Enabling the Safe Use of Biotechnology

Principles and Practice

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Contents

Foreword

The World Bank has a long-standing interest in fostering the safe and effective use of a wide variety of new technologies that enable environmentally sustainable development in its member countries. It recognizes that modern biotechnologies are a key component in the production of food, fiber, and fuel, especially in developing countries. These technologies are critical in protecting the environment, including safely disposing of environmental waste, and in better characterizing and conserving biological diversity.

In fostering wider use of modern biotechnologies, the World Bank is concerned that these powerful new tools be used safely and that they protect human health and the environment. In this context it has commissioned a review of the principles and procedures for the safe use of biotechnology, developed by a variety of governments and international agencies.

This volume describes the enabling environment required by a country to acquire the powerful new tools of biotechnology and to assess their potential usefulness. It is designed to guide policymakers through the issues and scientific principles that underlie the assessment of any risk associated with the use of the products and the processes of modern biotechnology.

Model practices are included for planned introduction of organisms with novel traits and their products into the environment. Containment systems are recommended for the safe use of such organisms. The principles of good laboratory and industrial large-scale practice for use in individual countries, as recommended by the Organisation for Economic Co-operation and Development, are summarized.

This volume is intended to be a reference for those responsible for implementing research and development programs on the use of modern biotechnology. It is also a reference for the regulatory framework that governs the development and use of the products and processes of biotechnology. We trust that this information will be helpful to those responsible for establishing and implementing regulatory systems that enable the safe use of biotechnology, especially in developing countries.

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The work was conducted under the general direction of Michel Petit, Director of the Agricultural Research and Extension Group of the World Bank. The study originated from work undertaken in Kenya with the National Council for Science and Technology (NCST) and the Kenyan Agricultural Research Institute (KARI). We acknowledge the encouragement given to the work by Professor Karega Mutahi, Permanent Secretary of the Ministry of Research, Technical Training, and Technology; Dr. David Andere, Secretary of NCST; Dr. Cyrus Ndiritu, Director of KARI; and Professor Norah Olembo, Chair of the Kenya-Netherlands Biotechnology Platform.

In a review of the approaches taken by various countries, the information resources now available on the Internet through the Biosafety Information Network and Advisory Service of the United Nations Industrial Development Organization (UNIDO) were found to be especially valuable. The series of documents on biosafety, commissioned and published by the Organisation for Economic Co-operation and Development (OECD) and the U.S. National Institutes of Health, were also particularly helpful. Examples of good laboratory practice and good industrial large-scale practice, included in chapter 7, are adapted from the OECD's 1992 report *Safety Considerations for Biotechnology.*

Special acknowledgment is given to the Australian Genetic Manipulation Advisory Committee (GMAC) for allowing us to use as examples in chapters 5 and 6 a number of sections from their *Guidelines for the Planned Release of Genetically Manipulated Organisms, Guidelines for Small-Scale Genetic Manipulation Work,* and *Guidelines for Large-Scale Genetic Manipulation Work.* Copyright for the GMAC's guidelines rests with the Commonwealth of Australia.

The editorial assistance of Pamela George Reginal MacIntyre, Amy Smith, Alicia Hetzner, and Virginia Hitchcock is warmly acknowledged. The volume was desktopped by Christian Perez. Tomoko Hirata designed the cover.

Abbreviations and Acronyms

BINAS	Biosafety Information Network and Advisory Service (of the United Nations Industrial Development Organization)
CBD	Convention on Biological Diversity
COP	Conference of the Parties (to the CBD)
CSD	Commission for Sustainable Development
DNA	deoxyribonucleic acid
ESD	environmentally sustainable development
FAO	Food and Agriculture Organization of the United Nations
GILSP	good industrial large-scale practice
GMAC	Genetic Manipulation Advisory Committee
IACSD	Inter-Agency Committee on Sustainable Development
IBC	institutional biosafety committee
IICA	Inter-American Institute for Cooperation on Agriculture
ISNAR	International Service for National Agricultural Research
ISNAR/IBS	ISNAR/ Intermediary Biotechnology Service
NBC	national biosafety committee
OECD	Organisation for Economic Co-operation and Development
ONT	organism with novel traits
OTA	Office of Technology Assessment of the U.S. Congress
PCR	polymerase chain reaction
rDNA	recombinant DNA
UNCED	United Nations Conference on the Environment and Development
UNEP	United Nations Environment Programme
UNIDO	United Nations Industrial Development Organization
USDA/APHIS	U.S. Department of Agriculture/Animal and Plant Health Inspection Service
WHO	World Health Organization

Introduction

The purpose of this publication is to provide a practical guide for policymakers and research managers who are responsible for making decisions on ensuring the safe use of modern biotechnology; producing new products in medicine, agriculture, and the environment; and promoting environmentally sustainable development.

This publication continues from the 1990 study *Agricultural Biotechnology: Opportunities for International Development*, sponsored by the World Bank, the International Service for National Agricultural Research (ISNAR), and the Australian government. In 1992 ISNAR and the World Bank published the study *Biosafety: The Safe Application of Biotechnology in Agriculture and the Environment* as a brief for policymakers prior to the United Nations Conference on the Environment and Development (UNCED). The study summarized the development of modern biotechnology, reviewed the accumulating documentation on the safe use of biotechnology in agriculture and the environment, and suggested a series of steps required to establish a national biosafety system. *Biosafety* is a term used to describe the policies and procedures necessary to ensure the safe application of modern biotechnology.

Since 1992 the World Bank has worked with a number of countries to assist in the formulation of national biosafety practices as part of the enabling environment necessary to underpin their investments in biotechnology. This document reports on the steps required to establish a national regulatory framework for biotechnology that will enable safe use of new products emerging in the fields of agriculture, the environment, and human health, especially in developing countries. It also reviews material accumulating on the introduction and commercial use of new biotechnology products. It describes how the regulatory requirements in these countries are being modified in light of increasing familiarity with the products and processes of modern biotechnology. The publication also summarizes the international context in which national biosafety systems are developed.

Chapter 1 reviews the current trends in biotechnology and identifies the areas in which new developments in modern biology are likely to contribute to environmentally sustainable development.

Chapter 2 outlines the enabling environment, including the national regulatory framework necessary to ensure safe use of biotechnology without endangering public health or the environment.

Chapter 3 describes risk assessment procedures to identify possible risks in research and development involving organisms with novel traits, plants, and animals.

Chapter 4 illustrates risk management procedures for the contained use and planned introductions of organisms with novel traits and other products of biotechnology.

Chapters 5, 6, and 7 present possible models for planned introductions, contained experimental use, and small-scale introduction and large-scale use of novel organisms, drawn from the

experience gained by the Genetic Manipulation Advisory Committee.

Consideration is being given to making this publication available in electronic media to facilitate ease of access and use. It is also intended that a decision support system for biosafety will be developed from this document and other relevant data in the future.

PART ONE

Principles

1. Overview

Biotechnology refers to any technique that uses living organisms or substances to make or modify a product, to improve plants or animals, or to develop microorganisms for specific uses (box 1.1).[1]

Biotechnology consists of a gradient of technologies, ranging from the long-established and widely used techniques of traditional biotechnology (for example, food fermentation and biological control) to novel and, in some cases, unproven techniques of modern biotechnology.[2] This includes the use of new techniques of recombinant DNA (rDNA) technology (often called genetic engineering), monoclonal antibodies, and new cell- and tissue-culture methods (figure 1.1).

During the 1970s scientists developed new methods for precise recombination of portions of deoxyribonucleic acid (DNA), the biochemical material in all living cells that conveys the instructions that govern inherited characteristics, and for transferring portions of DNA from one organism to another. This set of enabling techniques is referred to as rDNA technology or genetic engineering.

Over the past two decades the number of significant advances made in genetic engineering has increased dramatically. It is this increase in the use of new techniques for understanding and modifying the genetics of living organisms that has led to greatly increased interest and investment in biotechnology.

The applications developed from these new methods place them firmly within the continuum of techniques used throughout human history in industry, agriculture, and food processing. Thus, while modern biotechnology provides powerful new tools, these tools are used to generate products that fill essentially the same roles as those produced with more traditional methods. The properties of these products do not differ substantially from those that are already available and familiar.

The most striking differences between the techniques of modern biotechnology and those that have been used for many years lie in the increased precision with which the new techniques may be used and the shorter time required to produce results.

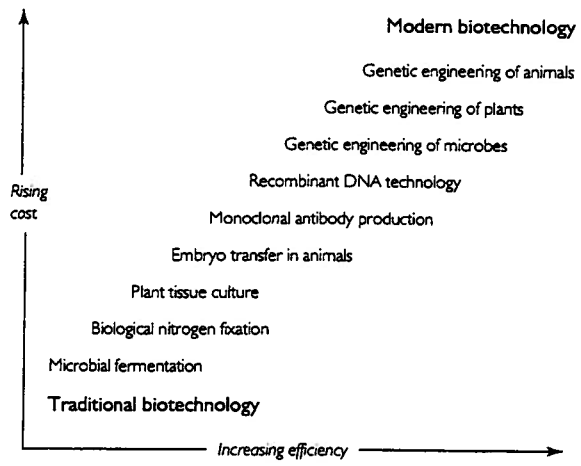
Box 1.1 What is biotechnology?

Biotechnology, broadly defined, includes any technique that uses living organisms, or parts of such organisms, to make or modify products, to improve plants or animals, or to develop microorganisms for specific use. It ranges from traditional biotechnology to the most advanced modern biotechnology.

Biotechnology is not a separate science but rather a mix of disciplines—genetics, molecular biology, biochemistry, embryology, and cell biology—transmuted into productive processes by coupling with such practical disciplines as chemical engineering, information technology, and robotics.

Biotechnology should be seen as an integration of new techniques emerging from modern biotechnology with the well-established approaches of traditional biotechnology, such as plant and animal breeding, food production, fermentation products and processes, and production of pharmaceuticals and fertilizers.

Figure 1.1 Gradient of biotechnologies



Source: Gabrielle J. Persley, ed., *Beyond Mendel's Garden: Biotechnology in the Service of World Agriculture* (Wallingford, United Kingdom: CAB International, 1990).

For example, modern rDNA technology enables plant breeders to collaborate with molecular biologists and transfer to a popular and highly developed crop variety one or two specific genes needed to impart a new characteristic, such as a specific kind of pest resistance. This increased precision in plant breeding translates into improved predictability of the resulting products in desirable areas, such as performance and survival.

Because modern biotechnology provides enormous potential power that is far reaching, and the number and variety of new products is so great, it is important to provide appropriate regulatory mechanisms to ensure that products produced by the use of new techniques are as safe as the products of traditional biotechnology, especially when those products are organisms that might interact with the environment.

Economic Significance of Biotechnology

Biotechnology is recognized as a major growth industry worldwide. Biotechnological advances underpin novel growth and development opportunities in a diverse range of industries. These include pharmaceuticals, food processing, plant production, animal production, and environmental management. Biotechnology has been identified as one of the key carriers of the next wave of technological development in the same way as computers are key carriers of the current information and communication technology wave.

Examples of the economic significance of biotechnology include the following:

- Advanced biological technologies underpin the development of half of the investigational new drugs in the United States (compared with traditional chemical technologies). The worldwide market for the top four biopharmaceutical products was $5.5 billion in 1993 and is growing at a rate of 14 percent a year. One pharmaceutical alone, erythropoietin, is estimated to have generated worldwide sales of more than $2 billion in 1994.
- Sales of biotechnology companies in the United States rose from $10 billion in 1993 to $11.2 billion in 1994. They are projected to grow to $50 billion by 2000.
- In the United States there have been more than 2,200 field trials of novel bioengineered crops, focused mostly on economically and agriculturally important species.

International Recognition of the Importance of Biotechnology

Many countries have recognized the potential of these techniques to contribute to economic growth in an environmentally safe manner. For example:

- India is using biotechnology in a wide variety of areas, including crop improvement, forestry, biopesticides, and biofertilizers.
- Indonesia recognizes biotechnology as a priority area and is one of four technology focuses for the country.
- Kenya, in accordance with national policy, is developing through international collaboration both transgenic plants with resistance to pathogens and environmental stress and vaccines to better protect livestock against disease.
- Malaysia has earmarked $20 million for the Malaysian National Biotechnology Directorate.
- Singapore has embarked on a biotechnology initiative and intends to focus on the biotechnology needs of its near neighbors.
- The United Kingdom has embarked on a major initiative—"biotechnology means

business"—to stimulate business use and application of biotechnology in the United Kingdom.

Emerging Biotechnologies and Sustainable Development

The concept of environmentally sustainable development is based on the conviction that it should be possible to increase the basic standard of living of the world's growing population without unnecessarily depleting the world's finite natural resources and further degrading the environment. If yields per hectare cannot be increased significantly over current levels, then more wilderness areas, which are only marginally suitable for agriculture but are a rich source of biodiversity, will be sacrificed to feed the growing world population. Emerging biotechnologies offer novel approaches for striking a balance between development needs and environmental conservation. A wider diffusion of the technology is critical to giving the world access to its positive impacts. Biotechnology is continuously and rapidly developing in an increasing number of sectors that improve the effectiveness of the way in which products and services are provided. However, the transfer and development of biotechnology in an environmentally sound manner requires a variety of conditions, including capital inputs that, in the case of many developing countries, are not readily available.[3]

All countries require appropriate infrastructures that permit them to acquire, absorb, and develop technology; to manage it efficiently and systematically; and to build up local scientific and technological competence. The ability of any country—of a developing country in particular—to discern, choose, and adapt environmentally sound emerging biotechnology can serve as a measurement of sustainable self-reliance that will allow it to fully participate in worldwide efforts to achieve sustainable development. The creation of enabling conditions poses new challenges that must be addressed in order for developing countries to realize the potential benefits of biotechnology and to minimize any possible adverse socioeconomic or environmental effects.

Environmentally Sound Management of Biotechnology

Agenda 21, which is a participatory plan of action jointly formulated and agreed upon by the world community at the Earth Summit in Brazil in June 1992, proposes a number of interrelated programs and program actions aimed at environmentally sustainable development. The Inter-Agency Committee on Sustainable Development (IACSD) designated the United Nations Industrial Development Organization (UNIDO) as the task manager for chapter 16 of Agenda 21, which deals with environmentally sound management of biotechnology.

In 1995 UNIDO reviewed the progress achieved on the implementation of this program. UNIDO notes that many of the issues discussed in chapter 16 are also reflected in other chapters of Agenda 21. Recognized as a cross-sectoral issue, biotechnology is particularly linked to the issues discussed in chapters 6, 11, 14, 15, 17, 18, and 21 of Agenda 21. The overall Agenda 21 program on biotechnology is outlined in box 1.2.

Since the United National Conference on the Environment and Development (UNCED) was held in 1992, considerable progress has been achieved in raising awareness among the scientific community, policymakers, and the general public of the potential benefits and risks of

Box 1.2 Agenda 21 and biotechnology

The program areas named below seek to foster the international principles to ensure the environmentally sound management of biotechnology, to engender public trust and confidence, to promote the development of sustainable applications of biotechnology, and to establish appropriate enabling mechanisms, especially in developing countries, through the following activities:

- Increasing the availability of food, feed, and renewable raw materials
- Improving human health
- Enhancing protection of the environment
- Enhancing safety and developing international mechanisms for cooperation
- Establishing enabling mechanisms for the development and the environmentally sound application of biotechnology.

biotechnology and the need for environmentally sound management. It is now widely recognized that, if properly managed, biotechnology can play an essential role in supporting the economic and social development of both industrial and developing countries. Biotechnology development and applications continue to grow at a rapid rate, leading to an expanding range of products and processes across several sectors that began with pharmaceuticals and health care and now extends to agriculture and the environment.

In the health industry many biotechnology products, such as insulin, diagnostics, and vaccines, have already been placed on the market, and products such as recombinant hepatitis B vaccine have gained widespread international use. Two new biotechnology-based cholera vaccines have recently been licensed in some countries. Currently, more than 2,000 clinical trials of biotechnology-related products are in progress. In agriculture such products as diagnostics and biopesticides are commercially used. Other products and technologies being developed include improved plant varieties, new animal vaccines, novel food ingredients, biotechnology-based techniques for the rapid detection and identification of toxic materials, and several bioprocessing technologies.

The trend in most developing countries is to acquire biotechnologies that are aimed at improving agriculture, food, and pharmaceutical production, and converting low-cost or marginalized raw materials into high value-added products. Tissue culture, vaccines, and some new diagnostics are currently available for immediate application in developing countries. These technologies are being used in several countries to increase crop yield, improve human and animal health, and reduce agrochemical inputs. In addition to the appropriate use of traditional and intermediate biotechnologies, an increasing number of developing countries are seeking to integrate more advanced biotechnologies into national development plans and programs, either as part of the relevant traditional sectors or as new biotechnology programs.[4]

With regard to the progress in enhancing safety and developing international mechanisms for cooperation, significant progress in regional consultation and cooperation has been made. Significant strides have been made building on the experience of the UNIDO/UNEP/WHO/FAO Informal Working Group on Biosafety and other recent international initiatives, such as the International Service for National Agricultural Research/Intermediary Biotechnology Service (ISNAR/IBS) and the Biotechnology Advisory Commission of the Stockholm Environment Institute. Another important initiative underway, under the auspices of UNEP, is to develop further international technical guidelines on safety in biotechnology, jointly put forward by the governments of the United Kingdom and the Netherlands. The recent launching by UNIDO of the Biosafety Information Network and Advisory Service (BINAS) within the United Nations system encouraged an increasing number of developing countries to participate as national focal points and to cooperate within the regions to establish regional nodes and networks. Currently, the absence of established biosafety procedures in many developing countries constitutes a major constraint to field testing and product development.

The Convention on Biological Diversity is considering the need for and the modalities of a possible protocol on biosafety under the Convention.

In view of this overarching use of biotechnology UNIDO notes that the Commission on Sustainable Development is uniquely placed to set the issue of safe use of biotechnology in the context of sustainable development in its widest possible sense.

Biotechnology and Biodiversity

Among the considerable ongoing international activity related to biosafety, one surprising venue, given the broad relevance of biotechnology to Agenda 21, is increasingly prominent: the Convention on Biological Diversity. One provision of the Convention, Article 8(g), required contracting parties to:

> establish or maintain means to regulate, manage, or control the risks associated with the use and release of living modified organisms resulting from biotechnology which are likely to have adverse

> environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health.

Another provision, Article 19 (3), stipulates that:

> The Parties shall consider the need for, and modalities of, a protocol setting out appropriate procedures, including, in particular, advance informed agreement in the field of the safe transfer, handling, and use of any living modified organism resulting from biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity.

The first Conference of the Parties (COP) agreed to a process of expert consideration and consultation. Under this process a group of fifteen experts convened in Cairo in May 1995 and prepared a report for the consideration of a larger meeting in Madrid some months later. The Madrid group reviewed the Cairo report and attached it to its own review document, which was prepared to inform deliberations by the COP in Jakarta from November 6–17 in 1995. The COP decided to embark on a negotiation process to develop in the field of safe transfer, handling, and use of living modified organisms a protocol on biosafety, specifically focusing on transboundary movement of any living modified organism resulting from biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity. In particular, the COP set out for consideration appropriate procedures for advanced informed agreement.

Much has been made in some quarters of the potential threats to biodiversity from modern biotechnology products, particularly organisms with novel traits. While the term is nowhere clearly defined, the way in which it is used suggests it is most commonly meant to refer to plants, animals, and microbes that have been modified with rDNA techniques to give them additional characteristics. The largest category of such organisms is new agricultural varieties of existing crop plants.

The threats to biological diversity worldwide are well-known. The single largest threat stems from the conversion of native lands to agriculture, the replacement of wildlands with monocultures, or their ecological near-equivalents, to feed the burgeoning world population. After this threat comes the dangers from habitat degradation through such agents as pollution or unsustainable extractive practices such as clear-cut logging and overfishing. Against this backdrop the threat to biological diversity from products of modern biotechnology, of which no more than the smallest handful are presently on the market, is infinitesimal. But what if all the products presently in the research and development pipeline were now on the market? Would the threats to biological diversity be increased or decreased?

