but not transduction. The presence of wide host-range, high copy number plasmids may provide more opportunity for dispersal, and relatively large number of donor cells facilitate transfer to recipients. In addition, other factors that affect transfer are spatial, temporal, and physiological separation of bacteria; immobilization through adherence to soil particles, organic materials, and other living organisms; genetic barriers such as restriction systems and plasmid incompatibility; and environmental conditions.

80. On the basis of similar considerations, estimates have been made of the transfer frequencies likely to be observed in specific environments. However, the frequencies at which genetic transfer is likely to occur and the significance of such transfer, in comparison to transfers which occur in nature, will, for the moment, likely have to be evaluated on a case-by-case basis.

EFFECTS OF FIELD RESEARCH ON THE ENVIRONMENT

81. The OECD report "Recombinant DNA Safety Considerations" describes the factors that should be considered in evaluating potential effects on the environment as "(1) effects on other organisms such as pathogenicity, infectivity, and effects on competitors, prey, hosts, symbionts, etc., (2) known or predicted involvement in biogeochemical processes such as mineral cycling, nitrogen fixation, etc., (3) genetic or phenotypic stability of released organisms, (4) probability of transfer of genetic material to other organisms in the ecosystem, (5) the effect of excessive increase in numbers of organisms following the application".

82. However, the limitations which would be placed on field research are specifically addressed at reducing the possibility of environmentally adverse effects.

ROUTES OF TRANSMISSION FOR VIRUSES, BACTERIA, AND FUNGI

83. Many bacteria are remarkably well adapted to passive transmission by wind-splashed rain, seeds, insects, vegetatively propagated stock, irrigation water, cultural implements or other

agents. Atmospheric dispersal for the most part occurs as a consequence of adventitious physical disturbance, and, for the most part appears to be equivalent to the dispersal of the particles and organisms to which the bacteria are attached. Mycoplasmas may be dispersed by similar mechanisms.

84. Plant viruses are dispersed primarily through mechanical means, either by plant propagation, by human contact, or by insect vectors. Most plant viruses have no specific mechanism for aerial transport.

85. Algae and protozoa have no special mechanisms for aerial dispersal, but are dispersed by animal vectors, or adventitious physical disturbance. The processes by which yeasts disperse aerially have not been in general studied, although they are abundant in the air. The characteristics of the experimental microorganism affect the probability of the experimental microorganism being transported from the research plot.

APPENDIX VI

INFORMATION TO BE REQUESTED IN APPLICATIONS FOR FIELD TESTING OF GENETICALLY MODIFIED PLANTS

Source: Kalous M.J.; Duke L.H. (1989), The Regulation of Plant Biotechnology in Canada, Part 2, The Environmental Release of Genetically Altered Plant Material, Seed Division, Agriculture Canada, Ottawa, Ontario, pp. 20-26

INFORMATION REQUESTED

The information requested in applications for the field testing of genetically altered plant material is based on information that the scientists will have gathered in laboratory experiments and environmental release protocol preparations. By excluding extravagant testing requirements, the financial burden that could be incurred by completion of an application is greatly decreased. The information in an application is considered confidential and is not released to other industry representatives or researchers. In cases where there is public inquire, the company or researcher will be contacted to identify proprietary information and this will not be disclosed.

Plant Material

The plant material must be fully characterized. The species and a brief botanical description should be included on the application. The ability to cross pollinate with members of the same species and native relatives as well as seed dispersal mechanisms and dormancy periods must be noted.

Gene Donor, Gene and Gene Product

A brief description of the donor species of the plant gene must be included when appropriate. The description should be sufficient to allow the regulator to identify any potential problems such as toxin production or weedy traits.

The gene inserted and its promotors and terminators should be identified. There is currently debate among federal regulatory agencies as to whether base pair sequences must be provided. There is also some question as to whether promotor and terminator sequences should also be provided as little is known of possible effects of integrated viral or microbial regulators on integrated or native DNA.

If known, the gene product and the affected pathway should be identified. The impact of the gene product on the plant material (e.g. insect resistance), tissue specificity and secondary metabolites should also be identified to allow assessment of plant material which may enter the food chain.

Transformation System

The transformation system must be detailed, e.g., protoplast fusion, mutagenesis or DNA transformation. Vectors must be identified and a plasmid map must be included if this type of system was used. Plasmid characteristics such as marker gene, and whether disarming has occurred (which genes have been removed) must be included.

The characteristics of the transformation system allow the regulator to decide what questions to ask when reviewing the application. This is not an attempt to regulate the process but the process must be considered for a proper assessment. Generic reviews will not work, specifically for this reason. It would be impractical to ask all researchers to answer the same questions because the applicability of the questions would vary greatly and a set of questions that covered every possible circumstance would be burdensome.

Laboratory and Greenhouse Assays

Any chemical, biochemical, and progeny tests and analyses performed on the plant material should be described. These types of tests will denote gene copy number, stability, and any adverse effects to the plant.

The researcher should provide data on any greenhouse experiments. Of particular importance are tests for weediness. Weediness can be seen in the greenhouse by excessive seed shattering, weak seed dormancy, and a great degree of competitiveness.

Test Site(s)

A description of the proposed test site(s) including exact map location(s), size and number of plot(s), quantity of seed (grams) to be imported and planted, proposed control variety(s) and isolation distances. The regulator must know of all field trials taking place within the country with the material in question. If the plants are grown to flowering, the current isolation distance required between the genetically altered plant material and similar species and weedy relatives is twice the isolation distance required for pedigreed seed production. These distances may be adjusted for individual trials depending on the protocol. For example, if an outcrossing study is being performed, the genetically altered plant

material will be close to plants of the nontranformed species. But, the transgenic plants must still be twice the isolation distance from plants not directly involved in the experiment. If isolation distances must be reduced, the company or researcher is required to provide proper disposal of all plant material within isolation distances. Scientific data are currently being collected to facilitate a reassessment of appropriate isolation distances.

Reproductive Isolation

Genetically altered plant material may be isolated reproductively, as well as physically, from similar species or related weedy relatives. This may be achieved in several ways.

1. Reproductive isolation may be obtained by placing glascine bags over flowering plants to prevent the dispersal of pollen.

2. Many weeds will flower well before the agricultural crops. This allows weeds to be easily identified and removed from the field, thus achieving isolation. This type of isolation requires more work because the fields must be continually monitored and rouged. In this case, isolation distances are still required to non- transformed plant species because they will flower at the same time as the genetically altered plant material.

3. The genetically altered plant material may be harvested before the flowering stage. In this case, there is little concern over outcrossing and the isolation distances recommended are not required as long as adequate monitoring procedures are used to ensure that the plant material does not reach the flowering stage.

Post Harvest Land Treatment and Site Monitoring

Plant material remaining after the trial should be treated in some manner. Usually the area is treated with herbicides or tilled to destroy any plant material that might have remained. This must be identified in the trial protocol. It is possible that volunteer plants will arise at the field trial site. In order to assure that these plants are destroyed, a procedure

for monitoring the site after the trial is complete must exist. This allows for the easy identification of volunteer plants resulting from the trial. During this period the site(s) will be monitored not only for volunteers but weedy relatives that are related to the genetically altered plant material that was grown.

Fate of Harvested Plant Material

Finally, the protocol must indicate the treatment of harvested plant material and seed. Seed and plant material is either kept for laboratory testing of further field testing, or exported to another country. A brief description of the types of analyses that will be performed on the plant material and seed should be included. This information allows regulators to monitor the disposal of transgenic plant material that has undergone field testing.

