SAFETY CONSIDERATIONS FOR BIOTECHNOLOGY 1992

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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FOREWORD

This report is a follow-up to the 1986 publication *Recombinant DNA Safety Considerations* which set out the first international safety guidelines for biotechnology applications to industry, to agriculture and to the environment. A Recommendation of the OECD Council adopted the conclusions and recommendations made in the report and instructed the Committee for Scientific and Technological Policy to review, in consultation with other interested Committees of the Organisation, the experience and action of Member countries in connection with the principles contained therein.

Following the Council Recommendation and instructions, and in response to the unanimous interest expressed by Member countries, the Committee for Scientific and Technological Policy decided at its 46th session, on 10-11 February 1987, to continue to keep safety issues under review and mandated its subsidiary body, the Group of National Experts on Safety in Biotechnology, to carry out a follow-up programme in co-operation with the Environment Committee.

This report deals with two priority issues of this programme which are connected with the important development of biotechnology industrial production and field experiments in Member countries. Namely, it elaborates the initial scientific criteria set forth in 1986 for the safe development, under "Good Industrial Large-Scale Practice" (GILSP), of fermentation-derived biotechnology products and defines "Good Developmental Principles" (GDP) for the design of safe small-scale field research with plants and micro-organisms with newly introduced traits.

On 28 November 1991 the OECD Council agreed to derestrict this report.

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PREFACE

The issue of safety in biotechnology has been a priority concern of the Committee for Scientific and Technological Policy for a number of years.

In 1983, this committee created a Group of National Experts to consider safety in the use of r-DNA organisms in industry, agriculture and the environment. As a result, general guidelines were published in 1986 in a report entitled *Recombinant DNA Safety Considerations*.

A follow-up programme to that report was started in 1988 and undertaken by the Directorate for Science, Technology and Industry in co-operation with the Environment Directorate of the OECD. The main task of the Group of National Experts was to update and further develop the safety considerations set out in the 1986 report.

This report sets out general principles and criteria for safe large-scale industrial production and small-scale experimental field research in biotechnology, two areas to which the Group of National Experts accorded priority attention in its Mandate.

The report consists of two parts :

- Part One further develops the Good Industrial Large-scale Practice criteria and reviews the fundamental principles, identified in the 1986 report, for the handling of low-risk r-DNA organisms in industrial production.

- Part Two provides guidance on the design of low or negligible risk (small-scale) field research with genetically modified plants and micro-organisms. It introduces general principles for such research, or «Good Developmental Principles» (GDP) applicable to the continuum of testing from laboratory to production release. This continuum is represented diagramatically in a number of stages (Stage 1, Stage 2, Stage 3) on page 20. Although, in general, each stage may be revisited several times, as indicated in the diagram, it was recognised that in some situations satisfactory results may be obtained in a single stage. Therefore, it should be understood that in some cases, and in the light of current experience and adequate knowledge, it may not be necessary to proceed in a progressive step-wise fashion from Stage I to Stage 3.

The scientific principles presented in this report should facilitate the process, started in 1986, of developing consensus on the scientific basis for safe use of biotechnology.

Whilst the Group of National Experts continues to review other issues included in their Mandate, among which biotechnology applied research and food safety, there was agreement that this report should be published without delay to provide Member countries with timely guidance for their growing number of industrial applications and experimental field research.

Part One

ELABORATION OF CRITERIA AND PRINCIPLES FOR GOOD INDUSTRIAL LARGE-SCALE PRACTICE (GILSP)

BACKGROUND

The OECD report, *Recombinant DNA Safety Considerations,* published in 1986, set out a concept called "Good Industrial Large-Scale Practice (GILSP)" applicable to intrinsically low-risk r-DNA organisms used in industrial production. The concept encompassed certain criteria which an r-DNA organism must meet in order to be given GILSP status. It stated that r-DNA GILSP organisms can be handled, on a large scale, under the same conditions of minimal controls and containment procedures as would be used for the host strains. The key principle for GILSP is that the r-DNA organism should be as safe as the low-risk organism from which it is derived.

An internal survey carried out in 1988 in OECD countries on the use of the GILSP concept or its underlying principles showed that it had been adopted in national guidelines in a number of countries and was being considered for implementation in others. Furthermore, in some countries significant numbers of r-DNA micro-organisms from a limited range of species as well as some r-DNA cell cultures had been assigned GILSP status.

The survey established that:

- there are now a number of examples worldwide of GILSP micro-organisms;
- there appears to be some variation in the extent to which the concept has been taken up in practice;
- there is a general need for a better understanding of the way the concept can be applied and, in particular, for further elaboration of the GILSP criteria.

Part One develops further the criteria and principles for Good Industrial Large-Scale Practice (GILSP) identified in the 1986 report. It is based on current knowledge and experience with use of GILSP organisms. It takes into account the findings of the survey and draws, in part, upon an analysis of 25 examples submitted by Member countries which illustrate application of the GILSP criteria to specific cases. As part of this GILSP update the "Fundamental Principles of Good Occupational Safety and Hygiene", originally given in the 1986 report, have also been further elaborated and entitled "Fundamental Principles of Good Occupational and Environmental Safety".

The material presented in Part One is intended to assist Member countries to identify low-risk organisms that meet the GILSP criteria and to select appropriate practices consistent with GILSP principles. It is anticipated that increasing knowledge and experience with the various applications of the GILSP concept and its underlying principles will allow continued evolution of these safety criteria. Existing information demonstrates that a wide range of organisms are grown safely on a large scale with low risk. Low-risk organisms that do not qualify under the criteria for GILSP may be designated for handling under the same conditions, based on considerations set out in Section I (p. 8).

I. GENERAL CONSIDERATIONS

An important general point made in the 1986 OECD report is that hazards associated with r-DNA organisms can be assessed and managed like those associated with any other organisms. It is expected that the vast majority of r-DNA organisms to be used in industrial large-scale production can be handled using GILSP.

Irrespective of' the intrinsic safety of the organisms concerned, zero risk is not realistic even for GILSP organisms.