The vast majority of new crop cultivars being produced with the techniques of modern biotechnology have been modified to sustain or increase yields, whether through imparting to them resistance to insect pests or disease agents—such as viruses, bacteria, or fungi—or through increasing their ability to withstand competitive pressures (or to eliminate such pressures) from, for example, weeds. It has been argued that if the genes added to existing cultivars to impart such characteristics were to move (generally by sexual recombination) into wild or weedy relatives, weed problems could be exacerbated or wild, pristine gene pools could become contaminated.

In the vast majority of cases, however, the pests or diseases detrimental to agricultural yields are not the limiting environmental constraints on the wild relative receptive to outbreeding from the domesticated cultivars. Experience shows that selection pressures found in nature do not favor such gene flow from modified crops to wild relatives.

A far more likely path through which potential characteristics or traits from genetically modified crops could have an impact on biodiversity is as follows: most modified crops are intended to have higher yields. Habitat loss (the principal threat to biodiversity) is a direct function of the conversion of native lands to agriculture, in response to greater food needs driven by growing human population. There are two

obvious ways to decrease the rate of habitat loss: either constrain the growth of human population or increase yields. Therefore, the most likely impact on biodiversity from novel crop varieties is to act to alleviate the main threats.

Enabling Environment

In order to capture present and future benefits of the application of biotechnology in the production of new products and processes, a nation must provide an environment in which the regulatory framework ensures the safe, expeditious, and economic development and use of such products and processes. In turn, this will attract the requisite investments in human resource development and the necessary capital.

The objective of such a regulatory framework will be to ensure that any potential risks to human health and the environment—through the use of biotechnology in the development of a new product or process from research to product development and use—are identified and minimized through a process of scientific review, guidance, monitoring, and evaluation. Experience in North America, Europe, and Australia in the past decade has shown that such a framework is most effective if it is flexible in nature and able to be modified in light of accumulating knowledge, with the objective of reducing product development costs using biotechnology while ensuring product safety.

The regulatory framework has been shown to be most effective and efficient when based on the scientific assessment of the risk to human health and the environment of the product created by the use of biotechnology, rather than by the process by which it was created. In part this is because of the fact that in many countries legislation exists that relates to the safety and efficacy of existing products (for example, legislation relating to foods and feeds, human and animal health, seeds, pest control, plant and animal quarantine, and plant protection). This legislation can be equally applied to similar products created through the use of biotechnology.

The provisions of such legislation are enacted by ministries that develop and apply appropriate regulations and guidelines and, when necessary, authorize appropriate inspectorates to ensure adherence to the regulations and guidelines by the users of biotechnology. When there is partition of responsibilities between ministries for different parts of the production process and the utilization of products incorporating the use of biotechnology (for example, the production of a genetically engineered food plant and the subsequent sale of food from such plants), it is clearly necessary that a coordinating mechanism be put in place.

Technological support is often provided to such regulatory authorities in the form of a technical advisory committee (for example, some form of a national biosafety committee, NBC, specifically charged with the development and application of national biosafety guidelines and appropriate practices, including such specifications for facilities as licensing and inspection).

Such regulations, guidelines, and practices are invoked and followed by institutions using biotechnology through an institutional biosafety committee (IBC), which interacts closely with a national biosafety committee as instructed in the regulations and guidelines outlined in box 1.3. Institutions write their own codes of practice based on the national regulations, guidelines, and practices to which individual investigators adhere.

Developing a product using biotechnology requires a continuum of activities at different levels of scale and containment as the product moves from the laboratory to production or manufacture and release of the final product. Appropriate regulations and guidelines are developed for each stage of the process. Important stages are small-scale genetic manipulation work, typically in contained laboratory or greenhouse conditions; large-scale genetic manipulation work also in contained conditions; and eventual release of the product and its derivatives in accordance with relevant legislation. When the product is an organism with novel traits, introduction will only be permitted following the findings of an environmental impact analysis showing that the organism would not present a significant risk to the environment or the community at the site or sites of its introduction.

In such cases the process of construction and introduction of organisms with novel traits and

Box 1.3 An example of the functions of a national biosafety committee

The Australian Genetic Manipulation Advisory Committee shall undertake the following functions in accord with the directions of the minister for administrative services:

- Maintain an overview of the biosafety factors associated with innovative genetic manipulation techniques
- Identify and keep under review classes of work which have undefined risk levels
- Alert Australian authorities, whether Commonwealth- or state-based, to the existence of novel risk factors
- Provide specialist technical advice on specific biosafety matters to organizations and regulatory agencies using these techniques
- Prepare or assist, as appropriate, with the preparation of codes, standards, or guidelines for the assessment and management of biosafety risk factors, whether for the committee to oversee activities or to assist regulatory agencies
- Participate in public discussions about the biosafety of these techniques
- Meet with agencies overseas to ensure that, as far as practicable, Australian guidelines and regulations are in harmony with international practice.

their products is based on the principles of good laboratory practice; good industrial large-scale practice; good developmental principles for the design of small-scale field research with genetically modified plants and microorganisms; and good agricultural practice. Good agricultural practice can be implemented by researchers and plant breeders in field trials involving the introduction of new plant materials.[5]

Standards and practices representing good laboratory practice have been promulgated by both international organizations such as WHO[6] and national organizations such as the National Institutes of Health of the U.S. Department of Health and Human Services, among others.[7] Standards and practices for good industrial large-scale practice have been promulgated and refined by international organizations such as the OECD[8] and incorporated into national biosafety guidelines by countries such as Australia.[9]

A generic approach to the safety assessment of low- or negligible-risk, small-scale field research using organisms with novel traits has been developed by OECD as a set of good developmental principles and could equally as well apply to agricultural and other types of environmental testing, such as mineral leaching or waste degradation. The underlying assumption is that a set of general experimental principles can now be identified, given the body of experience acquired to date, under which small-scale field research of low or negligible risk can be conducted with a specific genetically modified organism.

These concepts have been extended to biosafety considerations involved in the scale-up process, which describes the continuum of research and development and involves increasing the scale from preliminary field testing to general use.[10]

Considerations are identified that can be used to provide a framework to evaluate the safety of scale-up of new plant lines, crop cultivars, and other products. There is now, for example, considerable knowledge and familiarity with the procedures for managing the introductions of crop plants developed by a wide range of breeding methods, as well as experience with plants developed by rDNA methods (organisms with novel traits), which can be used to address the issue of scale-up. Experience comes from the knowledge available to conduct a risk and safety analysis for new plant lines or crop cultivars. It can be used to identify hazards, determine the magnitude of risk, and indicate appropriate management. Experience can also be used to recognize when more information is needed to analyze the risk and safety factors.

Biosafety

One of the major issues relating to the role and application of biotechnology in agriculture is the safety of organisms with novel traits and the appropriate regulatory measures for research and development, field testing, and marketing of organisms with novel traits. This is because uncontrolled introduction of organisms with novel traits might cause undesirable changes in ecological or genetic relationships in some communities. Hence careful design and review of organisms with novel traits, along with proper

planning and regulation of environmental introductions, is advisable to ensure that organisms with novel traits do not pose unacceptable risks to the environment. In performing risk assessment and risk management a distinction should be made between evaluation of organisms intended for contained use and those for planned introduction into uncontained settings.

Familiarity

Organisms with novel traits intended for introduction may differ from their parental organisms in their ability to survive and reproduce under varying environmental or climatic conditions. Organisms with novel traits should be evaluated on the basis of scientific principles for their potential to interact in unexpected and undesirable ways with local biological communities.

The concept of *familiarity* can assist decisionmakers in evaluations by providing a context in which to apply accumulated experience with such products. A wealth of experience has been acquired through traditional practices. Experience with the existing cultivars gives valuable insights as to the expected behavior of the new products.

Since the mid-1980s a substantial body of experience has also been accumulating with products to which new characteristics have been added through biotechnology. Field tests and data from laboratories and other facilities provide relevant information about phenotypic expression of the new characteristics in modified organisms and their interactions with the environment. By accumulating such experience it is expected that the performance of entire classes of organisms with novel traits will become familiar enough to require minimal regulatory attention. When familiarity with a plant or microorganism reaches such a level that there is reasonable assurance that the organism is essentially similar to known introductions, and when these present negligible risk, the introduction is assumed to be suitable for field testing, according to established practice.

Familiarity does not necessarily imply that the organism is safe—it does mean that accumulated knowledge and experience suggest appropriate and adequate approaches to risk assessment and management. In evaluating the potential risks associated with products of the new technologies, the aim is to introduce regulatory mechanisms to monitor any risks so that humanity can safely benefit from these new organisms and products.

Risk Assessment

Risk assessment is the process of gathering diverse data to identify possible risks in research and development involving genetically modified microorganisms, plants, and animals. Risk assessment should focus on the characteristics of the product itself rather than on the techniques used to produce it, provided standard safety measures are employed during production.

Following the framework put forward by the National Research Council of the U.S. National Academy of Sciences, the following factors are recognized to be important in assessing risks of organisms with novel characteristics:

For the environment:

- Properties of the organism and of the environment into which it may be introduced
- Possibility of containing and controlling the organism
- Probable effect on the environment should the organism or genetic trait persist longer than intended or spread to non-target environments
- Risks to human health and the environment that are associated with introduction of organisms with novel traits.

For the organism with the novel trait, taking into account:

- The recipient host or parental organism that receives the new trait
- The donor organism from which the trait is derived
- The vector used to transfer the trait from donor to recipient
- The inserted or introduced trait, including potential toxicity of a gene product or its metabolites
- Empirical data on the novel organism
- The intended application, for example, contained use or planned production (see chapter 5)
- The potential receiving environment (see chapter 5).

Another way of looking at risk assessment is to distinguish the following two parameters, namely *hazard* and *exposure*. *Hazard assessment* means evaluating whether an organism can be harmful and assessing whether it is a pest or a pathogen or if it will introduce new pests or pathogens or enhance existing ones. *Exposure assessment* involves evaluating the extent to which the environment or humans might be exposed to organisms with novel traits. The degree of exposure depends on the following parameters:

- The route of introduction
- The survival and reproductive potential of the organisms with novel traits
- The mode and rate of dispersal beyond the site of introduction (by wind, water, and insects)
- The location and size of any receptive or susceptible population.

Finally, basic questions to be asked in performing risk assessment include:

- Are the potential risks of the organism in the environment acceptable, compared to the relative benefits of such an organism?
- Is the public regulatory mechanism adequate to ensure safety?

Possible types of risk to be assessed in agricultural biotechnology include the likelihood that the plant product or plant material may show any of the following characteristics:

- The potential to become weeds or to transmit weediness properties
- The potential to show undesirable toxicity
- The potential for microorganisms or viruses, including those used in vaccines, to exhibit undesirable pathogenicity
- The potential for insects to become pests.

Data obtained from studies performed in laboratories, greenhouses, and contained animal facilities can provide an indication of the degree of risk, but the inability to fully simulate environmental processes in contained facilities is in itself a justification to move to field trials in the open environment under appropriate conditions of management and containment. In the meantime, accumulating evidence from studies in greenhouses or contained animal facilities, as well as in field trials, indicates that many of the perceived risks are remote. As a consequence, guidelines should form a flexible structure in order to allow for adaptation in light of learned experience.

Risk Management

The type of risk management for contained use and planned introductions of organisms with novel traits depends on the organism involved and the intended application. The process involves reviewing alternatives and selecting the most appropriate regulatory actions based on the findings of the risk assessment. Measures to be taken to minimize risk include physical and biological containment. Questions to be posed include:

- What are the risks?
- How probable is it that they will occur?
- How serious is the damage if they occur?
- What can be done to minimize the risks and contain the damage?
- Do the benefits outweigh the risks?

Containment

The term *containment* is used to describe safe methods for maintaining control over the distribution of organisms with novel traits in the laboratory and in the environment into which they are introduced. The purpose of containment is to minimize unnecessary exposure of laboratory workers and the environment to potentially hazardous organisms.

Biological containment of microorganisms principally involves the use of specific combinations of vector and host in such a way that the probability of transfer of a vector to an unintended host and subsequent survival of the host-vector combination in the environment is limited. The growth of plants, which require special environmental conditions for their survival (for example, biological containment), can be achieved in either the greenhouse or field. Similar results can be obtained with studies using contained animal facilities.

Physical containment involves physical constraints on the movement of organisms of uncertain risk or potential hazard. The aim of physical containment is to prevent inappropriate exposure of humans and the environment to

organisms. Physical containment is achieved by following the principles of good laboratory practice, occupational safety, and hygiene; by involving well-qualified and competent personnel who follow safe, standard procedures; and by having a working environment designed to prevent the unintended spread to the environment of organisms with novel traits.

Biosafety Levels

Biosafety levels are described as a series of constraints on the handling and dissemination of organisms graduated according to the level of potential risk. Different biosafety levels are reached by different combinations of laboratory practice and techniques, safety equipment, laboratory facilities appropriate for the operations performed, and the hazards posed by different organisms. Four biosafety levels have been defined, depending on the characteristics of the organisms involved. The proposed safety levels for work with organisms with novel traits take into consideration the results of the risk assessment described above. For microorganisms these levels and conditions are summarized below. Similar levels and conditions have been established for transgenic plants and animals.

- *Biosafety level one* requires safety equipment and facilities as appropriate for undergraduate and secondary training laboratories and is suitable for work with strains of viable organisms not known to cause disease in humans, animals, or plants.
- *Biosafety level two* is similar but includes specific personnel training, limited access to the laboratory and physical containment facilities, and is suitable for work involving moderate potential hazards to personnel and the environment.
- *Biosafety level three* is suitable for work with indigenous or exotic agents that may cause serious or potentially lethal disease as a result of exposure.
- *Biosafety level four* is required for work with those agents that pose a high individual risk of life-threatening disease.

Biosafety levels three and four are characterized by additional safety measures involving, among other things, further personnel training, strict working practices, qualified supervision, and strict physical containment in specially designed facilities and buildings.

Notes

1. Office of Technology Assessment of the U.S. Congress, *New Developments in Biotechnology. Three Field Testing Engineered Organisms: Genetic and Ecological Issues* (Washington, D.C.: U.S. Government Printing Office, 1988). Reprinted by Technomic Publishing Co., Lancaster, Pa.
2. Organisation for Economic Co-operation and Development (OECD), *Biotechnology, Agriculture and Food* (Paris: OECD, 1992) and material adapted from United Nations Industrial Development Organization (UNIDO), *Environmentally Sound Management of Biotechnology* (Vienna: UNIDO, 1995).
3. UNIDO, *Environmentally Sound Management of Biotechnology.*
4. UNIDO, *Environmentally Sound Management of Biotechnology.*
5. U.S. Department of Agriculture/Animal and Plant Health Inspection Service (USDA/APHIS), *Genetically Engineered Organisms and Products; Simplification of Requirements and Procedures for Genetically Engineered Organisms.* Federal Register 60 (Washington, D.C.: Government Printing Office, 1995) 43,567.
6. World Health Organization (WHO), *Laboratory Biosafety Manual* (Geneva: WHO, 1993).
7. U.S. Government Department of Health and Human Services, *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)* (Rockville, Md.: National Institutes of Health, 1994).
8. OECD, *Safety Considerations for Biotechnology* (Paris: OECD, 1992).
9. Genetic Manipulation Advisory Committee (GMAC), *Guidelines for Large-Scale Genetic Manipulation Work* (Canberra, Australia: GMAC, 1994).
10. OECD, *Safety Considerations for Biotechnology.*

2. An Enabling Environment for Biotechnology

The first step for a government in creating a suitable environment to realize the potential of biotechnology, to improve conditions and services for humankind, and to mitigate concerns about potential adverse effects to human, animal, and environmental safety is to provide a regulatory framework that ensures safe development of biotechnology products in a timely and effective manner. Such principles might include:

- Regulations based on the characteristics of the product
- Science-based risk assessment
- Protection of health and the environment
- Building on existing legislation and experience.

This approach recognizes and builds on the knowledge, expertise, and infrastructure already existing in regulatory areas. Therefore, it is both economically and scientifically sound because it allows regulators to build on existing knowledge and experience and to incorporate additional experiences gained through application of the regulations to biotechnology products.

Every product goes through several stages of development before it may be introduced for testing in the environment, and before it is licensed, approved, or registered for commercial sale and use. These may include:

- Import
- Laboratory research
- Environmental release
- Determination of product safety under relevant legislation
- Commercial release and use.

According to the nature of the product and its developmental stage, appropriate health and environmental considerations may be regulated under different legislative acts, together with specific regulations and published guidelines, and administered by different agencies. Identification of relevant legislation and responsibilities for its administration by different agencies and the construction of an effective coordinating mechanism at the national level are therefore the first steps in developing an enabling environment for safe use of biotechnology. The number of relevant acts and regulatory agencies varies from country to country, but there is a general need to identify the acts, agencies, and guidelines that relate to the different products of biotechnology and to define the nature of such products.

Given that biotechnology is the application of science and engineering to the direct or indirect use of living organisms (or parts or products of living organisms) in their natural or modified forms, such organisms with novel traits are the products of a process whereby their genetic material is altered by both natural processes and genetic engineering. Legislation to regulate and control the safety of such genetic modification is generally divided into two main areas: (a) the construction and contained use of organisms with novel traits and (b) the deliberate release of organisms with novel traits or the marketing of products containing them.

Therefore, there is a need to establish at the national and institutional levels appropriate

policies, regulations, and enforcement mechanisms for the control of introductions, methods for field testing, export and import, and commercial releases of organisms with novel traits. Furthermore, the potential of biotechnology for creating diverse products calls for an assessment of the product's safety and efficiency and the application of standards and regulatory measures based on public health, food safety, and quarantine laws. Existing regulations should be suitably strengthened to render them effective to encourage the use of new products and to ensure human health and environmental safety.

Appropriate regulatory reviews for organisms with novel traits are carried out by a variety of officials in various ministries and agencies in different nations. An effective means of coordinating the various regulatory components, including the development and application of appropriate guidelines, is through the establishment of a single national technical advisory committee with responsibility for both areas. Some countries, however, have a technical committee for each area. The Australian experience is most helpful in this regard in which the Genetic Manipulation Advisory Committee, the equivalent of a national biosafety committee, was established in 1987 and reported to the minister for administrative services. This was followed by the formation of institutional biosafety committees and the definition of the responsibilities of individual investigators and producers. This straightforward system requires the minimum in terms of new institutions and organizations and recognizes that adapting existing systems and approaches is the easiest way to quickly set up practical review mechanisms and tap existing relevant expertise, which in most countries is substantial. The system lends itself to countries seeking to develop their own regulatory process.

National Biosafety Committees

National biosafety committees (NBCs), supported by scientific subcommittees, are typically responsible to the appropriate minister(s) to:

- Maintain an overview of the biosafety factors associated with innovative genetic manipulation techniques.
- Identify and keep under review classes of work that have undefined risk levels.
- Alert national authorities to the existence of novel risk factors.
- Provide specialist technical advice on specific biosafety matters to organizations and regulatory agencies using these techniques.
- Prepare or assist with the preparation of codes, standards, or guidelines for the assessment and management of biosafety risk factors, whether for the committee to oversee activities or to assist regulatory agencies. These codes, standards, and guidelines are intended to assist countries in benefiting from the products of biotechnology without undue risk to human health and the environment.
- Participate in public discussions about the biosafety of these techniques.
- Meet with overseas agencies to ensure that, as far as practicable, national guidelines and regulations are in harmony with international practice.

It is then the responsibility of all organizations and individuals engaged in research, development, introductions, or applications of organisms with novel traits to familiarize themselves and comply with the relevant portions of such guidelines at each stage of their work.

Compliance with biosafety guidelines does not relieve those who wish to use organisms with novel traits from any other obligations which they may have under legislation dealing with particular types or uses of products. The introduction of new products for trade and commerce will continue to come under the jurisdiction of ministries with relevant sectoral responsibility. It is the responsibility of those engaged in particular fields of work to ascertain and abide by the relevant legal requirements.