APPENDIX VII

INFORMATION REQUIREMENTS FOR OVERSIGHT OF FIELD TESTING OF GENETICALLY MODIFIED MICROORGANISMS

Source: Major D.W.; Hart D.R.; Lush D.L. (1988), Release of Genetically Engineered Microorganisms into the Environment, Beak Consultants Limited, Canada

AIMS OF THESE INFORMATION REQUIREMENTS

The general aim of these proposals for information requirements is to ensure provision of sufficient information on the rGEM(s) or GEM(s) so as to permit the oversight agency to assess the relative safety and/or merits of releasing, for field trial purposes, rGEM(s) or GEM(s), and to provide the researcher(s) petitioner(s) a guide concerning the factors that will be used to reach a decision. The term rGEM(s) in this document will apply to those microorganism that have been genetically altered by rDNA techniques involving insertion or deletion of specific nucleotides sequences such that these inserted/deleted sequences can be defined in terms of nucleotide sequences and function. Whereas, the term GEM(s) will apply to microorganism that have been genetically modified by cell fusion or other technologies resulting in genetic modificatios or transfer, that are not documented to occur in nature, which cannot be completely discerned or described genotipically. Recipient refers to microorganism that genetic material will be inserted into, donor refers to the microorganism that serves as the source of genetic materials, and parent refers to either recipient or donor microorganisms. These guidelines should be considered as general information requirements only and individuals submitting information should do so taking into consideration:

- the nature of the product that they are intending to produce;
- its intended application; and
- the nature of the open environment into which it is likely to be tested.

These three considerations will help determine what specific information is appropriate in order to allow field trial approval, which in turn will determine the appropriate protocols that will be employed to evaluate the safety and environmental risks of releasing the rGEM(s) or GEM(s). Evaluations will be tiered, with increasing testing of the rGEM(s) or GEM(s) required if initial tests indicate a question of safety to human/-animal or environment.

Much of the information that is requested here should already be available to the individuals or company producing the rGEM(s) or GEM(s) as a result of the research leading to product development. Thus the time required should be no more than a few weeks for anyone

familiar with the rGEM(s) or GEM(s) to provide the agency with the requested background information. Depending upon the nature of the rGEM or GEM, the genetic alteration carried out, and the intended application, microcosm or contained greenhouse tests may be required prior to field test approval.

The release of rGEM(s) of GEM(s) will be considered in light of the balance between the rationale for their use, feasibility, efficacy and potential benefits in comparison to other available approaches, and analysis of potential human and environmental hazard potential.

The information required will help address these issues and can be arranged in the following categories:

- identification and characterization of the recipient/donor wild-type strain(s) in terms of physiology, ecology, genotype and phenotype. This will also ensure proper registration of the rGEM(s) or GEM(s);

- description of the inserted or deleted genetic material (for rGEMs) in terms of its source, nucleotide sequence, function, expression, current or previous location in the parent (i.e. chromosomal, plasmid) and insertion or deletion points (i.e. flanking nucleotides, restriction markers) and description of any other associated genetic material or properties that may be used for identification (e.g., marker genes which code for specific enzymes or which can be detected by genetic probes) or environmental control (e.g., suicide genes);

- comparison of the rGEM(s) or GEM(s) to the recipient and/or donor(s) in terms of identification and characterization;

- impact of the rGEM(s) or GEM(s) on the environment with emphasis on the ecological contrast between the recipient/donor(s) and the rGEM(s) or GEM(s); and

- quality assurance/quality control (QA/QC).

SPECIFIC INFORMATION REQUIREMENTS

1. Wild-type or Recipient Identification and Characterization

Rationale

A complete as possible understanding of the physiology, ecology, genotypic and phenotypic expressions of the recipient microorganism is essential since the dominant traits of the rGEM(s) will be that of the recipient. Thus as much information as possible on the recipient will aid in the prediction of the fate of the rGEM(s) after field trial release. This information will serve as baseline data for comparison to the rGEM(s). This information is also applicable to the donor microorganisms used in cell fusion or other techniques that transfer large amounts of DNA, non-specifically (i.e., GEM(s). The information required will include:

- **Identification:** This is a key issue since accurate identification permits available information (habitat/physiology, genetic characteristics, pathogenicity) on the organism to be collected from the available literature. Identification of the microorganism must include a taxonomic name and a reference to a culture that is available either as part of a culture collection or maintained in-house. How the recipient microorganism(s) is maintained and identified must be included as part of the QA/QC documentation. If the host microorganism is a new isolate it must be classified and a culture must be placed in a recognized culture collection or maintained in-house.

- **Habitat/Physiology:** Geographical distribution, habitat, factors that affect growth such as maximum-minimum pH, Eh (redox potential) and temperature, role in biogeochemicalcycling, nutritional requirements, predators, competitors, survival under various physicochemical conditions, dispersal mechanisms and dispersal rates are required. This information can usually be supplied by a search of the literature and laboratory experience.

- **Genetic Characteristics** (when applicable): Strain history with respect to previous genetic manipulations is required, with presence or absence of mobile genetic elements such as plasmids and transposons indicated. Information is also requested on the transfer rates of the inserted genetic material between the recipient and known or suspected organisms that conjugate with the recipient or have in common the same

vectors. Information is also requested on the transfer rates of the inserted genetic material between the recipient and any other organisms with which the recipient can form a symbiotic relationship. The conditions during transfer, and the mechanisms of transfer should be provided along with the transfer rates.

- **Pathogenicity:** This characteristic of the recipient is a key issue and serves as a flag for pathogenicity testing of the rGEM(s) or GEM(s). Accurate identification of the recipient or donor(s) will determine if the recipient of donor(s) are placed in a group of microorganisms that are known to be pathogens or closely related to plant, animal or human pathogens. It will also indicate if the recipient or donor(s) is a non-pathogenic strain of a pathogenic organism, in which case toxicity/pathogenicity testing of the rGEM(s) or GEM(s) will be required. If the recipient or donor(s) is a known pathogen or is related to a known pathogen then information on its host range, route of transmission and mechanism of infection should be provided.

2. Characterization of Inserted Genetic Material (where applicable)

Rationale

Genetic engineering utilizing rDNA technology allows the excision of specific nucleotide sequences and insertion of excised or synthesized sequences into the recipient microorganism. Since the new trait(s) that will be expressed in the recipient arises only from the information transferred to the recipient, it is necessary only to characterize the transferred genetic material. This is only true, however, if sufficient QA/QC exists to prove the characteristics of the inserted genetic material. The information requested on the genetic material includes:

- description of the function (i.e. non-coding regulatory region, coding region, proteins coded for);
- nucleotide sequence including any flanking nucleotides;
- source of genetic material;
- description of the anticipated or actual expression of the

genetic material in the rGEM(s);

- determination that inserted/deleted genetic material is the smallest possible number of nucleotides needed to give the desired phenotypic expression; and

- documentation of the location of the inserted genetic material in the host genome (i.e., chromosomal, plasmid, flanking nucleotides, other nearby coding genes, transposons, mobile genetic elements and regulatory non-coding regions that could affect the function, expression or transfer of the inserted genetic material).

3. rGEM or GEM Characterization and Comparison to the Recipient

Rationale for rGEM

Ideally, a rGEM should represent the sum of its parts, (i.e. the phenotypic expression of the rGEM should be the phenotypic expression of the recipient plus the expression of the inserted genetic material from the donor) or, in the case of deletion of genetic material, the phenotypic expression would be that of the parent minus the phenotypic expression of the deleted genetic material. However, the insertion or deletion of new material may well allow the rGEM to behave differently than expected. The information required to address this concern includes:

- comparison of the expression of the inserted genetic material in the recipient to its expression in the donor and description of any differences;

- documentation and description of any changes in the rGEM that are different from the recipient in terms of the recipient's baseline phenotypic expression, whether intended or not; and

- taxonomic classification of the rGEM to determine if the inserted genetic material causes a shift, as defined by a referenced taxonomic protocol, into a new species or genus of the recipient.