Central to the concept of GILSP are:

- the assessment of' the recombinant organism according to identified criteria to determine that it is as safe as the low-risk host organism;
- the identification and adoption of practices ensuring the safety of the operation.

r-DNA organisms which meet the GILSP criteria and are therefore of low-risk can thus be handled under conditions already found to be appropriate for the relevant hosts.

GILSP therefore lies within the framework of existing safety practices and provides an equivalent to established national and international definitions of the lowest risk category of organisms. It should be emphasized that in the conception and especially in the application of GILSP to date there has been great flexibility as to how the criteria described in Appendix F of the 1986 report are met in individual cases.

GILSP applies to organisms considered to be of low-risk and classified in the lowest risk class. In order to ensure that, for each individual case, an r-DNA organism merits the designation of GILSP, the criteria elaborated in Section II (pp. 10-13) must be taken into consideration in an integrated way. Two clear examples of other classes of organisms that warrant the GILSP designation, provided they are non-pathogenic and without adverse consequences for the environment, are:

i. those constructed entirely from a single prokaryotic host (including its indigenous plasmids and viruses) or from a single eukaryotic host (including its chloroplasts, mitochondria or plasmids -- but excluding viruses); and

ii. those consisting entirely of DNA segments from different species that exchange DNA by known physiological processes.

Organisms that do not meet all the criteria for GILSP are not GILSP organisms. However, after the case-by-case evaluation, they may be found to be of low risk. In such circumstances, these organisms may be handled using GILSP. Care must be taken, when extrapolating GILSP to other organisms, to evaluate whether specific practices in addition to GILSP are required to mitigate a specific concern.

Organisms which can be handled on a large scale under conditions of mininial controls and containment procedures will be:

- those meeting the criteria of Section II (pp. 10-13);

- those other classes of organisms described under points *i)* and *ii)* of the, abovc paragraph;
- other organisms not meeting either of these sets of criteria but which have been demonstrated to be of low-risk, as described above.

When handling GILSP and other low-risk organisms, established principles of good occupational and environmental safety as described in Section III (p. 14) must be followed.

II. ELABORATION OF CRITERIA FOR R-DNA GILSP (GOOD INDUSTRIAL LARGE-SCALE PRACTICE) MICRO-ORGANISMS AND CELL CULTURES

The criteria outlined below are relevant to micro-organisms and are equally appropriate for cell cultures. It is important that all of the criteria be considered in relation to one another in evaluating the GILSP status of an organism.

Host[1]

Non-pathogenic

The identity of the host must be established and the taxonomy well understood. The host must be evaluated to determine that it is not pathogenic. The host should not appear in national or other recognised lists of human pathogens. Member countries may have additional listings of plant and animal pathogens which may be a useful source of information in assessing the potential of the host to behave as a pathogen. In cases where uncertainty remains for the potential pathogenicity of an organism or an attenuated strain, further data must be developed to confirm its safety and hence its suitability for handling under GILSP conditions. In addition, some organisms not found in pathogen lists may produce toxic substances in amounts which require further evaluation[2].

Examples of hosts that are currently used in GILSP practice are listed below. It should be noted that, in some instances, entire species may qualify for GELSP host status, whereas in other cases only some strains or types may be so designated:

Saccharomyces cerevisiae
Escherichia coli K-12
Bacillus subtilis
CHO (Chinese Hamster Ovary) cells
Aspergillus oryzae

No adventitious agents

This is mainly relevant to cell cultures where harmful micro-organisms, in particular harmful viruses and mycoplasma, should not be present at detectable levels. Bacterial cultures should not contain unwanted phages.

Extended history of safe use

There should be adequate and documented experience of the safe use of the host organism, i.e. without harm to humans or to the environment. Historical and other data on the host, its progenitors or closely related strains may be appropriate for evaluation.

Such evidence may be obtained from applications such as food, enzyme and antibiotic production including from discharge practices used in such applications. Laboratory use and/or pilot scale fermentations under conditions of minimal containment could also provide useful data.

Built-in environmental limitations permitting optimal growth in industrial setting but limited survival without adverse consequences in the environment

The possibility of adverse effects can be reduced by restrictions on the organism's ability to multiply, disseminate or survive. This can be achieved by using built-in stable biological limitations which, without interfering with growth in the bio-reactor, dintinish survivability and prevent adverse consequences in the environment.

Examples of organisms with biological limitations include: auxotrophic strains, asporogenic strains, strains with built-in sensitivity to environmental factors, such as UV light, etc.

Vector/Insert

Well characterised and free from known harmful sequences

Vector: For the vector to be well-characterised, the function of the genetic material on the vector should be known.

Vectors can be characterised by a combination of reference to the literature, National Institute of Health (NIH) and/or other listings and a knowledge of the derivation and construction of the vector and subsequent experimental confirmation of the construct.

The characterisation should ensure that the vector is free from sequences that result in a phenotype harmful to humans or the environment; for example, through production of substances which can have harmful effects, such as toxins or factors known to be involved in pathogenicity and/or colonisation.

Insert: The source and the function of the DNA that is being inserted and its position on the vector should be known. Experience has shown that in many cases, this means the nucleotide sequence of the inserted DNA is known. This would include knowledge of whether more than one function is encoded in the sequence of the insert. In addition, the insert should not result in a phenotype harmful to humans or the environment as exemplified in the above paragraph.

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)

The vector/insert should be limited in size as much as possible to the genetic sequences required to perform the intended functions. This decreases the probability of introduction and expression of cryptic functions, or the acquisition of unwanted traits.

In some cases, the vector or the insert may affect the stability of the construct in the environment. For example, introduction of resistance genes may affect the ability of the recipient to survive in the environment (see below).

Should be poorly mobilisable

One consideration arising from the use of vectors to introduce an insert is the rate at which the vector/insert could subsequently be transferred from the original recipient. For example, the rate of exchange of plasmid vectors can be lowered by the elimination of transfer functions.

Other approaches can also be used to reduce the frequency at which the inserted DNA would be transferred from the recipient to other organisms, e.g. stable integration into the chromosome.

Should not transfer any resistance markers to micro-organisms not known to acquire them naturally

Frequently, genes for resistance to a variety of substances (e.g. antibiotics, heavy metals) are introduced for selection purposes into the recombinant organism. When evaluating a specific resistance gene the following should be considered:

- Whether and with what frequency the resistance marker(s) can be transferred from the recombinant organism to other organisms (see above).