Terms of Reference for a National Biosafety Committee

In regard to a government's need for disseminating technical and biosafety advice to ministers and other appropriate governmental authorities on the continuing assessment of the risks and benefits associated with the production and application of biological materials pro-

duced in laboratories and which occur in nature, a national biosafety committee shall:

- Establish and review, as necessary, guidelines for physical and biological containment and control procedures appropriate to the level of assessed risk involved in relevant research, development, and application activities.
- Review relevant proposals, except those that relate to research under contained laboratory conditions, and recommend any conditions under which this work should either be carried out or not be undertaken.
- Consult with relevant government agencies and other organizations as appropriate.
- Report to the minister and other responsible government authorities at least annually and promptly report any breaches of the above guidelines and any other relevant matters referred to them.
- Establish contact and maintain liaison with such monitoring bodies in other countries and with international organizations as appropriate.
- Advise on personnel training with regard to safety procedures as necessary.
- Collect and disseminate information relevant to the above, having due regard to the special circumstances relating to proprietary information.
- Establish and oversee the work of a scientific subcommittee, whose guidelines follow and whose role and function include not only participation in the relevant items above but also in research performed under contained laboratory conditions.

In support of an NBC a scientific subcommittee shall:

- Be formed to support the work of the NBC. It shall enter into discussions directly with scientists and their host institutions and with funding bodies in determining the conditions under which research should be carried out.
- Review proposals for such research and recommend any conditions under which experiments should be carried out or that work not be undertaken.
- Provide technical advice to the NBC and contribute to its functions.

In drawing up relevant codes, standards, and guidelines, the NBC and its scientific subcommittee will take cognizance of the large body of expertise which exists worldwide on the development, content, and application of such codes, standards, and guidelines. These guidelines generally relate to large- and small-scale genetic manipulation work and to the introduction of organisms with novel traits or their products into the environment. More specific guidelines relating to the nature of the product are also often required (for example, the *Guidelines for Regulations of Veterinary Biologics* produced by Agriculture Canada).[1]

Institutional Biosafety Committees

Institutional biosafety committees (IBCs) are essential to the overall monitoring and surveillance of genetic manipulation work and to the administration of the various guidelines. The caliber and experience of members on the IBC should be such that the IBC can competently carry out its duties. The chair of the committee should be of sufficient standing in the organization to ensure that IBC decisions and advice are implemented effectively. Appropriate arrangements shall be made when the chair is on leave. The NBC will consider the advice and assessment of the IBC to be of fundamental importance in its decisionmaking process.

The IBC shall ensure that staff recruited to work in laboratories, production facilities, or field trials in which work is conducted with organisms with novel traits are informed of potential hazards, have adequate training to ensure that their work is carried out under institutional guidelines, and have access to the IBC for advice. It shall inform new staff members of NBC and institutional guidelines and of the need to comply with them. Organizations of appropriate size conducting such work shall have a biological safety officer.

Composition

The IBC shall include:

- Staff with the requisite knowledge and expertise to assess, evaluate, and oversee work being carried out in the institution

- A biological safety officer
- An individual with expertise in testing biological safety facilities and equipment
- At least one person not associated with the institution who is in a position to consider the wider community interests.

A molecular biologist, a population biologist, or a geneticist shall be included among persons with requisite expertise. IBC membership shall include an ecologist with expertise relevant to the organism if introduction of a live modified organism is envisaged. In general, the scientific disciplines need only to be represented when work falling in that area is performed in the institution. For example, an institution working only on plants need not have an animal geneticist represented. Roles and responsibilities may be combined in the same person when appropriate.

Biological Safety Officer

It is recommended that institutions either appoint a biological safety officer or, in the case of smaller institutions or organizations, assign such duties to the IBC. The officer should have experience working with containment conditions. The officer shall be adequately trained and be able to offer advice on, or participate in, training of new staff or laboratory personnel. Appropriate deputizing arrangements shall be made when the officer is on leave.

The biological safety officer or the IBC chair shall act as adviser to the head of the institution or firm in all matters relating to containment, biological hazards, and staff safety. Regular safety audits and the supervision of a regular testing program for appropriate pieces of equipment shall be undertaken by the biological safety officer or the IBC.

Conflicts of Interest

The composition of the IBC is such that they often include members with specialist expertise who originate proposals themselves. Project supervisors should not assess their own proposals as IBC members. It is necessary that the IBC have sufficient scientific members, or add them as required, so that it is not dependent on the advice of the person submitting the proposal. To ensure that no conflicts of interest arise, final decisions on proposals shall be made in the absence of the originator.

Monitoring Work

The IBC shall ensure that its advice and that of the NBC on specific proposals is conveyed to the principal investigator(s) and is acted upon. Members of the IBC shall inspect the laboratories, facilities, or introduction sites from time to time to adequately monitor safety aspects of ongoing projects and production.

The IBC may draft whatever rules it considers appropriate to supplement the guidelines of the NBC or give effect to their intent. It shall have appropriate powers to ensure that the guidelines and rules are observed. Such rules may relate to containment procedures and operations for managing the project and to handling, transport, and storage of transgenic plants and animals. The IBC will keep minutes of its discussions and decisions.

Duties

The main functions of the IBC relevant to planned work are to:

- Assess and review all the proposals it receives in order to identify potential hazards to personnel, the community, and the environment and provide advice to the project supervisor on these hazards and their management.
- Ensure that all relevant data are included in the project proposal.
- Send the proposal to the NBC for review and assessment when required under the appropriate guidelines and ensure that NBC advice is complied with.
- Ensure that the appropriate institutional animal experimentation ethics committee has been consulted for proposals involving vertebrate animals.
- Monitor any changes to work within the organization and make recommendations to project supervisors from time to time.
- Review the qualifications and experience of personnel involved in projects to ensure that they are adequate for good professional practice and for the supervision of staff.

- Keep records for each planned introduction project.
- Provide an annual report to the NBC.
- Submit a final report, if required under the appropriate guidelines to the NBC, at the end of the project.

Records

The organization shall ensure that records are kept of all procedures, decisions, and staff involved in respect to each project.

Reporting Requirements

At the time of establishment the IBC is to provide the NBC with complete information on its composition and membership (box 2.1).

Accidents and Incidents

If unexpected results arise from a project, the IBC chair or the biosafety officer shall record accidents and any action taken. If the IBC chair believes that an accident or incident occurred that was directly attributable to work with genetic manipulation and was of sufficient concern, he or she shall make a report to the NBC and the head of the institution. An example of such an incident might be a deliberate failure to comply with the appropriate guidelines or any incident or accident that may have resulted in a risk to human health or the environment.

Box 2.1 Reporting requirements of the institutional biosafety committee to the national biosafety committee

Institutional biosafety committees (IBCs) are required to report once a year to the national biosafety committee (NBC) details of the following:

- Chair and secretary
- Biological safety officer, when applicable
- Membership of committee
- List of current proposals, including any changes of project supervisors
- List of certified facilities
- Any unexpected results from planned introduction work, including any animal welfare problems
- Any significant accident or incident relating to the health of workers, the community, or the environment, which may reasonably be directly attributed to the conduct of projects subject to these guidelines
- Any other matter that the IBC may wish to draw to the NBC's attention.

Sanctions

In general, governments require compliance with biosafety guidelines as a condition for funding research. Registration for tax incentives for private sector funding of research may also be conditional on compliance with the guidelines. A substantive breach of the guidelines may result in prosecution under either public health or environmental legislation.

The ultimate responsibility for safe use of biotechnology lies with those who practice the science in the process of research and production. The following section outlines these responsibilities.

Responsibilities of the Principal Investigator and Executive Officer

For each project a principal investigator (or, in the case of production, a production manager) will be identified who accepts full responsibility for all aspects of work. This individual should be thoroughly familiar with the requirements of the guidelines and should ensure that any project for which he or she is responsible involving the use of organisms with novel traits complies with the guidelines and, when appropriate, with NBC advice. In particular he or she shall:

- Assess the proposal to determine if it falls within the guidelines. If in doubt, the project supervisor shall consult the IBC.
- Provide any information on the proposal and its conduct that the IBC may require for its assessment and monitoring activities.
- Comply with NBC and IBC advice and recommendations on proposals.
- Forward a new proposal to the IBC before any substantial change is made to the procedures or the organisms used in a project.
- Carry out work under the conditions approved by the IBC and as advised by the NBC. For work involving vertebrate animals prior and continuing approval must be

received from the institutional animal experimentation ethics committee.
- Ensure that all workers, including subordinates and other co-workers, are aware of the nature of any potential hazards of the work and have received appropriate training in safety and emergency procedures.
- Notify all changes in the project team to the IBC.
- Report all unexpected results, accidents, and unexplained illnesses or absences immediately to the IBC.
- Advise the IBC of any intention to import or transport biological material that falls under the guidelines.
- Keep records, as appropriate, for each project.
- Submit a report to the IBC at the end of the project.

Note

1. Agriculture Canada, *Guidelines for Regulations of Veterinary Biologics* (Ottawa: Agriculture Canada, 1994).

3. Risk Assessment

The principles of risk assessment have been given in chapter 1 and the central concept of familiarity to this process emphasized. In terms of the development and use of organisms with novel traits in small-scale contained conditions, the experience of the past decade has shown that the initial regulations have proven unnecessarily complex. In general, the regulations in the OECD countries, for example, have been gradually relaxed over time in accord with the principles of good laboratory practice, which recognizes that the risks involved in working with organisms with novel traits are similar to those of working with unmodified organisms of equivalent hazard. The most recent revisions of guidelines for small-scale genetic work in OECD countries now make reference to the national and international codes of conduct for good laboratory practice, rather than containing specific sections referring to individual practices for risk assessment and avoidance. There are now additional sections dealing with the specific risks associated with working with live viral vectors and potentially hazardous fragments of DNA.

Live Viral Vectors

A variety of live viral vectors have been developed for efficient transfer of genetic material into cells. Hazards associated with these vectors depend on:

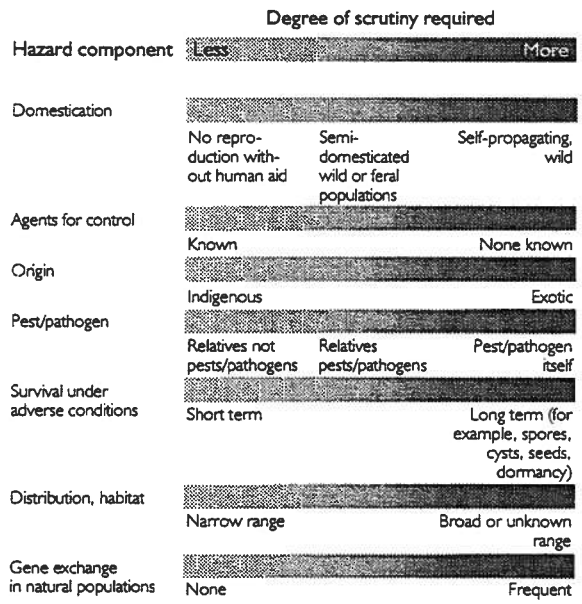
- The host range of the virus
- Its infectivity and availability for repeated rounds of infection
- Whether its genetic material becomes inserted into the host cell
- The nature of foreign genetic material inserted into the virus.

The major considerations are the nature of the inserted gene and whether the virus has the capacity to infect and propagate in human cells. Sequences of known oncogenes (tumor suppressor genes) of either viral or cellular origin—or of any gene encoding molecules that play a role in growth regulation of mammalian cells and genes encoding toxic or potentially toxic products—warrant special attention.

These changes indicate the need for a process to be put in place by which national biosafety guidelines are continually updated to take into account learned experience and to identify new potentially hazardous processes that may result from the research process.

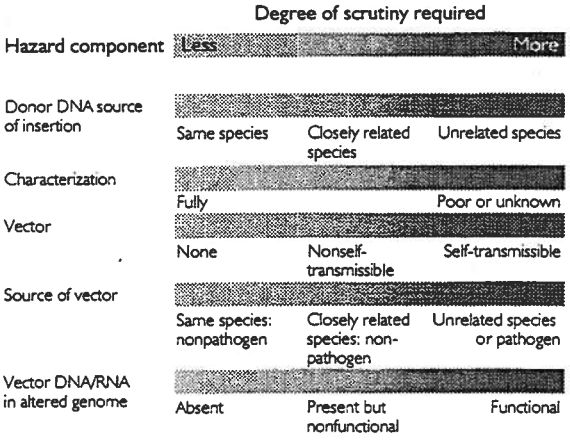
In a similar manner guidelines for large-scale genetic manipulation work have also been simplified in light of experience. Such guidelines apply to large-scale or industrial work, including scale-up in pilot plants with organisms with novel traits, and are based on the principles of good industrial large-scale practices.[1] At present there are no known hazards unique to genetic manipulation. Hazards related to microbiological work, such as the possibility of infection, allergenicity, or toxicity, may also be applicable to work with organisms with novel traits. Guidelines for large-scale genetic manipulation work are designed to minimize such risks.

Figure 3.1 Parent (wild type) organism



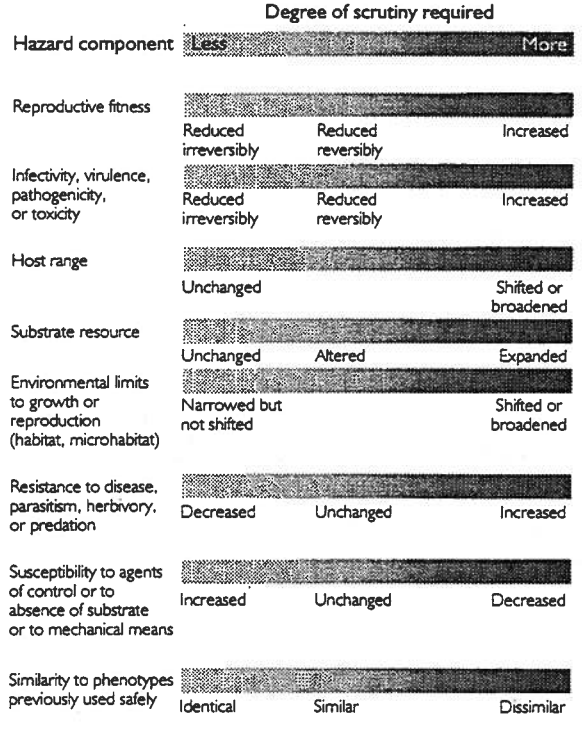
Source: See endnote 2.

Figure 3.2 Genetic constituents



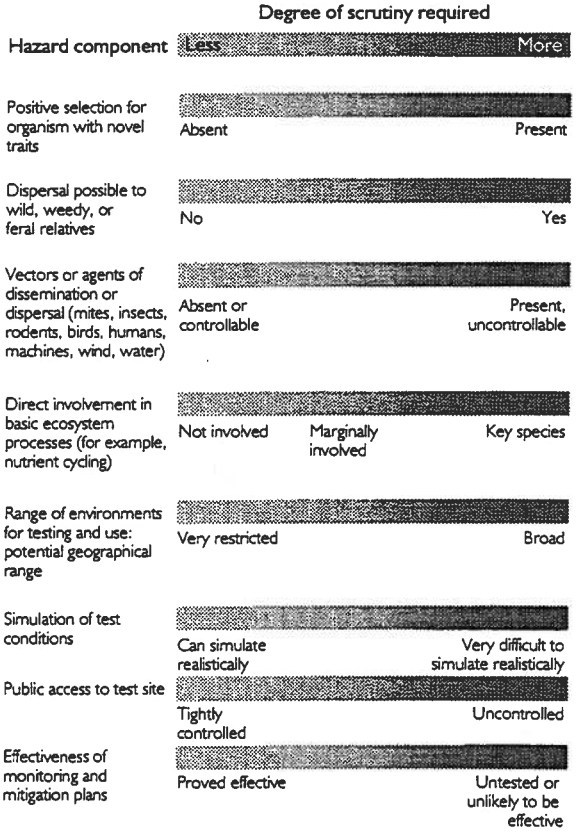
Source: See endnote 2.

Figure 3.3 Phenotype of organism with novel traits



Source: See endnote 2.

Figure 3.4 Attibutes of the environment



Source: See endnote 2.

Guidelines for risk assessment for the contained and uncontained introduction of organisms with novel traits into the environment are also undergoing a process of simplification in light of accumulated knowledge. The United States—the country with the most experience in the regulation of the release of organisms with novel traits—is proposing to allow the use of the notification procedure of most genetically engineered plants that are considered regulatory articles, provided that the introduction is conducted in accordance with specified eligibility requirements and performance standards. The United States believes that an expansion of the notification system to new plant species would simplify oversight procedures for new agricultural biotechnology products, while continuing to ensure their safe development.

These changes would streamline key provisions for field testing and for the review of the regulatory status of many regulated articles. There are three major components to the proposed changes.

- *Extensions to determination of nonregulated status.* The proposed regulations would allow the extension of a previously issued determination of nonregulated status to certain additional regulated articles closely related to an organism already determined not to be a regulated article in the initial determination.
- *Expansion of notification.* On the basis of experience under other notifications and under permit, species eligibility would be expanded to all plants not considered to be weeds at the site of introduction, so that most plant field trials currently performed under permit would be eligible for notification.
- *Increased flexibility through the use of guidelines.* The intent is to issue guidelines, the first of which would be intended to help applicants establish relatedness between two organisms to enable a new organism to be included within an extended determination of nonregulated status.

Factors in Risk Assessment

Risk assessment includes the use of scientific data to estimate the effects of exposure to hazardous materials or conditions. Derived from such a factual base, risk assessment identifies and characterizes the magnitude of potential adverse factors, either their quality or quantity. Important factors in risk assessment are the degrees of hazard posed by:

- The parent (wild type) organism
- Genetic constituents-donor DNA
- Phenotype of organisms with novel traits
- Attributes of the environment.

Figures 3.1 through 3.4 indicate the process of risk assessment and provide the reasoning behind the questions posed in the guidelines for release given in chapter 5.[2]

Notes

1. OECD, *Safety Considerations for Biotechnology.*
2. Material in figures 3.1–3.4 is adapted from the Australian Government's Recombinant DNA Monitoring Committee's *Procedures for Assessment of the Planned Release of Recombinant DNA Organisms* (Canberra: Australian Government Publishing Service, 1987) and from J. M. Tiedje and others, "The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations," *Ecology* 70 (1989) 298–315.

4. Risk Management

Risk management is the process of weighing alternatives to select the most appropriate regulatory strategy or action. It integrates the results of risk assessment with technical, social, economic, and political concerns. Carried out by regulatory agencies under legislative mandates, risk management is a decisionmaking process that determines reasonable control costs by requiring value judgments that compare potential risks and benefits. Risk management of the products of biotechnology involves comparison of their benefits and risks against those associated with the products they replace.

Risk management must also consider the economic and social costs of regulation that add to the costs of product development and the ultimate cost to the purchaser. The combination of cost and efficacy should be such that benefits are available to the most needy and spur economic development at all levels, given that any associated risks can be managed acceptably. Therefore, the process of risk management depends not only on scientific findings of risk assessment but also on public opinion.

The principles of risk management both for the contained use and planned introduction of organisms with novel traits are described in chapter 1, as also were the concepts of biological and physical containment and biosafety levels. The process of risk management follows the process of risk assessment at each stage of development and use of an organism with novel traits and its products as discussed in chapter 3.

As is the case with risk assessment there is now a considerable body of experience in risk management in the production and use of organisms with novel traits and their products. Acceptable practices and guidelines concerning biological and physical containment, levels of biosafety, and small-scale field testing are given in chapter 6.

As noted in chapter 1 the process of construction and release of organisms with novel traits and their products is based on the principles of good laboratory practice, good industrial large-scale practice (see chapter 7), good development principles for the design of small-scale field research with genetically modified plants and microorganisms (box 4.1), and good agricultural practice.[1]

Standards and practices of good laboratory practice have been promulgated both by international organizations such as WHO and by national organizations such as the National Institutes of Health, among others.[2] Standards and practices for good industrial large-scale practices have been promulgated and refined by international organizations such as OECD (box 4.2) and incorporated into national biosafety guidelines by such countries as Australia.[3]

A generic approach to the safety assessment of low or negligible risk small-scale field research using organisms with novel traits has been developed by OECD as a set of good development principles (see box 4.1), which could equally as well apply to agricultural and other types of environmental testing (for

example, mineral leaching or waste degradation). The underlying assumption is that a set of general experimental principles can now be identified, given the body of experience acquired to date, under which small-scale field research of low or negligible risk can be conducted with a specific organism with novel traits. The principles described are intended as scientific guides to the performance of low or negligible risk small scale field research, including basic and applied research. The application of good development principles should help the safety of small-scale field research 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.[4] In the case of scale-up for crop plants the OECD has also developed the relevant principles for risk assessment and management.