Rationale for GEM

Since there is little control over the outcome of the genetic modification in fusion, microinjection or other similar technologies that produce GEMs, it is currently impossible to characterize the genotypic changes that have occurred in the produced GEM. Thus the GEM as a final product must be characterized in terms of its phenotypic expression. The information that should be provided will contrast the GEM to the parent organism(s) and includes:

- taxonomic classification of the GEM to determine what relationship it has with naturally occurring microorganisms; and

- documentation and description of any changes in the GEM that are different from the parent organisms in terms of the parents' phenotypic expression, whether intended or not.

4. Comparison of the rGEM to the recipient or GEM to the parent organisms in terms of environmental considerations.

Environmental Fate of rGEM or GEM.

There must be a comparison carried out between the rGEM and the recipient and the GEM and the parents in their respective abilities to survive, grow and disseminate. The information obtained will aid in the prediction of the fate of the rGEM(s) or GEM(s) in the environment. If the rGEM(s) or GEM(s) can survive and proliferate in the environment as well as or better than the recipient or parents, respectively, then the questions that must be addressed include:

- comparison of the rGEM(s) to the recipient or the GEM(s) to the parents as to survival, proliferation and dissemination in various environments under various physico-chemical conditions; and

- determination of what factors will allow establishment of the rGEM(s) or GEM(s) in the environment where applied.

Environmental Impact of the rGEM of GEM

There must be information on the impact of the rGEM(s) or GEM(s) on the ecosystem in the area in which field testing is to be carried out. The questions that must be addressed include:

- whether the presence of the rGEM(s) or GEM(s) causes a significant, non-target, disruption of the ecology in the short-term; and

- whether there are unintended long term effects on important ecological processes.

The information to answer the above questions will require, first, microcosm and then subsequent field testing. Before the approval of a field test, microcosm and/or contained greenhouse experiments will have to provide the following information:

- effect of the rGEM(s) or GEM(s) on ecological communities in terms of structure and function (e.g., diversity, productivity and biogeochemical processes). The target function of the rGEM of GEM must be considered since their function may be to disrupt the ecology in a specific manner;

- effect of the frequency and size of rGEM or GEM applications over extended periods;

- transfer and consequence of transfer and expression of genetic material from the rGEM(s) to other organisms. This should be addressed if the likelihood or rate of transfer of genetic material is high, or the stability of the inserted genetic material is high;

- information on target and non-target organisms in terms of known and predicted effects; and
- stability of the inserted genetic material in the rGEM in terms of expression and changes in expression over time under various physico-chemical conditions

QUALITY ASSURANCE/QUALITY CONTROL

The following information is requested to help the oversight agency to better understand and assess the potential impacts and adequacy of release/monitoring methods associated with the rGEM(s) or GEM(s) application for field testing:

- product purity test methods and identity of any biological con-

taminants and byproducts which have been found;

- construction methods of all components and the final construction of the rGEM(s) or GEM(s);

- method, sensitivity and reliability of detection in the environment of the containment, transport, contingency, eradication and disposal procedures for all areas of production, distribution and application.

PROPOSED FIELD TRIALS

The information required pertains to the nature, application, methods and magnitude of the rGEM(s) or GEM(s) application, and the containment and decontamination procedures that will be used at the field trial site.

1. Site(s) information:

   - exact location;

   - environmental nature of the site including surrounding area (significant climatic, geological, edaphic and other environmental aspects);

   - physical and biological proximity to human and/or other significant biota; and

   - description of the dominant native flora/fauna.

2. Crop(s), animals etc. and number of hectares and/or organisms to be treated at the site.

3. Experimental design

4. Methods and arrangements for producing the required quantity of microorganism(s) product(s) and for transport to the site.

5. Date(s) and/or period(s) of the test(s).

6. Introduction Protocol:

- formulation to be tested;
- total quantity to be applied;
- method of introduction including rates(s), frequency, and (where applicable) duration of application;
- target organism(s); and
- crop/animal husbandry practices (e.g. plans for mechanical disruption of the soil, irrigation etc.).

7. On-site safety procedures including containment, decontamination, accidental release plans, microbial and inserted DNA monitoring. The microbial and inserted DNA monitoring information should include sensitivity and method of detection.

8. Security and contingency plans.

APPENDIX VIII

ENVIRONMENTAL ASSESSMENT REQUIREMENTS FOR LIVING BIOTECHNOLOGICAL VETERINARY BIOLOGICS

Source: Agriculture Canada (1989), Guidelines for the Regulation of Veterinary Biologics produced by Biotechnology

ENVIRONMENTAL ASSESSMENT (EA) REQUIREMENTS

I. Purpose and need

Describe proposed action.

- Include concise statement of problem identified and agency program/action designed to deal with problem.

II. Describe the organism and its properties compared to the non-engineered parents

Characteristics of parent.

a) Identification, sources, and strains.

b) Reproduction and capacity of genetic transfer.

- Source, description, and function of foreign genetic material.
- Method of accomplishing genetic modification.
- Genetic stability, expression, and potential for recombination of the vaccine microorganism.
- Advantages and disadvantages of the modified organisms compared to conventional products.
- Comparison of the modified organisms to parental properties.
- Route of administration.

III. Human safety

- Probability of human exposure.
- Possible outcomes of human exposures.

- Pathogenicity of parent microorganisms in man.
- Effect of gene manipulation on pathogenicity in man.
- Risk associated with widespread use of the vaccine.

IV. Animal safety

- Fate of the vaccine in target and non-target species.
- Potential of shed and/or spread from vaccinate to contact target and non-target animals.
- Reversion to virulence resulting from back passage in animals.
- Effect of overdose in target and potential non-target species.
- Relative safety when compared to conventional vaccines.
- The extent of the host range and the degree of mobility of the vector.
- Safety in pregnant animals and to offspring nursing vaccinated animals.

V. Affected environment

- Identify.
- Discuss effects of alternatives on each aspect of the environment.
- Ecological concerns.
- Extent of release into the environment.

- Persistence of the vector in the environment.
- Extent of exposure of non-target species.
- Behavior of parent microorganisms and vector in non-target species.
- Potential of vector to infect non-vertebrate organisms.
- Physical and chemical factors which can affect survival, reproduction, and dispersal.

VI. Environmental consequences

- Discuss program action problems/benefits/potential hazards.
- When considering R-DNA live vaccines, compare benefits and problems of the experimental vaccine with licensed products.
- Identify proposed alternatives.
- Discuss selection criteria and weight given to each.
- Justify selection of proposed alternatives.

VII. Consultation and coordination with other agencies, organizations, and persons

- List all contacts.
- Identify comments, if any.

VIII. Conclusion and summary

IX. References

- List all references cited or relied upon.
- Include personal communications.

ADDITIONAL REQUIREMENTS

In addition to above information for environmental assessment the manufacturer must submit all necessary information, including results from the restricted field trials, to demonstrate that the product is pure, safe and potent and efficacious.

Each submission must contain the following information:

1. A description of the product, serial numbers of product to be used, recommendations for use, and results of preliminary research conducted in containment, including satisfactory purity and safety test results for each serial of product to be used.

2. A proposed general plan covering the methods and procedures for evaluating the products and for maintaining records of the quantity of experimental product prepared, shipped, and used. In the event of unanticipated adverse effects of the modified organism, proposed methods of biological or physical control and retrieval should be included in this plan.

3. A tentative list of the names and addresses of the proposed recipients and quantity of experimental product which is to be shipped to each individual.

4. Copies of labels or label sketches with the statement "Notice! For Experimental Use Only - Not For Sale" or equivalent.