- Whether such acquisition can compromise the use of a therapeutic agent or lead to environmental perturbations. Markers for substances such as antibiotics, not currently in commercial use should also be evaluated to determine whether the marker exhibits cross-reactivity or linked resistance.

- Whether selection pressure might exist for the specific marker. For example, selection in the environment of an organism carrying a resistance gene may be enhanced if the selecting agent in question is present in adequate concentration in the environment. This may occur, for example, as a result of the use of antibiotics in livestock feed, or of pollution by environmental contaminants such as heavy metals.

r-DNA organism

Non--pathogenic

The nature and, where appropriate, the source of the inserted genes must be considered. The type of gene product and its function must be examined in the context of the characteristics of the host. If, for instance, the gene product has no known role in pathogenicity and the host is not pathogenic, then the r-DNA organism is expected to be non-pathogenic.

As safe in industrial setting as host organism or with limited survival, and without adverse consequences in the environment

This includes safety to both man and the environment. In general, the approach taken should be to consider the nature of the host and to focus on the nature of the inserted genes and the resulting products. Their effects on biological fitness and adaptability, including attributes such as the ability to colonise new niches, should be taken into account. Adverse consequences can be avoided, for example, by using r-DNA organisms of limited survival in the environment in relation to the wild strain. In some cases it may be necessary to generate and/or collect data on specific properties, for example, through monitoring of environmental discharges.

III. FUNDAMENTAL PRINCIPLES OF GOOD OCCUPATIONAL AND ENVIRONMENTAL SAFETY FOR PROCESSES USING GILSP ORGANISMS

The central objective is to identify appropriate good and prudent practices for handling GILSP and other low-risk organisms as described in Section I (pp. 8-9). These practices must be based on good principles of occupational hygiene and environmental management and on the use of physical controls where necessary.

Recombinant DNA-containing as well as other organisms used in industry will generally have been developed in the laboratory under the conditions specified by codes of good practice, guidelines or legislation governing research. Experience gained in using these organisms in the laboratory is one factor to be taken into account when determining the appropriate practices for large-scale production.

The fundamental principles of good occupational and environmental safety listed below should be applied for Good Industrial Large-Scale Practice, as well as for all levels of containment. These principles represent an attempt to describe the end to be achieved rather than an attempt to specify the technical means of implementation:

i) keep workplace and environmental exposure to any physical, chemical or biological agent including cellular products and debris to a level appropriate to the characteristics of the organism, the product and the process;

ii) exercise engineering control measures at source and to supplement these with appropriate personal protective clothing and equipment if necessary;

iii) test adequately, and maintain, control measures and equipment. The frequency of examination and testing will depend on the nature of the modified organism, the product and the process;

iv) test, as appropriate, for the presence of viable process organisms outside the process equipment, both in the workplace and in the environment;

v) ensure personnel have adequate training and experience;

vi) as required, to establish biological safety committees and/or consult with worker representatives and to consult with regulatory authorities;

vii) establish and implement a code of practice in the workplace for the safety of personnel and for the protection of the environment[3].

NOTES

1. In Part One of this document the term "host" is used to describe the recipient organis m.

2. The concept of toxicity should not be limited to lethality, but should include mutagenicity, carcinogenicity neurotoxicity, etc.

3. The type of topics covered could include but are not limited to: prohibition of eating, drinking, smoking, mouth pipetting and application of cosmetics in the workplace; training and supervision of staff in safety and hygiene procedures; disposal of biological and other wastes; guidance for ancillary and maintenance staff; operation of bioprocessing and associated equipment; medical or health surveillance; incident response procedures.

Annex

Suggested Criteria for r-DNA GILSP (Good Industrial Large-Scale Practice) Micro-organisms and Cell Cultures

(Revised Appendix F to *r-DNA Safety Considerations, 1986)*

Host Organism	Vector/Insert	r-DNA Organism
Non-pathogenic	Well characterised and free from known harmful sequences	Non-pathogenic
No adventitious agents	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)	As safe in industrial setting as host organism, or with limited survival, and without adverse consequences in the environment
Extended history of safe use **OR**	Should be poorly mobilisable	
Built-in environmental limitations permitting optimal growth in industrial setting but limited survival without adverse consequences in the environment	Should not transfer any resistance markers to micro-organisms not known to acquire them naturally	

Part Two

GOOD DEVELOPMENTAL PRINCIPLES (GDP): GUIDANCE FOR THE DESIGN OF SMALL-SCALE RESEARCH WITH GENETICALLY MODIFIED PLANTS AND MICRO-ORGANISMS

BACKGROUND

The 1986 OECD report *Recombinant-DNA Safety Considerations* concluded that "assessment of potential risks of organisms for environmental or agricultural applications is less developed than the assessment of potential risks for industrial applications". It went on to say that "the means for assessing r-DNA organisms can be approached by analogy with the existing data base gained from extensive use of traditionally modified organisms in agriculture and the environment generally". The 1986 report also suggested that because of "step-by-step assessment during the research and development process, the potential risk to the environment of the applications of r-DNA organisms should be minimised.

The recommendations in this area noted that "considerable data on the environmental and human health effects of living organisms exist and should be used to guide risk assessments", and that "research to improve the prediction, evaluation, and monitoring of the outcome of applications of r-DNA organisms should be encouraged". Any development of general international guidelines governing such applications was judged to be "premature" in 1986. It was recommended that "review of potential risks should be conducted on a case-by-case basis, prior to application. Case-by-case means an individual review of a proposal against assessment criteria which are relevant to the particular proposal; this is not intended to imply that every case will require review by a national or other authority since various classes of proposals may be excluded."

In April 1988, the OECD's Group of National Experts on Safety in Biotechnology met to consider the need for a follow-up programme to the 1986 report. The group decided that part of its programme would be to develop general principles that would identify a generic approach to the safety assessment of low -- or negligible risk small-scale field research. The principles, labelled Good Developmental Principles (GDP), would be developed while countries continued to use the general case-by-case approach as defined in the 1986 report.