Box 4.1 Good development principles: a working assumption

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

- The *first* working assumption is that certain 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 the risk of an experiment can be reached by evaluating the relevant factors, their interaction under conditions of the experiment, including existing data from greenhouse and laboratory studies, when available.
- 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 close monitoring, easier assessment and analysis, and the possibility of more effective containment measures in the event of unforeseen and potentially damaging occurrences.

Source: Organisation for Economic Co-operation and Development (OECD), *Safety Considerations for Biotechnology* (Paris: OECD, 1992).

Box 4.2 Key safety factors

Key factors in determining the safety of any experiment are:

- Characteristics of the organism(s) used, including the introduced genetic material
- Characteristics of the research site and surrounding environment
- Use of appropriate experimental conditions.

Source: OECD, *Safety Considerations for Biotechnology* (Paris: OECD, 1992).

The concept of familiarity is again central to the application of risk management including:

- Indicating when standard cultural practices are adequate to contain a potential hazard
- Indicating when other management practices are needed
- Indicating when not to scale up because practices to achieve safety are not available or acceptable
- Indicating when more information is needed.

The scale-up of plant lines and crop cultivars results in increased interactions with the environment. Such large-scale tests may reveal scale-dependent effects not detected in small-scale tests. The OECD report *Safety Considerations for Biotechnology: Scale-Up of Crop Plants* contains recommendations for management practices with respect to potential risks associated with:

- Gene transfer
- Weediness
- Trait effects
- Biological vector effects and genetic material from pathogens
- Worker (human) safety.

National biosafety guidelines relating to scale-up of agricultural crops should incorporate the principles and management practices as elaborated by the OECD.[5]

The OECD report concludes that there is considerable knowledge and experience (*familiarity*) with the procedures for managing the introduction of crop plants developed by a wide range of breeding methods. Such knowledge and experience gained with crop and transgenic plants developed by various breeding methods could be applied to address the safety of scale-up.

Specific tests can be designed to detect the occurrence of scale-dependent, safety-related effect as appropriate, following risk assessment.

This is borne out by the experience of the regulatory authorities in the United States where, between 1987 and 1994, thirty-nine different plant species have been field tested under permit. The list includes transgenic plants from twenty-two plant families, including flowering plants, monocots, dicots, gymnosperms, herbs, shrubs, and trees. No event in any field trial has resulted in any known dissemination of the regulated article.[6]

Similar familiarity is being acquired in the use of the products of biotechnology in the areas of human/animal health and the management of the environment.

Conclusion

The experience that has accumulated on creating an enabling environment in many countries for safe use of biotechnology shows that the most efficient and effective regulatory systems have evolved in those countries that have developed regulatory mechanisms within their existing legislative and regulatory structures, rather than in those that have created separate arrangements for biotechnology.

This means that the organization of national biosafety systems will differ, depending on their existing legislative and regulatory structures. There are, however, common policies and procedures for safe use of biotechnology that would be of value to all countries developing national biosafety systems, tailored to their specific needs and to those of their neighbors and trading partners.

A major lesson that has emerged from the use of biotechnology is the need to retain flexibility in the national regulatory system in order to be able to respond to accumulating experience in-country and to benefit from the experience of other countries. An increasing number of biotechnology products are becoming available in the fields of medicine, agriculture, and the environment, and experience is accumulating in many countries on the most efficient and effective regulatory systems for handling these new products.

Continuing review and modification of regulatory systems will be necessary to most effectively capture the developments of biotechnology.

Notes

1. USDA/APHIS, *Genetically Engineered Organisms and Products; Simplification of Requirements and Procedures for Genetically Engineered Organisms.*
2. World Health Organization (WHO), *Laboratory Biosafety Manual* (Geneva: WHO, 1993); and U.S. Department of Health and Human Services, *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).*
3. GMAC, *Guidelines for Large-Scale Genetic Manipulation Work.*
4. OECD, *Safety Considerations for Biotechnology.*
5. OECD, *Safety Considerations for Biotechnology: Scale-Up of Crop Plants* (Paris: OECD, 1993).
6. USDA/APHIS, *Genetically Engineered Organisms and Products; Simplification of Requirements and Procedures for Genetically Engineered Organisms.*

Part Two

Practice

5. Model Practices for Planned Introductions

A matter of immediate concern to nations establishing an enabling environment for safe use of biotechnology is the question of release of organisms with novel traits (ONTs) into the environment. As an example, the guidelines developed by the Genetic Manipulation Advisory Committee (GMAC) in Australia for such planned release is given in the following sections.[1]

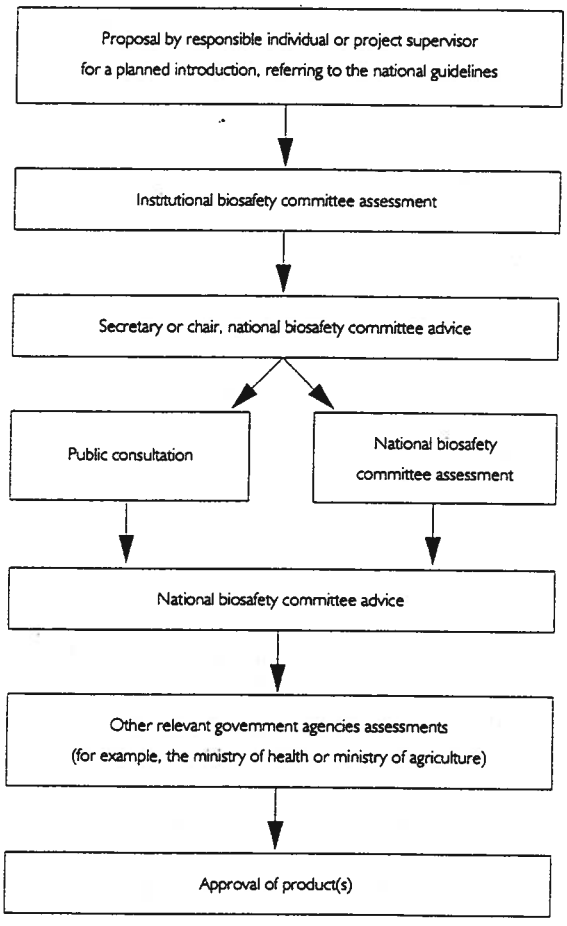
Introduction and Scope

- The scope of the guidelines, including definitions of some terms used, is presented in this section.
- The procedures to be followed by proponents are described in the next section.
- Questions to be answered when submitting a proposal are set out in the third section.
- The roles and responsibilities of the responsible individual, the institutional biosafety committee (IBC), and the project supervisor are detailed in this chapter.

Summary of Procedures

Figure 5.1 may assist proponents in following the procedures laid down in the guidelines. It is a summary and for initial assistance only and is not to be taken as a substitute for the detailed requirements of the guidelines.

Figure 5.1 Summary of procedures



Source: See endnote 1.

Scope

These guidelines apply to the planned introduction into the environment of viroids, viruses, cells, or whole ONTs that are either dissimilar to or otherwise functionally nonequivalent to others already found in the country or that may pose a hazard to public health or the environment.

Introductions into the environment of whole plants generated from interfamily cell fusion experiments may fall under these guidelines.

Definitions

In these guidelines, unless otherwise indicated, certain terms are defined as follows:

- *ONT*—Organism with novel traits.
- *NBC*—National biosafety committee.
- *IBC*—Institutional biosafety committee.
- *Project supervisor*—Project supervisor appointed in accordance with these guidelines.
- *Proponent*—Any legal person (including any firm, company, institution, or organization) that proposes to carry out any introduction to which these guidelines apply.
- *Responsible individual*—The individual bearing primary responsibility for the conduct of the planned introduction in accordance with NBC requirements. He or she may be the project supervisor, the proponent, or any other individual with day-to-day supervisory responsibilities.
- *Secretary*—The secretary or chairperson of the NBC.

Application of Guidelines

These guidelines apply to the introduction into the environment in the country of an ONT (including an imported ONT), whether by way of field trial or otherwise, unless the introduction is exempted under these guidelines. They do not apply to contained work carried out under appropriate institutional guidelines in accordance with international standards.

If the project supervisor is uncertain if a proposed introduction falls under these guidelines, or considers that there is any possibility that it might, a description of the proposed work should be submitted to the IBC or directly to the NBC for written clarification.

If there is no IBC in the home organization, and the project supervisor is uncertain if a proposed introduction falls under these guidelines, a detailed description of the proposed work should be submitted to the NBC. If the work does fall under these guidelines, an IBC must be established to coordinate work with the NBC.

Exemptions

The introduction of an ONT that has been approved for general marketing or commercial use by an authority having lawful power to do so is exempt from these guidelines. The introduction of an ONT is not exempt from these guidelines merely because prior work with the organisms was exempt because it was conducted under approved containment conditions.

The release of ONTs in effluent from contained laboratories in accordance with good industrial large-scale practice, under conditions specified by the NBC for the particular ONT and project, is exempt from these guidelines.

Through a written decision signed by the secretary or chairperson the NBC may make exempt a planned introduction from regulation under these guidelines of: (a) an ONT that has been inactivated or devitalized (the NBC may require full details of the method of inactivation and data showing the extent of inactivation), or (b) an ONT that contains no nucleic acid capable of replication.

An ONT that has previously been approved for planned introduction by the NBC may be exempted from these guidelines if, in the judgment of the IBC and NBC, experience demonstrates no unreasonable risk. An exemption may be unconditional or subject to conditions.

Procedures

Responsibility for Compliance

The responsible individual (usually the person applying for permission to carry out the introduction) is accountable for ensuring that these guidelines are complied with in relation to a proposed introduction of an ONT. The responsibility

includes the naming of an appropriately qualified project supervisor (who may be the responsible individual), ensuring that the work is monitored by an appropriately constituted IBC that is familiar with these guidelines, and ensuring that all persons involved in the proposed introduction are made aware of and directed to observe these guidelines and the advice of the NBC.

Whenever an IBC becomes aware of a proposed introduction it must ensure that these guidelines are complied with. It is the responsibility of the IBC, or any member thereof, to see that the NBC is advised of any failure to comply.

Accidental Release

All procedures for handling ONTs shall be designed to ensure as much as possible that no accidental release of an ONT occurs and that all introductions are planned and carried out in accordance with these guidelines.

Should any accidental release of an ONT occur, which should have been introduced in accordance with these guidelines, it shall be reported immediately to the IBC and NBC, along with details of mitigating action taken (if appropriate) and the names of persons or authorities who have been notified. Reporting a matter to the NBC does not relieve the proponent of any other obligations it may have under common law or statute to notify relevant authorities or persons who may be affected.

Preparation of a Proposal

Before any planned introduction of an ONT takes place, the proponent shall submit a written proposal to NBC. As soon as the introduction of an ONT is contemplated, it becomes the responsibility of the responsible individual or project supervisor to give full consideration to all the possible effects of the proposed introduction, in particular, the necessary steps for compliance with these guidelines. The secretary or chairperson of the NBC is available to consult with the project supervisor on any matter in relation to these guidelines. Compliance with these guidelines does not exempt the proponent from any other relevant guidelines or requirements relating to the ethics of animal or human work.

When the proposal has reached an appropriate stage, the responsible individual or project supervisor shall prepare answers to the questions set forth in the next section (Core Questions for Proponents) as well as answers to questions in other sections. The answers shall be forwarded to the IBC where the IBC shall assess the proposal. In doing so the IBC shall consider whether the data from prior contained work provide a basis for proceeding with confidence to the planned introduction. The IBC shall engage in continuing consultation with the responsible individual or project supervisor and make any suggestions for revision of the proposal or further inquiry as necessary.

Submission of Proposal

When the IBC is satisfied with the proposal, it shall forward it to the NBC, together with a completed cover sheet (appendix 5.A) and a public information sheet (appendix 5.B).

When a proposal includes commercially sensitive information, the proponent may mark relevant portions as *Commercial-in-Confidence* and include substantial reasons why the marked passages should be treated as such. When material is clearly marked as confidential, the NBC will treat it as such, unless it forms the view that some disclosure is necessary. In that event the NBC will notify the proponent in writing and will negotiate a mutually agreed resolution. If an agreement is not reached, the proposal may be withdrawn without disclosure or prejudice at any time prior to the necessary disclosure in pursuit of approval.

Consideration by a National Biosafety Committee

When the NBC receives a proposal, it will (a) publicly announce receipt of the application and a brief description of the proposed introduction; (b) circulate the description to interested individuals and organizations registered with the NBC for this purpose; and (c) send a description of the proposed introduction to the municipal council or other relevant authorities for the area

of the introduction. It is suggested that the proponent consider issuing a press release to a widely circulated newspaper that describes the proposed introduction in the area—both at the time of submitting the proposal and after receiving NBC advice on the proposal.

The public will have thirty days to comment to the NBC on the proposed introduction. To expedite NBC consideration of the proposal, the NBC will forward to the proponent any substantive comments it receives from the public. The proponent may respond to these comments by writing the NBC.

Each proposal may be considered by ad hoc subcommittees of the NBC with the appropriate expertise, which are established for the purpose. Proponents are entitled to and will be provided with information about dates of any meetings from the secretary. Eight weeks will normally be required between receipt of a proposal and its initial consideration by the committee. Proponents may be asked to attend meetings to answer questions on the proposal.

NBC advice on a proposal is sent to the IBC within four weeks after final consideration. If the NBC considers that the proposed introduction is likely to have a significant effect on the environment, the proposal is referred to the appropriate ministry. The ministry may require an environmental impact statement or recommend that certain conditions be attached to the proposal.

After the NBC has advised that a planned introduction may proceed, it will issue the public information sheet that is submitted by the proponent as a press release. Copies of the public information sheet will also be forwarded to the members of the public who commented on the initial notification of the introduction and to relevant local officials in the area of the introduction.

Conduct of the Planned Introduction

Project supervisors and responsible individuals shall comply with monitoring protocols approved by the IBC in accordance with NBC advice. Any unexpected problem or incident shall be reported immediately to the IBC and NBC, together with details of any action taken and the names of persons or authorities who have been notified. Reporting a matter to the NBC does not relieve the proponent of any other obligations it may have under common law or statute to notify relevant authorities or persons who may be affected.

Within six months of the completion of the planned introduction the responsible individual or project supervisor shall submit a detailed report to the IBC for review. The IBC shall review the report to determine whether:

- Protocols were properly observed during the trial
- Aims of the trial were achieved
- Adverse effects occurred
- Survival and dissemination characteristics of the organism were as expected.

On completion of its review the IBC shall submit a report to the NBC in accordance with appendix 5.C.

Core Questions for Proponents

All proposals for the introduction of organisms with novel traits under these guidelines shall include answers to the core questions set out in section A and in the other sections relevant to the proposal. The proposal shall be prepared by the responsible individual or project supervisor and the IBC as previously described.

Questions relating to possible risks may not be answered with certainty. However, it is the responsibility of those engaged in the preparation of the proposal to give the fullest and best consideration of which they are capable regarding possible impacts of the proposed introduction and to make full disclosure of relevant matters to the IBC and NBC. Impacts to be considered include those on public health and safety, agricultural production, other organisms, and the quality of the environment. Full regard is to be paid to the experience gained in contained work on the organism and to the results of a search of the relevant literature and consultation with appropriate experts and public authorities.

Answers are to be supported by appropriate data and references. If none are available, the basis on which the answer is given should be stated. When any doubt exists about the appropriate answer to a question, the nature of the doubt is to be stated. When a potential hazard is noted, the clearest possible explanation of the rel-

ative risks involved shall be provided and possible steps to eliminate or manage the hazard are to be considered and suggested, when appropriate.

Species to Be Introduced

A1 What is the species of organism to be introduced? When relevant, give information on the strain, cultivar, and population.
A2 Is the ONT capable of causing disease or other ill health in humans, plants, or animals? If so, what are the possible effects?
A3
- What is the origin of any exogenous hereditary material?
- Does the exogenous hereditary material come from an organism that causes disease or other ill health in humans, plants or, animals? If so, what are the possible effects?

Purpose of Planned Introduction

A4
- What is the aim of the proposal and the intended eventual use of the ONT?
- What are the advantages and disadvantages of the chosen strategy compared with other methods?

Location

A5 Describe the size of the introduction and, when relevant, the area and location of land to be used. Include a map when relevant.
A6
- What are the reasons for the choice of location?
- Describe in detail relevant features of the physical environment, particularly those that may minimize or exacerbate any undesirable effects.
- How close is the site to population centers, centers of agricultural activity, or the habitat of biota that might affect, or be affected by, the planned introduction?

Habitat and Ecology

A7
- What is the natural habitat of the parent organism and its range?
- Where was the parent organism originally isolated?
- What is the distribution of the parent organism in the country?
- Is the parent organism already present at or near the site of the planned introduction? If so, provide available data on populations.
- Is the parent organism exotic to the country?

A8 Are there any known predators or parasites of the organism in the country? If so, describe.
A9 Could the introduction of the ONT prejudice any beneficial function of the parent organism in the environment?
A10 Describe any anticipated direct or indirect ecological effects of the introduction that are not covered in subsequent sections.

Genetics of the Organism with Novel Traits

A11 What genetic modification has been made? Give a detailed description of the steps undertaken.
A12 Does the ONT have a potentially unstable genotype?
A13
- To what extent is the genetic modification characterized? Provide data to show the extent of characterization.
- What is the location of the inserted DNA in the final construct. How many copies are present?
- What markers or sequences will enable the ONT to be identified in the laboratory and under field conditions?

A14
- What type of vector was used in the transformation? Provide a description of the vector showing the position of the inserted DNA and any other control sequences or markers in the vector.
- Can the vector transfer to other hosts? If so, provide information on its host range.
- Is the recombinant vector present in the final construct? If not, how was it removed?

A15 If no vector was involved:
- If exogenous nucleic acid was introduced, how was this accomplished?
- How many copies of the gene were inserted?
- What secondary genetic effects may be anticipated?

A16 How does the modification change the phenotype of the organism to be introduced? Present data to demonstrate the effect of the modification, including the level of expression and regulation of the genetic insert.

A17

- What intrinsic genetic features, if any, of the ONT regulate its survival and dissemination in the environment? How stable are these features?
- What genetic changes, if any, have been included in the ONT to limit or eliminate its capacity to reproduce or transfer its genes to other organisms?

Data from Contained Work and Other Studies on Stability, Survival, Dissemination, and Transfer

A18 On the basis of contained experiments or other relevant experience, provide data on:

- The survival times of the ONT in habitats relevant to the planned introduction
- The growth rate (or generation time) of the parent organism and ONT in the ranges of environmental conditions characteristic for the place and date of the planned introduction
- The frequency of reversion or loss of the genetic change.

A19

- What is the capability of the ONT to disperse from the area of the planned introduction? What are the dispersal mechanisms in air, water, and soil?
- Can the parent organism form long-term survival structures, such as seeds or spores?

A20 Is there any evidence that the novel trait can be transferred to other organisms found at the site of the planned introduction and surrounding environment? If so:

- To what organisms and at what frequencies? List the species that have been tested or otherwise evaluated for receptivity and explain the rationale for this choice.
- What transfer mechanisms are involved?
- What techniques have been used to demonstrate receptivity or transfer?
- What are any possible adverse effects of the transfer?

A21 Does the modified trait confer a selective advantage on the ONT under certain conditions? If so, what are these conditions? Provide data on growth rates with and without selection pressure.

A22 Would the ONT be expected to show any competitive advantages over its unmodified parent in mixed populations under the conditions in the test site? If so, what are the advantages?

Experimental Procedures, Monitoring, and Contingency Planning

A23 Describe in detail the overall experimental protocol for the introduction, including protocol for control, test, and challenge organisms, if appropriate.

- How many organisms are to be introduced?
- How many introductions of the ONT are proposed?

A24

- What are the arrangements for producing the ONT in quantity, transporting it to the site, and accounting for the transported organisms? (See appendix 5.E for transport requirements.)
- How will the ONT be introduced?

A25

- What methods are to be used, if appropriate, to test for batch to batch consistency if large-scale production is required to produce ONTs for introduction?
- What specific measures have been taken or will be taken in the production process to ensure the quality and purity of the ONT to be introduced?

A26

- How will the survival of the ONT be monitored? Provide a description of techniques for monitoring the presence of ONTs or transferred genetic material beyond the primary site, including specificity, sensitivity, and reliability of detection methods?
- If the introduction is likely to affect the characteristics or abundance of other species, how will this be monitored?
- How will transfer of the introduced gene to other species be monitored?