Appropriate animal health authorities at the national level will negotiate with the appropriate regional authorities in which the trial is going to be conducted. If needed, the necessary advice will be sought from other federal regulatory agencies. Restricted field trial studies will be conducted under quarantine conditions acceptable to the Animal Health Authorities where there is adequate evidence of biological and/or physical control of the recombinant-DNA organism. Note that each study in this category will be conducted only in quarantine facilities already approved. Facilities shall be maintained and operated in an appropriate way to prevent the dissemination of any communicable disease. Test facilities may be inspected by a Veterinary Officer of the regulatory agency to determine whether they comply with the following guidelines:

Physical requirements of the facility

Generally the facility should be located and constructed so as to prevent test animals from having physical contact with other animals outside the facility. The holding area should be of sufficient size to prevent over-crowding of animals in quarantine. The facilities should be constructed with materials which can withstand cleaning and disinfection. Doors, windows and other openings should be provided with screen to prevent birds and insects from entering. A safe and effective program will be required to control insects, ectoparasites, and avian and mammalian pests. Facility specifications shall comply with applicable Federal, Provincial and local laws, and regulations relating to pollution control and the protection of the environment.

Sanitation and security

The applicant shall arrange for a water supply adequate for use in cleaning and disinfecting the facility. All feed and bedding used in the approved quarantine facility shall originate from an area not under quarantine and shall be stored in the test facility in a vermin-proof storage area. If needed, facilities must be adequately equipped to incinerate, or effectively disinfect bedding and solid waste at the conclusion of the study.

The application for the field trial will be evaluated and the decision to approve or reject the acceptance of the trial will be made within 60 days after the submission of the complete information. If approved, the field trial can be initiated 30 days following the approval.

For the granting of the license for the manufacture and sale of the live recombinant product, the applicant must submit all necessary information including test results of the limited field trial. The data must demonstrate that the product is safe, potent and efficacious and meets all the requirements of the Regulatory Agency. Once the product license is issued the product is accepted for distribution throughout the country without restriction and can be used in target species according to the approved label instructions.

APPENDIX IX

SUGGESTED CRITERIA FOR rDNA GILSP (GOOD INDUSTRIAL LARGE SCALE PRACTICE) MICROORGANISMS

Source: OECD (1986), Recombinant DNA Safety Considerations, Paris.

Host Organism	rDNA Engineered Organism	Vector/Insert
- Non-pathogenic;	- Non-pathogenic;	Well characterized and free from known harmful sequences
- No adventitious agent	- As safe in industrial setting as host organism, but with limited survival without adverse consequences in environment.	Limited in size as much as possible to the DNA required to perform the intended function; should not increase stability of of the construct in the environment (unless that is a requirement of the intended function).
- Extended history of industrial use; or		Should be poorly mobilisable
- Built-in environmental limitations permitting optimal growth in industrial setting but limited survival without adverse consequences in environment.		Should not transfer any resistance markers to microorganisms not known to acquire them naturally (if such acquisition could compromise use of drug to control disease agents).

APPENDIX X

FRAMEWORK TO ASSESS FIELD TESTING OF GENETICALLY MODIFIED PLANTS

Source: National Research Council (1989), Field Testing Genetically Modified Organisms: Framework for Decisions, Wash. D.C.

Most of the extensive past experience on field research of plants that have been genetically modified by classical techniques is relevant to field research of plants modified by molecular and cellular techniques. Procedures of confinement, monitoring, and mitigation work equally well, regardless of how the plant was produced.

The types of modifications that have been seen or anticipated with molecular techniques are similar to those that have been produced with classical techniques. No new or inherently different hazards are associated with the molecular techniques. Therefore, any oversight of field tests should be based on the plant's phenotype and genotype and not on how it was produced. The power of the molecular methods, however, does present the possibility that plants with unfamiliar but desired phenotypes may be produced. In some cases, new genes sources may be used, but familiar phenotypes will result. Plants with unfamiliar phenotypes should be subject to oversight until their behavior is predictable and shown to be nondetrimental to the environment.

In this section, a decision-making framework (Fig. 1) that allows experimental field testing based on (1) familiarity with the plant and genetic modification (Fig. 2), (2) the ability to confine the plant (Fig. 3), and (3) the perceived environmental impact if the plant should escape confinement (Fig. 4) is proposed.

Situations that are familiar and considered safe on the basis of past experience or experimentation should be classified as manageable by accepted standards (MAS). MAS plants would include, for example, classically produced plants and other plants with familiar phenotypes. These plants should be field-tested in a manner that is most appropriate based on past experience in traditional plant breeding.

All plants can be confined, some more readily than others. The use of sterile plants is probably the best example of easy confinability, providing that attention is paid to the dissemination of vegetative propagules. The other extreme would be to confine an open-pollinated plant in the presence of cross-hybridizing wild relatives. In this situation, confinement may be as strict as physical containment in a quarantine greenhouse. It is clear that the appropriate level of confinement depends on the plant and the geographic area for the field test. If confinement is difficult or uncertain, attention needs to be given to the potential environmental impact of the introduction. If there is potential for considerable

negative environmental impact, confinement procedures should be rigorous, as with screened cages. If potential impact is low, less stringent procedures should be called for.

As data based on field tests accumulate, it may be desirable to lessen the confinement requirements so that a plant can be used in a crop improvement program. Field-test results need to be assessed for potential negative environmental impact as a result of altered characteristics of weediness, toxicity, or pest resistance. Data obtained through field testing provide the best way to assess the presence of undesirable characteristics accurately.

The committee has also included a set of example questions (Figs. 1 through 4) that might need to be asked at each phase in the decision-making process. This is not a comprehensive list. The importance attached to each of these questions should be determined on a case-by-case basis.