At that meeting, there was agreement that GDP should apply equally well to both agricultural and other types of environmental testing (i.e. mineral leaching or waste degradation) and that a single document could appropriately describe principles for both these kinds of applications.

Given the importance and complexity of the subject, and its widespread interest, an earlier version of this part was made available for discussion and public comment in March 1990.

The present version results from the review and assessment of comments received, including those from environmentalists, industry, trade unions, the public and policymakers in general.

1. PURPOSE AND SCOPE OF THE REPORT

Part Two describes scientific principles for the design of small-scale field research with genetically modified plants and micro-organisms. The principles described, Good Developmental Principles (GDP), are intended as scientific guides to the performance of low -- or negligible risk small-scale field research, including basic and applied research. They are not intended to bypass or prejudice any regulatory action on field research with plants and micro-organisms. These principles will allow flexible national approaches to the design and conduct of small-scale field research.

Also addressed are plants and free-living and plant-associated micro-organisms. Future work may extend the application of GDP to other organisms as well as to animal vaccines.

II. INTRODUCTION

In general, the progression in the development of a genetically modified organism[1] for use in the environment involves research conducted through a continuum of testing from laboratory to greenhouse/glasshouse to introduction into the environment. These stages can be represented as in the following diagram. In the research and development process, controlled experiments are conducted in properly designed facilities prior to release. Each stage may be revisited several times, e.g. to construct organisms with better field performance, or to accumulate additional data. In some situations, satisfactory results may be obtained in a single stage.

Codes of good practice or guidelines have been established, both nationally and internationally, for safe conduct of research in the first stage of the diagram, research in the laboratory/greenhouse/glasshouse. These primarily address human health and worker safety.

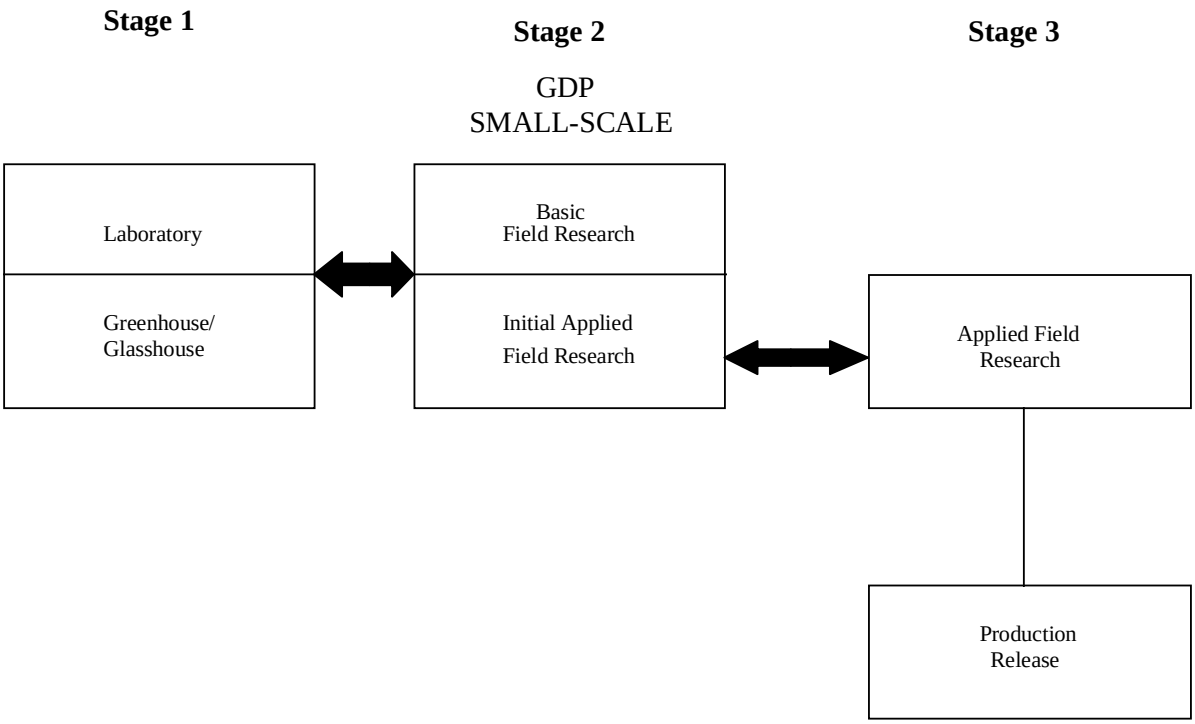
However, similar codes of practice and principles referring to environmental safety have not been compiled for the small-scale basic and applied field research stage (Stage 2).

This part presents general principles for the design of small-scale field research in Stage 2. The 1986 OECD publication *Recombinant-DNA Safety Considerations* provided, in Appendices, lists of considerations to be used in assessing field research involving genetically modified organisms. What follows describes how these considerations may be used in the design of low -- or negligible risk small-scale field research.

The application of GDP should help ensure the safety of small-scale field research with genetically modified organisms by providing guidance to investigators on selecting organisms, choosing the research site, and designing appropriate experimental conditions. It should assist in the review of proposals for small-scale field trials which in turn should provide data to predict the safety of large-scale trials as part of the step-by-step process. Annexes 1 and 2 (pp. 27 and 32) discuss the

interaction of experimental conditions with characteristics of plants and micro-organisms respectively.

Figure 1. **GDP in the context of field research**



III. GOOD DEVELOPMENTAL PRINCIPLES (GPD): WORKING ASSUMPTIONS

The underlying assumption of GDP is that a set of general experimental principles can be identified under which small-scale[2] field research of low or negligible risk can be conducted with a specific genetically modified organism.

- The *first* working assumption is that certain general scientific principles related to the organism, the research site, and experimental conditions have varied relative importance in determining whether an experiment is of low or negligible risk.

- *The second* assumption is that a conclusion regarding that risk of an experiment can be reached by evaluating the relevant factors and their interaction under the conditions of the experiment including, when available, existing data from greenhouse and laboratory studies.

- The *third* assumption is that the interaction of these factors is easier to address in small-scale field experiments than in large-scale experiments because of their limited scope, which permits closer monitoring, generally easier assessment and analysis and

the possibility of more effective containment measures in the event of unforeseen and potentially damaging occurrences.