A27

- What, if any, potential hazards or deleterious effects can be postulated and how are these to be evaluated during the introduction?

- Describe any structures or procedures that will be in place to reduce dissemination of the ONT.
- If transfer of the inserted genetic trait to other organisms with adverse consequences is possible (see A20), what methods will be used to minimize these effects?

A28
- Will the ONT remain in the environment after the introduction? If so, for what period of time, and what might be the consequences?
- Will measures be taken to reduce populations or dispose of the ONT once the introduction is completed? If so, provide details.
- What monitoring is to be undertaken after the introduction is completed?

A29 What contingency measures will be in place to remove the ONTs if a hazard becomes evident during the course of the planned introduction?

A30 Describe site supervision procedures and any safety procedures undertaken by staff.

Other Assessments

A31 Has NBC assessed a small-scale proposal for the development of the ONT? If so, what were the results of such previous work?

A32
- Have the same or similar introductions been made before, either within or outside the country? If so, what were the beneficial or adverse consequences? Provide references or reports of previous assessments.
- Has another country rejected an application for the planned introduction of this organism? If so, on what basis?
- What factors might suggest greater or less risk for the proposed introduction in the country as compared to other countries?

A33 Has the ONT been imported? If so, provide documentation of quarantine clearance or assessment.

A34 Is there any reason to think that the ONT, if introduced, could constitute a hazard in the area designated or elsewhere in the country? If so, please explain. Provide any other information you may have that could assist NBC assessment of this proposal.

Plants

If the plant is intended for human or animal consumption, answer the questions in section M.

B1 Is there familiarity with the parent plant through an extended history of cultivation and of safe use? If not, explain.

B2 What, if any, unintended pleiotropic effects, including undesirable effects on agronomic characteristics of the plant, may result from the expression of the transgene in the ONT (for example, reduced fertility, increased disease incidence, production losses, and grain shedding)? Indicate the likelihood of these events.

B3
- Describe the mechanism of pollen dispersal (by insect vectors or other means) in the plant.
- Provide any available data on pollen viability.
- Indicate any potential pollinators and their range and distribution in the country.

B4
- Are any members of the genus of unmodified parent plants known to be weeds in any environment? If so, specify.
- Are there any literature reports on cross-pollination between the species to which the ONT belongs and wild relatives known to be weeds? If so, please provide.

B5
- Provide quantitative data on successful cross-pollination between the plant and any wild relatives.
- If you know that sexually compatible plants live near the site of the planned introduction, give details and quantify the chances for cross-pollination.
- If cross-pollination occurred, would the resulting progeny be likely to enjoy a reproductive advantage? If not, why not?

B6
- Will the plants in this introduction be allowed to set seed? If not, is this planned for subsequent introductions?
- If plants are allowed to set seed, does the mature seed normally remain contained within an ear, capsule, or pod so that practically all of the seed can readily be harvested, or is the seed shed soon after it matures?
- Can the seed be dispersed by natural mechanisms? If so, describe.

- Are the seeds capable of surviving in a dormant condition for a long time? If so, how long?

B7 Can the plant be dispersed by vegetative propagation? If so, describe possible mechanisms.

B8

- What is the likelihood that the imparted characteristic could be transferred to other species with adverse consequences?
- If there is any possibility of such transmission, would it have the potential to affect the distribution and abundance of the other species? If so, specify.
- If there is any possibility of such integration, has any attempt been made to minimize the risk (for example, by imparting male sterility or other means of reproductive isolation)? If not, why not?

B9 How might the plant's competitive advantage (reproductive fitness) be changed by the novel trait in the agricultural setting or in the natural environment? Explain.

B10 Does the novel characteristic change the capacity of the plant to add substances to or subtract substances from the soil (for example, nitrogen, toxic compounds)? If so, describe the change.

B11

- Is there any likelihood that the introduced gene could cause an increase in toxicity of the plant for animals and humans? If so, provide available data.
- Could any products of the plant concentrate in the natural or human food chain to levels that become toxic? If so, explain.
- Is the biodegradability of the plant changed? If so, how?

B12 What secondary ecological effects might result from introduction of the ONT (for example, effect on endangered native species, resistance of insect populations to an insecticide, reduction or increases in numbers of prey or parasites)?

B13 If the construct involves resistance to a chemical agent (other than selective agents, such as antibiotics, used in strain construction):

- Provide data on the degradability, selectivity, and toxicity of the chemical concerned.
- What is the agronomic significance of the chemical?
- What is the biological activity of the chemical?
- How is the chemical applied and used?

B14 If the construct involves resistance to an herbicide, explain whether the introduction will:

- Result in more effective use of the herbicide
- Result in better weed control in the crop
- Result in a more efficient overall farming operation
- Allow a change to a program that involves environmentally friendly chemicals or practices.

Microorganisms Living in or on Animals

Questions here relate to organisms such as gut biota living in larger hosts and microorganisms applied externally to animals (for example, bacteria to prevent fleece rot). Issues included here should also take into account the ecological interactions and behavior of host organisms that could have environmental impacts.

C1 What is the animal host species?

C2 Does the parent organism have an extended history of use in agriculture? If so, please elaborate.

C3 Is there any evidence that the ONT might be capable of establishing in or on other animals, including feral animals? If so, what are these animals and what are the possible or probable effects?

C4

- What new capacity will the ONT provide for the host species (for example, ability to degrade plant or pasture toxins)?
- What secondary effects can be postulated from conferring that capacity on the host?

C5 Will the competitive advantage or reproductive fitness of the host be altered? Explain, providing data to support your answer.

C6 What effects (including secondary effects) are likely on other plants or animals in the agricultural and natural environments? (Please include in your answer any likely effect on non-host animals or feral populations.)

C7 What secondary effects can be postulated from the introduction of the ONT into or onto the host? For example, is there a possibility of the genetic insert being transferred to other organisms in the host or to host cells?

C8 For ONTs living in animals will the ONT be excreted or otherwise leave the animal? If so, how long does it survive outside the animal?

C9
- What is the survival and dispersal of the ONT in natural waters and soil?
- What are any possible or probable effects of the ONT on water quality?
- Does the ONT produce spores?
- Is the ONT resistant to desiccation?

C10
- What sterilizing and antimicrobial agents are active against the ONT?
- Is the ONT susceptible to ultraviolet and ionizing radiation?

Microorganisms as Live Vaccines

D1
- What disease is to be controlled by the use of this vaccine?
- On what host species is the vaccine to be used?
- What is the host range of the parent organism from which the vaccine was constructed?

D2 If the vaccine is intended for humans, what are the proposed target groups for the vaccine? Specify age range, risk factor groups, and geographic area of residence, if applicable.

D3
- Provide data regarding level and duration of immunity produced in the host species after vaccination with the ONT.
- Over what period can the vaccine organism be detected in vaccinated animals or their excretions? Provide supporting data.

D4 Can the vaccine organism spread from vaccinated to nonvaccinated animals or to other species, including humans? If so, what is the mechanism and frequency? Provide data, if available.

D5 Is there any evidence to indicate whether the susceptibility of the host to the vaccine organism could be affected by the current state of the host (for example, immunosuppression or superimposition of other disease) or by other treatments (for example, drugs)? If so, elaborate.

D6 Does the genetic material of the vaccine organism have the potential to become incorporated in whole or in part into the genome of any cells of the vaccinated host? (If the answer is yes and the vaccine is for human use, answer the questions in section J.)

D7 If this is a viral vaccine, can the nucleic acid of the virus in the vaccine be rescued or restored to wild type by recombination or complementation with intracellular viruses?

D8
- In trials is it proposed to dispose of waste that contains vaccine organisms? If so, describe the arrangements.
- What is the fate of the vaccinated animals at the conclusion of the trial?

D9 Will the vaccinated humans or animals carry live vaccine organisms at the end of the trial? If so:
- Will they be likely to disseminate the live vaccine organisms to their family contacts or to the general population?
- What measures, if any, will be taken to minimize this possibility?
- Will the organisms be able to cross the placenta?

D10 Is the use of this vaccine organism likely to preclude its subsequent use for vaccination against other diseases? Will its usefulness for other vaccinations be affected?

D11
- Is the vaccine likely to have any deleterious effects on pregnant humans or animals? If so, specify. For humans, provide appropriate data from animal models.
- Is the vaccine teratogenic (causing developmental defects) for the fetus at any stage of gestation? If so, elaborate.

D12
- Does the ONT produce spores?
- Is the ONT resistant to desiccation?
- What sterilizing and antimicrobial agents are active against the ONT?
- Is the ONT susceptible to ultraviolet and ionizing radiation?

Microorganisms Not Falling into Sections C or D

Questions here relate to microorganisms associated with plants and microorganisms which might be applied to modify the physical or chemical environment (for example, microorganisms to modify soil properties).

E1 For microorganisms associated with plants, what is the partner species of plant? Describe the specificity of the interaction and indicate the range of plant species with which the ONT can interact similarly.

E2 Does the parent organism have an extended history of use in agriculture? If so, please elaborate.

E3 For microorganisms associated with plants:

- What is the effect of the ONT on the partner plant species and how will this be monitored?
- What other secondary effects might the ONT have on the plant?
- Does the modification cause any change to the range of host plant species available to the organism?
- What effect of the ONT, if any, is anticipated on the distribution and abundance of the host plant species and other species with which the organism can interact?

E4 If the ONT is associated with plant species that are food crops, could it affect the suitability of the resultant produce for human or animal consumption? If so, explain.

E5 Are there any effects expected on soil chemistry (for example, pH, mineral leaching, chelation, nutrient levels)?

E6

- What is the survival and dispersal of the ONT in natural waters and soil?
- What are any possible or likely effects of the ONT on water quality?
- Does the ONT produce spores?
- Is the ONT resistant to desiccation?

E7 What effects might the ONT have on soil organisms that are known to be beneficial to plants (for example, *Rhizobium*, *Azospirillum*, *Frankia*, and mycorrhizal fungi) and are likely to be in the test area?

E8 What is known about interactions between the ONT and closely related microorganisms in the partner plant (if applicable) or the environment of the site of introduction?

E9 For ONTs associated with plants, what effect might the ONT have on insects, birds, and animals (including humans) which may eat the plant?

E10 Does the ONT exchange genetic material with known plant pathogens? If so, elaborate.

E11

- What sterilizing and antimicrobial agents are active against the ONT?
- Is the ONT susceptible to ultraviolet and ionizing radiation?

Animals (Vertebrates, not Including Fish)

If these are to be consumed as food, also answer the questions in section M.

Questions here relate to all animals except fish. Please note that all work involving animals should be conducted according to standard principles for the safe and humane treatment of experimental animals, and may therefore require review by institutional animal experimentation ethics committees and authorities administering animal welfare legislation.

F1

- What unintended effects (environmental, animal welfare) may result from the planned introduction, and what is their likelihood?
- Are any of the intended gains directly linked to changes in other characteristics of the species? If so, specify.

F2 What effects might the expression of the modified trait have on the physiology, behavior, and reproduction of the animal? Explain with data (for example, studies from model animals).

F3

- Will the animals in this experiment be allowed to breed? If not, is breeding planned for later experiments or in the commercial phase?
- Are the arrangements for handling any offspring the same as those for the experimental animals? If not, please specify the arrangements.

F4 Do feral populations of the experimental species exist in the country? If so:

- Do the feral populations cause agricultural, environmental or disease-control problems? Specify the problems.
- Has any experimental work been done on the expression of the novel genetic material in feral animals (for example, cross-breeding of ONTs with captive feral animals)? If so, what were the results?
- What is the likelihood of the novel genetic material entering the feral gene pool (for example, by interbreeding with modified farm animals)?
- What effect might the entry of the novel genetic material into a feral gene pool have on the distribution and abundance of the feral population or on its ability to cause agri-

cultural or environmental problems, or to contribute to the spread of infectious disease? Provide data to support your answer.

F5 If no feral populations exist in the country, comment on the likelihood that the novel characteristic may enhance the ability of the species to establish feral populations.

F6 Can the ONT interbreed with any species native to the country?

F7 What management procedures and environmental factors, if any, are required for optimal expression of the novel trait? Provide data to support your answer.

Fish and Aquatic Organisms Such as Crustaceans

If the organism is to be consumed as food, also answer the questions in section M.

G1

- Could the ONT produce any new metabolites or toxins likely to have deleterious effects on parasites or predators? If so, elaborate.
- What other unintended effects may result from the planned introduction? Your answer should include consideration of the effect of the ONT on community ecology at the site of the planned introduction.
- Are any of the likely gains directly linked to losses in other characteristics of the organisms?

G2

- Will the ONTs in this introduction be allowed to breed? If not, is breeding planned for later introductions or commercial use?
- Are the arrangements for handling any offspring the same as those for the experimental organisms? If not, please specify the arrangements.

G3 Can the changed or added genetic material be transmitted by means other than by reproduction normal for the species or to any other species? If so, specify and elaborate its effects.

G4 Do natural populations of the parental organism exist in the country (including in rivers, lakes, or coastal waters)? If so, do the natural populations cause problems with other organisms? Specify the organisms and the problems.

G5 If no natural populations of the organism to be modified exist in the country, could the modified characteristics enhance the ability of the species to establish populations in aquatic habitats?

G6 Has any experimental work been done on phenotypic expression of the novel genetic material in naturally occurring organisms (for example, cross-breeding of ONTs with wild or farmed stocks)? If so, what were the results?

G7 What is the likelihood of the novel genetic material entering the gene pool of natural populations?

G8 Could the entry of the novel genetic material into the gene pool of a natural organism have any effect on the distribution and abundance of the organism or on associated fisheries, the environment or public health? If so, please explain.

G9 What mechanisms will be used to prevent dispersal of the ONT into other ecosystems?

Invertebrates

If the organism is to be consumed as food, also answer the questions in section M.

H1

- What effects might the ONT have on the food chain?
- Could the ONT produce any new metabolites or toxins likely to have deleterious effects on parasites or predators? If so, elaborate.
- What other unintended effects (other than those covered in section G) may result from the introduction? Your answer should include consideration of the effect of the ONT on the community ecology at the introduction site.

H2

- Will the ONTs in this introduction be fertile? If not, is it intended to use fertile organisms in later introductions?
- Are the genotype and phenotype of the offspring the same as those of the ONTs to be introduced? If not, please specify the differences.

H3 Do populations of the parental organism exist in the country? If so, do these populations cause agricultural, environmental or public health problems or benefits? Specify the problems or benefits.

H4

- Can the changed or added genetic material be transmitted by means other than

reproduction normal for the species? If so, specify, and elaborate its effects.
- What is the likelihood of the novel genetic material entering gene pools of natural populations?
- Can the changed or added genetic material be transmitted to any other species? If so, specify the mechanism of transfer and list the species.

H5 Has any experimental work been done on the phenotypic expression of the novel genetic material in other genetic backgrounds (for example, cross-breeding of modified strains with wild or caught stock)? If so, what were the results?

H6 Could the entry of the novel genetic material into the gene pool of natural populations of the organism have any effect on the distribution and abundance of the natural populations? What would be the effect of this change?

H7 What mechanisms will be used to prevent dispersal of the ONT into other ecosystems?

Insertion of Hereditary Material into the Genome of Human Subjects

The transfer of cloned DNA into a human subject may be a form of planned introduction and researchers working in this area must therefore abide by these guidelines. Institutional ethics committees should seek NBC advice on technical aspects of a proposal and on the appropriateness of the disease and subjects proposed for such experiments at the current stage of knowledge.[2]

J1
- What are the target cells?
- Will the cells be transformed in vitro or in vivo?
- When answering the questions in A13, provide the complete nucleotide sequence of the DNA to be inserted.

J2 Will a vector be used to introduce the DNA into the target cells? If so:
- Describe the vector and its key characteristics (in more detail, if possible, than in the answer to questions in A14).
- Is the vector capable of replicating in the target cells? If so, can the vector be introduced, with or without the cloned DNA in a form capable of infecting other cells of the treated person or other persons?
- Is there any possibility that the vector will infect nontarget cells, specifically germ cells? Provide detailed evidence on this point.

J3 With regard to the incorporation of the DNA into the genome of the target cells:
- Is any degree of targeting to specific sequences within the genome anticipated?
- What evidence is there regarding the likelihood of disturbance of the function of vital cellular genes?
- What long-term animal experiments have been conducted to analyze the risk of oncogenesis in DNA insertions of this type?

J4
- What risks will be explained to patients and how will these risks be monitored to evaluate the accuracy of this advice? Provide a draft of the consent form.
- What other risks of introduction of cloned DNA into the genome of human cells can be anticipated and how have these risks been evaluated?

J5
- If the cloned DNA is not expected to be inserted into the genome of the target cells, how is it going to bring about its desired effect and what evidence is there of adequate persistence of the DNA in the target cells?
- What untoward effects could be anticipated from persistence of unintegrated DNA within the target cells? How have these risks been evaluated?

J6 Describe the experimental basis (derived from tests in cultured cells and animals) for claims about the efficacy and safety of the proposed system for gene delivery, and explain why the model chosen is the most appropriate.

J7 If a retroviral system is used:
- How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation?
- What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells?
- What steps have been taken in designing the vector to minimize instability or variation?
- What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

- Has a protocol similar to the one proposed for a clinical trial been carried out in nonhuman primates or other animals? If so, what were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

Organisms for Biological Control

K1
- What is the species targeted for biological control?
- What direct effects does the parent organism have on the target species?
- What direct effects does the ONT have on the target species?

K2
- What is the host range of the ONT? If the host range of the ONT is likely to be different from that of the parent organism, explain why.
- What nontarget organisms have been tested for susceptibility to the ONT?
- What is the rationale for the choice of species tested?

K3 How is the ONT transferred from one target individual to another and what factors affect this transferability?

K4 What secondary effects can be envisaged on predators, prey, or parasites of the target species?

K5
- Explain the consequence of the removal or reduction of the target species on the management of agriculturally significant plants or farm animals.
- Predict any change in the ecosystem resulting from a reduction in the population of the target organism.

K6 Does the ONT produce metabolites that may have deleterious effects directly on other organisms or indirectly through concentration in the food chain? If so, elaborate.

K7 If the modified genetic traits can be transmitted to other organisms that are likely to be in the environment (see A20), are these other organisms likely to affect nontarget species?

K8 What genetic response might be invoked in populations of the target organism as a result of the use of the ONT (for example, increased resistance to the modified organism)? What evidence is there for this response?

Organisms for Biomediation

L1
- What is the target substrate for bioremediation?
- What effect does the parent organism have on the target substrate?
- What effect does the ONT have on the target substrate?

L2 What other substances can be metabolized by the ONT that cannot be metabolized by the parent organism?

L3 Will the ONT be self-sufficient once exposed to the target substrate or will additional measures be required (for example, provision of supplementary nutrients and growth factors or other environmental modifications)?

L4 Does the ONT produce metabolites that may have deleterious effects directly on other organisms or indirectly through concentration in the food chain? If so, specify.

L5 What effects might the ONT have on water, air or soil quality?

L6 What effects might the ONT have on organisms that ingest it?

L7 Will the ONT be dispersed from the site of application? If so, describe the mechanisms involved and the possible or probable consequences.

Organisms to Be Consumed as Food

Please note that organisms to be consumed as food require clearance by the appropriate national regulatory body.

M1 Is the parent organism or the donor organism already used in food production or eaten as food? If so, at what consumption levels and is any processing needed or commonly used before consumption?

M2
- Does the ONT produce metabolites that may have adverse effects on the consumer (humans or animals)? If so, elaborate. Provide available data on toxicology, allergenicity, and other possible adverse effects.

- Can any products of the ONT concentrate in the food chain to levels that may become toxic? If so, elaborate.

M3 Will the nutritional quality of the food be changed by the genetic modification? If so, how?

M4 Is the ONT to be processed during the production of the food? If so, elaborate.

Notes

1. This chapter is based on material in GMAC, *Guidelines for the Planned Release of Genetically Manipulated Organisms* (Canberra: GMAC, 1993).

2. Some of the questions in section J have been adapted from sections of the U.S. *Federal Register* relating to human gene therapy proposals.

APPENDIX 5.A ASSESSMENT OF A PLANNED RELEASE PROPOSAL COVER SHEET

The cover sheet should be typed in black ink and attached to the front of the proposal after the institutional biosafety committee's (IBC) evaluation. The IBC should send the proposal and this cover sheet to the national biosafety committee (NBC), together with any supplementary information it considers relevant (see question 14).