FIGURE 1

FRAMEWORK TO ASSESS FIELD TESTING OF GENETICALLY MODIFIED PLANTS

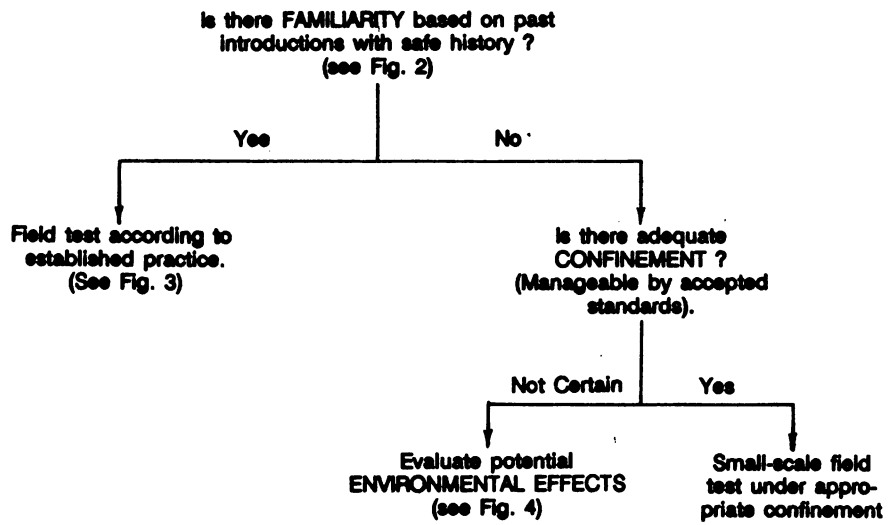


FIGURE 2

FAMILIARITY

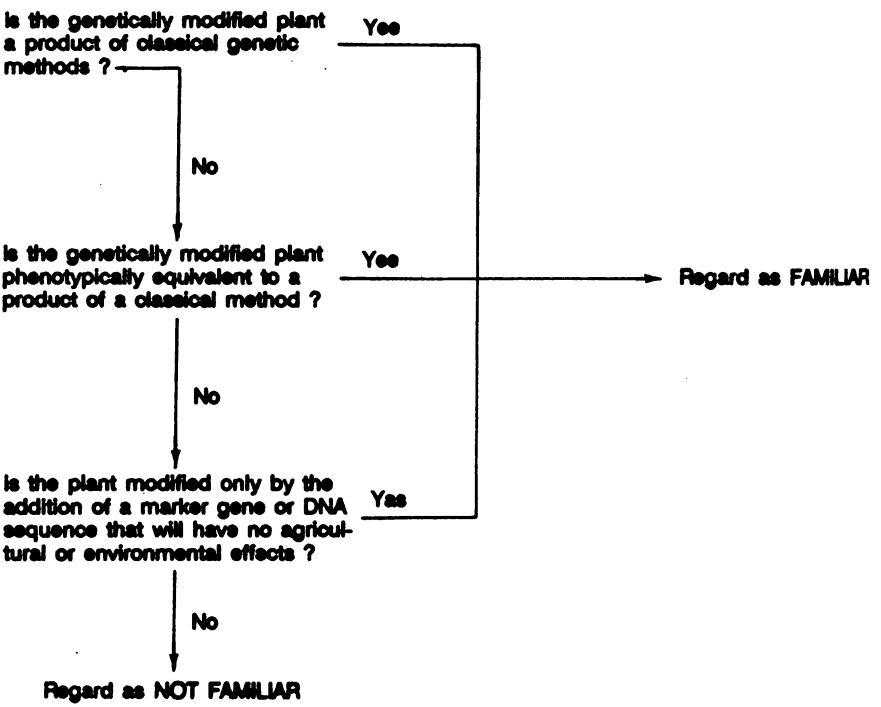


FIGURE 3

CONFINEMENT

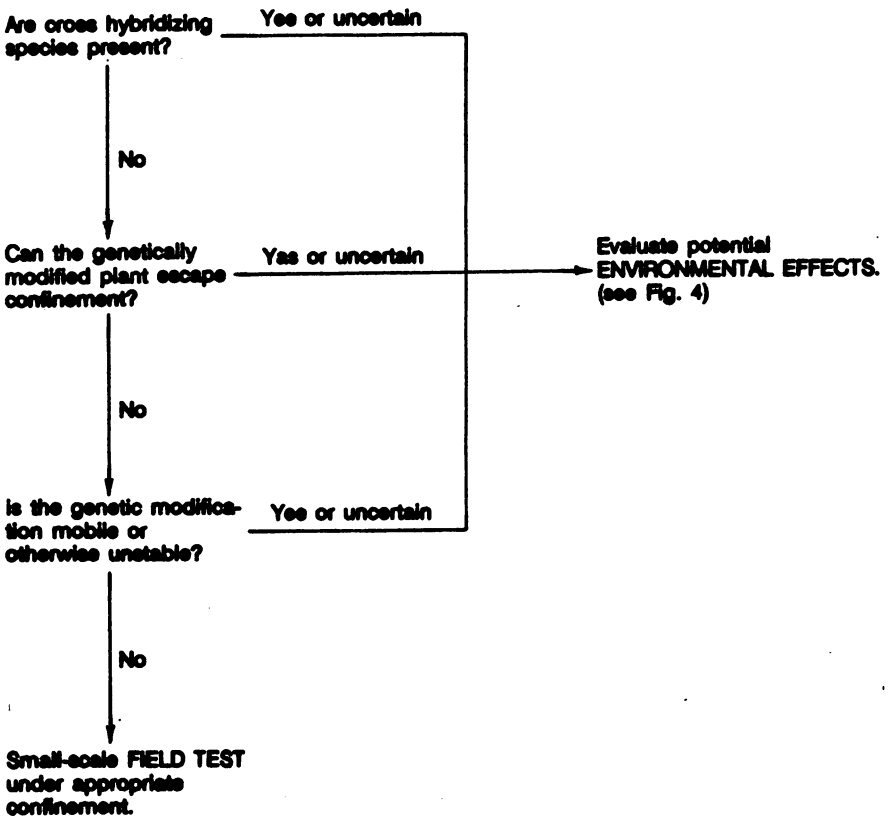
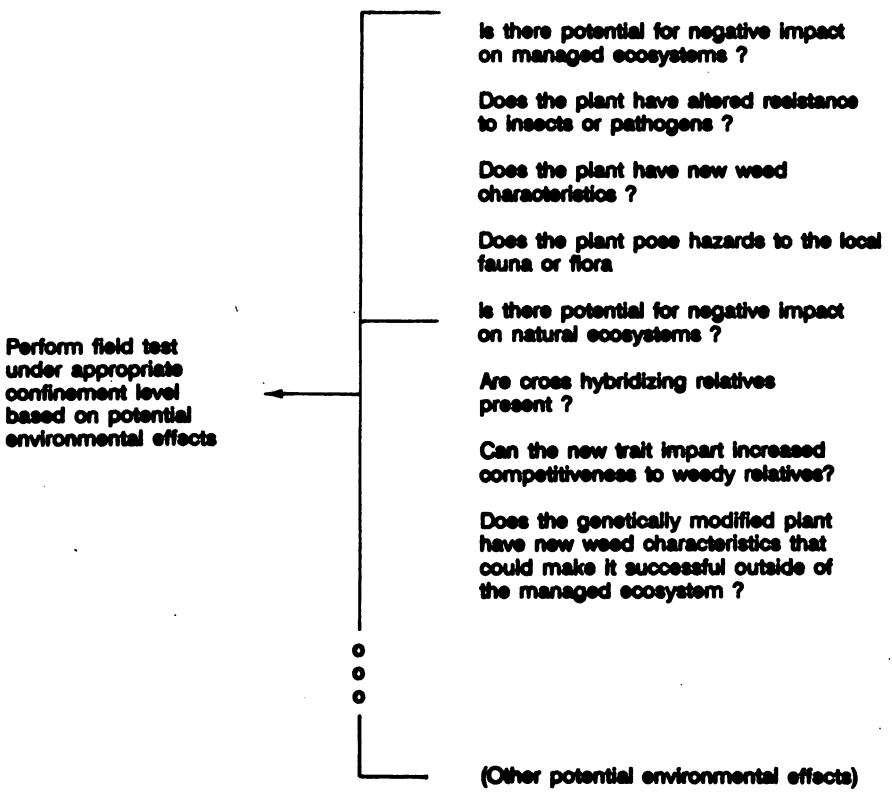


FIGURE 4

POTENTIAL ENVIRONMENTAL EFFECTS.
APPROPRIATE QUESTIONS FOR SPECIFIC APPLICATIONS
TO BE ADDED BY USERS OF THE FRAMEWORK



APPENDIX XI

FRAMEWORK TO ASSESS FIELD TESTING OF GENETICALLY MODIFIED MICROORGANISMS

Source: National Research Council (1989), Field Testing Genetically Modified Organisms: Framework for Decisions, Wash. D.C.

Mankind has a long history of using microorganisms in food processing, agriculture, waste treatment, and in other beneficial applications. New molecular methods for genetically modifying microorganisms will expand the range of beneficial applications, for example, in control of plant disease and in biodegradation of toxic pollutants.

In many respects, molecular methods resemble the classical methods for modifying particular strains of microorganisms, but many of the new methods have two features that make them even more useful than the classical methods. Precision allows scientists to make genetic modifications in microbial strains that can be characterized more fully, in some cases to the level of the DNA sequence. This reduces the degree of uncertainty associated with any intended application. The new methods have greater power because they enable scientists to isolate genes and transfer them across natural barriers.

The power of these new techniques creates the opportunity for new applications of microorganisms. Despite some initial concerns over the use of recombinant methods in laboratory research, it is now clear that these methods in themselves are not intrinsically dangerous.

The next step after laboratory experimentation is to test modified microorganisms in the field, and establishing a scientifically based framework for decisions on field testing has been a primary purpose in this report. No adverse effects of introductions have been seen and an extensive body of information documents safe introductions of some microorganisms, such as the rhizobia, mycorrhizal fungi, baculoviruses, *Bacillus thuringiensis*, and *Agrobacterium*. However, less is known about field tests of microorganisms than of plants. Thus, for unfamiliar applications, it is prudent to prepare for the control of the introduced microorganisms.

Questions concerning the effects of an introduced microorganism arise whenever the intended introduction differs substantially from those with an established record of safety. Such questions as unintended persistence and possible adverse effects should be addressed scientifically, and as the scientific community continues to accumulate information regarding the safety or risk of environmental applications of microorganisms in field tests, levels of oversight can be tuned to the needs of particular situations.