A number of assumptions are also made concerning the key factors which determine the safety of any specific experiment.

IV. KEY SAFETY FACTORS

The key factors in determining the safety of any specific experiment are:

- the characteristics of the organism(s) used, including the introduced gene/genetic material;
- the characteristics of the research site and surrounding environment; and
- the use of appropriate experimental conditions.

Characteristics of organisms

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 reduce the likelihood of these adverse effects by mitigation methods and/or confinement of the research organism or its genetic material to a restricted research site. However, it must be recognised that mitigation and confinement are more readily accomplished with higher plants than with most micro-organisms.

Characteristics of the research site

The research site should 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.

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.

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

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

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

Experimental conditions

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 introduction of organisms, monitoring and mitigation; a precise description of the design of experiments, including planting density and treatment patterns; and description of specific data to be collected, and of methods for analysis to test for statistical significance.

The design of low -- or negligible risk small-scale fieid research includes: choosing an appropriate geographical location in relation to proximity to significant biota that could be affected; characterising the research site, including, for example, size and preparation, climatic features; designing introduction protocols including quantity and frequency of application; choosing methods of site preparation and cultivation; choosing methods for confinement, decontamination, monitoring and mitigation; designing treatments applicable to the research; developing suitable safety and handling procedures for application and contingency plans in the event of the need for early termination of an experiment.

Additional precautions may be required when considering a particular organism or trait or for particular environments such as aquatic environments.

Researchers designing and conducting these field experiments should carefully consider the following in the development of protocols and codes of practice for conducting small-scale field research with plants and micro-organisms:

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

2. Exercise measures to limit dispersal and establishment beyond the test site and supplement these measures when appropriate.

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

V. APPLICATION OF GDP

Experiments with plants[3]

The safety of small-scale field research with plants can be determined by analysing the characteristics of the organism and the research site and by designing appropriate, scientifically and environmentally acceptable, experimental conditions. The following discussion of GDP for plants includes characteristics of the organism and assumes the prudent choice of research site and experimental conditions.

The plants most likely to be tested are domesticated crop species. In many cases, there is extensive experience with their reproductive isolation and with the prevention of spread of plants outside the test area. Most domesticated crop plants cannot persist or thrive in non-cultivated environments.

Characteristics of plants to be considered include:

- the biology of the reproductive potential 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;
- the mode of action, persistence, and degradation of any newly acquired toxic compound;
- the nature of biological vectors used in transferring DNA to plants;
- interactions with other species and/or biological systems.

GDP should facilitate the design and conduct of field experiments so that: *i)* the experimental genetically modified plants remain reproductively isolated from the gene pool represented by sexually compatible plants outside the experimental site; **AND** *ii)* genes or genetically modified organisms will not be released into the environment beyond the research site; **OR** *iii)* plants are used which, even without reproductive isolation, will not cause unintended, uncontrolled adverse effects.

GDP can be applied in one or both of the following ways:

1. The experiment allows for the control of reproduction:
   - an experimental restriction or intrinsic biological limitation makes the plant incapable of reproduction;

OR

2. The experiment limits the likelihood of harm to (or significant impact on) the environment:

– there is minimal likelihood that the plant will survive, disperse, or become established beyond the research site;

AND

– any toxic compound newly acquired or enhanced by the plant has a minimallikelihood of detrimental effect on managed or natural ecosystems;

AND/OR

– 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.

The interaction of experimental conditions with the characteristics of plants is discussed in more detail in Annex I (p. 27). The scientific considerations described therein are derived from experience gained in field research with new plant varieties obtained by conventional and new plant breeding techniques.

Experiments with micro-organisms[4]

The safety of small-scale field research with micro-organisms can be determined by analysing the characteristics of the organism and the research site, and by designin 9 4p ,propriate scientific and environmentally acceptable experimental conditions.

As distinct from plants, tests with micro-organisms usually involve large populations, some portion of which may persist. The individual organisms in that population cannot always be genetically isolated, e.g. the possibility of horizontal DNA transfer p4nnot always be excluded in micro-organisms. Micro-organisms must be thought of in Statistical terms that consider the probability of an event occurring in a given population/ pnvironment.

Characteristics of micro-organisms to be considered include:
containment measures

– dispersal, survival and multiplication;

– interaction with other species and/or biological systems;

– potential for gene transfer;

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

GDP should facilitate the design and conduct of field experiments so that: *i*) transfer of genetic material of interest is controlled **AND** *ii*) dissemination[5] of micro-organisms containing that genetic material is controlled; *OR iii*) there are no unintended, uncontrolled adverse effects on other organisms even though transfer and dissemination may occur.

GDP can be applied in one or both of the following ways:

1. The experiment allows for control of transfer of genetic material and dissemination beyond the research site:

– The biology of the organism minimises the probability of horizontal gene transfer, or measures are taken to prevent or minimise it;

– The biology of the organism minimises the probability of horizontal gene transfer, or measures are taken to prevent or minimise it;

AND

– The organism has limited ability to compete;

AND

– Measures are taken to rninimise movement/dispersal of the micro-organism frorn the test site;

OR

– Measures are taken to prevent or mitigate establishment beyond the test site if necessary.

2. The experiment limits the likelihood of harm to (or sipfficant impact on) areas beyond the research site:

– There should be no adverse environmental effects beyond the research site, even if the micro-organism should disseminate from the site, as shown by knowledge and previous experience (e.g. characteristics of the organism including the introduced gene/genetic material, 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

The experiment should be designed to detect, as appropriate, 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, as appropriate, should they occur.

The ability of a micro-organism 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. The interaction of experimental conditions with the characteristics of micro-organisms are discussed in more detail in Annex 2 (p.32).

NOTES

1. The term “Genetically modified organism” is employed here in a broad sense. Its scope may evolve over time with the progress of science and technology, and vary from country to country and agency to agency, depending on the various responsibilities and purposes involved.

2. In the context of this part, “small” refers to the minimum size required to fulfill the objectives of the experiment while maintaining GDP.

3. The principles developed here apply to gymnosperms and angiosperms. Principles for other plants including saprophyfic fungi have yet to be developed.

4. The principles developed here apply to micro-organisms which include: viruses, bacteria, microalgae, protozoa and fungi.