Trial Release

At the conclusion of the field trial the investigator should submit a comprehensive report to the IBC. The IBC should provide at least a summary of the report to the NBC.

Confidential Business Information

Proposals containing confidential business information (CBI) should be clearly indicated, and the confidential information itself should be unambiguously highlighted. An additional copy of the proposal with deleted information should also be submitted, clearly marked *CBI Deleted.* Proponents should also provide justification to explain how disclosure of the information claimed as CBI would be harmful.

Public Information Sheet

A completed public information sheet should be attached to the proposal.

Press Release

It is suggested that proponents consider issuing a press release to a newspaper circulated in the area of the planned introduction, both at the time of submitting the proposal and after receiving NBC advice on the proposal.

Approval

When appropriate approval has been received, a copy of the instrument of approval (for example, permit and registration number) should be forwarded to the NBC.

Additional Information

Contact the secretary of the NBC. See the guidelines for planned introductions of organisms with novel traits outlined in chapter 5 for detailed requirements.

1. Reference numbers (NBC or IBC identification numbers of previous proposals from which this proposal has been developed).
2. Project title.
3. Name of host institution or sponsoring organization.
4. Address or contact information for supervising IBC.
5. Name, position, and address of responsible individual or project supervisor or manager.
6. Proposed location of field trial.
7. Name of municipality in which field trial is planned.
8. When is the field trial scheduled to begin?
9. When is the field trial scheduled to conclude?
10. Give specific details describing the size of the field trial (area and number of organisms involved).
11. Describe briefly the schedule and scale for planned future trials.
12. What government authorities have been consulted about this project? Give names of agencies and officials contacted.
13. Give names and addresses of authorities to whom the NBC should forward its advice on this proposal. List agencies that have legal responsibilities for approving the end-use of products that might result.
14. *IBC assessment.* Provide an evaluation of the project, including comments on the project supervisor's capability to manage the work, the adequacy of the project design, site selection, and contingency plans. Use additional pages as necessary.
15. *IBC request for advice.* On what specific points does the IBC seek the committee's advice?
16. Will a press release on the project be distributed? If yes, when and to whom?

17. Provide details of any action taken to inform or consult the public (for example, the local community) about the project.
18. The information herein provided is, to the best of my knowledge, complete, accurate, and truthful (Name and signature of responsible individual and date).
19. *Endorsement of the IBC.* The IBC has assessed and endorsed this proposal (Name and signature of IBC chairman and date).
20. Senior administrative official (or delegate) to countersign (Name and signature of senior administrative officer and date).

APPENDIX 5.B PUBLIC INFORMATION SHEET

Please note that the information provided on this sheet is for public distribution. It should be written in plain language. Do not include any confidential business information. You should ensure that information you provide does not prejudice your rights to patent protection.

Name of organization:_____

Address of organization:_____

Name of contact person: _____

Telephone number of contact person:_____

Fax:_____

E-mail: _____

World Wide Web: _____

Organism to be introduced: _____

Location and size of planned introduction:_____

Purpose of planned introduction: _____

Brief summary of the nature and results of any genetic modification. Use of technical terms should be minimized.

Agencies consulted before introduction (list approvals obtained): _____

APPENDIX 5.C INSTITUTIONAL BIOSAFETY COMMITTEE REPORT ON PLANNED INTRODUCTION AFTER ITS COMPLETION

Institutional biosafety committee (IBC) name: _____

National biosafety committee (NBC) project review number: _____

Project title: _____

Project supervisor: _____

Agency or agencies approval received (date): _____

Location of planned introduction: _____

Date of commencement: _____

Date of completion: _____

Summary of report. Include answers to the following questions:

- What monitoring procedures were undertaken?
- Were the procedures undertaken according to the protocol submitted for NBC review? Describe.
- Were the aims of the planned introduction achieved? Describe.
- Were there any unexpected effects? If there was any adverse effect, a report should be made immediately to the agency concerned and to the NBC at the time of the occurrence and reiterated at the time of writing this report.
- What is the number of organisms with novel traits surviving at the site of the introduction? What will be the fate of these organisms?
- Will the project be continued to a further stage? If so, provide details

Signature of IBC chair: _____

Date: _____

APPENDIX 5.D REQUIREMENTS FOR TRANSPORT OF ORGANISMS WITH NOVEL TRAITS

Transport of Animals with Novel Traits

With regard to transportation arrangements for animals with novel traits, two principles must be paramount:

- The need to prevent the animals from escaping, especially with regard to reasonable contingencies such as accidents en route, so that they will not interbreed with feral populations
- The need to ensure that they are properly identified and duly arrive at the intended destination and to ensure that a competent biologist with some experience in handling animals with novel traits takes delivery of them.
- Accounting procedures should be in place to ensure that the same number of animals sent is also delivered.

The institutional biosafety committee (IBC) may institute whatever procedures or rules it considers appropriate to meet these conditions. It may be necessary for the IBC to inspect the arrangements for transportation to satisfy itself that the above principles are adhered to and that any additional conditions that the IBC considers appropriate are met.

It may be helpful to arrange for the purchase of animal boxes that are approved by international airlines for the transport of specific pathogen-free animals. These may be adapted for specific needs.

Transport of Insects with Novel Traits and Their Pathogens

With regard to transportation arrangements for insects with novel traits (including live insects and insect cell cultures infected with pathogens manipulated to contain novel traits):

- The insects should be in a clearly labeled, unbreakable holding container that is adequately sealed to prevent escape.
- The holding vessel should be placed in another, clearly labeled and well-sealed container for transport.
- Insects should be transferred from the holding vessel to a new container immediately upon arrival at their destination.
- All transport materials should be decontaminated by autoclaving after transfer of the transported insects into new containers.
- Accounting procedures should be in place to ensure that the same number of containers sent is also delivered.
- Requirements are the same for insect pathogens with novel traits as for human and vertebrate pathogens.

Transport of Plants with Novel Traits

With regard to transportation arrangements for plants with novel traits:

- Vegetative plant material from plants with novel traits to be transported within and between institutions should be carried in a primary container (for example, a plastic bag for cuttings and an envelope for seeds) that is packed in a secondary, unbreakable container.
- The outer container should be labeled to indicate that it contains propagative material from plants with novel traits, and the label should include the telephone number of a contact person should the package be lost or damaged. Labels on seed packets should include the number of seeds being transported.
- Whole plants should be netted and deflowered before transport. They may be transported in pots, contained in boxes or crates.
- Plants should not be transported once they have set seed.
- Accounting procedures should be in place to ensure that the same number of plants or containers sent is also delivered.

APPENDIX 5.E FOOD AND FEED SAFETY ASSESSMENT

Food Safety Issues

The appropriate authorities will regulate the final products of biotechnology before they are released for human consumption or animal feed. There are, however, important food safety issues that pertain to the host plant, donor organisms, and new substances that will be introduced into the food that developers should address. Potential new substances considered in this safety assessment are proteins, carbohydrates, fats, and oils because these are the substances that will be introduced or modified in the first plant varieties developed by recombinant DNA (rDNA) techniques.

The principal investigators should consider the food safety issues mentioned in the following sections.

Host Plant

- Potential adverse effects of an altered metabolic pathway in the plant
- The inheritance of the introduced genetic material as a single mendelian trait
- Genetic stability of the new plant variety
- Changes in the concentrations or bioavailability of important nutrients for which a food is widely consumed
- Monitor toxicant concentrations to ensure they are within an acceptable range.

Donor

- History and derivation of molecular constructs
- Activities of any introduced regulatory sequences
- Potential for inadvertently introducing undesirable substances (for example, as a result of the expression of extraneous open reading frames)
- Donor-derived toxicants that could potentially end up in food and feed products.

Proteins

- Safe history of use in food
- Similarity to proteins used as food components
- Toxicity, allergenicity, and dietary exposure
- In the case of enzymes, ascertain that they are not involved in production of toxic substances.

Carbohydrates

- Elevated concentrations of an indigestible carbohydrate that normally occurs at low concentrations
- Conversions of normally digestible carbohydrate to an indigestible form.

Fats and Oils

- Presence of fatty acids of known toxicity (for example, erucic acid)
- Presence of fatty acids with chain lengths greater than C_{22}
- Alterations in the ratio of saturated to unsaturated fatty acids.

Genetically Engineered Pesticides

To ensure clarity, a pesticide is legally defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest, or intended for use as a plant regulator, defoliant, or desiccant. Pesticides can be classified as either chemical pesticides or biological pesticides. Biological pesticides are subdivided into three groups: microbial pesticides, biochemical pesticides, and transgenic plant pesticides.

Pesticide regulation falls under the jurisdiction of the pest control products authorities who register new pesticides. The following guidelines are meant to assist developers of potential data requirements for toxicological evaluation of genetically engineered plant pesticides.

Transgenic Plant Pesticides

The following information will be required when registering new pesticides:

SOURCES OF PESTICIDAL GENETIC MATERIAL
- Identification of the donor organism(s)
- Identification of the pesticidal genetic material.

PESTICIDE PRODUCTS
- Identification and characterization of the protein or peptides encoded by the inserted genetic material
- Identification and characterization of the nonproteinous active pesticidal ingredients resulting directly from the introduction of the genetic material.

VECTOR SYSTEM
- A description of the vectors
- The identity of the organisms used for cloning of the vectors
- A description of methodologies used for assembling all vectors.

RECIPIENT PLANT
- Identity and taxonomy of recipient plant to cultivar, line, or variety
- Life cycle, mode of reproduction, and dissemination
- Description of methods that are used to deliver the gene sequence(s) to the recipient plant.

GENE EXPRESSION IN THE PLANT
- Whether the inserted genes are expressed constitutively or if the genes are inducible
- Localization and expression in plant parts
- Estimation of the number of gene copies
- Gene expression during the plant's life cycle.

PRODUCT ANALYSIS AND RESIDUE CHEMISTRY
- Mode of action of the pesticidal product
- Concentration of pesticidal product in the plant.

PHYSICAL AND CHEMICAL PROPERTIES
- Information required in the event that genetic manipulation is for the purpose of producing de novo nonproteinous products.

MAMMALIAN TOXICOLOGICAL REQUIREMENTS (PROTEIN PRODUCTS ONLY)
- *Food products.* Data on oral studies (acute, subchronic, chronic feeding, or other studies) is required as are reporting of observed dermal toxicology or irritation effects and pulmonary studies.
- *Nonfood.* Report any observed dermal toxicity or irritation effects; pulmonary studies in case of volatile pesticide products.

In general, for well-characterized proteins introduced by rDNA techniques that do not exhibit unusual functions, safety testing will not be necessary. However, for certain groups of proteins known to be toxic to vertebrates—for example, bacterial and animal toxins, hemaglutinins, enzyme inhibitors, vitamin-binding proteins (avidin), vitamin-destroying proteins, enzymes that release toxic compounds, and selenium-containing proteins—testing is necessary to ensure safety.

Microbial Pesticides

The following information will be required:
- The potential for toxicity of the microbial ingredient together with the fermentation medium in laboratory animals
- Taxonomic characterization of the active microbial ingredient
- A description of the manufacturing (growth) process, including measures taken to minimize the presence of contaminating organisms
- Toxicological data from subcutaneous injection of rodents in case of B. thuringiensis products.

Under the pesticide act the pest control products authorities can exempt plant pesticides from the requirement of a tolerance if such tolerance is not necessary to protect the public health. In the future, as more knowledge is obtained, the authorities may consider exempting some plant pesticides under the act.

Appendix 5.F Recommended Crop Isolation Distances for Production of Certified Seed

	Crop	Isolation distance (meters)		Crop	Isolation distance (meters)
1.	Maize	500	16.	Courgettes (zucchini)	1,500
2.	Beans	150	17.	Watermelons	1,200
3.	Irish potatoes	50	18.	Lettuce	30
4.	Wheat	150	19.	Swiss chard	1,000
5.	Rice	150	20.	Radishes	1,000
6.	Peas	100	21.	Celery	500
7.	Pigeon peas	1,000	22.	Beet roots	500
8.	Carrots	1,600	23.	Cabbage	1,600
9.	Onions	1,000	24.	Kale	1,600
10.	Spinach	500	25.	Cauliflower	1,600
11.	Tomatoes	50	26.	Broccoli	1,600
12.	Brinjals (eggplant)	50	27.	Brussels sprouts	1,600
13.	Cucumbers	1,500	28.	Turnips	500
14.	Melons	1,200	29.	Chilies	1,000
15.	Okra	500	30.	Barley	150

6. Guide to the Contained Use of Organisms with Novel Traits

This chapter consists of four parts, each of which covers areas relevant to the development and application of national and institutional biosafety guidelines. They include the development of national and international guidelines for genetic manipulation work, specifications for biological safety cabinets, importation guidelines for organisms with novel traits, and classification of etiological agents and oncogenic viruses on the basis of hazard.

Development of National and International Guidelines for Genetic Manipulation Work

One of the first tasks of national regulatory agencies will be to develop, publish, and ensure compliance with guidelines for small- and large-scale genetic manipulation work. Such guidelines should be continually revised to take into account current knowledge and practices in relation to the existing regulatory systems. There are many examples of both international and national guidelines available for study, and the UNIDO/BINAS network, which is accessible on the Internet, is a useful source of information in this respect. Although such guidelines include many technical details and appropriate forms, which may vary from nation to nation, it is critically important to define the scope of the guidelines and the various categories of risk and containment and levels of approval, supervision, monitoring, and reporting. An example of such definitions is given below.[1]

Section 1: Scope and Exemptions

1.1 *Scope*

1.1.1 *Scope of guidelines.* Such guidelines should apply to any experiment involving the construction and propagation of viroids, viruses, cells, or organisms[2] of novel genotype[3] produced by genetic manipulation that are either unlikely to occur in nature or likely to pose a hazard to public health or to the environment.[4]

Categories of small-scale work that specifically fall under the scope are described in section 2 below.

1.1.2 Proposals for human gene therapy work are assessed by the appropriate national authority. Any proposals involving the introduction of DNA into human subjects shall be submitted to that authority. If the introduction of the DNA involves an infectious agent, the authority, as part of its assessment, must seek advice from the national biosafety committee (NBC). However, when the infectious agent carries determinants intended to induce protection against infection only (for example, vaccines), the proposal should be submitted directly to the NBC. The NBC may seek the advice of the appropriate national authority on such applications.

1.1.3 If an investigator is uncertain if the proposed work falls within the scope of these guidelines, a description of the proposed work should be submitted to the institutional biosafety committee (IBC) for written clarification before work commences. If there is no IBC

in the firm or institution, a description of the work proposed should be sent to the NBC.

1.2 *Exemptions*

The types of experiments described in sections 1.2.1 to 1.2.6 are specifically exempt from the guidelines unless they also fall into Categories A and B of section 2, in which case the guidelines apply.

1.2.1 Any experiment involving the fusion of mammalian cells that does not generate a viable organism (for example, the creation of hybridomas to produce monoclonal antibodies).

1.2.2 Protoplast fusion between nonpathogenic microorganisms.

1.2.3 Protoplast fusion or embryo-rescue involving plant cells.

1.2.4 Any experiments involving the production or use of *gene knockout mice* (that is, mice in which the genetic modification involves deletion or inactivation of a specific gene), whether or not the mice also carry a selectable marker gene, provided that the selectable marker gene does not confer an advantage on the adult animal. Subsequent genetic manipulation work using knockout mice is not exempt from the guidelines and falls under Category B(i).

1.2.5 Any experiments involving approved host-vector systems provided that the donor DNA:

- Is not derived from microorganisms able to cause disease in humans, animals, or plants
- Does not code for a toxin for vertebrates with an LD50 of less than 100µg/kg and is not an oncogene
- Does not comprise or represent more than two-thirds of the genome of a virus and is not being used in an experiment in which the genetic material missing from the viral genome, and essential for producing infection, is available in the cell into which the incomplete genome is introduced or is made available by subsequent breeding processes.

1.2.6 Any experiment granted a Category C *Special Exemption* by the NBC. (See section 2.3 for the procedures to be followed in applying for a Category C *Special Exemption.*)

1.2.7 Exempted work should be carried out under conditions of standard microbiological laboratory practice. If pathogenic organisms are used, the containment level appropriate to the pathogen should be employed, and personnel should have appropriate training and recommended vaccinations.

1.2.8 Work with oncogenes should be carried out in accordance with specific guidelines and practices developed by the NBC.[5]

1.2.9 If work is considered to be exempt from these small-scale guidelines under sections 1.2.1, 1.2.2, 1.2.3 or 1.2.4, it is also exempt from the NBC large-scale guidelines. Work falling under sections 1.2.5 or 1.2.6 is not exempt from the large-scale guidelines.

1.2.10 Exemptions under sections 1.2.1 to 1.2.6 do not apply to planned releases of genetically manipulated organisms. These experiments fall under the NBC's *Guidelines for the Planned Release of Genetically Manipulated Organisms* (see chapter 5).

1.2.11 Exemption from the guidelines does not provide exemption from any statutory provisions that may apply to any aspect of a project involving genetic manipulation work (for example, quarantine legislation).

1.3 *IBC Notification*

1.3.1. The IBC shall be notified of, and approve, all work that falls under the guidelines. As well, scientists who believe that their work falls into any of the exemptions in sections 1.2.1 to 1.2.5 are nevertheless required to notify their IBC of the proposed project. If the institution does not have an IBC, scientists should notify the NBC by submitting a description of the proposed work before commencement. Request for Category C *Special Exemption* (section 1.2.6) shall be made by submission of a proposal form and detailed submission to the IBC (see section 2.3).

1.3.2 Forms for IBC notification for work exempted under sections 1.2.1 to 1.2.5 may be devised by IBCs. A copy of the form should be retained by the investigator. A sample of such a form is included below:

> I, (name of principal investigator) believe the experiment(s) I am proposing to do (short description of work) is exempt from the NBC *Guidelines for Small-Scale Genetic Manipulation Work* under sections 1.2.1–1.2.5, because it is (description of specific exemption).
>
> Signature of investigator: _____
> Date: _____

1.3.3 Any substantive change in an experiment already granted a Category C *Special Exemption*, which may affect its exemption status, requires submission to the IBC of a new *Proposal Form for Assessment of Small-Scale Genetic Manipulation Work*. If the IBC endorses the change in the proposed work, it shall forward the form to the NBC, together with the IBC's assessment of the proposal.

Section 2: Categories of Small-Scale Work

These categories refer to the NBC's scope of review of genetic manipulation work.

Work involving more than 10 liters of culture or any industrial-scale work will fall under the NBC *Guidelines for Large-Scale Genetic Manipulation Work*, which should be developed and published separately.[6] Proposals for planned release of organisms falling under the scope, regardless of the way in which the genetically manipulated organism is obtained, shall comply with the NBC *Guidelines for the Planned Release of Genetically Manipulated Organisms*, which should also be developed and published separately by each national biosafety committee (see chapter 5).

2.1 *Category A: Experiments Requiring NBC Advice and IBC Approval*

This category includes work that may cary a hazard to researchers, the community, or the environment. Included in this category is work for which the nature or the degree of hazard may be uncertain. The level of physical containment required will vary according to the nature of the work and its assessed hazard. In some cases biosafety level two containment may be considered sufficient, while in others higher containment levels may be necessary. This category of work requires assessment and advice from the NBC to the IBC. Principal investigators shall not begin work on proposals assessed as Category A until specifically advised by the IBC.

A proposal on an NBC proposal form for assessment of small-scale genetic manipulation work shall be submitted to the IBC for assessment. The IBC shall forward it to the NBC together with the IBC's recommendations or comments.

The following types of experiments fall into Category A:

A(i) Work with toxin producers:

- Work with DNA encoding a toxin for vertebrates having an LD50 of less than 100 µg/kg.
- Work involving high-level expression of toxin genes even if the LD50 is greater than 100 µg/kg. Work with uncharacterized DNA, which may include toxin sequences from toxin-producing organisms, also falls within this subcategory. However, work with DNA that has been characterized and shown not to code for a toxin, from a toxin-producing organism as donor, is not included in the subcategory.

A(ii) Experiments involving viral vectors whose host range includes human cells, and which contain one or more inserted DNA sequence coding for a product known to play a role in the regulation of cellular growth or to be toxic to human cells.