In the recommendations that follow, a framework has been developed as a basis for a workable and scientifically based evaluation of the safety of microorganisms intended for field testing. This framework has been developed from consideration of three criteria: (1) familiarity with the history of introductions similar to the proposed introduction; (2) control over persistence and spread of the introduced microorganism as well as over exchange of genetic material with the indigenous microflora, and (3) environmental effects, including potential adverse effects associated with the introduction.

The framework does not distinguish between classical and molecular methods of genetic manipulation, nor between modified and unmodified genotypes. The framework is product rather than process oriented, focusing on the properties of the microorganism rather than on the methods by which it is obtained. Knowledge of the methods used may nonetheless yield useful information concerning the precision of genetic characterization of the microorganism, which in turn may be relevant for assessment of its similarity to previous applications, persistence, and possible effects after introduction.

The framework has not focused on other variables, often suggested as criteria for oversight, because they convey relatively less scientifically useful information for assessments: the sources of genes, whether recombinants are intra-or intergeneric, and whether coding or noncoding regions of the genome have been modified. The necessity of using, whenever possible, simple and readily identifiable criteria for oversight is recognized.

Terms such as "uncertainty", "sufficient", and "significant" are used in the framework without precisely defining their quantitative limits. Any specific numerical values assigned would be arbitrary and subject to disagreement, as some underlying variables may be difficult to quantify precisely. In the final analysis, assignment of risk categories must include a rational examination of the relevant scientific knowledge for each introduction.

In the framework, assessments of potential risks arising from the introduction of microorganisms into the environment are made according to the three major criteria of familiarity, control, and effects. Upon evaluation of these three criteria, a proposed introduction can be field-tested according to established practice or it can be assigned to one of

three levels of concern: low, moderate, or high uncertainty (Fig. 1). The framework is inherently flexible, allowing an application to be reassigned to a different category as additional scientific information is obtained that is relevant to any of the three criteria.

Small-scale field tests can proceed according to established practice if the microorganism used, its intended function, and the target environment are all sufficiently similar to prior introductions that have a safe history of use (Fig. 2). *Rhizobium* used for enhancement of nitrogen fixation in leguminous crops provides a familiar example.

If an introduction does not satisfy the familiarity criteria, it is evaluated with respect to both our ability to control the microorganism's persistence and dissemination and the microorganism's potential for significant adverse effects (Fig. 1). For example, *Rhizobium* modified to encode an insecticidal toxin would not be a familiar introduction, even though it might well prove to be safe. An introduction is considered to be in the low uncertainty category if it satisfies appropriate criteria with respect to both controllability and low potential result in adverse effects. An introduction is considered to be in the moderate-uncertainty category if it satisfies criteria for either controllability or potential effects, but not both. An introduction is considered to be in the high-uncertainty category if it satisfies neither the control nor the effects criterion (Fig. 1). The high uncertainty status implies that potential adverse effects exist and are coupled with potential inability to control the microorganism, and hence its potential effects.

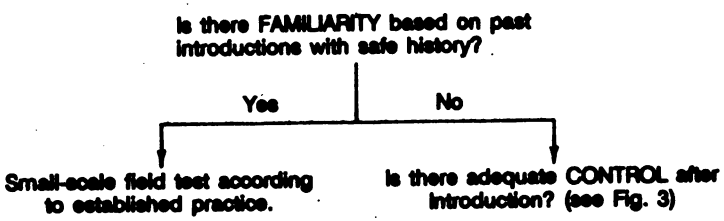
Specific criteria for evaluating control of the microorganism after it is introduced must include the potentials for persistence of the introduced microorganism, genetic exchange between the introduced and indigenous microorganisms. and spread of the introduced microorganism to non-target environments (Fig.3). A series of questions to be addressed in evaluating the potential for unwanted persistence of an introduced microorganism is illustrated in Fig. 4.

Criteria for evaluating effects must depend, at least in part, on the intended function of the introduced microorganism in its target environment (Fig. 5). Thus, a proposed field test of a bacterium to be used for biodegradation of a toxic pollutant should be preceded by definitive laboratory experiments and should be designed to determine whether toxic by-products of the degradation may be created and persist.

As the agencies grant permission to introduce genetically modified microorganisms in field tests, they will receive advice from panels of experts who can utilize the decision framework described here. With experience, familiarity will increase, and we anticipate this will be accompanied by adjustments in the rigor of oversight.

FIGURE 1

FRAMEWORK TO ASSESS FIELD TESTING OF GENETICALLY MODIFIED MICROORGANISMS



Have significant adverse environmental effects been reasonably excluded? (see Fig. 5)

	Yes	No
Yes	Low uncertainty	Moderate uncertainty
No	Moderate uncertainty	High uncertainty