5. Dissemination comprises the concepts of “movement/dispersal” and “establishment” beyond the test site.

Annex 1

Scientific Considerations for Small-Scale Field Research with Plants

The following text describes scientific considerations underlying Good Developmental Principles (GDP) 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 practised in some form for thoUnited Statesnds of years, it was after the rediscovery of Gregor Mendel's work in 1900 that the systematic breeding now practised by plant breeders became widely used. 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 help to identify plant characteristics and experimental conditions that allow the safe conduct of small-scale field research.

Small-scale field research with genetically modified plants is conceptually 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 ionising radiation; crosses between cultivars of a crop species; and interspecific crosses, including crosses between cultivated species and crosses between cultivated species and related non-cultivated species. When conducting conventional plant breeding research, attention is often given to preventing possible genetic influx from any sexually compatible plants into the research plot. It has not been demonstrated to date that natural transfer of genetic material from plants to organisms other than plants occurs.

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 in breeding experiments are of no practical use to the breeder and are eliminated, with no further effect on either the environment or on subsequent plant breeding. Only a very small proportion of new germplasm lines produced by plant breeders warrants further research or eventual commercial release. This practice, however, does not imply that new plants are competitively unfit to survive in a variety of ecological niches.

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, United States, as a forage plant in the 1830s, water-hyacinth (*Eichhornia crassipes*) introduced into Florida, United States, as an aquatic ornament and the Asian weed kudzu (*Pueraria lobata*) introduced as a stabiliser of soil embankments and as crop forage on unproductive land. Many other important weeds (Canada thistle, yellow starthistle, field bindweed), now present in the United States, are the result of the accidental

introduction of foreign plant species. In Europe, there have been similar problems as a result of intentional or accidental introduction of foreign plant species such as sunflower (*Helianthus annuus*), common ragweed (*Ambrosia artemisiifolia*) and giant hogweed (*Heracleum mantagazzianum*) which causes severe dermatitis in man. 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 using GDP should not be considered analogous to uncontrolled introduction of foreign plants into entirely new environments, but experience from such introductions may provide relevant information.

Reproductive isolation of genetically modified plants

Conventional plant breeding experiments utilise reproductively isolated plants in the research plots in addition to limiting the size of the plots. Employing practices that ensure reproductive isolation of the modified plants is an excellent method for preventing dissemination of genetic material from the test plant into other members of the same or related species.

In considering natural mechanisms for reproductive or genetic isolation in 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.

The practice of maintaining a considerable degree of reproductive isolation is currently used by plant breeders and by seed producers to produce genetically pure seed. 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 principle of reproductive isolation applies. This principle can be applied successfully to reduce the likelihood of dispersal of genetic material from the experimental plot.

The practices currently employed by plant breeders and 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 that it will have no significant adverse effect on the environment.

To provide some guidance in determining the types of practices that are appropriate for reproductive isolation, a list of examples is provided in the next paragraph. 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 should be that experimental genetically modified plants are reproductively isolated.

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

- The most common method used to isolate plants from sexually compatible plant populations is spatial separation. Most requirements for growing pedigreed or 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.

- In the case of some 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 detasselling 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.

- 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.

- 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 means such as covering of flowers (bagging) prior to anthesis.

- When the objectives of a field test do not require that seed be produced, 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 might otherwise be difficult to isolate.

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 should 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

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.

There is some possibility of environmental risk in small-scale field research involving plants modified to contain toxins, even if the plant genetic material remains confined to the experimental site. This is due to the fact that these plants might affect organisms entering the site (e.g. by making the toxin available to organisms not usually encountering the toxin in their ecosphere/niche) or have some residual, unintended effect on non-target organisms that are 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 systems

Various physical, chemical, and biological means are available to transform plants with new genetic material. These techniques include the use of electroporation, micro-injection, ballistic microprojectiles, organisms or molecular vectors. The first three techniques 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. 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.

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: *i*) well characterised and unlikely to be transmitted after entering the plant (disarmed *Agrobacterium tumefaciens* Ti plasmid meets this specification); and *ii*) transferred from the same or closely related species (as the recipient plant); and/or *iii*) transferred from non-pathogenic prokaryotes or non-pathogenic lower eukaryotic plants; and/or *iv*) transferred from plant pathogens only if the sequences capable of producing disease or damage in plants have been deleted.

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/glasshouse conditions establishing the safety of this vector system. In most of the field research with genetically modified 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, except the border sequences, 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 (see Section V, p. 23).

Annex 2

Scientific Considerations for Small-Scale Field Research with Micro-organisms

The following sections describe the scientific considerations underlying Good Developmental Principles (GDP) for field research with genetically modified micro-organisms. Small-scale field research presents a situation where the issues to be addressed are constrained by the relatively small size of the experimental plot. Such research would normally occur at only a single or a few geographic locations as opposed to large-scale testing or use, or to unlimited application. 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 micro-organism will become established and what the effect of the introduced micro-organism on the environment will be.

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

Application in the environment

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).

Micro-organisms are generally applied in small-scale field research as soil amendments, as foliar sprays, as seed treatments, 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 most cases. Therefore, the discussion of scientific principles can focus on these few as the most commonly used.

Greater dispersion of the micro-organisms 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 minimised by the choice of drip application and drip irrigation, rather than spray applications and spray irrigation.

Dissemination, including survival and multiplication, in the environment

"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).

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 a few micro-organisms. Limited information is available on the dissemination of saprophytic organisms (except for some that interact with plant pathogens, e.g. *Agrobacterium radiobacter*).

These studies show that dissemination depends on three factors: *i*) the inoculum (size, fitness, infectivity, viability); *ii*) the movement[1]/dispersal[2] properties of the population; and *iii*) 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."

In evaluating field research, it is not possible to separate completely 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 would 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.

Inoculum

The survival of an experimental micro-organism 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 micro-organism in the environment. However, some prediction of likely behaviour can be made based on existing knowledge and empirical observations generated from a number of sources: greenhouse testing, microcosm testing, knowledge of the behaviour of closely related organisms (e.g. parental organisms and the intended function of the introduced trait if the experimental organism is genetically modified).