A(iii) Experiments using, as host or vector, microorganisms that are able to cause disease in plants, humans, or animals, except:

- When microorganisms are listed as approved hosts or vectors
- When the DNA to be introduced is fully characterized and will not increase the virulence of the host or vector.

Included in this subcategory are experiments using defective vector or helper virus combinations that have the potential to regenerate nondefective recombinant virus.

If the nonapproved host or vector is a pathogen, the work falls under Category A(iii) unless the DNA to be introduced is fully characterized and will not affect the virulence of the host or vector, in which case it is classified as B(iii).

The appropriate recommendations for vaccination and contra-indications should be observed for any work involving human pathogens.

A(iv) Introduction of genes determining pathogenicity into microorganisms other than previously NBC-approved hosts.

A(v) Cloning or transfer of complete viral genomes, viroids, or fragments of a genome capable of giving rise to infectious particles pathogenic to humans, animals, or plants. Work involving cloning of less than two-thirds of a

complete viral genome would fall outside this subcategory. Cloning of viral genome lacking activity for a vital component of replication or packaging, not supplied by the experimental system, would also fall outside this subcategory.

A(vi) Experiments involving recombinations between whole viral genomes, viroids, or complementary fragments of such genomes in which one or more fragments contain virulence or pathogenic determinants. This subcategory includes experiments that alter host-ranges of pathogens or which may increase their virulence or infectivity.

A(vii) Any experiment to inject a fragment or the whole genome of a virus into an embryo to produce a transgenic animal secreting or producing infectious viral particles.

A(viii) Experiments not falling into the above subcategories of Category A or into Category B but which fall under the scope of the guidelines as defined in section 1.

2.2 *Category B: Experiments for NBC Notification and IBC Approval*

This category includes work that carries a low level of hazard to laboratory personnel, the community, or the environment. Such work requires at least biosafety level two (laboratory, plant house, animal house, insectary, birdhouse, or aquarium) physical containment. Some work may require additional precautions or higher containment because the donor DNA or its components are by their nature hazardous or infectious. Other work may require special containment features.

The IBC and the investigator shall identify all potential hazards and their nature and determine any additional procedures and conditions appropriate for work in this category.

The following subcategories of work shall be submitted to the IBC for determination of the appropriate working and containment conditions and shall begin only after IBC assessment and approval. The IBC shall subsequently forward the proposal and assessment forms to the NBC for information.

If work falls into both Categories A and B, Category A takes precedence and Category A requirements apply.

B(i) Genetic manipulation work involving whole animals (including nonvertebrates, but excluding microorganisms):

- Genetic manipulation of somatic cells of animals in vivo.
- Genetic manipulation of the genome of the oocyte or zygote or early embryo by any means to produce a novel whole organism. For transgenic animal work, prior approval from the relevant institutional bioethics committee is required.
- Work involving injection of naked nucleic acid into animals.

B(ii) Genetic manipulation work involving the production of modified whole plants. The investigator should submit supplementary information as requested by the NBC for work involving whole plants.

B(iii) Work with non-NBC–approved host-vector in which the host or vector either:

- Does not cause disease in plants, humans, or animals
- Is able to cause disease in plants, humans, or animals but the DNA to be introduced is fully characterized and will not increase the virulence of the host or vector.

The appropriate recommendations for vaccinations and contra-indications should be observed for any work involving human pathogens.

B(iv) Work with approved host-vector systems, where the gene inserted is (a) a pathogenic determinant; (b) uncharacterized DNA from microorganisms able to cause disease in humans, animals, or plants; or (c) an oncogene.

Shotgun cloning of mammalian DNA in NBC-approved host-vector systems is not intended to fall into this category. Investigators wishing to have new host-vector systems added to the list are asked to make a detailed submission to NBC through their IBC.

Note that experiments not falling into the above subcategories of Category B or into Category A, but falling under the scope in section 1, require NBC advice and IBC approval (see subcategory A(viii)).

2.3 *Category C: Experiments for Special Exemption*

Investigators whose experiments fall under Category A or B, but who consider that their projects do not present a significant risk to occupational and public health or to the environment and that the genetically manipulated DNA does

not introduce any particular hazard, may request a Category C *Special Exemption.*

Such requests shall be accompanied by a detailed submission addressing the above points and shall include a completed proposal form for new proposals. Requests for Category C *Special Exemption* shall be forwarded to the IBC, which shall decide whether it wishes to endorse the exemption request and, if so, shall forward a copy of the request, the proposal form, and IBC assessment form to the NBC for recommendation.

Any significant changes in any of the parameters of the work that could affect its exempt status under this category will require the submission of another proposal by the investigator, IBC assessment, and if endorsed by the IBC, forwarding of the proposal and assessment forms to the NBC for recommendation.

Biological Safety Cabinets

The biological safety cabinets are classified in Class I, Class II, and Class III:

Class I

A Class I safety cabinet is a ventilated cabinet for personnel protection that has an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (a) with a full-width open front, (b) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and c) with an installed front-closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater.

Class II

A Class II safety cabinet is a ventilated cabinet that has has an open front with inward air flow for personnel protection and HEPA-filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater.

Class III

A Class III safety cabinet is a closed-front ventilated cabinet of gas-tight construction that provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure (at least 0.5 inches) water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

Importation of Organisms with Novel Traits

These guidelines govern the import of organisms with novel traits into the importing country. Information relating to the description of the organism is requested, together with the natural history of the organism, its laboratory of origin, its destination in the importing country, and its intended uses. Sufficient information is required to enable the authorities to undertake a risk assessment prior to approving or not approving the import.[7]

1. *Introduction of Regulated Materials*

1.1 Any introduction of regulated materials should be authorized by an import permit.

1.2 Approval or denial of an import permit shall be based on the following guidelines for evaluation.

1.3 Responsible person or persons involved:

- Name, title, address, telephone number, and signature
- Name, address, and telephone number of the person(s) who developed or supplied the regulated material.

1.4 Materials to be introduced:

- Quantity of the regulated material(s) to be introduced and proposed schedule and number of introductions
- All scientific, common, and trade names and all designations necessary to identify the regulated material
- Country and locality in which the regulated material was collected, developed, and produced

- Known potential to cause an epidemic (survival, reproduction, and dispersal rates)
- Known potential to cause losses
- Known potential hosts or alternative hosts
- Known ability to evolve
- Known vector of organisms
- Known mode of spread and conditions for epidemic
- History of epidemics.

1.5 Genetically modified microorganisms:

- Nomenclature and characteristics of donor, recipient, and vector organisms
- A detailed description of the molecular biology of the systems (for example, donor-recipient-vector) that is or will be used to produce the regulated materials
- A description of the anticipated or actual expression of the altered genetic material in the regulated materials; an explanation of how that expression differs from the expression in the nonmodified parental organism, such as morphological or structural characteristics, physiological activities and processes, and number of copies inserted in the genetic material; the physical state of this material inside the recipient organism (integrated or extra-chromosomal), products and secretions, and growth characteristics
- A detailed description of the processes, procedures, and safeguards that have been used or will be used in the country of origin and in the importing country to prevent contamination, release, and dissemination in the production of the donor organism, recipient organism, vector or vector agent, regulated materials, and a constituent of each regulated material which is a product.

1.6 Others:

- A detailed description of the uses and the purpose for introducing the regulated material, including a detailed description of the proposed experimental or production design
- History of similar introductions
- A description of transfer of the regulated material (for example, mail, common carrier, baggage, or hand carried)
- A detailed description of the intended destination (including final and all intermediate destinations), or distribution of the regulated material (for example, greenhouse, laboratory, growth chamber location, or field trial location; pilot project location; production; propagation and manufacture location; proposed sale and distribution location)
- A detailed description of the proposed procedures, processes, and safeguards that will be used to prevent escape and dissemination of the regulated material at each of the intended destinations
- A detailed description of any biological material (for example, culture medium or host material) accompanying the regulated material during movement
- A detailed description of the proposed method of final disposition of the regulated material.

Classification of Etiological Agents and Oncogenic Viruses on the Basis of Hazard

Each country will need to compile a classification of etiological agents and oncogenic viruses on the basis of hazard, dependent on its own requirements and legislation. However, the list given below can serve as a starting point for the compilation of such a classification.[8]

Appendix B-I. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes shall be considered Class 1 agents.

Appendix B-II. Class 2 Agents

APPENDIX B-II-A. CLASS 2 BACTERIAL AGENTS

Acinetobacter calcoaceticus
Actinobacillus—all species
Aeromonas hydrophila
Amycolata autotrophica
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Campylobacter fetus
Campylobacter jejuni
Chlamydia psittaci
Chlamydia trachomatis

Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diphtheriae, C. equi, C. haemolyticum, C. pseudotuberculosis, C. pyogenes, C. renale
Dermatophilus congolensis
Edwardsiella tarda
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen
Haemophilus ducreyi, H. influenzae
Klebsiella—all species except oxytoca
Legionella pneumophila
Leptospira interrogans—all serotypes
Listeria—all species
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycobacterium avium
Mycoplasma—all species except Mycoplasma mycoides and Mycoplasma agalactiae, which are in Class 5
Neisseria gonorrhoea, N. meningitides
Nocardia asteroides, N. brasiliensis, N. otitidis-caviarum, N. transvalensis
Pasteurella—all species except those listed in Class 3
Rhodococcus equi
Salmonella—all species and all serotypes
Shigella—all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae, S. pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio cholerae, V. parahemolyticus
Yersinia enterocolitica

APPENDIX B-II-B. CLASS 2 FUNGAL AGENTS
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides braziliensis

APPENDIX B-II-C. CLASS 2 PARASITIC AGENTS
Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Schistosoma mansoni
Toxocara canis
Toxoplasma gondii
Trichinella spiralis
Trypanosoma cruzi

APPENDIX B-II-D. CLASS 2 VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS
Adenoviruses—human, all types
Cache Valley virus
Coronaviruses
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis viruses—associated antigen material
Herpes viruses—except Herpesvirus simiae (Monkey B virus), which is in Class 4
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Pox viruses—all types except Alastrim, Smallpox, and Whitepox, which are Class 5, and Monkey pox, which depending on experiments is in Class 3 or Class 4
Rabies virus—all strains except Rabies street virus, which should be classified in Class 3
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except Herpesvirus simiae (Monkey B virus) and Marburg virus, which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus

Vesicular stomatitis virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

APPENDIX B-II-E. CLASS 2 ONCOGENIC VIRUSES (SEE APPENDIX B-VI-C)

APPENDIX B-II-E-1. LOW-RISK ONCOGENIC VIRUSES
Adenovirus 7-Simian virus 40 (Ad7-SV40)
Adenovirus
Avian leukosis virus
Bovine leukemia virus
Bovine papilloma virus
Chick-embryo-lethal orphan (CELO) virus or fowl adenovirus 1
Dog sarcoma virus
Guinea pig herpes virus
Lucke (frog) virus
Hamster leukemia virus
Marek's disease virus
Mason-Pfizer monkey virus
Mouse mammary tumor virus
Murine leukemia virus
Murine sarcoma virus
Polyoma virus
Rat leukemia virus
Rous sarcoma virus
Shope fibroma virus
Shope papilloma virus
Simian virus 40 (SV-40)

APPENDIX B-II-E-2. MODERATE-RISK ONCOGENIC VIRUSES
Adenovirus 2-Simian virus 40 (Ad2-SV40)
Epstein-Barr virus (EBV)
Feline leukemia virus (FeLV)
Feline sarcoma virus (FeSV)
Gibbon leukemia virus (GaLV)
Herpesvirus (HV) ateles
Herpesvirus (HV) saimiri
Simian sarcoma virus (SSV)-1
Yaba

Appendix B-III. Class 3 Agents

APPENDIX B-III-A. CLASS 3 BACTERIAL AGENTS
Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium bovis, M. tuberculosis
Pasteurella multocide type B—"buffalo" and other foreign virulent strains (see appendix B-VI-B)
Pseudomonas mallei (see appendix B-VI-B)
Pseudomonas pseudomallei (see appendix B-VI-B)
Yersinia pestis

APPENDIX B-III-B. CLASS 3 FUNGAL AGENTS
Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. duboisii

APPENDIX B-III-C. CLASS 3 PARASITIC AGENTS
None

APPENDIX B-III-D. CLASS 3 VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS
Monkey pox virus—when used in vitro (see appendix B-VI-D)
Arboviruses—all strains except those in Class 2 and 4. (Arboviruses indigenous to the United States are in Class 3 except those listed in Class 2. West Nile and Semliki Forest viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus—when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Rickettsia—all species except Vole rickettsia when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used in vitro

Appendix B-IV. Class 4 Agents

APPENDIX B-IV-A. CLASS 4 BACTERIAL AGENTS
None

APPENDIX B-IV-B. CLASS 4 FUNGAL AGENTS
None

APPENDIX B-IV-C. CLASS 4 PARASITIC AGENTS
None

APPENDIX B-IV-D. CLASS 4 VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS
Ebola fever virus

Hemorrhagic fever agents—including Crimean hemorrhagic fever, (Congo), Junin, and Machupo viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Monkey pox virus—when used for transmission or animal inoculation experiments (see appendix B-VI-D)
Tick-borne encephalitis virus complex—including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Venezuelan equine encephalitis virus, epidemic strains—when used for transmission or animal inoculation experiments
Yellow fever virus-wild—when used for transmission or animal inoculation experiments

Appendix B-V. Class 5 Agents (see appendix B-VI-E)

APPENDIX B-V-A. ANIMAL DISEASE ORGANISMS THAT ARE FORBIDDEN ENTRY INTO THE UNITED STATES BY LAW
Foot and mouth disease virus

APPENDIX B-V-B. ANIMAL DISEASE ORGANISMS AND VECTORS THAT ARE FORBIDDEN ENTRY INTO THE UNITED STATES BY THE U.S. DEPARTMENT OF AGRICULTURE POLICY
African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Mycoplasma mycoides—contagious bovine pleuropneumonia
Mycoplasma agalactiae—contagious agalactia of sheep
Nairobi sheep disease virus
Newcastle disease virus—Asiatic strains
Rhinderpest virus
Rickettsia ruminatium—heart water
Rift valley fever virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Theileria parva—East Coast fever
Trypanosoma evansi
Trypanosoma vivax—Nagana
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

APPENDIX B-V-C. ORGANISMS THAT MAY NOT BE STUDIED IN THE UNITED STATES EXCEPT AT SPECIFIED FACILITIES
Alastrim (see appendix B-VI-D)
Smallpox (see appendix B-VI-D)
Whitepox (see appendix B-VI-D)

Appendix B-VI. Footnotes and References of appendix B

APPENDIX B-VI-A.
The original reference for this classification was the publication *Classification of Etiologic Agents on the Basis of Hazard*, 4th edition, July 1974, U.S. DHHS, Public Health Service, Centers for Disease Control and Prevention, Office of Biosafety, Atlanta, Georgia 30333. For the purposes of these *NIH Guidelines* this list has been revised by the National Institutes of Health.

APPENDIX B-VI-B.
A U.S. Department of Agriculture permit, required for import and interstate transport of pathogens, may be obtained from the U.S. Department of Agriculture, ATTN: Animal and Plant Health Inspection Service, Import-Export Products Office, Room 756, Federal Building, 6505 Belcrest Road, Hyattsville, Maryland 20782.

APPENDIX B-VI-C.
National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses, U.S.

Department of Health, Education, and Welfare Publication No. (NIH) 75-790, October 1974.

APPENDIX B-VI-D.
All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization Collaborating Center for Smallpox Research, Centers for Disease Control and Prevention, Atlanta, Georgia).

APPENDIX B-VI-E.
U.S. Department of Agriculture, Animal, and Plant Health Inspection Service.

Notes

1. These definitions are based on parts 1 and 2 of the Genetic Manipulation Advisory Committee's *Guidelines for Small-Scale Genetic Manipulation Work* (Canberra: GMAC, 1995).

2. At the time of drafting these guidelines the exact nature and mechanism of action of the etiological agents of spongiform encephalopathies of humans and of several species of domestic and wild animals (prions) had not been resolved. Any genetic manipulation work involving prions or their precursor proteins should be reported to the national biosafety committee (NBC). Note that the importation of these substances is controlled by quarantine legislation.

3. In the case of multicellular organisms *novel genotype* refers to all or some of the cells that make up the organism.

4. This statement on the scope of work reviewed by the NBC applies to large- and small-scale work and the planned release of genetically manipulated organisms. The *Guidelines for Large-Scale Genetic Manipulation Work* and the *Guidelines for the Planned Release of Genetically Manipulated Organisms* need to be developed by each national biosafety committee as separate documents.

5. See, for example, appendixes 5.5 and 5.6 of GMAC's 1995 *Guidelines for Small-Scale Genetic Manipulation Work* for guidelines and practices developed by the NBC.

6. In some situations it may be desirable to work with volumes greater than 10 liters (for example, to establish procedures for downstream processing). If such work does not involve commercial production, applications to vary the 10 liter limit will be considered by the NBC on a case-by-case basis.

7. This section is adapted from material in the *Philippines National Biosafety Guidelines*. (Manila: Department of Science and Technology, 1986).

8. U.S. Government Department of Health and Human Services, *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).*

7. Guide to Good Laboratory Practice and Industrial Large-Scale Practice

The following examples of guidelines for good laboratory practice and good industrial large-scale practice are taken from the Organisation for Economic Co-operation and Development (OECD) study *Safety Considerations for Biotechnology*.[1]

Good Laboratory Practice

Human error, poor laboratory practice, and misuse of equipment cause the majority of laboratory accidents and related infections. This chapter provides a compendium of techniques designed to correct or minimize the most commonly reported accidents caused by these factors.

Techniques in the Use of Pipettes and Pipetting Aids

- Cotton-wool–plugged pipettes will reduce the possibility of contaminating the pipetting aid.
- Air should never be blown through a liquid containing infectious agents.
- Infectious material should not be mixed by alternate suction and expulsion through a pipette.
- No infectious material should be expelled forcibly from a pipette.
- To avoid the hazards of accidentally dropping infectious cultures from pipettes, a disinfectant-soaked cloth should be placed on the working surface and autoclaved after use.
- Mark-to-mark pipettes are preferable to other types because they do not require expulsion of the last drop.
- Fluids should be discharged down the inner wall of the tube or bottle or beneath the surface of the liquid in the container.
- Contaminated pipettes should be completely immersed in a suitable disinfectant before being autoclaved.
- A discard pan for pipettes should be placed within the biological safety cabinet, not outside it.
- A syringe fitted with a sharp hypodermic needle must not be used as a pipetting device. Blunt cannulas should be substituted for needles.

Techniques to Avoid Dispersal of Infectious Material

- A pipetting aid should always be used. Mouth pipetting should be prohibited.
- The circle of a microbiological loop should be completely closed and the arm not more than 6 centimeters long.
- When there is risk of spatter of infected material in a Bunsen flame, a micro-incinerator should be used. Plastic disposal loops are a safe alternative.
- Catalase tests should not be done on slides. Tube methods should be used or cover-glass methods used in an exhaust protective cabinet. Catalase tests may also conveniently be performed by touching a microhematocrit

capillary tube loaded with hydrogen peroxide on to the surface of a colony.
- Discarded specimens and cultures should be placed in leak-proof containers for disposal.
- Working areas must be cleaned with a suitable disinfectant when each work period is finished.
- Horizontal outflow cabinets (clean air work stations) are not microbiological safety cabinets and should not be used as such.

Techniques in the Use of Biological Safety Cabinets

- The use and limitations of cabinets must be explained to all potential users.
- The cabinet must never be used unless the fan is switched on and the air flow indicator is in the safe position.
- If it has an openable glass viewing panel, this must be raised when the cabinet is in use.
- Apparatus and materials must be kept to a minimum during operation.
- A Bunsen burner must not be used in the cabinet. The heat produced might distort the air flow and the filters might be burned. A micro-incinerator is permissible, but disposable plastic loops are preferable.
- All work must be done in the middle to the rear of the cabinet and be visible through the glass window.
- It must be understood that the cabinet will protect neither the hands nor the worker from gross spillage, breakage, or poor technique.
- The cabinet fan should be run for at least fifteen minutes after completion of work in the cabinet.