FIGURE 2

FAMILIARITY

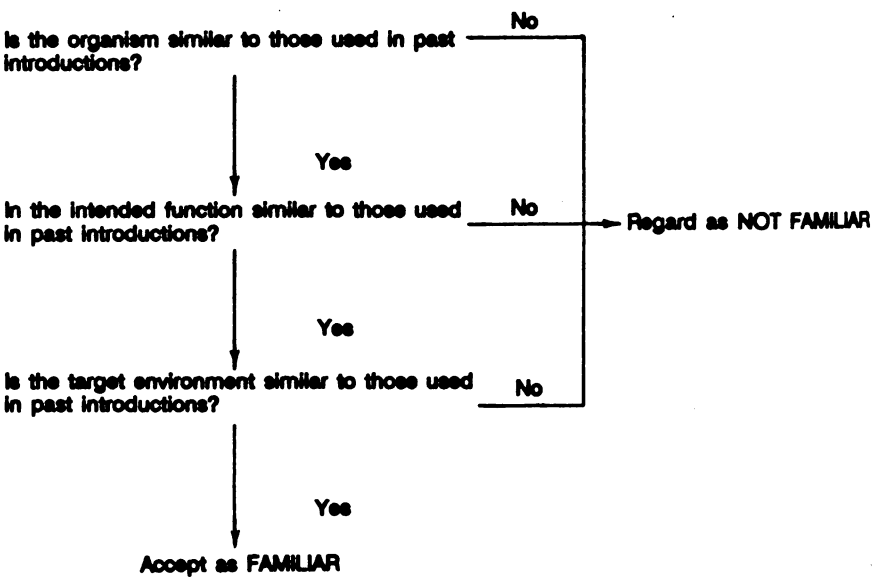


FIGURE 3

CONTROL.
APPROPRIATE QUESTIONS FOR SPECIFIC APPLICATIONS
TO BE ADDED BY USERS OF THE FRAMEWORK

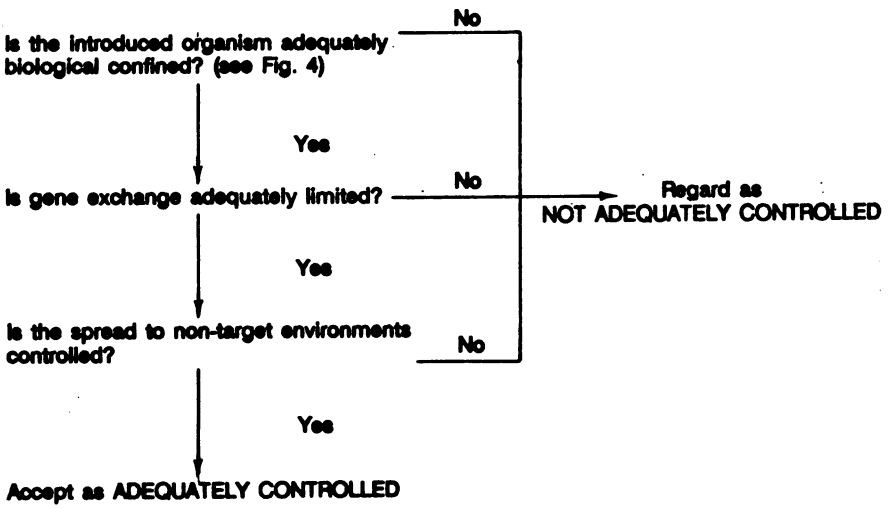


FIGURE 4

BIOLOGICAL CONFINEMENT,
APPROPRIATE QUESTIONS FOR SPECIFIC APPLICATIONS
TO BE ADDED BY USERS OF THE FRAMEWORK

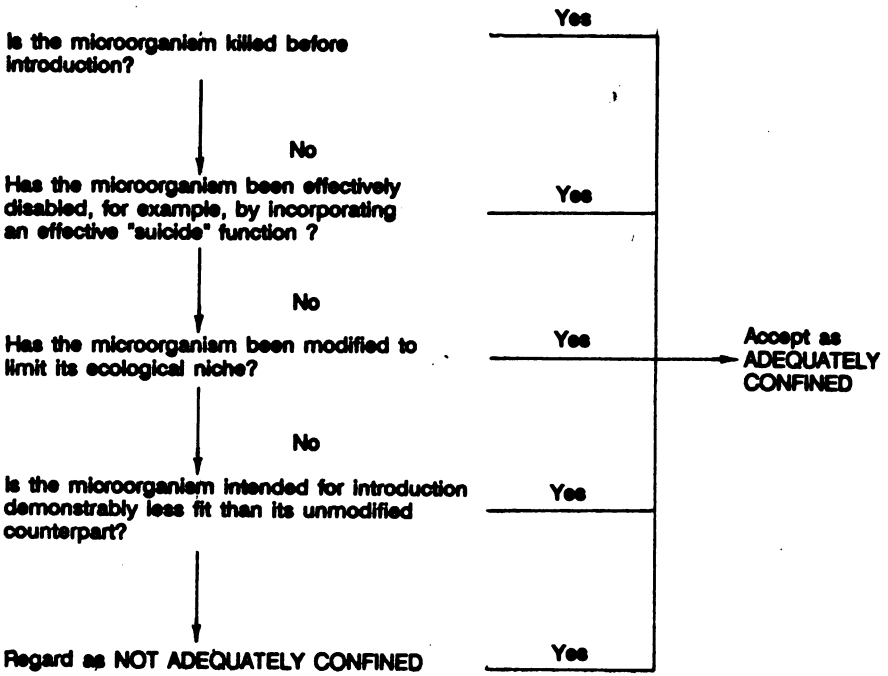
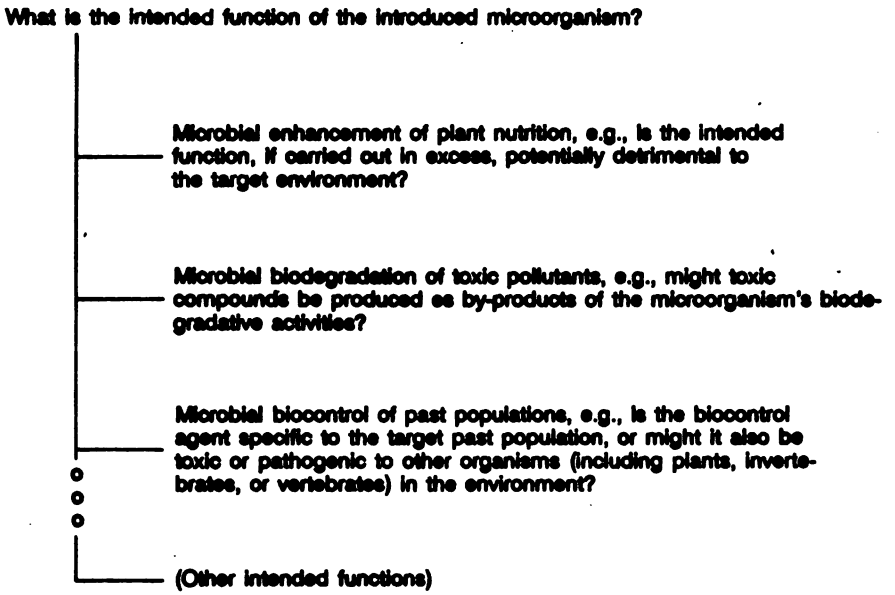


FIGURE 5

POTENTIAL ENVIRONMENTAL EFFECTS.
APPROPRIATE QUESTIONS FOR SPECIFIC APPLICATIONS
TO BE ADDED BY USERS OF THE FRAMEWORK



APPENDIX XII

REQUIREMENTS FOR LICENSING OF VETERINARY BIOLOGICS PRODUCED BY NEW TECHNIQUES OF BIOTECHNOLOGY

Source: Agriculture Canada (1989), Guidelines for the Regulation of Veterinary Biologics Produced by Biotechnology

GENERAL LICENSING REQUIREMENTS

Preparation and certification of master seed(bacterial or virus) stocks

Manufacture of experimental product to minimum outline specifications

Host animal efficacy (immunization and challenge)

Preparation of three consistency serials

Field safety tests

Satisfactory completion of all test requirements in "Outline of Production"

Submission of samples to Veterinary Biologies Evaluation Laboratory for confirmatory testing

Accept labels

Licensing

Release of prelicensing serials

Veterinary biologies produced by new techniques of biotechnology such as rDNA, chemical synthesis, hybridoma technology may require special assays for potency and stability determinations. Additional tests may be required to assure safety, especially when live microorganisms are present.

In order to maintain uniformity of production, manufacturers are required to obtain seed materials for production from a lot of seed material which is defined as the Master Seed. Master Seed and final product are tested to assure purity, safety, identity and immunogenicity.

The Master Seed for rDNA derived products will consist of a plasmid or virus carrying the inserted gene. The constructed plasmid is

then introduced into the appropriate eukaryotic or prokaryotic expression system selected for vacine production. Genomic DNA may also be transferred directly into a variety of mammalian cells. Alternatively, in such cases, the stable transfected cell will be considered as the Master Seed.

Recombinant DNA Master Seeds will be characterized by providing a construction map of the bacterial plasmid containing the new gene. Background information concerning the rDNA procedures used to isolate, purify and identify genetic material from one source and the modification used for the insertion of this material into a new host is required. The manufacturer must provide a nucleotide sequence analysis in order to characterize adequately the foreign DNA used to code for a particular antigen.

Immunogenicity of vaccines must be supported by statistically valid host animal immunization and challenge studies.

The manufacturer must prepare "Outlines of Production" that include procedures to ensure consistency and recovery of specific antigenic material. Recovery procedures must include removal of excessive antibiotic levels and undesirable fermentation by-products such as excessive bacterial endotoxins. Some in-process test procedures which might be used for monitoring purposes are: growth rate, SOS gel mapping, antibiotic resistance, metabolic markers, molecular weight, activity, and percent protein.

For each serial release of the final biotechnology products testing for purity, safety and potency will be required. Standard procedures will be applicable for purity, potency and efficacy. Safety procedures may entail expanded laboratory and field testing programs. In addition to these tests, product characterization will be required to demonstrate gene expression. Some examples of the techniques which can be used are: partial sequence analysis, high performance liquid chromatography, peptide mapping, polyacrylamide gel analysis and molecular weight determination.

ANNEX I

MEMBERS OF THE INTERAMERICAN STUDY GROUP OF THE NEW BIOTECHNOLOGY IN AGRICULTURE AND HEALTH WHICH PARTICIPATED AT THE MEETING HELD IN BRASILIA, MAY 30 - JUNE 1, 1990.