An inoculum must contain sufficiently high numbers of micro-organisms at the research site in order that a minimum level is present for effective dispersal to other sites. In addition, it is likely that some dilution of inocula will occur as the micro-organism leaves the research site. Dilution would probably increase as the micro-organism 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 micro-organisms of the test strain in the original research site).

It should be noted that the number constituting a 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. Dispersal by vectors or by mechanical transport may lower the minimum effective inoculum load; this must be taken into account. It can be assumed that for some organisms, a small number of organisms would be an effective inoculum, while for other

organisms very large numbers are necessary. 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, therefore, be determined on a case-by-case basis.

Instituting measures to lower the number of micro-organisms 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.

Movement, dispersal and transport

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.

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. Therefore, biological characteristics of the test micro-organism must be considered in evaluating the safety of field research.

While some micro-organisms are dispersed by several means, others may be restricted to one or a few modes of movement. In general, the more highly adapted a micro-organism 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.

Micro-organisms are transported by a variety of routes as described below: *i*) by wind; *ii*) by water; *iii*) by mechanical means (e.g. humans and animals); and *iv*) by biological vectors.

i) Wind

Effectiveness of aerial dispersal is influenced by several factors. These include: mechanisms of entering the atmosphere (take-off), particle shape, ability to survive environmental stress (e.g. desiccation, uv light), ability to adhere to soil and other particles. Some micro-organisms have adaptations which permit them to disperse aerially.

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

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. When the test micro-organism possesses a high potential for dispersal by the aerial route, the positioning of the small-scale research plot on an off-shore island may provide acceptable security.

ii) Water

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

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.

Rain splashes can throw droplets, potentially micro-organism-laden, from plant surfaces into the air. Splash dispersal occurs when water droplets impinge on plant surfaces covered with micro-organisms; for example, certain plant pathogenic bacteria can be spread for kilometres by driving rain.

The research plot can be designed to address and limit dispersal through these potential routes. For example, border strips around the research site can be used to isolate plants within the research plot and thus prevent micro-organisms 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 with suitable decontamination systems in the test plot can be implemented.

Moreover, the research plot can be situated so as to limit access of the test micro-organism to groundwater or open bodies of water under both average and exceptional climatic conditions, and it is possible to control water flow through the use of drainage, collection and physical barriers.

iii) Mechanical means

Human activities: Humans disperse all kinds of micro-organisms over short and long distances in a variety of ways: 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.

Mechanical disturbances such as tillage may loft “rafts” of soil bearing clumps of micro-organisms 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 micro-organisms contained in the aerosol droplet.

In small-scale field research, care can be taken to limit dispersal of micro-organisms 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 micro-organisms on contaminated materials can be restricted by use of appropriate decontamination procedures.

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

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, screening or fencing of the experimental site. Maintenance of such physical barriers is essential, as is their continued monitoring, to ensure their effectiveness.

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

There are other methods by which passive dispersal can occur. For example, micro-organisms that colonise 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.

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

iv) Biological vectors

Micro-organisms can be transmitted by insects during feeding and movement of the insect from plant to plant. By definition, the insect vector and the micro-organism establish a specific relationship. A vector carries the micro-organism 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/micro-organism relationship, the less likely in general the micro-organisms will be moved by other vectors.

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

The common insect vectors are aphids and leafhoppers, but white flies, mealy bugs, beetles, dipterans, psyllids, 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).

Insects can vector micro-organisms for both short and long distances. Insects like leafhoppers are strong fliers. Some anthropods, such as mites, cannot fly but can be carried passively by wind.

Even insects which are not strong fliers can disperse micro-organisms over long distances since these airborne insects can be carried hundreds of kilometres by wind.

Small-scale field research design can be used to address potential vectoring of test micro-organism by insects. For example, if it is known that the test micro-organism 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. These arrangements are rarely totally effective in eliminating vector activity and are highly dependent on the climatic situation of the particular season concerned.

Availability of suitable habitats

One of the most important considerations in determining whether a micro-organism will be disseminated is whether habitats[3] and/or niches[4] in which the micro-organism will become established are available.

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

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 the pathogen does not find a sufficient density of suitable habitats (susceptible plants), it does not disseminate in an epidemic fashion. When such strategies are employed, the micro-organism may proliferate within the experimental plot, but it would not disseminate outside the plot if it does not find suitable hosts.

Experimental design in a small-scale field trials 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 is termed "geographic isolation".

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 metre radius of land surrounding the research site. However, such procedures require thorough monitoring as most soils contain weed-seed banks capable of germinating.

Multiplication and survival

As noted in the previous section, survival and multiplication of the experimental micro-organism 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 micro-organism 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.

Clearly, in attempting to evaluate the probability that an introduced micro-organism will be an effective competitor, be favoured 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 micro-organism will be experimented with in the agro-ecosystern from which it or its parental micro-organisms were isolated. In such a situation, neither the introduced gene nor the introduced micro-organism will be new or unique in that environment, although the frequency at which the gene/micro-organism combination occurs in that site subsequent to application may differ from that generally observed.

The added gene/micro-organism combination would be in competition with the indigenous population of micro-organisms. While this does not guarantee that the added gene/micro-organism 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 of the behaviour of the parental organisms can be used to predict the likely response of the gene/micro-organism combination to factors such as competition for nutrients, predation and environmental stress, selection, and antibiosis.

Given present knowledge, however, it is usually only through actual field trials that behaviour can be assessed, and the competitive ability of the experimental micro-organism will frequently have to be tested empirically. Data generated in the laboratory, greenhouse/glasshouse or microcosm may, in addition, form an important element in an evaluation of small-scale field research.

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/micro-organism combination. When relatively small numbers of the gene/micro-organism 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 in the inoculum from which genotypes can be selected is less.

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

Competition and selection are important considerations in evaluating a submission and designing safe small-scale field research. The phenomenon of “finding a new niche” will be treated here as a facet of selection.

i) Competition

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. 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 substrates, intense competition occurs.