Techniques to Avoid Ingestion of Infectious Material

- Larger particles and droplets (>5 micrometers) released during microbiological manipulations settle rapidly on the bench surfaces and the hands of the operator. Hands should be washed frequently. Workers should avoid touching their mouth and eyes.
- Food and drink should not be stored or consumed in the laboratory.
- There should be no smoking or gum-chewing in the laboratory.
- Cosmetics should not be applied in the laboratory.

Techniques to Avoid Injection of Infectious Material

- Injection may result from accidents with hypodermic needles, Pasteur pipettes, and broken glass.
- Accidents with hypodermic needles can be reduced only by greater care and making less use of syringes and needles. If syringes must be used for measurement, blunt cannulas should be substituted for needles.
- Accidental inoculation with Pasteur pipettes and broken glass may be avoided only by greater personal care.

Techniques for the Separation of Serum

- Only properly instructed laboratory staff should be employed for this work.
- To prevent splashes and aerosols, good microbiological technique should be observed. Potentially infected fluids, including blood, should be pipetted carefully, not poured. Mouth pipetting must be forbidden.
- Pipettes should be discarded and completely submerged in hypochlorite or some other suitable disinfectant. They must remain in the disinfectant at least overnight before disposal.
- Discarded specimen tubes containing blood clots and the like should be put in suitable leak-proof containers (with the caps replaced) for autoclaving or incineration.
- A solution of sodium hypochlorite should be provided for cleaning splashes and spillage of blood and serum.

Techniques for the Use of the Centrifuge

GENERAL. The following precautions should be observed:
- Mechanical safety is a prerequisite in the use of clinical centrifuges.
- Infectious airborne particles may be ejected when centrifuges are used improperly. These particles travel at speeds too high to be captured and retained if a centrifuge is placed in a traditional Class I or Class II safety cabinet.

- The centrifuge should be operated according to the manufacturers' instructions.
- Good centrifuge technique and sealed centrifuge buckets offer adequate protection from microorganisms and agents in Risk Groups 3 and 4.

CENTRIFUGATION OF RISK GROUP 2 MICROORGANISMS, AGENTS, AND MATERIALS. The following precautions should be observed:

- Centrifuge buckets and trunions should be paired by weight and should be properly balanced with tubes in place.
- To avoid dislodging trunions and spilling the contents of the tubes, the motor should be started slowly and speed increased gradually.
- Centrifuges should be placed at such a level that workers of less than average height can see into the bowl to place the trunions correctly on the rotor.
- Centrifuge tubes and specimen containers to be used in the centrifuge should be made of thick-walled glass or plastic and should be inspected for defects before use.
- The interior of centrifuge bowls should be inspected daily for evidence of bad techniques, indicated by staining or soiling at the level of the rotor, and should be cleaned if necessary.
- Angle head should be used for microbiological work except in special high-speed centrifuges. With ordinary angle heads some fluid, even from capped tubes, may be ejected because of the geometry of the machine.
- Except in ultracentrifuges and with small prothrombin tubes, a space of at least 2 centimeters should be left between the level of fluid and the rim of each centrifuge tube. Tubes containing infectious material should be capped.

CENTRIFUGATION OF RISK GROUPS 3 AND 4 MICROORGANISMS, AGENTS, AND MATERIALS. The following precautions should be observed, in addition to those above:

- Centrifugation should be done in batches separate from other material.
- Centrifuge tubes or bottles should have screw caps and should be marked in a way agreed locally to indicate that the contents are in Risk Groups 3 and 4.
- Sealed centrifuge buckets (safety cups) should be used.
- The sealed buckets should be loaded, sealed, and opened in a biological safety cabinet.

Techniques for the Use of Homogenizers and Shakers

- Caps and cups or bottles should be sound and free from flaws or distortion. Caps should be well-fitting and gaskets must be in good condition.
- Aerosols containing infectious particles may escape from shakers and homogenizers between the cap and the vessel. A pressure builds up in the vessel during operation. Teflon homogenizers are recommended because glass homogenizers may break releasing infectious material and possibly wounding the operator.
- Machines should be covered when in use by a transparent plastic housing of strong construction. This should be disinfected after use. When possible, these machines, under their plastic covers, should be operated in a biological safety cabinet.
- After shaking or homogenization, all containers should be opened in a biological safety cabinet.
- Sonicators should be used inside biological safety cabinets. Hearing protection should be provided.

Techniques for the Use of Tissue Grinders Such as Griffith's Tubes and TenBroek Grinders

- Grinders should be held in a wad of absorbent material in a gloved hand when tissues are ground.
- They should be operated in a biological safety cabinet.

Techniques for Opening Ampoules that Contain Lyophilized Infectious Materials

Care should be taken when ampoules of freeze-dried materials are opened as the contents are in a vacuum and the sudden inrush of air may disperse the contents into the atmosphere. Ampoules should always be opened in safety cabinets.

The following procedure is recommended for opening ampoules:

- The outside of the ampoules should be decontaminated before use.
- A file mark is made on the tube near the middle of the cotton-wool plug.
- A red-hot glass rod is applied to the file mark to crack the glass.
- The top is removed gently and treated as contaminated material.
- The cotton-wool plug, if still above the contents of the ampoule, is removed with sterile forceps.
- Liquid for resuspension is added slowly to the ampoule to avoid frothing.

Storage of Ampoules that Contain Infectious Material

- Ampoules containing infectious material must never be immersed in the liquid phase of liquid nitrogen because cracked or imperfectly sealed ampoules may break or explode on removal.
- If very low temperatures are required, ampoules may be stored in the vapor phase only (that is, above the level of the liquid nitrogen).Whenever possible, infectious agents should be stored in mechanical deep freeze cabinets or on dry ice rather than in liquid nitrogen.
- The outside of ampoules stored in these ways should be decontaminated when they are removed from storage.

Techniques for Care, Use, and Operation of Refrigerators and Freezers

- Refrigerators, deep freeze, and dry-ice chests should be checked, cleaned out, and defrosted periodically to remove any ampoules or tubes containing hazardous materials that may have broken during storage. Rubber gloves should be worn during cleaning.
- All materials, especially infectious or toxic materials, stored in refrigerators or deep-freeze should be labeled with the scientific name of the material, the date stored, and the name of the individual storing the material.
- Do not store flammable solutions in non-explosion-proof refrigerators.

Good Industrial Large-Scale Practice

The OECD study on safety considerations for biotechnology worked out the principles for handling organisms with novel traits in industrial use. This report sets out the principles and criteria recommended for the safe use of such organisms in industry and is an appropriate basis for regulating this sector.

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

Recombinant DNA organisms that 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 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, a rDNA organism merits the designation of GILSP, the criteria elaborated by the OECD 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 nonpathogenic and without adverse consequences for the environment, are:

- 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)
- 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 that can be handled on a large scale under conditions of minimal controls and containment procedures will be:

- Those meeting the criteria of GILSP organisms
- Those other classes of organisms described above
- 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 must be followed.

Note

1. OECD, *Safety Considerations for Biotechnology.*

Glossary

aerosol	Suspension in air of finely dispersed solids or liquids.
Agrobacterium tumefaciens	A bacterium that infects plants and contains a **plasmid** that can be used to introduce foreign DNA into plant cells.
amphotropic retrovirus	A **retrovirus** that will grow in the cells from which it was isolated and also in cells from a wide range of other species.
amplify	To increase the number of copies of a gene or DNA sequence.
autoclave	A device in which materials are sterilized using steam under high pressure.
bacterium	A single-celled **prokaryotic** organism.
bacteriophage	A virus that infects bacteria; also called phage.
batch record	The record kept for each batch or run of a project which includes details of processing, maintenance, accidents, and disposal.
baculovirus	A group of viruses that infect insects and can be used as **vectors** to produce foreign proteins in insect cells.
biological safety cabinet, biosafety cabinet	Specially constructed cabinets that are designed to protect workers and the environment from dangerous agents, especially bacteria and viruses.
cell	The smallest structural unit of living organisms that is able to grow and reproduce independently.
chloroplast	The pigment (chlorophyll)-containing, photosynthesizing organelle of plants.
chromosome	A structure in the cell, consisting of DNA and proteins, that carries the organism's genes.
clone	As a noun: a group of genes, cells, or organisms derived from a common ancestor and genetically identical. As a verb: to generate replicas of DNA sequences or whole cells using genetic manipulation techniques.
conjugative plasmid	A **plasmid** that codes for its own transfer between bacterial cells by the process of conjugation (mating).

construct	As a noun: genetically manipulated DNA.
containment	Prevention of the spread of genetically manipulated organisms outside the laboratory. Physical containment is accomplished by the use of special procedures and facilities. Biological containment is accomplished by the use of particular strains of the organism that have a reduced ability to survive or reproduce in the open environment.
containment level	The degree of physical containment provided by a laboratory, which depends on the design of the facility, the equipment installed, and the procedures used. Physical containment levels are numbered from one to three, three being the highest level.
decontamination	Physical or chemical process that kills or removes unwanted infectious agents (does not necessarily result in sterility).
defective virus	A virus that is unable to reproduce in its host without the presence of another (helper) virus.
DNA	Deoxyribonucleic acid, the molecule that carries the genetic information for most organisms; consists of four bases and a sugar-phosphate backbone.
donor	The organism or cell from which DNA is derived for insertion into another organism (the host).
Drosophila	A genus of flies whose genetics have been extensively studied.
ecotropic retrovirus	A **retrovirus** that will grow in cells of the species from which it was isolated, but to a very limited or undetectable level in cells of other species.
effluent	Liquid (or gaseous) industrial waste.
embryo-rescue	The process in plant breeding whereby tissue from young embryo plants is excised and propagated in vitro for subsequent growth as differentiated plants.
***Escherichia coli* (*E. coli*)**	A bacterium that inhabits the intestinal tract of humans and other animals.
***Escherichia coli* K12**	A strain of *E. coli* that has been maintained in culture in laboratories for many years. It has lost the ability to colonize the intestinal tract of humans and animals, is well-characterized genetically, and is often used for molecular cloning work.
***Escherichia coli* B**	Another well-characterized laboratory strain of *E. coli*.
eukaryotic	Belonging to the group of organisms whose cells contain a true nucleus. Eukaryotic organisms include animals, plants, and fungi.
expression	Manifestation of a characteristic that is specified by a gene; often used to mean the production of a protein by a gene that has been inserted into a host organism.
fungi	Nonphotosynthetic eukaryotic organisms, including molds, that feed on organic matter.
fusion	Joining of the cell membranes of two cells to create a daughter cell that contains the genetic material from both parent cells.
GAP	Good agricultural principles.

gamete A reproductive (egg or sperm) cell.

GDP Good development principles.

gene A hereditary unit of nucleic acid that specifies the structure of a protein or RNA molecule.

gene therapy The replacement of a defective gene in a person or other animal suffering from a genetic disease.

genetic engineering See **genetic manipulation.**

genetic manipulation A technology used to alter the genetic material of living cells or organisms in order to make them capable of producing new substances or performing new functions.

genome The total genetic complement of a given organism.

genotype The genetic makeup of an organism, as distinguished from its physical appearance (the phenotype).

germline cells Gametes and the cells from which they are derived. The genetic material of germline cells, unlike that of **somatic cells**, can be passed to succeeding generations.

growth factor A protein that stimulates cell division when it binds to its specific cell-surface receptor.

helper virus A virus that, when used to infect cells already infected by a **defective virus**, enables the latter to multiply by supplying something the defective virus lacks.

HEPA filter High efficiency particulate air filter with trapping efficiency greater than 99.99 percent for particles of 0.3 micrometers in diameter.

HIV Human immunodeficiency virus (a retrovirus).

host A cell or organism into which foreign DNA is introduced to enable production of proteins or further quantities of the DNA.

host range For a virus, the range of species that can be infected by that virus.

host-vector system Combination of **host** and the **vector** used for introducing foreign DNA into the host.

hybridoma A hybrid cell used in production of monoclonal **antibodies** that is produced by fusing an antibody-producing cell (B lymphocyte) with a tumor cell.

in vitro Literally in glass; performed in a test tube or other laboratory apparatus.

in vivo In a living organism.

LD50 The dose of a toxin or infectious agent that will kill half of a population of organisms.

microorganism An organism that can be seen only with the aid of a microscope.

mitochondrion A self-reproducing organelle that occurs in the cytoplasm of all cells in most **eukaryotes**. *Plural* mitochondria.

monoclonal antibody An antibody that is derived from a single **clone** of **hybridoma** cells and recognizes only one antigenic site.

oncogene An activated (modified) cellular gene that causes normal cells to become cancerous.

oocyte	A cell that divides to form the female reproductive cell.
packaging	In the process of virus replication, the assembly of the components of the virus to form the complete virus particle.
pathogen	An organism that causes disease.
PCR	See **polymerase chain reaction.**
phage	See **bacteriophage.**
phenotype	The observable properties of an organism as distinguished from its genetic makeup (the genotype).
planned release	Intentional release of a genetically modified organism into the open environment.
plasmid	A small, self-replicating molecule of DNA that contains a specific origin of replication. Plasmids are often used as cloning **vectors**.
plasmid mobility	The rate at which the vector-insert could subsequently be transferred from the original recipient.
polymerase chain reaction	A technique for generating in vitro an increased quantity of a target segment of DNA.
prion	An infectious agent of unknown etiology that causes spongiform encephalopathies in humans and animals.
prokaryotic	Belonging to the group of microorganisms whose DNA is not enclosed within a nuclear membrane.
promoter	A DNA sequence, located in front of a gene, that controls expression of the gene. It is the sequence to which RNA polymerase binds to initiate transcription.
protein	A molecule composed of amino acids.
protoplast	A plant or bacterial cell that has had the outer cell wall removed.
receptor	Cell-surface protein to which molecules, such as hormones and growth factors, bind to exert their effects on the cell, or to which viruses bind to gain entry to the cell.
recombinant	Organisms, cells, viruses, and the like that contain **recombinant DNA**.
recombinant DNA	DNA formed by joining in vitro segments of DNA from different organisms.
recombination	The occurrence or production of progeny with combinations of genes other than those that occurred in the parents.
replication	Reproduction.
resistance marker	A gene that confers antibiotic resistance to the recipient microorganism.
retroviral vector	A **retrovirus** that is used to introduce foreign DNA into animal cells, usually by replacing part of the viral genome with the foreign DNA of interest.
retrovirus	A virus that uses the enzyme reverse transcriptase to copy its RNA genome into DNA, which then integrates into the host cell genome.
RNA	Ribonucleic acid, a molecule similar to DNA whose functions include decoding the instructions for protein synthesis that are carried by the genes; comprises the genetic material of some viruses.

sharps — Sharp laboratory items, such as syringe needles, scalpels and razor blades, and broken glass.

shotgun cloning — The production of a large random collection of cloned fragments of the DNA of an organism from which genes of interest can later be selected.

somatic cell — Any cell of a multicellular organism other than **germline cells**.

SOP — Standard operating procedure.

sterilization — Act or process that kills or removes all infectious agents; applied particularly to bacteria and molds, their spores, and viruses.

taxonomy — The study of the classification of living things.

Ti plasmid — A large plasmid of the bacterium ***Agrobacterium tumefaciens*** that carries genes for tumor induction in some plants. A disarmed form of the plasmid that lacks the tumor-inducing genes is often used as a vector to introduce foreign DNA into plant cells.

tissue culture — In vitro growth of tissue cells in nutrient medium.

toxin — A poisonous substance, produced mainly by microorganisms but also by some fungi, plants, and animals.

transgenic (organism) — An organism whose cells, including the germline cells, contain foreign DNA; transgenic animals are produced by the insertion of the foreign DNA into the newly fertilized egg or embryo.

tumor suppressor gene (or anti-oncogene) — A gene that encodes a protein thought to be necessary for the controlled growth of normal cells. When the gene is functionally inactivated by either deletion or mutation, the cell exhibits unregulated growth resulting in neoplasia.

vector — A self-replicating agent (for example, a plasmid or virus) used to transfer foreign DNA into a host cell.

viroid — A disease-causing agent of plants that is smaller than a virus and consists of a naked RNA molecule.

virulence — Ability of an organism to cause disease.

virus — A submicroscopic infectious particle, containing genetic material (DNA or RNA) and protein, which can replicate only within the cell of an organism (plant, animal, or bacteria).

xenotropic retrovirus — A **retrovirus** that is endogenous to a species but cannot replicate well in that species, generally because of a receptor block. Xenotropic retroviruses tend to have a wide range for replication in cells of heterologous species.

zygote — The cell produced by the union of the male and female gametes.

Bibliography

Agriculture Canada. 1994. *Guidelines for Regulations of Veterinary Biologics.* Ottawa: Agriculture Canada.

Committee on the Scientific Evaluation of the Introduction of Genetically Modified Microorganisms and Plants into the Environment, National Research Council. 1989. *Field Testing Genetically Modified Organisms: Framework for Decisions.* Washington, D.C.: National Academy Press.

Department of Science and Technology. 1986. *Philippines National Biosafety Guidelines.* Manila: Department of Science and Technology.

Genetic Manipulation Advisory Committee (GMAC). 1993, 1996. *Guidelines for the Planned Release of Genetically Manipulated Organisms.* Canberra: GMAC.

———. 1994. *Guidelines for Large-Scale Genetic Manipulation Work.* Canberra: GMAC.

———. 1995. *Guidelines for Small-Scale Genetic Manipulation Work.* Canberra: GMAC.

Inter-American Institute for Cooperation on Agriculture (IICA). 1991. *Guidelines for the Release into the Environment of Genetically Modified Organisms.* San Jose, Costa Rica: IICA.

Office of the Federal Register, National Archives and Records Administration. 1995. *Codes of Federal Regulations. Agriculture, 7, parts 300 to 399.* Revised as of January 1. Washington, D.C.: Government Printing Office.

Office of Technology Assessment of the U.S. Congress. 1988. *New Developments in Biotechnology, Three Field Testing Engineered Organisms: Genetic and Ecological Issues.* 1988. Washington, D.C.: Government Printing Office. Reprinted by Technomic Publishing Co., Lancaster, Pa.

Organisation for Economic Co-operation and Development (OECD). 1992a. *Biotechnology, Agriculture and Food.* Paris: OECD.

———. 1992b. *Safety Considerations for Biotechnology.* Paris: OECD.

———. 1993a. *Field Releases of Transgenic Plants, 1986-1992, an Analysis.* Paris: OECD.

———. 1993b. *Safety Considerations for Biotechnology: Scale-Up of Crop Plants.* Paris: OECD.

———. 1993c. *Safety Evaluation of Foods Derived by Modern Biotechnology: Concepts and Principles.* Paris: OECD.

———. 1993d. *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology.* Paris: OECD.

Persley, Gabrielle J., ed. 1990a. *Agricultural Biotechnology: Opportunities for International Development.* Wallingford, United Kingdom: CAB International.

———. 1990b. *Beyond Mendel's Garden: Biotechnology in the Service of World Agriculture.* Wallingford, United Kingdom: CAB International.

Persley, Gabrielle J., Luther V. Giddings, and Calestous Juma. 1992. *Biosafety: The Safe Application of Biotechnology in Agriculture and the Environment.* The Hague: International Service for National Agricultural Research.

Recombinant DNA Monitoring Committee of the Australian Government. May 1987. *Procedures for Assessment of the Planned Release of Recombinant DNA Organisms.* Canberra: Australian Government Publishing Service.

Tiedje, J. M., R. K. Colwell, Y. L. Grossman, R. E. Hadson, R. E. Linski, R. N. Mack, and P. J. Regal. 1989. "The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations." *Ecology* 70: 298–315.

United Nations Industrial Development Organization (UNIDO). 1991. *Voluntary Code of Conduct for the Release of Organisms into the Environment.* Vienna: UNIDO.

———. 1995. *Environmentally Sound Management of Biotechnology.* Vienna: UNIDO.

United Nations Environment Program (UNEP). 1995 forthcoming. *International Technical Guidelines for Safety in Biotechnology.* Nairobi: UNEP.

U.S. Department of Agriculture/Animal and Plant Health Inspection Service. 1995. *Genetically Engineered Organisms and Products; Simplification of Requirements and Procedures for Genetically Engineered Organisms.* Federal Register 60, No. 162, 43,567–73. Washington, D.C.: Government Printing Office.

U.S. Department of Health and Human Services. 1994. *Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).* Rockville, Md.: National Institutes of Health.

World Health Organization (WHO). 1993. *Laboratory Biosafety Manual.* Geneva: WHO.

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