Elisa Barahona Nieto
Secretaría General del Medio Ambiente, MOPU
C/P Castellana 67
28071 Madrid, España
Tel. (1) 253-1600, Fax 533-0111, Telex 22325

Luiz Antonio Barreto De Castro
Coordenador de Biotecnología
EMBRAPA / CENARGEN
SAIN Parque Rural
Caixa postal 70770
Brasilia D.F., Brasil
Tel. 273-0101, Fax 248-0557, Telex 1622

Willy Becak
Director General
Instituto Butantan
Av. Vital Brasil, 1500
Caixa postal 65
Sao Paulo, Brasil
Tel. 211-8381

Alejandro Blanco Labra
Investigador
Centro de Investigación y de
Estudios Avanzados
Kilómetro 9.6 Libramiento Norte
Carretera Irapuato - León
Apartado 629
Irapuato Gto. México
Tel. 51-600, Fax 51-282, Telex 122224 CIUIME

Felipe Canale
Director
Comité Sanidad Vegetal Cono Sur
Av. Millan 4703
Montevideo, Uruguay
Tel. 29-8720, Fax 39-6508, Telex 26236 UY

Jerry Callis
Consultant IICA
P.O. Box 537
Paradise Pt. Rd.
Southold, N.Y. 11971, U. S. A.

Raúl Casas Olascoaga
Director
Centro Pan Americano de Febreo Aftosa
Caixa Postal 589
Río de Janeiro RJ, Brasil
Tel. (021) 771-3128, Telex 021-30253 COFA BR.

Edgar Julian Duncan
Senior Lecturer
The University of the West Indies
Department of Plant Science
St. Augustine, Trinidad and Tobago
Tel. 662-4991, Telex 2520 UWI NG.

Mario González Pacheco
Asesor Regional de Biológicos
Organización Panamericana de Salud/OMS
525, 23rd St. N.W.
Washington D.C., U. S. A.
Tel.(202) 861-3216

Oscar Grau
Coordinador Técnico
Programa Regional de Biotecnología, UNESCO
Apartado Postal CC 111 - CP 1876
Bernal, Argentina
Tel. 54-21-21-5556, Fax 54-21-3-2978, Telex 31151

Marcel Gutiérrez Correa
Profesor
Laboratorio de Micología y Biotecnología, Facultad de Ciencias
Universidad Nacional Agraria La Molina
Apartado 456
Lima, Perú
Tel. 352-035 Ext. 273 y 271

Jean Hollebone
Director,
Issues, Planning and Priorities
Pesticide Directorate
Agriculture Canada
P.O. Box K1A 0C6
Ottawa, Canada
Tel. (613) 993-4544, Fax (613) 998-1312

Juan Izquierdo
Oficial Regional Producción Vegetal
Av. Santa Marta, 6700
Apartado 10095
Santiago, Chile
Tel. 228-8056, Fax 562-48-4312, Telex 340279 FAOCHI CK

Walter Jaffé
Technical Secretary of the Interamerican Study Group
Especialista en Generación y Transferencia de Tecnología
IICA - Sede Central
Apartado 55-2200 Coronado, Costa Rica
Tel. 29-0222, Fax 29-4741, Telex 2144 IICA

David T. Kingsbury
Professor
The George Washington University
Medical Center
2300 Eye Street, N.W.
Tel. (202) 994-3484, Fax (202) 994-0875

José La Torre
Director
Centro Virología Animal (CEVAN)
Serrano 661 (1414)
Buenos Aires, Argentina
Tel. 854-5602/8209, Fax 54-1-8564-495

Miguel Laufer
Director de Ciencia y Tecnología
1889, F. St. N.W.
Washington D.C.. U.S A.
Tel. (202) 458-3368

Gloria León
Profesora Titular Bioquímica
Universidad Austral de Chile
Instituto de Bioquímica
Independencia 567 - Casilla 567
Valdivia, Chile
Tel. (063) 21-3911 ext. 1333

Licda. Patricia Londoño
Investigadora Biología Molecular
Instituto de Inmunología
Hospital San Juan de Dios
Av. 01, número 1001, Bogotá, Colombia
Tel. 2334255/233-9006

Terry L. Medley Jr.
Director
Biotechnology, Biologics and Environmental Protection
U.S. Department of Agriculture
Animal and Plant Health Inspection Service
6505 Belcrest Rd.
Room 850 -Federal Building
Hyattsville, M.D., U. S. A.
Tel.(301) 436-7602, Fax (301) 436-8669

Henry I. Miller
Director
Office of Biotechnology, FDA
HE-6, 5600 Fishers Lane
Rockville, MO , U.S.A.
Tel. (301) 443-7573, Fax (301) 443-7005

Harry Mussman
Deputy Assistant Secretary
U.S. Department of Agriculture
Science and Education USDA
14th and Independence
Washington D.C., U. S. A.
Tel. (202) 447-8885

Eduardo Palma
Coordinador Programa Biotecnología
Instituto Nacional de Tecnología Agropecuaria
Rivadavia, 1439
Apartado Postal CC 17, Buenos Aires, Argentina
Tel. 621-0899, Telex 17518 INTA AR

Rodolfo Quintero Ramírez
Director General
Programa Regional de Biotecnología, UNIDO
Masaryk 2910, Col. Polanco
México D.F. 1570, México
Tel. 250-1550 - Ext. 152, Fax 1771055

William Roca
Director
Biotechnology Research Unit, CIAT
A.A. 67 - 13, Cali, Colombia
Tel. 57-23-6750, Fax 57-23-64-7243, Telex 05769 CIAT CO.

Bakhshish S. Samagh
Senior Staff Veterinary
Veterinary Biologics and Biotechnology
Agriculture Canada
801 Fallowfield Road
P.O. Box 11300 - Station H
Nepean, Canadá
Tel. (613) 998-9320

Alejandro Silva Rodríguez
Vice Director de Productos Farmacéuticos
Centro de Ingeniería Genética y Biotecnología
Av. 25, e/ 148 e 150 - Playa
P.O. Box 6162, La Habana, Cuba
Tel. 201-401, Fax 537-218 070, Telex 512330 ING. GEN. CU

Maro Sondahl
Senior Research Director
DNAP
2611 Branch Pire
Cinnaminson, N.J., U. S. A.
Tel. (609) 786-2809, Fax (609) 829-5087, Telex 750493

Eduardo J. Trigo
Director del Programa de
Generación y Transferencia de Tecnología
Sede Central - IICA
Apartado 55-2200 Coronado, Costa Rica
Tel. 29-0222, Fax 29-4741, Telex 2144 IICA

Pieter Van der Meer
Ministry for the Environment of the Netherlands
Dr. V/d Stamstraat 2
P.O. Box 450 - 2260 MB
Leidschemdam, The Nertherlands
Tel. 703-209367, Fax 703-175077

Anne Vidaver
Professor
University of Nebraska
68583 Lincoln NE, U. S. A.
Tel. (402) 472-2858, Fax (402) 472-2853

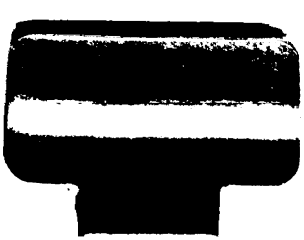
Rodrigo Zeledón
Profesor Investigador
Universidad Nacional
Heredia
Apartado 816
San José, Costa Rica
Tel. 25-5586, Fax (506) 38-1298

Joseph L. Zelibor
Industrial Development Officer
United Nations Industrial Development Organization
Biotechnology and Genetic Engineering Unit
Vienna International Center
Room D-1901
P.O. Box 300
A-1400 Vienna, Austria
Tel. (0222) 21131-5351, Fax (0222) 230-7355

Autor

Título Guidelines for the release into the environment of genetically modified organisms

Fecha Devolución	Nombre del solicitante



INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
P.O.Box 55-2200 Coronado, Costa Rica/Tel.: 29-02-22/Cable: IICASANJOSE/Telex: 2144 IICA CR
Electronic Mail EIES: 1332 IICA SC / FAX (506) 29-47-41, 29-26-59 IICA COSTA RICA