Free-living soil micro-organisms: Most of the information on free-living soil micro-organisms is derived from experience with *Rhizobium* species and microbial amendments used as biological control agents. This experience shows that at the end of the growing season, the added micro-organism does not usually predominate. To explain these observations, it has been hypothesised that the organisms of the microbial amendment must compete with an indigenous flora well adapted to local conditions and are not always effective in this competition. This pattern of competition may differ with micro-organisms that have significant resting spores, such as many soil-borne fungal pathogens and semi-saprophytes.

A micro-organism 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.

Micro-organisms 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 dormant vegetative cells.

Antibiosis occurs when one microbial population produces a substance that is inhibitory to other populations. Examples of antibiosis include production of substrates to suppress competitors and production of substances such as lactic or sulfuric acid, alcohol, acetic acid, and low-weight organic acids. Antimicrobial agents probably have a significant function in competitive interaction in micro-environments. 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.

Predation may also be a factor influencing microbial survival and population levels. Free-living nematodes, protozoans and bacteria act as predators on micro-organisms in the soil. Although the impact of such predators on microbial populations is unclear, it is likely that micro-organisms have developed strategies for dealing with predation.

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 create a balance of life in the soil which will affect the comparative competitive ability of the applied micro-organism.

Host obligate micro-organisms: Micro-organisms that depend on a host for survival are termed in this paper host obligate micro-organisms. Most available information addressing the factors

affecting competitive ability in host obligate micro-organisms was generated from studies of micro-organisms as biological control agents, and in plant pathology as well as in plant breeding.

In the micro-organism/plant interaction, host obligate micro-organisms may be epiphytic (on the surface of the plant) or endophytic (inside plant tissues) or both.

The endophytes have few competitors (other plant pathogens or possibly secondary invaders of diseased tissue), when compared to the epiphytes or free-living soil micro-organisms. Endophytes such as viruses, viroids and some prokaryotes (e.g. rickettsia-like bacteria, mycoplasmas and spiroplasmas) multiply entirely within their host or vector and generally are considered labile when exposed to the outside environment. There are, however, certain types of plant viruses that are known to survive in water, soil and crop debris. The environment in which they must compete is, thus, determined to a great extent by the host. Although they may have fewer microbial competitors, endophytes must deal with host defenses.

Plant obligate epiphytic micro-organisms may be categorised 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 micro-organisms 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.

In a second type of nutritional relationship, the host-obligate micro-organism obtains nutrients from a plant by killing the host tissue in advance of colonisation.

Many of the factors affecting competition among free-living soil micro-organisms can be seen in host-obligate micro-organisms. 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 micro-organisms must also find and colonise/infect suitable hosts. The need for host-obligate micro-organisms to find suitable hosts is a factor which can be used in designing an experimental protocol to test these organisms safely.

ii) Selection

Selective pressure is exerted by the environment and favours organisms possessing adaptive features. The best known example of selection in micro-organisms is the emergence of bacterial strains resistant to antibiotics. Selection of resistant strains is promoted by the use of antibiotics for treatment of human and animal microbial infections, in animal feed, and for agricultural purposes. Another example of selection is the increase in the numbers of micro-organisms 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 into the environment.

For the purposes of this part, “discovery of a new niche” is treated as a form of selection. It occurs when a micro-organism develops the capability to perform a “new” function within an ecosystem. It can also occur when a micro-organism performing a function which the indigenous community does not perform is introduced into an ecosystem.

Selective pressures affect the ability of an organism to survive, multiply and 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.

Interaction of the Micro-organism with other Species and/or Biological Systems in the Environment

Experimental micro-organisms in small-scale field research can interact with other species in a number of ways. In Recombinant-DNA Safety Considerations, two specific kinds of interaction are noted in the outline. These are: *i*) the effects of the micro-organisms on target or non-target organisms; and *ii*) the potential for and effect of horizontal transfer of genetic material. The following paragraphs address these two types of interaction, and an initial attempt is made to examine them. These considerations can also be related to field research design.

Target or non-target organisms

Many of the micro-organisms that are tested in field plots are intended to have effects on another organism, the target organism. For decades, plant pathologists have used micro-organisms 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 micro-organisms. Micro-organisms used as biocontrol agents are specifically selected or modified to affect a target pest organism. Some micro-organisms such as *Bacillus thuringiensis* are used routinely in the environment as biological control agents for some lepidopteran insects. Research using unmodified micro-organisms has been conducted with little adverse effect on the environment even though the micro-organisms have known effects on other organisms in the environment being reported on. The issues that are routinely considered in these tests are instructive in testing genetically modified micro-organisms.

When a micro-organism 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 micro-organisms to act as biological control agents, the genes that are inserted may encode toxins or they may broaden the host range or increase virulence on the micro-organism for a particular target organism. The effect of any new trait on the host range of the micro-organism should be evaluated 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 micro-organism 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.

These concepts call 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 micro-organism and in limiting the exposure of significant populations of sensitive beneficial insects.

Gene transfer

The gene transfer capability of an engineered micro-organism or the stability of the genetic construct may affect the micro-organism's interaction with other micro-organisms. Gene transfer refers to the dissemination of genetic material through natural genetic mechanisms.

The factors to be considered in analysing the effects of gene transfer on the safety of a genetically modified micro-organism 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. What is the known function of the new genetic material?

4. If the modified micro-organism 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?

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. 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 or simulated natural settings have been documented.

Several factors that may affect transfer are the presence or absence of: *i*) large bacterial densities that enhance mating; *ii*) free DNA that may promote transformation; and *iii*) 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 numbers of donor cells facilitate transfer to recipients. In addition, other factors that affect transfer are spatial, temporal, and physiological separation of bacteria; immobilisation through adhesion to soil particles, organic materials, and other living organisms; genetic barriers such as restriction systems and plasmid incompatibility; and environmental conditions.

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, have to be evaluated on a case-by-case basis.

NOTES

1. "Movement" refers to an active process that may involve behavioural choices such as a relationship with an insect vector.

2. "Dispersal" refers to a passive process such as rain splash.

3. A "habitat" is the physical location where an organism is found. The physical and chemical characteristics of habitats influence the growth, activities, interaction, and survival of the micro-organisms found in them.

4. A "niche" is broader than a habitat. A niche describes not only the physical habitat but also the functional role and the actions of the micro-organisms within that space. As used in this document, the term "niche" describes a functional role of an organism within an ecosystem.

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